Characterization of Aquatic Nonindigenous Species for Department of Defense Vessels: Bacteria, Algae, and Small Microfauna (< 80 μm) in Ballast Water

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Approved for public release
This study characterized the bacteria, phytoplankton, and small microzooplankton (< 80 µm) in ballast tanks of DoD vessels operating from harbors on the U.S. East and West Coasts. From Sept. 2002-July 2004, 62 ballast tanks were sampled aboard 28 ships operated by the MSC and MARAD. A high percentage of the tanks (94%) had adequate records to determine the source locations and age of the ballast water, and 90% had had ballast exchange with open-ocean waters. Sources of variability in microbiota abundances were evaluated within and among ships that used different ballasting practices; an electronic Atlas of Phytoplankton Species was created; a database was designed to assist in risk analysis of microflora and microfauna species introductions by DoD ships; and laboratory-bench-scale experiments were conducted to examine the effectiveness of heat treatment on survival of selected species. The data were used to make recommendations to improve ballast water management practices, and to further advance understanding about the potential for introductions of aquatic invasive microbiota by DoD ships.

Nonindigenous species, ballast water, bacteria, phytoplankton, microzooplankton

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Administrative Information

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1. Executive Summary

- Invasions of nonindigenous species continue to present a significant threat to marine environments worldwide. Invasions can adversely affect regional biodiversity, public health, energy and food supplies, and local economies. Currently the movement of ballast water is regarded as the most important mechanism for transfer of aquatic nonindigenous species. Although many aquatic microflora and microfauna are known to have a cosmopolitan distribution, ballast water exchange practices can alter the abundances of harmful species and set up conditions where previously rare populations proliferate.

- The overall goal of this study was to expand on research on the contents of ballast water of U.S. Navy vessels conducted by Ruiz et al. (1999a), by characterizing the bacteria, algae, and microzooplankton (maximum dimension < 80 µm) inhabiting ballast tanks of Department of Defense (DoD) vessels operating from harbors on the both coasts of the United States. The specific objectives were: (1) Analysis of the types and quantities of phytoplankton, microzooplankton, and bacteria in ballast water of DoD vessels, including potentially harmful taxa and considering vessel travel routes in data interpretation; (2) Focused assessment on known harmful microbes (selected taxa that may pose environmental or public health risks); (3) Assessment of intra-vessel variability, i.e. the variability among ballast tanks from one vessel; (4) Experimental determination of impacts of heat treatments on harmful algal species; (5) Design of an Atlas of phytoplankton species identified from the ballast tanks; and (6) Construction of a relational database for data organization, storage, and export for statistical analyses and for use in risk assessment.

- Between September 2002 and July 2004, 62 ballast tanks were sampled aboard 28 ships, including 16 ships at 9 ports on the U.S. West Coast, and 12 ships at 4 ports on the U.S. East Coast. Vessels were operated by the Military Sealift Command (MSC) and the Maritime Administration (MARAD), and included 11 fleet oilers, 15 roll-on/roll-off carriers, 1 container ship, and 1 lighter. Ballast tanks were screened for suitability of sampling using a decision tree developed in collaboration with SERDP project CP-1245. The tank water had been held for 2-176 days.

- Physical and chemical conditions in the ballast tank waters were characterized in situ at 0.5-m, 1-m, 2-m, 5-m, and 10-m depths. Samples were also collected in duplicate for nutrient and chlorophyll a analyses, and for characterizing assemblages of bacteria, phytoplankton, and microzooplankton (maximum dimension < 80 µm). Phytoplankton and microzooplankton were quantified from composite water-column samples using published standard techniques, and were identified to the lowest possible taxonomic level. Bacteria were quantified using flow cytometry, and harmful taxa were identified using taxa-specific molecular probes. Taxa were evaluated for their geographic distribution based on historical records, and were categorized as cosmopolitan if distributed across estuarine/coastal and oceanic waters.

- A total of 280 analyses were conducted for each of the following parameters: TKN, NO₃⁻, TN, TP, TOC, and chlorophyll a concentrations. In addition, 185 preserved phytoplankton samples and 122 preserved microzooplankton samples were analyzed for taxonomic composition. A total of 372 phytoplankton microcultures (6 per tank) and 124 zooplankton
microcultures (2 per tank) were prepared from fresh (unpreserved) sub-samples, and examined weekly for 8 weeks. Cultured species were used to assist in species identifications. Total bacteria abundance was assessed from 183 samples using flow cytometry; molecular screening for bacterial pathogens was conducted on 62 samples (1 per tank); and 62 samples were plated in triplicate for detection of selected *Vibrio* species.

- In separate project efforts, (i) 18 laboratory bench-scale experiments (each 24 hours, simulating the duration of intra-coastal voyages) were conducted using representative cultured algal and microzooplankton taxa, to examine the effectiveness of various durations and levels of heat treatment on survival of selected species in ballast water samples; (ii) an Atlas of phytoplankton species identified from the ballast water samples was created; and (iii) a database was constructed in Microsoft ACCESS 2000, which allows any given biological observation within the database to be mapped in terms of its voyage-specific ballasting history to facilitate risk determinations.

- Of the 62 tanks sampled, a high percentage (94%) had adequate records to enable determination of the source locales and age of the ballast water. Most tanks (~90%) had undergone some form of ballast exchange, and contained at least a portion of ballast water from an open-ocean source by the time ships entered a U.S. port. The extent of the exchange was variable; for 37% of tanks we could not determine the full extent of exchange from ships’ logs, and for ~10% of tanks we could not determine whether management practices had been applied. Two ballast tanks sampled had not undergone exchange or treatment, but the water in these tanks had been loaded in the open ocean so that no management was required.

- Most ballast water tanks were not depth-stratified, indicated by uniform temperature, salinity, pH, and dissolved oxygen. Turbidity and chlorophyll *a* levels were negligible, and nutrient concentrations were low to moderate. No significant relationships were detected between physical/chemical parameters and phytoplankton, microzooplankton, or bacterial abundances, except for a statistically significant positive relationship between centric diatom densities and nitrate concentrations.

- A total of 100 phytoplankton species were identified from the ballast tanks, including 19 potentially harmful taxa defined as capable of causing disease or death of humans or beneficial aquatic life. Nearly all species are known to have a broad geographic range; most are cosmopolitan, and all have previously been reported from coastal U.S. waters. Phytoplankton assemblages were dominated by chain-forming diatoms and dinoflagellates. Species diversity (richness) was higher in ballast tanks with coastal water, and in tanks containing Atlantic or Pacific Ocean source waters rather than Indian Ocean water. Diversity generally decreased with ballast water age.

- Phytoplankton abundance was highly variable, with viable organisms comprising about half of the total cells. Median phytoplankton abundance was comparable in tanks with Atlantic and Pacific Ocean source waters, whereas abundance in ballast tanks with Indian Ocean source waters was ~10-fold lower. Abundance was significantly higher in tanks with recently added coastal water than in tanks without coastal sources, but highly variable in waters held less than 30 days. Tanks with ballast water age more than 33 days did not produce culturable phytoplankton.
Microzooplankton assemblages were dominated by tintinnid ciliates with cosmopolitan distribution, which were found in 84% of the ballast tanks. Nematodes and copepod nauplii were subdominant. Other groups represented were amoebae, Cladocerans, foraminiferans, molluscan larvae, radiolarians, rotifers, turbellarians, invertebrate eggs, and other invertebrate larvae. Eggs and larval stages could not be identified to species. Microzooplankton abundance was highly variable, and comparable in tanks with Atlantic, Pacific, and Indian Ocean source waters.

Bacterial abundances were surprisingly consistent among ballast tanks. Abundance was significantly different in ballast tanks with Atlantic versus Pacific Ocean water, but was unrelated to vessel type, exchange status, age of water, environmental conditions measured, or other factors. At least 1 of 4 pathogenic eubacteria (*Listeria monocytogenes, Escherichia coli, Mycobacterium spp., Pseudomonas aeruginosa*) was detected in 48% of the ballast tanks. Toxigenic strains of *Vibrio cholerae* were not detected in any tanks.

There were no similarities in abundance patterns among phytoplankton, microzooplankton, and bacterial assemblages. Moreover, despite a general pattern of reduction in abundance of the three microbiota groupings with ballast age, regression analyses indicated that age accounted for negligible variation in abundance. Thus, microbiota abundances are more strongly influenced by other factors, possibly including spatial/temporal variations in microbiota during ballasting, differential effects of ballast water management, and variation in sources of stress factors among ballast tanks and ships.

Sources of variability in abundances of phytoplankton, microzooplankton, and bacteria were evaluated within and among ships that used different ballasting practices. For ships with tanks of similar ballasting history, the largest source of variation was among ships. For ships with tanks of differing ballasting histories, and for all ships/tanks considered collectively, the largest source of variation in biota abundances was between or among ballast tanks within ships. Duplicate samples per tank contributed little to the variation in the data. Although tanks with similar ballasting history yielded similar abundances of microbiota, phytoplankton species diversity sometimes differed markedly between paired tanks, and Holm et al.’s (2005) companion study reported that mean abundances of macrozooplankton were not significantly correlated between paired tanks. Thus, significant differences in biota abundances even in paired tanks can occur, likely related to environmental factors that diverge in one tank versus another over time.

In the heat treatment experiments, except for a toxigenic raphidophyte flagellate (*Heterosigma akashiwo*, 36°C), all phytoplankton taxa tested were killed at 34°C or less. Based on previous research, this temperature can be attained in ballast tanks, at least for some ship designs, by using waste engine heat.

The Phytoplankton Atlas was designed to include micrographs or drawings of phytoplankton species that were identified, using light or scanning electron microscopy, from the ballast tanks assessed in this project. Each species description also includes taxonomic information, harmful impacts, the geographic distribution, source waters in this project if known, and key references.
The ACCESS database was constructed to include all chemical, biological, vessel, and ballast management data from this project, and it is also linked to the Phytoplankton Atlas. It was designed to be useful in risk determinations; for example, the mapping coupled with environmental data may indicate that a given ship location at a given time of year represents a high risk for uptake of water with a diverse, abundant flora and fauna.

Overall, the data from this study have important implications in efforts to assess how ballasting practices can affect the potential for introductions of aquatic microbiota species including bioinvasive taxa. Further actions are recommended as follows:

♦ Reliable estimates of microbiota abundances at a vessel scale will require sampling multiple ballast tanks that encompass the variation in the tank histories. Efforts to obtain replicate samples from individual tanks should be minimized in favor of sampling additional tanks or additional ships. For ships with tanks containing ballast water of similar history, a single tank can often serve as a reasonable predictor of microbiota for all tanks, but multiple tanks should be sampled if possible.

♦ Considering organisms with maximum dimension > 50 µm, quantities of viable phytoplankton in 47% of the ballast tanks and microzooplankton in 31-39% of the ballast tanks exceeded proposed standards. The data suggest that development, evaluation, and adoption of treatment technologies such as heat treatment, or alternative management strategies, will be necessary to enable DoD vessels to comply with proposed standards for ensuring safe and environmentally sound operations.

♦ Amphibious vessels of the U.S. Navy carry a major proportion of the ballast water transported by DoD ships, but could not be sampled because of operational commitments. Characterization of the microbiota in their ballast tanks is recommended, when operations allow. Further study is also needed to assess the effects of ballast water exchange on coastal microbiota, which will be possible when ships holding coastal water that has not been exchanged (not available in this study) can be included for comparison with tanks containing exchanged water.

♦ To complete the description of ballast tank microbiota assemblages of DoD ships, two additional efforts are recommended. First, this study indicates that operations of DoD vessels may present a low risk for transfer of pathogenic bacteria such as toxigenic strains of *Vibrio cholerae*. Additional research encompassing a broader suite of microbial pathogens (bacteria, viruses, and protozoans) would help to strengthen insights about the potential for transport of pathogenic microorganisms. Second, research should be undertaken to assess the importance of DoD ship-fouling for introducing aquatic non-indigenous species.

♦ To minimize the risk of introduction of harmful microbiota, DoD vessels should be encouraged to conduct ballast water exchange as far offshore as possible. To improve compliance with applicable instructions or regulations, DoD vessels should be encouraged to maintain detailed records of their ballasting activities as standard operating procedure.
2. Introduction

2a. Background

The discovery that marine species could be transported in the ballast water of modern steel ships was first reported by Ostenfeld (1908), but the process has been ongoing since exploration and trading activities by much earlier civilizations such as the Phoenicians and Vikings. In the first half of the 1900s, scientific reports were published sporadically about introductions of non-native species via ballast water, but scientific understanding and public concern about this environmental problem have increased especially within the past two decades (Hay et al. 1996). The long-distance dispersal of marine microorganisms, plants, and animals in the ballast water of ships constitutes a potential environmental problem because, although the oceans are continuous, coastal marine life is geographically discontinuous. For example, many marine species found in along U.S. coasts do not occur in European or South American waters (Ruiz et al. 1997). The fact that many microflora and microfauna species presently have cosmopolitan distribution may reflect a long history of global transport by ships, migratory birds and animals, winds, and other mechanisms. Nevertheless, based upon a few well-studied waters such as Chesapeake Bay, San Francisco Bay, and the Hudson Estuary, the effects of human activities in nonindigenous species introductions and resulting economic and ecological impacts are so major that entire ecosystems have been completely changed. The San Francisco Bay ecosystem, for example, now hosts ~230 species of nonindigenous plants, animals, and microorganisms, and in some areas all of the common species are nonindigenous, with introductions continuing at an estimated rate of one new species every 14 weeks (Cohen and Carlton 1995, 1998). Chesapeake Bay and the Hudson River Estuary have sustained ~200 and ~120 known or possible introductions, respectively (Ruiz et al. 1997, 1999b; Miller 2000).

Undesirable introduced aquatic species typically overgrow systems because environmental conditions are conducive while natural predators are often lacking. They dramatically alter ecosystem structure and diversity, typically greatly reducing or eliminating desirable species; they can also adversely affect public health, energy and food supplies, and local economies, costing billions of dollars in the U.S. annually (e.g. Rayl 1999, Pimentel et al. 2000). Of the introductions that have occurred in the last 20 to 30 years, most are believed to have occurred via ballast water (Carlton 1985, Carlton and Geller 1993, Ruiz et al. 1997, Cohen 1998, Cohen and Carlton 1998). Thus, the movement of ballast water (or “clean ballast”) and ballast tank sediments is regarded as the most important mechanism at present for transfer of aquatic nonindigenous species (Ruiz et al. 1997, 2000). Considering the enormous potential for nonindigenous species introductions, it is remarkable that relatively few apparently have been successful: For example, about 1 billion organisms, including ~280 species, were estimated to be released from one bulk carrier cargo in deballasting activities (Smith et al. 1996).

Scientific understanding about nonindigenous species introductions via ballast water mostly has been based upon studies of commercial vessels. The U.S. Department of Defense (DoD) operates a large fleet, as well. Moreover, DoD vessels must cover all oceans and require unrestricted access to national and international waters to facilitate domestic commerce, and to protect and project national interests (United States Department of Defense [DoD] 2000). This project was designed to strengthen understanding about the extent to which DoD vessels may be introducing non-indigenous, harmful species to our nation’s waters. This research was therefore
targeted to assist in the enhancement of DoD mission readiness, by reducing the environmental risks of transport of non-indigenous organisms by DoD vessels while simultaneously enhancing public safety and health protection (U.S. DoD 2000). As international attention on these matters increases, this information will aid in determining preemptive mitigation strategies to help maintain international vessel access for protection and projection of US interests.

2b. Regulatory Drivers

This section was largely taken or modified from the companion study of Holm et al. (2005), with permission. Ballast water exchange is defined as replacement of ballast water taken up in coastal areas with water from the open ocean, either by completely deballasting and reballasting, or by continuously flushing the ballast tanks (National Research Council 1996). The goal is to discard from the ballast tanks coastal organisms that were taken up in the port of departure. Oceanic organisms, from an environment characterized by more constant, dependable salinity, temperature, and low pollutant concentrations, generally do not survive when released into the coastal or fresh waters of the destination port (National Research Council 1996). The exchange process can be highly variable in affecting abundances of bacteria, phytoplankton, and zooplankton in the ballast water (e.g. Smith et al. 1996, Wonham et al. 2001, Drake et al. 2002).

Although ballast water exchange is, at present, the only widely used measure for mitigating ballast water transport (e.g. International Maritime Organization [IMO] 1998, 2001, 2004; National Invasive Species Act [NISA] 1996; U.S. Coast Guard [USCG] 2002), there are known shortcomings associated with exchange including lack of applicability to intra-coastal voyages; difficulty in assessing trans-vessel efficacy; lengthy time required for exchange; hull stress; and the potential for large amounts of inocula to remain in tanks (e.g. Carlton et al. 1995, Hallegraeff 1998, Zhang and Dickman 1999). These issues likely will prevent use of ballast water exchange as the primary means of controlling ballast water-related species invasions in the long-term.

As mentioned, DoD vessels require unlimited access to national and international waters in order to successfully execute U.S. defense and foreign policy. To be afforded such access, DoD vessels must be compliant with environmental regulations that may otherwise restrict operations. Regulatory pressure to control introductions of invasive, nonindigenous species via ballast water continues to increase at state, federal, and international levels (Cohen 1998, U.S. DoD 2000), including from DoD ships. For example, the Uniform National Discharge Standards process (U.S. EPA 1999) identified non-indigenous species as a potential constituent of concern for discharges from DoD vessels. The following information summarizes related major legislation and regulations:

- **Nonindigenous Aquatic Nuisance Prevention and Control Act (NANPCA, P.L. 101-646)** – was largely originated in response to the zebra mussel invasion of the Great Lakes in the 1980s, and focused on the Great Lakes. Goals were to prevent unintentional introductions of nonindigenous species transported in ballast water; to develop methods and plans to monitor and control introductions arising from vectors other than ballast water; to initiate and coordinate research on management and control options; and to evaluate ecological and economic impacts of aquatic nonindigenous species invasions. Vessels entering the Great Lakes from outside the Exclusive Economic Zone (EEZ) were required to exchange their clean ballast outside the EEZ,
to exchange their ballast at alternative sites within the EEZ where such exchange would not
result in the introduction of invasive species, or to employ alternative management options or
treatment technologies that were evaluated as similar to ballast water exchange in effectiveness
of species removal. For other United States waters, this act required voluntary guidelines
(including ballast exchange or comparable treatment, and records maintained on ballast water
management) by October 1997 for vessels carrying ballast water into this country from outside
the EEZ. The voluntary regulations became mandatory in September 2004 (33 CFR Part
151.2035). DoD vessels were exempted, except that they were required to implement some form
of management program for ballast water that minimized nonindigenous species introductions.

- National Invasive Species Act (NISA, P.L. 104-332; focus, the Great Lakes, Hudson River,
and Alaska’s North Slope) – This act required vessels that used ports in the Great Lakes or the
Hudson River, and vessels that exported oil from the North Slope of Alaska, to exchange their
ballast water in the open ocean (= waters more than 200 nautical miles [n.m.] offshore, or greater
than 2000 meters in depth). It also required all vessels that brought ballast water into other
United States ports from outside the EEZ to report information on the volumes of ballast water
carried, and how it had been managed. Voluntary actions were also requested, including removal
of ballast tank sediments and removal of fouling organisms from hulls and seachests,
management of the timing and location of ballasting operations, and open-ocean ocean exchange
of ballast water or treatment by other procedures that had been approved by the U.S. Coast
Guard. The voluntary regulations became requirements in September 2004 (33 CFR Part
151.2035): Vessels carrying ballast water that originated outside the EEZ and less than 200 n.m.
from shore must be completely exchanged in an open-ocean area greater than 200 n.m. from
shore treated or otherwise managed using a substitute method approved by the U.S. Coast Guard.
DoD vessels were exempted, except that they were required to implement some form of
management program.

DoD ballast water management plans accordingly were developed by the U.S. Navy (NENRP
1994) and the U.S. Army. The U.S. Navy’s (1994) ballast water management program requires
(as of October 2002) surface ships that had loaded ballast water in polluted areas or within 3 n.m.
from shore to complete a 200% exchange of that ballast water at a distance greater than 12 n.m.
from shore, by twice emptying and refilling the affected tanks. Required records in the ship’s
engineering log included the volume taken on, the position of the ship during deballasting, and
whether an exchange was completed. U.S. Army vessels follow Army Regulation 56-9, Surface
Transportation – Watercraft, Section 1-5 (Marine Policies), which requires vessels to be operated
in an environmentally sustainable manner, and to comply with the regulations in 33 CFR when
possible.

- National Defense Authorization Act – This act created the Uniform National Discharge
Standards (UNDS) program in 1996 by amending the Clean Water Act. The UNDS program
requires the DoD and the U.S. Environmental Protection Agency (EPA) to develop uniform
standards for non-sewage discharges from DoD vessels during routine operations. As a first
phase of this process, UNDS (1999) identified ballast water (Clean Ballast) from amphibious
assault ships, transport docs, and submarines operated by the U.S. Navy, fleet auxiliaries (e.g.
oilers) and special mission craft operated by the Military Sealift Command (MSC), and lighters
of the U.S. Army, as a discharge that could potentially introduce nonindigenous species (EPA
1999). Thus, in the future, the treatment and discharge of ballast water by some DoD vessels will
be regulated by the UNDS program.
Prospective regulations (e.g. international regulations proposed by the International Maritime Organization [IMO] 2004; and United States Senate Bill S.363, the Ballast Water Management Act of 2005) may limit the use of exchange as a treatment method for ballast water of commercial and DoD vessels. These regulations effectively would lead to replacement of ballast water exchange with more efficient means of species removal, by setting strict discharge standards based on the abundance or concentration of organisms (coastal or oceanic) in the clean ballast. For example, allowable concentrations of indicator bacteria would be < 1 colony forming unit (cfu) of toxigenic *Vibrio cholerae* per 100 mL or < 1 cfu of toxigenic *V. cholerae* per gram wet weight of zooplankton (to which *V. cholerae* can attach); < 126 cfu (S.363) or < 250 cfu (IMO) of *Escherichia coli* per 100 mL; and < 33 cfu (S.262) or < 100 cfu (IMO) of intestinal enterococci per 100 mL. Ships built before 2009 would be required to attain the mandated discharge standards by 2014-2016; ships built after 2009 would have to meet the standards at launch or by 2012, depending on the ballast capacity.

These proposed discharge standards would directly affect the operations of the cargo vessels and tankers maintained by the Maritime Administration (MARAD) for the DoD, that are not regulated by the UNDS program, and may also affect ships that fall under the UNDS program. Both S.363 and the IMO convention exempt military vessels, yet require that military vessels manage their ballast water to basically comply with the discharge standards. Thus, S.363 and the IMO’s regulations, if adopted, could potentially limit the operations of DoD vessels unless effective management or treatment measures can be implemented to meet the discharge requirements. Development of such measures requires knowledge of the abundance and diversity of organisms carried by DoD vessels; the spatial and temporal variability of nonindigenous species transport by DoD vessels; and how nonindigenous species transport is affected by present management practices.

2c. Previous Data on Species Transport by Ballast Water of DoD Vessels

Information on ballast water transport of organisms by DoD vessels previously was unavailable, with exception of one study on naval vessels in Chesapeake Bay (October 1994 – September 1996; Ruiz et al. 1999a). About 75 naval vessels entered the Bay during each quarter of the study, mostly consisting of amphibious or supply ships returning from the northwestern or west-central Atlantic Ocean. Assessment of 35 vessels indicated that they carried, on average, only ~20% of their maximum ballast water capacity. The potential contribution of DoD ships to introductions of nonindigenous organisms was minor: it was estimated that the naval vessels had transported only about 750,000 metric tonnes of ballast water to the Bay, or ~1% of the volume of ballast water discharged by commercial vessels. Based on analysis of ballast water samples from 18 ships, naval vessels contributed an order of magnitude lower abundance of macroplankton than did commercial vessels, apparently from an unknown source of high mortality (>80% of the macro-plankton, from comparison of 2 naval and 2 commercial vessels) in ballast tanks of naval vessels.

2d. Project Goals and Technical Objectives

The overall goal of this project (CP-1244) was to strengthen the database on transport of aquatic organisms by the ballast water of representative fleet oilers and cargo vessels operated by the Military Sealift Command [MSC] (Figure 1) and lighters operated by the U.S. Army. It should
be noted that naval amphibious vessels can carry large volumes of ballast water (UNDS 1999), but were not available for the study due to operational commitments. We emphasized bacteria, phytoplankton, and microzooplankton (maximum dimension, 20-80 µm). As mentioned, we partnered with another project (CP-1245, Holm et al. 2005, “Characterization of Aquatic Nonindigenous Species for Department of Defense Vessels: Large (> 80 µm) Zooplankton in Ballast Water”, Naval Surface Warfare Center, Carderock Division) that focused on the evaluation of multiple sampling techniques and analysis of macrozooplankton. The abundance and diversity of microorganisms in ballast water likely varies depending on the type of vessel, the departure/destination ports, season, and ballast water management practices. We implemented a sampling program designed to span this variation, and sampled vessels at destination ports on the East and West Coasts during each season. We additionally assessed relationships between microbial community abundance and diversity, and variables such as origin, ballast tank architecture, and management practices. Moreover, selected harmful species detected during these evaluations were exposed to an experimental heat regimen to assess the potential of waste engine heat as means of treatment. The specific technical objectives were as follows:

Objective 1 – Analysis of the types and quantities of phytoplankton, microzooplankton, and bacteria in DoD vessel ballast water. We developed detailed sampling and handling procedures to analyze ballast water. The data were interpreted considering vessel travel routes to determine the types and concentrations of organisms arriving from ports within and outside the U.S., for use in assessing risks associated with various ballast water management variables.

Objective 2 – Focused assessment on known harmful microbes. We screened for selected taxa of bacteria and microalgae transported by DoD vessels that may pose environmental or public health risks (for example, the presence of *Vibrio cholerae* and certain other pathogenic *Vibrio* spp.; and toxigenic dinoflagellates, diatoms, raphidophyte, and cyanobacteria species; Hallegraeff 1993, Burkholder 1998, Hurst et al. 1997).

Objective 3 – Assessment of intra-vessel variability. We intensively sampled all eight tanks of a vessel to assess the variability across tanks within a given vessel, and 4 tanks of a second vessel. The data allowed us to better determine the potential risk range that a given vessel (delivery device) may pose to a receiving system.

Objective 4 – Design of an Atlas of the phytoplankton species identified from the ballast tanks, including images, taxonomic information, geographic distribution, and source waters in this study;

Objective 5 – Construction of a relational database for data organization, storage, and export for statistical analyses and for use in risk assessments. We organized the data matrix (both ship- and sample-related), using a series of linked-data entry screens for ease of entry, viewing, and export of data for analysis and application. The database was linked to the Phytoplankton Atlas and can also be linked to provide data for GIS mapping to facilitate risk assessment.
Objective 6 – Determination of impacts of heat treatments on harmful species. We tested the effectiveness of various durations and levels of heat treatment on survival of selected phytoplankton and zooplankton species in ballast water samples. The basis for this objective was research by consultant Hallegraeff, who helped to develop a cost-effective heating technique using waste heat from the ships’ engines to kill many unwanted organisms such as toxic dinoflagellates and certain noxious microfauna in ballast water affecting Australian coasts (Hallegraeff 1998, Rigby et al. 1999).

3. Materials and Methods

3a. Operational Definitions

Potentially harmful phytoplankton or bacteria included taxa with strains that have been known to cause disease or death in humans or beneficial aquatic life (e.g. Table 1).

Table 1. Effects of harmful phytoplankton (here, including heterotrophic taxa) on humans and beneficial aquatic life (following Burkholder 1998, Shumway et al. 2003).

<table>
<thead>
<tr>
<th>Organisms Affected</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct Pathogenicity</td>
<td>Adverse impacts on metabolism, survival, recruitment; promotion of disease</td>
</tr>
<tr>
<td>Shellfish</td>
<td>Depressed feeding, reduced valve closure (increased vulnerability to predators); low recruitment; physical attack by some heterotrophic harmful taxa; death</td>
</tr>
<tr>
<td>Finfish</td>
<td>Respiratory distress; lethargia alternating with erratic behavior/spasms and other neurological signs; hemorrhaging (gills, liver, etc.); lesions and damaged epidermis; diseased organs, physical attack by some heterotrophic harmful taxa; mechanical abrasion of gill tissues; death</td>
</tr>
<tr>
<td>Aquatic mammals</td>
<td>Respiratory distress, lethargia, depressed feeding, depressed immune system functioning</td>
</tr>
<tr>
<td>Humans</td>
<td>Nausea, vomiting, respiratory distress, eye irritation, flu-like symptoms</td>
</tr>
<tr>
<td>Indirect Pathogenicity</td>
<td>Reduction of food source organisms that are killed by toxins or outcompeted by noxious species. Bioaccumulation of toxins (e.g. shellfish consumption and concentration of toxic phytoplankton cells) and consumption of contaminated organisms by higher trophic levels (e.g. consumption of toxin-laced seafood by humans). Alteration of habitat, causing increased physiological stress; death (e.g. hypoxia/anoxia from blooms of noxious or toxic species)</td>
</tr>
<tr>
<td>Shellfish</td>
<td>Neoplasias, gonadal tumors, other disease, death</td>
</tr>
<tr>
<td>Finfish</td>
<td>Respiratory distress/failure, increased susceptibility to disease, organ damage (e.g., liver), death</td>
</tr>
<tr>
<td>Birds</td>
<td>Depressed feeding, erratic behavior, neurological signs, death</td>
</tr>
<tr>
<td>Aquatic mammals</td>
<td>Increased disease (believed to be linked to suppressed immune system functioning), death</td>
</tr>
<tr>
<td>Humans</td>
<td>Respiratory distress/failure, impaired coordination and mobility, memory dysfunction, and other neurological signs; convulsions; organ damage; death</td>
</tr>
</tbody>
</table>
Coastal versus other major sources of ballast water — Tanks were divided into two major source groups based on whether they were known to contain a proportion as coastal water as defined by the regulatory EEZ limit of 200 n.m. These two sources were designated as coastal versus open-ocean (“other”).

Age of ballast tank water — The age, defined as the time water was held within ballast tanks was highly variable, and averaged ~35 (median 10) days and ~25 (median 13) days for coastal versus other ballast water sources, respectively (Table 2). It should be noted that voyages 7 and 9, considered within the coastal source group, strongly influenced the “age” data because the paired tanks that were sampled from those two voyages contained “old” water (voyage 7, 173 days old; voyage 9, 99 days old). Removal of these four tanks from the coastal group reduced the mean water age to ~10 days (median 6 days); thus, most tanks in the coastal group (16 of 20) contained water that was, on average, approximately half the age of the water in other tanks.

<table>
<thead>
<tr>
<th></th>
<th>Coastal (n = 20 tanks)</th>
<th>Open-Ocean (n = 38 tanks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (days held in tank ± 1 SD)</td>
<td>34.9 ± 55.0</td>
<td>24.7 ± 25.8</td>
</tr>
<tr>
<td>Range (days held in tank)</td>
<td>2-173</td>
<td>5-104</td>
</tr>
<tr>
<td>Coefficient of variation</td>
<td>150%</td>
<td>106%</td>
</tr>
<tr>
<td>Median days held in tank</td>
<td>10</td>
<td>12.5</td>
</tr>
</tbody>
</table>

Note: Of the 62 tanks assessed, 4 (from voyages 3 and 25) could not be classified as coastal or open-ocean (non-coastal or with negligible coastal sources) because there were no ballast records (also see Table 7).

Paired versus unpaired tanks — Paired (“homogeneous”) tanks on a vessel contained water of the same age or history; unpaired (“heterogeneous”) tanks contained water of differing histories.

3b. Sampling Design and Methods

3b.1. Ballast Tank Sampling

The intent of our sampling strategy, jointly developed in collaboration with SERDP project CP-1245, was to encompass the tank variability resulting from ballast age, season, region, vessel characteristics, and exchange status. The sampling period spanned ~2 years, from September 2002 through July 2004. Ballast tanks on various ships were screened for suitability of sampling using a decision tree developed in collaboration with SERDP project CP-1245 (Table 3). Prior to their arrival, ships coming into U.S. ports were no ballast that originated from outside the 200-nautical mile EEZ. Upon arrival, or as soon as possible thereafter, members of SERDP project CP-1245 boarded each vessel, and interviewed the crew about the volume and history of the ballast water on board, selected ballast tanks to be sampled, and completed the sampling procedure. At least two ballast tanks usually were sampled from each ship.

Vessels and tanks were represented numerically. A suffix was used to designate the voyage as opposed to the actual ship, because one vessel was sampled twice during the study. The voyage numbers were established chronologically and hyphenated with each tank designation. For example, Voyage-Tank 1-2P represented Voyage 1, Tank 2 port (e.g. Table 4). In each ballast tank, water depth was measured, and background environmental conditions in the ballast water (temperature, salinity, pH, dissolved oxygen concentration, turbidity) were profiled using a hand-held meter (YSI model 85, Yellow Springs Instruments, Inc.), or a Hydrolab...
Measurements were taken just below the surface (0.5 meter [m]) and at depths of 1 m, 2 m, 5 m, and 10 m depending on the depth of the water column and the tank architecture. These data were taken to assess for relationships between environmental variables and species abundance and diversity. Three major categories of samples were also collected in duplicate: chemical water quality samples, and net concentrated samples and whole-water samples for biota analyses. A detailed sampling protocol was developed and organized schematically (see Appendix 1).

Microzooplankton technically range from 20-200 µm in maximum dimension (Omori and Ikeda 1984). To avoid analytical overlap with project team SERDP CP-1245, microzooplankton analyzed in this study were collected from the 20-µm net tows and filtered through an 80-µm mesh sieve, yielding a size fraction ranging from 20-80 µm. Macrozooplankton were quantitatively collected first by members of SERDP CP-1245 using 80 µm-mesh plankton nets (100-cm length, 30-cm diameter; Sea Gear Corporation), followed by a quantitative tow for phytoplankton using a 20 µm-mesh net for phytoplankton and microzooplankton that were
datosonde sensor bundle (Hach, Inc.; Figure 2).

<table>
<thead>
<tr>
<th>Voyage</th>
<th>Event</th>
<th>Name</th>
<th>Tank</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>11</td>
<td>Lenthall</td>
<td>4S</td>
</tr>
<tr>
<td>6</td>
<td>13</td>
<td>Lenthall</td>
<td>6P</td>
</tr>
<tr>
<td>6</td>
<td>15</td>
<td>Lenthall</td>
<td>2P</td>
</tr>
<tr>
<td>6</td>
<td>16</td>
<td>Lenthall</td>
<td>2S</td>
</tr>
<tr>
<td>6</td>
<td>17</td>
<td>Lenthall</td>
<td>8P</td>
</tr>
<tr>
<td>6</td>
<td>18</td>
<td>Lenthall</td>
<td>8S</td>
</tr>
<tr>
<td>16</td>
<td>35</td>
<td>Soderman</td>
<td>2AS</td>
</tr>
<tr>
<td>16</td>
<td>36</td>
<td>Soderman</td>
<td>2CS</td>
</tr>
<tr>
<td>17</td>
<td>37</td>
<td>Grumman</td>
<td>2P</td>
</tr>
<tr>
<td>17</td>
<td>40</td>
<td>Grumman</td>
<td>2S</td>
</tr>
<tr>
<td>17</td>
<td>37</td>
<td>Grumman</td>
<td>8P</td>
</tr>
<tr>
<td>17</td>
<td>40</td>
<td>Grumman</td>
<td>8S</td>
</tr>
<tr>
<td>20</td>
<td>45</td>
<td>Cape Inscription</td>
<td>3U</td>
</tr>
<tr>
<td>20</td>
<td>46</td>
<td>Cape Inscription</td>
<td>7C</td>
</tr>
<tr>
<td>24</td>
<td>51</td>
<td>Cape Kennedy</td>
<td>6S</td>
</tr>
<tr>
<td>24</td>
<td>53</td>
<td>Cape Kennedy</td>
<td>7S</td>
</tr>
<tr>
<td>25</td>
<td>54</td>
<td>Yano</td>
<td>CP</td>
</tr>
<tr>
<td>25</td>
<td>55</td>
<td>Yano</td>
<td>BS</td>
</tr>
<tr>
<td>26</td>
<td>56</td>
<td>Cape Intrepid</td>
<td>3UD</td>
</tr>
<tr>
<td>26</td>
<td>57</td>
<td>Cape Intrepid</td>
<td>6S</td>
</tr>
<tr>
<td>27</td>
<td>58</td>
<td>Lenthall</td>
<td>2P</td>
</tr>
<tr>
<td>27</td>
<td>59</td>
<td>Lenthall</td>
<td>2S</td>
</tr>
<tr>
<td>27</td>
<td>60</td>
<td>Lenthall</td>
<td>8P</td>
</tr>
<tr>
<td>27</td>
<td>61</td>
<td>Lenthall</td>
<td>8S</td>
</tr>
</tbody>
</table>

Table 3. Ballast tank sampling decision tree.

1) Does the vessel have ballast water on board?
   No- Complete NSWC questionnaire; proceed to next vessel.
   Yes- Complete NSWC questionnaire and go to Step #2.

2) Is access to at least 2 ballast tanks available?
   No- Proceed to next vessel
   Yes- Go to Step #3.

3) Water column in ballast tanks > 3 m?
   No- Proceed to next tank or vessel.
   Yes- Go to Step #4.

4) Ballast water characteristics - ranking of ballast water source types
   No Exchange- Best Choice; note, and proceed to Step #5.
   Near-shore exchange- Note, and proceed to Step #5.
   Open-ocean exchange- Note, and proceed to Step #5.

5) At least 2 ballast tanks similar in source type?
   Yes- Best Choice; note, and proceed to Step #6.
   No- Note, and proceed to Step #6.

6) Are the ballast tanks used regularly?
   Yes- Best Choice; note, and proceed to Step #7.
   No- Note, and proceed to Step #7.

7) Sample the ballast tank.

Table 4. Examples of designation system for voyages and tank pairs with versus without the same ballasting history. The same voyage number indicates that all associated tanks were sampled (= events) on that voyage; unique pair numbers within a given voyage indicate that each of those tanks, although on the same ship, had a different ballasting history.

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evaluated as part of this study. Initially 5-µm-mesh nets were tested for use with phytoplankton and were found to be problematic, because of clogging and resulting poor reproducibility and overall performance. At a 5- to 10-µm mesh size, surface water tension may also become a factor in addition to rapid physical clogging. The approach selected was to augment the 20-µm-mesh net samples with a qualitative 5-µm-mesh net tow for species richness determinations. This approach was supported by a recent study (Gollasch et al. 2003) that compared phytoplankton sampling methodology in ballast tanks: the authors found that 20-µm-mesh plankton nets were a good choice for representative sampling of phytoplankton density and species richness. Certain pumps yielded phytoplankton densities approximately 50% higher than net-derived samples, but pump performance was highly variable and restricted to a single depth. Gollasch et al. (2003) additionally reported that the 10-µm-mesh net was slightly more effective than the 20-µm-mesh net in species richness and yielded low inter-replicate variability. Considering the nine different methods evaluated by those authors, overall the 20-µm net used in this study was considered to be a sound choice and in keeping with the recommended approach. Small phytoplankton that would have been missed by the 20-µm-mesh net were analyzed from whole-water samples and (for the cyanobacterium, *Microcystis aeruginosa*) using molecular techniques (see section 3b.6). Six tanks (from vessels-tanks 9-4P/S, 19-7C, 20-7C, 25-CP, 26-3U) had to be sampled with a manual diaphragm pump because tank architecture precluded use of the nets. These data were considered in species diversity metrics, but not statistical comparison of phytoplankton abundance among tanks.

Whole-water samples were collected using an air-displacement, whole-water composite sampler (Labline, Inc.). This sampling canister is capable of collecting equal aliquots of water at set depth intervals, combined for a 2-L vertical composite sample. The composite sample was used for chemical analyses, bacteria quantification and molecular identifications, and culturing of phytoplankton and zooplankton taxa of interest. This sampling device was selected for two reasons: First, the air displacement enabled dependable water collection over multiple depths, and collection could be triggered by simply removing plugs (fill and displacement) from the surface through a spring-loaded line, independent of the weight-bearing line. Thus, the device could be triggered on the bottom of the tank and retrieved with periodic stops at 1-m intervals (Appendix 1). Secondly, the smooth polypropylene surface comprising the interior canister wall could be thoroughly cleaned between sampling events, particularly important to ensure sample integrity for bacterial identifications and chemical analyses. Although the plankton nets were also cleaned between sampling events, the pliability and fine mesh of the nets would have been problematic for avoiding molecular cross contamination. The hardened, smooth interior surfaces of the air displacement canister were cleaned with bleach and then with 10% sulfuric acid, followed by thorough rinsing with deionized water prior to each scheduled boarding. Canisters were also rinsed prior to sampling with water from the subject tank.

Samples designated for phytoplankton enumeration and molecular identification of bacteria were preserved with acidic Lugol’s solution (Vollenweider 1974). Samples designated
for bacterial enumeration via flow cytometry were preserved with 50% gluteraldehyde, and those for microzooplankton enumeration were preserved with 10% buffered formalin. During warm months, cooler ice packs were included in the kit in order to maintain a temperature designed to avoid heat stress for organisms in samples designated for culture.

![Figure 3. Overview of sampling structure.](image)

Figure 3 provides an overview of samples collected using the above-described procedures. Several more ships were sampled on the U.S. West Coast, but the same numbers of tanks were sampled between the two major U.S. coasts (total, 62 tanks; note that 4 were eliminated from analysis because they lacked ballast records or because of a fuel leak that contaminated the water). We also intensively sampled all 8 tanks of a single vessel on the East Coast to access the variability across tanks within a given vessel. In total, ~280 analyses were conducted for total Kjeldahl nitrogen (TKN), nitrate+nitrite (here referred to as NO₃⁻), total phosphorus (TP), and total organic carbon (TOC). TN was determined as TKN + NO₃⁻. Chlorophyll a (Chla) was also assessed as an indicator of total phytoplankton biomass (Wetzel and Likens 2001). A total of 185 preserved phytoplankton samples and 122 preserved microzooplankton samples were analyzed for species composition (lowest taxonomic level) and abundance. A total of 372 phytoplankton culture wells (6 per tank) and 124 zooplankton culture wells (2 per tank), filled with appropriate media and feeder algae, were prepared for inoculation of fresh (unpreserved) sub-samples upon arrival (expressed overnight shipment after sampling). These cultures were examined weekly for 8 weeks. Identifiable phytoplankton and zooplankton species were isolated and maintained in monocolulture for species identification. From these, selected taxa were prepared for 18 heat treatment experiments (each 24 hours in duration) after range-finding studies. Total bacteria abundance was assessed for 183 samples using flow cytometry; molecular screening for bacterial pathogens was conducted on 62 samples; and one sample from each tank was plated in triplicate for selected *Vibrio* species.

### 3b.2. Water Quality Analyses

Samples for analysis of TSS were maintained at ≤ 4°C, filtered within 48 h, and measured gravimetrically (method 2540D, American Public Health Association [APHA] et al. 1992; practical quantitation limit, 2 mg L⁻¹). Chla samples were filtered under low vacuum (Whatman GF/C filters, 55-69 kPa) and low light (20 µmol photons m⁻² s⁻¹) within 12 hours of collection,
and were stored frozen (-20°C) with desiccant in darkness until analysis within two months. Chl
a was extracted in 90% basic acetone (U.S. EPA 1997a, Wetzel and Likens 2001), and
fluorescence was determined using a Turner 10-AU fluorometer. It should be noted that
measurement of TSS was terminated halfway through the study because samples consistently
were at or below the detection limit.

Nutrients were analyzed using a Technicon (Traacs 800) or Lachat Instruments
(Quickchem 8000) autoanalyzer. Variances from the U.S. EPA and the NC DENR-DWQ were
obtained to enable use of procedures for nutrient sample storage and analysis (substitution of
freezing at –20°C for acidification; two-month limit), that accommodated low-level analysis of
estuarine matrices (U.S. EPA 1992, 1997b). Water samples for total P (TP) analysis were frozen
at -20°C until analysis, using a variance of EPA method 365.1 (U.S. EPA 1992, 1993; practical
quantitation limit (APHA et al. 1992), 10 µg L⁻¹). Samples for NO₃⁻ analysis were frozen and
analyzed within two months, using a variance of EPA method 353.4 (U.S. EPA 1992; practical
quantitation limit, 6 µg NO₃⁻ L⁻¹). Samples for TKN (free ammonia [NH₃] + organic nitrogen
[N₆]) were assayed using a modification of EPA method 351.2 (U.S. EPA 1993; samples held at
-20°C and not preserved with sulfuric acid; practical quantitation limit, 140 µg N L⁻¹). TOC
concentrations were determined using high-temperature non-dispersive IR-combustion
techniques (NDIR; APHA et al. 1998).

3b.3. Phytoplankton Analyses

Each 250-mL preserved phytoplankton sample
was settled undisturbed for > 48 hours; the upper 20 mL
was then gently siphoned and removed; the remaining 50
mL was remixed, and 25 mL was settled in an Utermöhl
chamber (Lund et al. 1958, Wetzel and Likens 2001) for
24 hours. Total phytoplankton and phytoplankton cells
that appeared viable when collected (in preserved
material, with intact membranes and cytoplasm, and with
no signs of damage or decay; Burkholder and Wetzel
1989) were enumerated and identified to lowest possible
taxa using phase contrast light microscopy (600x),
following Wetzel and Likens (2001) (e.g. Figure 4).
More than 40 references were consulted for taxa
identifications, spanning the taxonomic literature on all
algal groups represented (Cyanobacteria; and protist phyla as Chlorophyta, Cryptophyta,
Dinophyta, Haptophyta, and Ochrophyta, as in Graham and Wilcox 2000).

Phytoplankton assemblages from each ballast tank (from both net and whole-water
samples) were cultured by incubating fresh (unpreserved) samples in 6-well plates for up to 8
weeks in f/2 marine growth media, ± hydrated silica (Guillard 1975) or other conducive media at
23°C, using a 14/10-hour light/dark photoperiod and light intensity (source, fluorescent tubes) of
100 µmol photons m⁻² s⁻¹. The media were prepared using ultra-filtered (0.22 µm pore size) tank
water or ultra-filtered natural seawater At weekly intervals, sub-samples were examined under
light microscopy (phase contrast, 600x) for actively growing algal taxa.
Scanning electron microscopy was used to identify cultured taxa, following Parrow et al. (2006). To discern the plates of armored dinoflagellates, cells first were treated with a 40% reduction in salinity for 30 minutes (i.e. 40% reduction in osmolality, monitored using a Vapor Pressure Osmometer – Wescor, Inc.) and then combined with an equal volume of fixative cocktail (1% OsO₄, 2% glutaraldehyde, and 0.1 M sodium cacodylate final concentration) at 4°C for 20 minutes (Parrow et al. accepted). Fixed cells of dinoflagellates and other taxa were filtered onto polycarbonate filters (3-µm porosity), rinsed in 0.1 M sodium cacodylate, dehydrated through an ethanol series, CO₂ critical-point-dried, sputter-coated with 25 nm Au/Pd, and viewed at 15 kV on a JEOL 5900LV scanning electron microscope.

Species identifications of potentially harmful taxa were also checked with species-specific molecular probes (18s rDNA), where available (Scholin et al. 2003). For DNA extraction, 50 mL of acidic Lugol’s-preserved sample was centrifuged and total DNA extracted from the pellet using an UltraClean Microbial DNA Isolation Kit (MoBio Laboratories, Solana Beach, CA). Extracted DNA was stored at -20°C until PCR was performed. This kit is suitable for extraction of both eukaryotic and bacterial DNA (below). For real-time PCR assays, each reaction contained the following: 0.1U Taq Pro (Denville Scientific), 1X PCR buffer, 4mM MgCl₂, 0.2µM forward primer, 0.2µM reverse primer, 0.3mM each deoxynucleotide triphosphosphate, 0.25 mg mL⁻¹ bovine serum albumin, 0.3uM Taqman probe, molecular grade water to 10ul and 1ul DNA template.

To assess clone diversity of potentially harmful algal taxa, conventional PCR methods (i.e. not real time) were applied; specifically, primers 4618 (modified from Medlin et al. 1988) and DINO (Oldach et al. 2000) were used to generate a 149-base pair PCR product spanning a highly variable region of the dinoflagellate 18S genome. The following cycling parameters were used for all conventional PCR with Takara Taq: 94°C for 5 minutes followed by 45 cycles of 95°C for 30 seconds, annealing temperature for 30 seconds, and 72°C for the appropriate extension time (which depended upon amplicon size). The PCR ended with a 72°C for a 10-minute step. For all reactions using Megafrag, the extension temperatures were changed to 68°C instead of 72°C and the denaturing times (those associated with 95°C) were decreased per manufacturer’s instructions. PCR products were analyzed on an ethidium bromide-stained gel. Bands were excised and DNA was extracted following manufacturer’s instructions provided with the MinElute Kit (Qiagen, Valencia, CA).

A series of diversity metrics was evaluated for use in analyzing the phytoplankton dataset, including basic richness (species number) and taxonomic (relatedness) metrics as described by Clarke and Warwick (1998). These metrics include indices that capture not only the distribution of abundance among species (richness and evenness, e.g. Shannon-Weiner), but also the taxonomic relatedness of the species in each sample. The taxonomic diversity index (Clarke and Warwick 1998) is determined as the average path length between all pair wise species occurrences in a given sample. Taxonomic paths lengths are established using a reference Linnean taxonomic hierarchy based on the structure of the Integrated Taxonomic Information System (ITIS), a web-based multinational taxonomic database. The Taxonomic Distinctness Index is determined as the average Linnean path length between any two randomly chosen individuals within a sample. The TDI is viewed as a relatively “pure” measure of taxonomic relatedness, with less emphasis on the abundance distribution. Community structure metrics were also considered, for example, the proportion of dinoflagellates and other groupings in the total phytoplankton community or assemblage. This information was potentially applicable; for
example, if the study indicated that certain ballast water exchange practices encourage survival of dinoflagellates, it would be desirable to avoid those practices based on the harmful characteristics of some dinoflagellate species, and their resistant life history stages (cysts) (Hallegraeff and Bolch 1992, Sonneman and Hill 1997, Hamer et al. 2001).

3b.4. Atlas of Phytoplankton Species

The Atlas deliverable for this project included phytoplankton that were identified to the species level. The Atlas was generated from Microsoft Access databases. The various sub-reports reside in a stand-alone database that is linked to the main data archive (Section 3c, below). Each species page was designed to include the following information:

1. *Species name* (conformed to Integrated Taxonomic Information System (ITIS) (United States Department of Agriculture 2005) database spelling if the ITIS naming was current; note that species were ordered alphabetically within phyla;
2. *Image(s)* of each species are provided as photographs or drawings, and the source;
3. *Known impacts* of the species, if any;
4. *Former names*, included for clarification, given ongoing, rapid changes in taxonomy;
5. *Distribution*, based upon the published literature;
6. *Source region*, based upon data from the ballast water management reports of each vessel’s Chief Engineer, when available; and
7. *References* consulted for taxonomic and supporting information.

3b.5. Microzooplankton Analyses

Microzooplankton (including foraminifers, ciliates, rotifers, and copepod nauplii, 20-200 µm in maximum dimension) were preserved in 10% buffered formalin and stained with Rose Bengal in an attempt to better discern organisms from detrital material (Wetzel and Likens 2001; Johnson and Allen 2005). The zooplankton samples were settled for at least 24 hours, after which they were concentrated by decanting off supernatant until a workable concentration was achieved. In several cases the original sample concentration was sufficient so that no further concentration was needed. Samples were counted by resuspending the decanted sample, withdrawing 1 mL by pipette, and placing it into a Sedgwick-Rafter (S-R) cell for examination of the entire cell at 200x using a compound microscope. If further detail was required for identification, subsamples were examined at 400x under phase contrast. Sufficient S-R cells were evaluated to count at least 200 organisms; in some cases the samples were so devoid of microzooplankton that the entire sample had to be examined in S-R chambers. Principal taxonomic keys utilized included Smith (1977), Alder (1999), Boltovsky (1999), Kemle-von Mucke et al. (1999), and Lee et al. (2000).

3b.6. Bacterial Analyses

Total bacterial abundance was quantified using flow-cytometry after DNA staining with SYBR® Green I nucleic acid stain (Molecular Probes, Eugene, OR) (Gasol and Del Giorgio 2000, Button and Robertson 2001). Bacterial populations were identified and individual cells counted on flow cytometric histograms of right-angle light scatter versus green DNA fluorescence. Samples were analyzed in duplicate, with periodic quality control matrix spikes. Spikes were made by injecting 10 µL of a *Vibrio* spp. bacterial culture into 1mL of sample and comparing the total cell
counts with and without spiking solution. Nominal spike value was determined by running 10µL *Vibrio* prepared with 0.2µm-filtered sheath fluid following the above protocol.

Molecular techniques were used to screen sampled for potentially harmful bacterial taxa. For DNA extraction, 50 mL of acidic Lugol’s-preserved sample was centrifuged and total DNA extracted from the pellet using an UltraClean Microbial DNA Isolation Kit (MoBio Laboratories, Solana Beach, CA). Extracted DNA was stored at -20°C until PCR was performed.

Various real-time PCR assays (Wittwer et al. 1997) were performed (Table 5), specific for harmful algal species and bacterial pathogens. Lower limits of detection were determined by serial dilution of purified bacterial DNA. The last dilution detected in the series was then regenerated by spiking into background DNA extracted from various ballast samples. This allowed assessment of potential inhibitory effects from other materials in the ballast water samples that were co-extracted. Although the cycle number at which the dilution was detected by real-time PCR varied with different background samples used, the dilution was still detectable, thus validating the assays as a qualitative tool.

Several of the real-time PCR assays incorporated SyBr Green as the detection platform, analyzed using a Lightcycler (Idaho Technology) or a Smartcycler (Cepheid). Assays performed on the Lightcycler used 94°C for 2 minutes to release the antibody from the hot start Taq polymerase, followed by 50 cycles of 94°C for 0.5 second, annealing temperature (varies for each assay) for 0.5 second, and 72°C for 10 seconds (fluorescence acquisition occurred after this step in each cycle). The following cycling was used to generate the melting curve: 97°C for 20 seconds, 50°C for 20 seconds, and then reactions were slowly ramped back up to 97°C at 0.2°C per second. Other assays performed in this research incorporated Taqman probes as the detection platform. The probes, specific for target loci, are fluorescently labeled on the 5’ end with a reporter dye (e.g. FAM [carboxy-fluorescein]) and at the 3’ end with a quencher dye (e.g. TAMRA [carboxytetramethylrhodamin] or BHQ [black hole quencher]). The following cycling parameters were used to run Taqman-based assays: 50 cycles of 94°C for 0.5 second, and annealing temperature for 20 seconds. Fluorescence acquisition occurred after each cycle. For real-time assays using either SyBr Green or Taqman probes, we used a hot-start Taq polymerase suitable for bacterial sequences that might be difficult to amplify (Takara Taq; Takara Bio, Shiga Japan). Each reaction contained the following: 0.05U Taq polymerase, 1X PCR buffer, 5mM MgCl2, 0.2 µM forward primer, 0.2 µM reverse primer, 0.3 mM each deoxynucleotide triphosphosphate, 0.25 mg mL-1 bovine serum albumin, molecular grade water to 10 µL, 1 µL of DNA template, and either 1X SyBr Green or 0.3uM Taqman probe.

Other species-specific assays for bacterial pathogens, as well as the assays used for the cloning and sequencing component of the project (below), utilized *conventional PCR* methods (Table 5). For generating clones from bacterial species, primers modified from Wilmotte et al. (1993) were used to generate about a 600-base pair product spanning several variable regions within the bacterial 16S genome. A high-fidelity Taq polymerase was used to minimize misincorporations of nucleotides during PCR cycling. Each reaction contained the following: 1U Taq polymerase (Megafrag, Denville Scientific for dinoflagellate clones or Takara Taq, Takara Bio for bacterial clones), 1X PCR buffer, 4 mM MgCl2, 0.8 µM forward primer, 0.8 µM reverse primer, 0.2 mM each deoxynucleotide triphosphosphate, 0.25 mg ml-1 bovine serum albumin, molecular grade water to 50 µL, and 1µL of DNA template. Cycling parameters for the conventional PCR techniques were as described for phytoplankton.
Table 5. Molecular techniques used to screen for potentially harmful microalgae and bacteria: target species, target loci, loci descriptions, PCR techniques used, and lower limits of detection. Specificity is for the organism listed unless otherwise indicated.

<table>
<thead>
<tr>
<th>Target Species</th>
<th>Target Loci</th>
<th>Loci Description</th>
<th>PCR</th>
<th>Lower Limit of Detection</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Algae</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Fibrocapsa japonica</em></td>
<td>LSU</td>
<td>large subunit</td>
<td>Taqman</td>
<td>n.d.</td>
<td>Bowers et al. (in prep.)</td>
</tr>
<tr>
<td><em>Heterosigma akashiwo</em></td>
<td>LSU</td>
<td>large subunit</td>
<td>Taqman</td>
<td>n.d.</td>
<td>Bowers et al. (in prep.)</td>
</tr>
<tr>
<td><em>Karlodinium micrum</em></td>
<td>18S</td>
<td>18S rRNA</td>
<td>Taqman</td>
<td>&lt;1 cell mL(^{-1})</td>
<td>Tengs et al. (2001)</td>
</tr>
<tr>
<td><em>Pfiesteria piscicida</em></td>
<td>18S</td>
<td>18S rRNA</td>
<td>Taqman</td>
<td>&lt;1 cell mL(^{-1})</td>
<td>Bowers et al. (2000)</td>
</tr>
<tr>
<td><em>Pfiesteria shumwayae</em></td>
<td>18S</td>
<td>18S rRNA</td>
<td>Taqman</td>
<td>&lt;1 cell mL(^{-1})</td>
<td>Bowers et al. (2000)</td>
</tr>
<tr>
<td><strong>Bacteria</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Listeria monocytogenes</em></td>
<td>hly</td>
<td>listeriolysin</td>
<td>Taqman</td>
<td>~58 fg</td>
<td>Rodriguez-Lazaro et al. (2004)</td>
</tr>
<tr>
<td><em>Microcystis aeruginosa</em></td>
<td>myc</td>
<td>encodes enzyme that makes microcystin; codes for DNA binding protein</td>
<td>Taqman</td>
<td>n.d.</td>
<td>Foulds et al. (2002)</td>
</tr>
<tr>
<td><em>Morganella morganii</em></td>
<td>16S</td>
<td>16S rRNA</td>
<td>Conventional</td>
<td>&lt;10 fg</td>
<td>Kim et al. (2003)</td>
</tr>
<tr>
<td><em>Mycobacterium spp.</em></td>
<td>16S</td>
<td>16S rRNA invasion plasmid antigen</td>
<td>Taqman</td>
<td>~58 fg</td>
<td>Oldach lab. (^b)</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>23S</td>
<td>23S rRNA</td>
<td>SyBr</td>
<td>74 fg</td>
<td>Ludwig et al. (2000)</td>
</tr>
<tr>
<td><em>Salmonella spp.</em></td>
<td>himA</td>
<td>protein – codes for hemolysin</td>
<td>SyBr</td>
<td>60 fg</td>
<td>Bej et al. (1994)</td>
</tr>
<tr>
<td><em>Shigella spp.</em></td>
<td>lpaH</td>
<td>H</td>
<td>Conventional</td>
<td>&lt;45 fg</td>
<td>Kong et al. (2002)</td>
</tr>
<tr>
<td><em>Vibrio alginolyticus</em></td>
<td>hsp</td>
<td>heat shock protein</td>
<td>SyBr</td>
<td>178 fg</td>
<td>Oldach lab. (^c)</td>
</tr>
<tr>
<td><em>Vibrio cholera</em></td>
<td>ctxA</td>
<td>toxin gene</td>
<td>SyBr</td>
<td>2 fg</td>
<td>Nandi et al. (2000) (^d)</td>
</tr>
<tr>
<td><em>Vibrio vulnificus</em></td>
<td>vvhA</td>
<td>hemolysin/ cytolyisn gene</td>
<td>Taqman</td>
<td>250 fg</td>
<td>Campbell et al. (2003)</td>
</tr>
</tbody>
</table>

\(^a\) Probe is specific for pathogenic *Leptospira* spp.
\(^b\) Probe detects *Mycobacterium marinum, M. ulcerans, M. chesapeakei, M. pseudoshottsii, and M. liflandii*.
\(^c\) Probe detects *Vibrio* spp.
\(^d\) Probe detects toxic strains of *Vibrio cholera*. 


3b.7. Molecular Techniques: Ligation, Cloning, and Sequence Analyses for Selected Dinoflagellates and Bacteria

The use of dinoflagellate-general 18S and bacterial-general 16S primers resulted in a pool of amplicons from each ballast water sample analyzed (n = 62). These amplicons were ligated into a plasmid vector and transformed into bacterial cells (pCR 2.1) using the TOPO TA Cloning kit (Invitrogen, Carlsbad, CA). Transformations were plated onto LB plates containing 50 µg mL⁻¹ ampicillin and 40 µg of 40 mg mL⁻¹ X-gal, and then were incubated overnight at 37°C. After incubation, 96 white colonies were picked (from each ballast water sample) with sterile pipet tips and resuspended in 20 µL of sterile water. The insertion of targets into bacterial cells supplied with the kit interrupted expression of the lacZ gene; thus, white colonies represented colonies with inserts, while blue colonies represented colonies without inserts.

To further screen colonies for inserts, PCR was performed as described above with primers targeted to the pCR 2.1 bacterial cells used for transformations. If a colony did not contain an insert, the resulting amplicon was approximately 300 base pairs, while those with inserts produced amplicons of either 450 base pairs or 900 base pairs for dinoflagellate and bacterial species, respectively. A high-fidelity Taq polymerase (Megafrag, Denville Scientific) was used for all PCR (as described above), with an annealing temperature of 60°C and an extension time of one minute. Five to ten reactions from each plate of clones (along with the negative controls) were analyzed on an ethidium bromide-stained gel. The DYEnamic ET Terminator Cycle Sequencing Kit (Amersham Biosciences, Piscataway, NJ) was then used to sequence all clones. The sequencing reactions contained the following: 2 µL of dye (diluted 1:5), 1 µL of desired primer (0.4 µM final concentration), 1.75 µL of sterile water, and 0.25 µL PCR product. Cycling parameters were as follows: 25 cycles of 95°C for 20 seconds, 55°C for 15 seconds, and 60°C for 1 minute. After cycling, sequencing reactions were centrifuged through Sephadex G50 to remove unincorporated dye. Sequencing was performed on the ABI 3100 automated capillary DNA Sequencer (Applied Biosystems, Foster City, CA).

For sequence analysis, overlapping sequence reads were assembled and inspected for ambiguities using Sequencher (version 4.1.2, Gene Codes Corporation, Ann Arbor, MI). The resulting contigs were aligned in MacClade and inspected to ensure they were truncated to the correct amplicon length, based on position of the primers. The sequences were then uploaded into the Ribosomal Database Project II (Cole et al. 2003) to determine the closest 16S bacterial match. The query was set to return the top three similarity scores for each clone, defined as the number of (unique) oligomers shared between the sample sequence and a given RDP sequence divided by the lowest number of unique oligomers in either of the two sequences.

3b.8. Heat Treatment Experiments

Thermal tolerances of selected phytoplankton taxa were experimentally assessed to determine the temperature regimes needed for lethality. The target organisms were obtained either from cultures of ballast water samples or from the NCSU CAAE culture collection. They were chosen to span the major taxonomic groupings that were observed in ballast water samples from this study. For each taxon after completing range-finding studies (Klaassen et al. 1991, U.S. Environmental Protection Agency 2002), the experiments included 4-5 temperatures (e.g. controls at 23°C, treatments at 30°C, 34°C, 38°C and, where necessary, 41°C) crossed with 5-6 time intervals (e.g. 0, 1, 4, 8, 12, and 24 hours). The assays were conducted in darkness within
enclosed, precision-temperature-controlled water baths. Phytoplankton viability was quantitatively assessed where possible using vital staining techniques and epifluorescence light microscopy or flow-cytometric analysis (e.g. nucleic acid-binding fluorescent dyes; Jochem 1999, Lage et al. 2001, Parrow et al. 2002; Figure 5, to characterize the proportion of cells killed over time at each temperature. Interpretations were confirmed by re-culture. For taxa that did not respond well to vital staining procedures, after being subjected to the experimental treatments, attempts were made to re-culture the organisms under conditions conducive for growth. These data were used to identify points in the heat x time treatments that corresponded to complete death of the taxon.

Figure 5. (A) Demonstration of flow-cytometric analysis (upper panel), using a toxic Heterosigma akashiwo strain from our Center’s culture collection, killed and stained with SYTOX green (nuclear stain; Parrow et al. 2002). Two distinct populations were differentiated for viability via stain uptake and fluorometric intensity of nuclear material. This permitted event counts via flow cytometry, and quantitative heat treatment response profiles. (B) A dead cell of the dinoflagellate Prorocentrum micans with a compromised membrane showing uptake of SYTOX green (nuclear stain). (C) A live cell of Prorocentrum micans, showing auto-fluorescence (red) indicative of intact, photosynthesizing chloroplasts, and negligible uptake of the nuclear stain.

3c. Construction of a Regional Database

The data matrix (both ship- and sample-related) was organized in Microsoft ACCESS 2000, using a series of linked-data entry screens for ease of entry, viewing, and export of data for analysis and application. The database has been linked to a taxonomic image library and, if desired, can provide data for GIS mapping of ship trajectories to facilitate risk assessment. It was designed, as well, to incorporate chemical, biological, vessel, and ballast management data. A series of key queries and reports was included that are responsive to the needs and nature of ballast water investigations and to this project.

Microsoft Access 2000 was selected as the database management system for several reasons:

1. Query by Example (QBE) is much more user-friendly than an alternative approach, Structured Query Language, and QBE allows non-technical researchers to use the database without support from database professionals;
2. Access can be divided into a data portion and a mechanical portion that allows each user to have a copy of the data without interfering with manipulation of the data by other researchers;
3. Access allows generation of the database with the mechanicals as an executable, without need for additional licenses; and
4. Ad hoc requests can be responded to quickly and easily.
Each biological sample was assigned a unique number with the barcode identifier for the associated sample or culture fraction. This feature is key to the ability of the application because it allows for linkage to an image library that was produced over the course of this project (see Phytoplankton Atlas, Appendix 2). The naming convention developed for the project imagery is tied to the biological sample number, allowing rapid display of the image as part of the various system queries. An example of the output is given using data from the USNS Henry J. Kaiser (Figure 6).

**Figure 6.** Example of a GIS map for a DoD ship track, here, the USNS Kaiser ship track created using ballasting logs obtained in association with the 5 Sept. sampling effort. If desired, this application can be linked to the project database to enable automated production of maps, based on a wide range of potential database queries. Coupled with data such as those in this study, this feature can provide visualization opportunities to facilitate risk analysis of various ballasting activities.

The database proved useful for other practical functions, such as labeling of all containers used in sampling at each stage of the project. A set of forms and queries generated labels for each container, uniquely numbered and, where useful, bar coded with the serial number for tracking and identification. In addition, the database enabled tracking processes involved in the project. A series of forms and input procedures was included for the entry, storage, and validation of processes including tracking to/from the vessels, sample breakout upon receipt of sampling kits to the laboratory, and receipt of various analyses performed on the samples. The core database also was linked with several databases for functions that were not directly tied to the primary purpose of data organization, archiving, and extraction. These included databases for taxonomic information and species nomenclature, literature citations, and Phytoplankton Atlas generation.

Thus, this ACCESS database has the capability to allow any given biological observation within the database to be mapped in terms of its associated voyage-specific ballasting history. This functionality, coupled with the query functions for biological and chemical data, in turn enables various mapping scenarios on multiple scales in efforts to characterize risk. In addition, the database has the capacity, if desired, to be web-linked with various remote-sensing resources which, when overlaid with the system mapping outputs, allow consideration of other factors that may be relevant to organism occurrence and density. This feature may be useful in risk determinations, as mentioned. For example, the mapping feature, coupled with the chemical and biological observations from this study and used together with NOAA sea surface temperature animations, may indicate that a given ship location at a given time of year represents a high risk for uptake of water with a diverse, abundant flora and fauna.
3d. Data Analysis

Taxa abundance and diversity data were subjected to exploratory statistical analyses such as scatter plots and box plots of various combinations of tank criteria and sampling information, including water quality and taxonomic data. PROC VARCOMP (or PROC MIXED; SAS Institute, Inc. 1999) was used to determine whether the more appropriate sampling unit in studies such as this is the tank or the vessel (i.e., is as much variability captured by sampling eight tanks on one ship as by sampling one tank on each of eight ships?). In addition, variance related to abundance data for phytoplankton, zooplankton, and bacteria was examined in isolation from the environmental- and age-related variables and compared within and across ships using the SAS MIXED procedure (SAS Institute, Inc. 1999). Given that many tanks were sampled as pairs with similar ballasting histories, the data were analyzed in total as well as in subsets in an attempt to characterize the relative influence of this sampling aspect on the apportioning of variance among the components. This approach resulted in four datasets comparing phytoplankton, zooplankton, and bacteria abundances as (1) the entire dataset; (2) subset of data including voyages wherein a single set of paired tanks with similar ballast exchange history was sampled; and (3) a subset of data including voyages in which two or more unpaired tanks were sampled. The objective was to determine which components demonstrated higher variance, thereby informing future sampling designs and possibly for incorporation in future regulatory based monitoring protocols.

Appropriate determinative analyses were applied to further characterize relationships among the measured variables. These included a standard set of GLM analyses (linear regressions, ANOVAs) to examine relationships among response variables (phytoplankton, zooplankton, bacteria abundance; phytoplankton diversity, zooplankton diversity) and explanatory variables (physical / chemical data, ballast management). A high volume of analyses was required (9,200 regressions; 650 ANOVAs, t-test matrices, and including log- and other transformations). Two sampling events were excluded from consideration: #27, Red Cloud, 2A starboard wing was excluded because addition of oil had contaminated the ballast water; and #44, Cape Island, tank 6P was excluded because a high volume of tap water had been added to the tank. In all, ~75 phytoplankton, zooplankton, and bacteria variables (including eight different diversity metrics, abundances, and proportions of taxonomic groupings) were regressed against minimum ballast age. Various transformations and variable filtrations were also applied, including independent analysis of tanks that lacked a coastal water source. Data presentations for phytoplankton, zooplankton, and bacteria were organized in graphics by source region and by ascending age, moving from left to right within each regional section. Within this organizational scheme, ballast tanks were differentiated by whether they contained a proportion of coastal water. The age of water within a given ballast tank was based upon the last documented ballasting event prior to sampling.

Patterns in phytoplankton and zooplankton assemblage (community) structure were compared considering all tanks collectively, and tanks with versus without coastal water sources. Principal components analyses considered physical/ chemical variables, organism abundances, and primary biodiversity metrics. PCA reduced dimensionality and was used in an attempt to assess linear variable combinations that explain most of the variance in the data set. Similarity matrices (e.g. the Bray-Curtis similarity matrix, based upon log(x+1) transformed abundance data; SAS Institute, Inc. 1999) were depicted through cluster analysis as dendograms, or by multi-dimensional scaling (MDS) patterns, for phytoplankton and zooplankton based upon groupings by exchange status, major source water, season, etc. The similarity matrices (MDS...
patterns) were also compared with a non-parametric Rank Sum procedure to compare how similarly phytoplankton and zooplankton assemblages clustered across tank groupings. ANOSIM analyses were also conducted, including some nested analyses that compared seasonal groupings within the previously described ballast water age classes.

4. Results

4a. Vessels Sampled and Ballast Water Management Data

A total of 28 vessels were sampled, including 16 from 9 ports on the U.S. West Coast and 12 vessels from 4 ports on the U.S. East Coast (Table 6). The study primarily included oilers and cargo vessels operated by or for the Military Sealift Command. One US ARMY lighter was also sampled. There were two main vessel operator or service groupings, the Military Sealift Command (i.e., vessels operated directly by MSC) and the Maritime Administration (MARAD – civilian contract operators). Four different vessel classes were sampled from among the MSC vessels, and 5 were sampled from the MARAD grouping. Those vessels designated in the exchange field as M (= Mandatory) fell under the mandatory exchange requirements of OPNAV 5030 1b, and those designated as V were subject to the voluntary requirements of NISA and NANPCA (see regulatory summary in Introduction by Holm et al. 2005 NSWC-CD, SERDP project number CP-1245).

<table>
<thead>
<tr>
<th>Voyage Number</th>
<th>Fleet 1</th>
<th>Operator 2</th>
<th>Vessel Type 3</th>
<th>Vessel Class</th>
<th>Exchange Rule 4</th>
<th>Sampling Port</th>
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<tbody>
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<td>P</td>
<td>MSC</td>
<td>UR-OILER</td>
<td>1</td>
<td>M</td>
<td>San Diego, CA</td>
</tr>
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<td>P</td>
<td>MSC</td>
<td>UR-OILER</td>
<td>1</td>
<td>M</td>
<td>San Diego, CA</td>
</tr>
<tr>
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<td>A</td>
<td>MSC</td>
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</tr>
<tr>
<td>4</td>
<td>P</td>
<td>MSC</td>
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<td>1</td>
<td>M</td>
<td>San Diego, CA</td>
</tr>
<tr>
<td>5</td>
<td>A</td>
<td>MARAD</td>
<td>V-RORO</td>
<td>2</td>
<td>V</td>
<td>Portsmouth, VA</td>
</tr>
<tr>
<td>6</td>
<td>A</td>
<td>MARAD</td>
<td>V-RORO</td>
<td>1</td>
<td>M</td>
<td>Norfolk, VA</td>
</tr>
<tr>
<td>7</td>
<td>P</td>
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<td>V-RORO</td>
<td>2</td>
<td>V</td>
<td>Alameda Point, CA</td>
</tr>
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</tr>
<tr>
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</tr>
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<td>MSC</td>
<td>LMS-RORO</td>
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<td>V</td>
<td>Norfolk, VA</td>
</tr>
<tr>
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<td>P</td>
<td>MSC</td>
<td>LMS-RORO</td>
<td>6</td>
<td>V</td>
<td>San Francisco, CA</td>
</tr>
<tr>
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<td>A</td>
<td>MSC</td>
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</tr>
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<td>16</td>
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<td>MSC</td>
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<td>Everett, WA</td>
</tr>
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</tr>
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<td>P</td>
<td>MARAD</td>
<td>RORO</td>
<td>8</td>
<td>V</td>
<td>Tacoma, WA</td>
</tr>
<tr>
<td>19</td>
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<td>MARAD</td>
<td>RORO</td>
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<td>V</td>
<td>Pt Hueneme, CA</td>
</tr>
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<td>LMS-RORO</td>
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<td>V</td>
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<td>MARAD</td>
<td>V-RORO</td>
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<td>San Francisco, CA</td>
</tr>
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<td>V-RORO</td>
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<td>V</td>
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</tr>
<tr>
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<td>LMS-RORO</td>
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</tr>
<tr>
<td>26</td>
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<td>RORO</td>
<td>8</td>
<td>V</td>
<td>Tacoma, WA</td>
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<tr>
<td>27</td>
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<td>1</td>
<td>M</td>
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</tr>
<tr>
<td>28</td>
<td>A</td>
<td>USA</td>
<td>MLSV</td>
<td>10</td>
<td>V</td>
<td>Fort Eustis, VA</td>
</tr>
</tbody>
</table>

1 – Vessel Member of Pacific or Atlantic Fleet
2 – Operator:  MSC – Military Sealift Command   MARAD – Maritime Administration Civilian Operated
3 – Service Type: UR-OILER – Underway Replenishment Oiler; V-RORO – Vehicle Roll-On-Roll-Off Cargo Vessel; LMS-RORO – Large Medium Speed RORO
4 – Indicates if vessel operates under voluntary or mandatory open ocean exchange rule
Of the 62 tanks analyzed, 94% (all but 4, from voyages 3 and 25) had ballasting records either in the form of ballast logs, U.S. Coast Guard ballasting forms, or watch logs/communiqués that provided sufficient information to identify the recent source location(s) and age of the related water. Most tanks analyzed (90%, or 56 of 62) were subjected to some form of ballast management practice designed to ensure that upon ships’ arrival at a U.S. port, at least a portion of the ballast water was from an open ocean source (Table 7, Figure 7). About 55% (34 of 62) of the tanks were subject to deliberate, complete exchanges (100-300%), but 35% (22 of 62) were exchanged in uncertain proportions (XU in Table 7, Figure 8). The latter uncertainty was due to inadequate specificity in records and/or the bundling of multiple tank pairs in log entries without maintaining tank specific tonnages. The 2 tanks from voyage 19 were carrying open ocean water or tap water and were “topped off” with an additional infusion of open ocean water. Overall, records were adequate to determine source locales and the age of most ballast tanks. Records of the MSC generally were more complete and had a structured ballast log with a ballasting history for the voyage.

Based on the ballast records and utilizing the 200 n.m. regulatory criteria, 32% of the tanks contained some coastal water (Figures 7, 8), from either full exchanges in coastal areas or partial ballasting of coastal sources prior to making port. Of the 20 tanks, 14 were within either the 200 n.m. defining the U.S. EEZ or the same Food and Agricultural Organization (FAO) maritime region as the sampling port. About 10% of the tanks (6 of 62, from voyages 18, 20, and

<table>
<thead>
<tr>
<th>Voyage</th>
<th>Tanks</th>
<th>Sampling Season</th>
<th>Exch. Rule</th>
<th>Exch Activity</th>
<th>Coastal*</th>
<th>Proximal*</th>
<th>Regional*</th>
<th>Shelf*</th>
<th>Distance (NM)</th>
<th>Age (days)</th>
<th>Sources or Exchange Location(s) and Comments</th>
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<td>1</td>
<td>2 P/S Wing</td>
<td>Summer</td>
<td>M</td>
<td>3E Y Y N N 120</td>
<td></td>
<td>6</td>
<td>00 - 200 nm off southern Mexican coast</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>2</td>
<td>6 P/S Wing</td>
<td>Fall</td>
<td>M</td>
<td>XU N N NA NA NA 10</td>
<td></td>
<td>10</td>
<td>Central Pacific between Hawaii and Midway</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>8 P/S Wing</td>
<td>Fall</td>
<td>?</td>
<td>M U ? NA NA NA ?</td>
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<td>?</td>
<td>?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>7 P/S Wing</td>
<td>Spring</td>
<td>M</td>
<td>XU N NA NA NA 10</td>
<td></td>
<td>10</td>
<td>Central Pac. &gt;200nm North of Wake Island</td>
<td></td>
<td></td>
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<tr>
<td>5</td>
<td>1 Center Line</td>
<td>Spring</td>
<td>V</td>
<td>1E Y Y Y 106 14</td>
<td></td>
<td>14</td>
<td>100 - 200 nm off Louisiana Coast</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>6</td>
<td>2 P/S Wing</td>
<td>Summer</td>
<td>M</td>
<td>XU N N NA NA NA 9</td>
<td></td>
<td>9</td>
<td>Mediterranean highly diluted with Gulf Stream waters</td>
<td></td>
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</tr>
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<td>4 P/S Wing</td>
<td>Summer</td>
<td>M</td>
<td>XU N N NA NA NA 13</td>
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<td>13</td>
<td>Mediterranean waters diluted with mid and eastern Atl. waters</td>
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<td></td>
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<td></td>
</tr>
<tr>
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<td>8 P/S Wing</td>
<td>Summer</td>
<td>M</td>
<td>XU Y Y Y N 44 5</td>
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<td>5</td>
<td>Mediterranean with significant coastal Carolina water</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>9</td>
<td>8 P/S Wing</td>
<td>Summer</td>
<td>V</td>
<td>XU Y Y Y 117 99</td>
<td></td>
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<td>100 - 200 nm off Louisiana Coast</td>
<td></td>
<td></td>
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</tr>
<tr>
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<td>Summer</td>
<td>V</td>
<td>XU Y Y Y 117 99</td>
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<td></td>
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</tr>
<tr>
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<td>Winter</td>
<td>V</td>
<td>XU Y Y Y 117 99</td>
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<td></td>
<td></td>
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</tr>
<tr>
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<td>Winter</td>
<td>V</td>
<td>XU Y Y Y 117 99</td>
<td></td>
<td>99</td>
<td>Mediterranean with significant coastal Carolina water</td>
<td></td>
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<td>Winter</td>
<td>V</td>
<td>XU Y Y Y 117 99</td>
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<td>99</td>
<td>Mediterranean with significant coastal Carolina water</td>
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</tr>
<tr>
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<td>Winter</td>
<td>V</td>
<td>XU Y Y Y 117 99</td>
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<td>99</td>
<td>Mediterranean with significant coastal Carolina water</td>
<td></td>
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<td>Winter</td>
<td>V</td>
<td>XU Y Y Y 117 99</td>
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<td>Winter</td>
<td>V</td>
<td>XU Y Y Y 117 99</td>
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<td></td>
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<td></td>
</tr>
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<td>17</td>
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<td>Winter</td>
<td>V</td>
<td>XU Y Y Y 117 99</td>
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<td>99</td>
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<td>Winter</td>
<td>V</td>
<td>XU Y Y Y 117 99</td>
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<td>XU Y Y Y 117 99</td>
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<td>25</td>
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<td>Winter</td>
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<td>XU Y Y Y 117 99</td>
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Table 7. Ballast management summary for the tanks examined (nm = nautical miles).
Table 7, cont’d.

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<tr>
<th>Voyage #</th>
<th>Tanks</th>
<th>Exchange Season</th>
<th>Exch. Rule</th>
<th>Exch Activity</th>
<th>Coastal</th>
<th>Proximal</th>
<th>Regional</th>
<th>Shelf?</th>
<th>Distance (NM)</th>
<th>Sources or Exchange Location(s) and Comments</th>
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<tr>
<td>19</td>
<td>6P</td>
<td>Spring</td>
<td>V</td>
<td>Top off</td>
<td>N</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>26</td>
<td>Indian Ocean with Tacoma tap water – top off</td>
</tr>
<tr>
<td>7C</td>
<td>Winter</td>
<td>V</td>
<td>Top off</td>
<td>N</td>
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<td>NA</td>
<td>NA</td>
<td>104</td>
<td>Pacific Ocean – top off</td>
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<td>20</td>
<td>3U</td>
<td>Spring</td>
<td>V</td>
<td>XU</td>
<td>Y</td>
<td>N</td>
<td>N</td>
<td>Y</td>
<td>14</td>
<td>2</td>
</tr>
<tr>
<td>7C</td>
<td>Spring</td>
<td>V</td>
<td>XU</td>
<td>Y</td>
<td>N</td>
<td>N</td>
<td>Y</td>
<td>26</td>
<td>5</td>
<td>Persian Gulf followed by Pacific near Hawaiian Island</td>
</tr>
<tr>
<td>21</td>
<td>2 CS</td>
<td>Winter</td>
<td>V</td>
<td>XU</td>
<td>N*</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>101</td>
<td>Open Atlantic 400 nm west of Gibraltar</td>
</tr>
<tr>
<td>22</td>
<td>1 P/S FD</td>
<td>Spring</td>
<td>V</td>
<td>1E</td>
<td>N</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>13</td>
<td>Open Atlantic</td>
</tr>
<tr>
<td>23</td>
<td>Fore</td>
<td>Spring</td>
<td>V</td>
<td>1E</td>
<td>Y</td>
<td>N</td>
<td>Y</td>
<td>Y</td>
<td>46</td>
<td>30</td>
</tr>
<tr>
<td>24</td>
<td>6 P/S</td>
<td>Spring</td>
<td>V</td>
<td>1E</td>
<td>N</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>43</td>
<td>Northern Indian Ocean – 800 nm west of Sri Lanka</td>
</tr>
<tr>
<td>7S</td>
<td>Spring</td>
<td>V</td>
<td>1E</td>
<td>N</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>45</td>
<td>N. Indian Ocean (~50%) Some recent open Pacific</td>
</tr>
<tr>
<td>25</td>
<td>CP, BS</td>
<td>U</td>
<td>V</td>
<td>?</td>
<td>?</td>
<td>?</td>
<td>NA</td>
<td>NA</td>
<td>11</td>
<td>Open Atlantic</td>
</tr>
<tr>
<td>26</td>
<td>3 UDT</td>
<td>Summer</td>
<td>V</td>
<td>1E</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>2.3</td>
<td>4</td>
</tr>
<tr>
<td>6S</td>
<td>Summer</td>
<td>V</td>
<td>3E</td>
<td>N</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>29</td>
<td>Open Atlantic</td>
</tr>
<tr>
<td>27</td>
<td>1P/S</td>
<td>Summer</td>
<td>M</td>
<td>XU</td>
<td>Y</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>41</td>
<td>15</td>
</tr>
<tr>
<td>8P/S</td>
<td>Summer</td>
<td>M</td>
<td>3E</td>
<td>N</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>8</td>
<td>Atlantic, 200 nm off Norfolk, VA</td>
</tr>
<tr>
<td>28</td>
<td>1P/S</td>
<td>Summer</td>
<td>M</td>
<td>1E</td>
<td>N</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>11</td>
<td>Atlantic</td>
</tr>
</tbody>
</table>

Note: P/S designates paired tanks. Four tanks (Voyage 3 – tanks 8P and 8S; Voyage 25 – tanks CP and BS) were eliminated from statistical analyses involving "coastal" vs. "non-coastal" source waters because there were no ballast records available (? below). Coastal status was designated as Y (yes); non-coastal (open-ocean) status was designated as N or N* (coastal water considered negligible).

1 – Exchange Rule

M = Vessel was under Mandatory Exchange rule.
V = Vessel was under Voluntary Exchange rule.
U = Could not determine if any exchange or open ocean dilution treatment was applied.

2 – Exchange Activity

XU = Exchange or open ocean dilution occurred but to an undetermined extent.
1E = 100% open ocean exchange.
2E = 200% open ocean exchange.
3E = 300% open ocean exchange.
? = No records or logs available.
Top off = Tanks contained tap water and were topped off with waters of the open ocean.

3 – Coastal

Indicates whether the tank possessed some coastal water.

4 – Proximal

Indicates whether the coastal waters were proximal to the sampling port.

5 – Regional

If not proximal, was it in the same FAO region?

6 – Shelf

Did the Coastal exchange occur on the continental shelf?

7 – Shelf

Distance in nautical miles exchange took place from nearest coastline.

Color Coding in Source Field provided for clustering of tanks into source regions based on most recent activity prior to sampling.

Indicates whether the coastal waters were proximal to the sampling port.

27) contained foreign coastal water. Distance to the adjacent coastline was calculated for all 20 “coastal source” tanks using GIS (Table 7). Voyages 1 and 9 included 4 tanks that were sampled in the Pacific Ocean ~100 n.m. off the southern coast of Mexico (Table 7, Figure 9A), putting...
the source waters in the same FAO region as the West Coast sampling ports. The remaining 10 tanks contained waters definitively from the coastal U.S. Of these, 6 tanks were ballasted with waters from or proximal to the U.S. sampling port, while 4 were ballasted in the Gulf of Mexico (Figure 9A). Moreover, of the 20 tanks containing some coastal water, 10 were ballasted within the bounds of the primary continental shelf of the adjacent land mass. Four of these tanks were sampled over coastal shelf zones from foreign locales (Voyages 18, 20 – Table 7); the remaining 6 were ballasted over shelf zones in U.S. waters. There was no indication or record that any of the foreign waters were discharged into the US receiving port.

The following brief descriptions are provided of the ballast management for each vessel. Figure 9 provides an example of mapping of coastal ballasting events and the means by which the coastal status of tanks was assigned. Voyages are shown that involved uptake of coastal water. Voyages 16 and 26 were not mapped at high resolution because their coastal sources were the arrival port in Puget Sound, WA. Similarly, voyage 18 included ballasting at the Guam Fuel Pier. There was no need to explicitly determine measure position for these voyages. Tanks were each sampled in duplicate.

**Voyage 1** (Fleet Oiler, sampled 5 September 2002) –

Two paired tanks (with ballast water of the same management history) on this MSC underway-replenishment oilier were sampled. The ballasting logs were complete and indicated that the last activity, 6 days prior to sampling, was 3 full exchanges 120 n.m. off southern Mexico (Figure 9A). The exchange area was within the FAO region, although not within the EEZ, and the exchange took place 100+n.m. off the coastal shelf. Neither tank showed evidence of depth stratification, and environmental conditions were as follows: Salinity, 33.2-33.3 ppt; temperature, 23.7-26.4°C, and dissolved oxygen (DO), 5.7-5.9 mg · L⁻¹.

**Voyage 2** (Fleet Oiler, sampled 22 November 2002) –

Two paired tanks were sampled 10 days prior to sampling. Records were complete although some tanks were grouped so that, in some instances, actual ballasting tonnage for a given tank could not be discerned (XU). Ballast was originally loaded in the eastern central Pacific Ocean and was managed in the same region: Two source tanks were filled with waters adjacent to Sri Lanka and Indonesia in the straits of Malacca, and ~640 tonnes (705 tons) of this water were transferred to the paired tanks that were sampled (6-P/S). Tanks 6-P/S were then diluted to < 5% coastal sources.
with waters of the open Pacific. Neither tank was depth-stratified, and environmental conditions were: salinity, 32.0-35.5 ppt; temperature, 18.6-20.5°C; and DO, 5.1-5.9 mg · L⁻¹.

**Voyage 3** (Fleet Oiler, sampled 23 December 2002) –
Two paired tanks were sampled. Ballast logs were inadequate to determine ballasting status. Interviews with the crew indicated that the ballast water was ~2 days old and its most recent source region was at an unknown location in the Atlantic Ocean, where management of unknown quality had occurred. Neither tank was depth-stratified. Environmental conditions were: salinity, 36.2-36.5 ppt; temperature, 12.8-18.2°C; and DO, 6.4-7.3 mg·L⁻¹.

**Voyage 4** (Fleet Oiler, sampled 18 May 2003) –
Two paired tanks were sampled. Records indicated that the last activity prior to sampling was an exchange in the open Pacific, 10 days before. Records were generally complete, but did not indicate the extent of the exchange (XU). Neither tank was depth-stratified. Environmental conditions were: salinity, 39.6-39.8 ppt; temperature, 15.3-15.7°C; and DO, 4.6-6.0 mg · L⁻¹.
**Voyage 5** (Roll-on/Roll-off Carrier, sampled 19 May 2003) –

A single tank was available for sampling. Records indicated that a single complete exchange (100% empty-refill) was completed in the western central Atlantic Ocean, 106 n. m. from the coast of Louisiana within U.S. waters, 14 days prior to sampling (coastal ballast category; Figure 9A). The exchanged ballast water originally had been loaded in the Indian Ocean. The tank was not depth-stratified, and environmental conditions were: salinity, 35.2-35.5 ppt; temperature, 18.2-18.5°C; DO, 7.3-7.8 mg · L⁻¹.

**Voyage 6** (Fleet Oiler, sampled 27 May 2003) –

To gain further insights about within-vessel variation in characteristics of clean ballast water, we sampled 8 tanks (four pairs of wing tanks) on this MSC underway-replenishment oilier, each in duplicate. Ballasting logs were structured and complete, although tanks were often grouped under various activity codes, making tank-specific activities difficult to discern in some cases. The ballast water in all tanks originated from the Mediterranean Sea. Tank pairs 2P/S, 4P/S, 6P/S, and 8P/S were the subject of multiple ballast exchanges in the Mediterranean, some of which were proximal to coastal areas, particularly for tanks 6P/S. Upon exiting through Gibraltar, about half of the combined capacity for all four tanks were discharged (proportion not specified), apparently as a concerted effort to deballast Mediterranean waters. Tanks 4P/S were also ballasted with 27% of their combined capacity in proximity to coastal Portugal. These activities were followed by varying degrees of uptake in the open Atlantic Ocean, although not a complete exchange for all tanks. Tanks 2P/S and 6P/S were then ballasted west of Bermuda with oligotrophic waters of the Gulf Stream at 60% (by volume) of their combined capacity. Tanks 6P/S and 8P/S were also ballasted with waters 44 n.m. off coastal North Carolina, proximal to the Norfolk, Virginia port (Figure 9B). This was the last ballasting activity prior to our sampling effort in Norfolk. Biological (abundance) and chemical data indicate that tank pair 2P/S was the likely recipient of the bulk of the Gulf Stream waters, whereas tank pair 8 P/S received the most of the water from coastal North Carolina. Thus, tanks had undergone management (exchange of varying extents) as the vessel crossed the Atlantic Ocean, yielding ballast tank waters that had been held for 5-17 days. Exchanges occurred in the western central Atlantic, northeastern Atlantic, and northwestern Atlantic Ocean. The tanks were not depth-stratified, and environmental conditions were: salinity, 31.9-36.9 ppt; temperature, 17.4-23.4°C; DO, 6.3-7.7 mg · L⁻¹.

**Voyage 7** (Roll-on/Roll-off Carrier, sampled 27 May 2003) –

Two paired tanks were sampled. Ballasting records were sparse and were in the form of a single communiqué that indicated the last ballasting activity for the target tanks was an exchange that took place 99 days earlier for both tanks, with the western central Atlantic as the water source (~117 n.m. from the Louisiana coast over the continental shelf). The tanks were not depth-stratified, and environmental conditions were: high salinity, 40.8-41.2 ppt; temperature, 14.7-14.9°C; and DO, 4.2-6.8 mg · L⁻¹.

**Voyage 8** (Roll-on/Roll-off Carrier, sampled 26 May 2003) –

A single forepeak tank was available for sampling. Coast Guard ballast forms indicated that the ballast water had been loaded in the western central Pacific Ocean, and underwent a complete exchange (100% empty-refill) in the eastern central Pacific east of the Marianna Islands, 16 days prior to sampling. The tank was not depth-stratified, and environmental conditions were as follows:
high salinity, 39.3-39.4 ppt; temperature, 14.6-14.7°C; DO, 6.2-7.0 mg · L⁻¹.

**Voyage 9** (Container Ship, sampled 8 June 2003) –

Two paired ballast tanks were sampled. This MARAD polar supply vessel visited many disparate locales. The ballast water was about 173 days old, and originally was loaded in the western central Pacific Ocean. Coast Guard records indicated that the last ballasting activity was an exchange of unknown extent in the eastern central Pacific ~116 miles off the Mexican coast, 173 days prior to sampling. This was within the FAO region of the sampling port, but based on the EEZ criteria, this constituted a coastal source outside U.S. waters. However, the exchange took place 100+ n.m. off of the coastal shelf. The tanks were not depth-stratified, and environmental conditions were: high salinity, 32.0-32.9 ppt; temperature, 12.2-13.0°C; DO, 5.1-7.7 mg · L⁻¹.

**Voyage 10** (Fleet Oiler, sampled 1 July 2003) –

Two paired tanks on this MSC underway-replenishment oilier were sampled. Records indicated that the last activity prior to sampling was a complete exchange (100% empty-refill) in the open western central Atlantic Ocean 5 days prior to sampling. The tanks were not depth-stratified, and environmental conditions were: salinity data not available; temperature, 26.4-26.9°C; DO, 6.5-6.6 mg · L⁻¹.

**Voyage 11** (Roll-on/Roll-off Carrier, sampled 13 August 2003) –

Two unpaired tanks (i.e. with ballast water differing in age) on this MSC RORO were sampled. The actual exchange disposition of tank 2CP was uncertain, although at least a partial exchange was completed in the northwest Atlantic 22 days before sampling. Tank 2 CS underwent a complete exchange (100% empty-refill) in the Indian Ocean, at least 76 days prior to sampling. The tanks were not depth-stratified, and environmental conditions were: salinity, 36.0-36.9 ppt; temperature, 25.3-26.4°C; DO, 4.7-5.6 mg · L⁻¹.

**Voyage 12** (Roll-on/Roll-off Carrier, sampled 20 August 2003) –

Two paired tanks on this MSC RORO were sampled. The last activity prior to sampling was a complete exchange (100% empty-refill) in the eastern central Pacific Ocean, 12 days before. The tanks were not depth-stratified, and environmental conditions were: salinity, 34.7-34.9 ppt; temperature, 18.7-19.7°C; and DO, 5.0-6.7 mg · L⁻¹.

**Voyage 13** (Fleet Oiler, sampled 3 September 2003) –

A single wing tank was available for sampling. The ballast water was at least 9 days old, and had undergone two complete exchanges (200% empty-refill) in the northwest coastal Atlantic Ocean after being loaded in the Mediterranean Sea. The tank was not depth-stratified, and environmental conditions were: salinity, 36.0 ppt; temperature, 25.8-27.0°C; and DO, 4.0-5.4 mg · L⁻¹.

**Voyage 14** (Fleet Oiler, sampled 16 September 2003) –

Two paired tanks on this MSC underway-replenishment oilier were sampled. The major source water apparently was ballasted as an exchange of unknown extent in the eastern central Pacific Ocean ~50-100 n.m. west of Hawaii, ~33 days prior to sampling. The tanks were not depth-stratified, and environmental conditions were as follows: salinity, 35.0-36.1 ppt; temperature, 20.8-21.7°C; and DO, 5.3-5.7 mg · L⁻¹.
**Voyage 15** (Roll-on/Roll-off Carrier, sampled 17 November 2003) –
Two unpaired ballast tanks were sampled. Coast Guard records indicated that a single complete exchange (100% empty-refill) of both tanks had occurred in the open Indian Ocean 46 days prior to sampling. The tanks were not depth-stratified, and environmental conditions were: salinity, 34.8-35.1 ppt; temperature, 16.8-17.6°C; DO, 4.6-5.7 mg · L⁻¹.

**Voyage 16** (Roll-on/Roll-off Carrier, sampled 21 November 2003) –
Two unpaired ballast tanks were sampled. Both tanks had undergone a complete exchange (100% empty-refill) in the eastern central Pacific Ocean ~250 n.m. off the northern California coast, 23 days before sampling at Washington State. The contents of tank 2A were ~5 days old because a valve leak forced uptake of ~172 tonnes (190 tons) of port water. Although a similar problem was not documented with certainty for tank 2C, salinity in that tank was lower than expected if the only source had been 250 n.m. offshore, suggesting that some coastal (port) water was introduced to that tank (nominally with water age ~23 days), as well. Tank 2A was classified as containing coastal water from within the sampling port. The tanks were not depth-stratified, and environmental conditions were: salinity, 29.2-32.2 ppt; temperature, 11.0-11.4°C; DO, 5.1-6.3 mg · L⁻¹.

**Voyage 17** (Fleet Oiler, sampled 11-12 February 2004) –
Four tanks (two pairs) on this MSC underway-replenishment oilier were sampled to gain further insights about within-ship variability in ballast tank contents. The ballast water in all four tanks originated from the Mediterranean Sea, and the four tanks were each subjected to three complete exchanges (300% empty-refill) in the eastern central Atlantic Ocean 5 days (1st pair of tanks, 2P/S) or 10 days (2nd pair of tanks, 8P/S) before sampling, respectively. The tanks were not depth-stratified, and environmental conditions were: salinity, 37.3-38.3 ppt; temperature, 9.5-19.8°C; DO, 6.1-7.2 mg · L⁻¹.

**Voyage 18** (Fleet Oiler, sampled 4 March 2004) –
Two paired tanks were sampled. Ballast water originally was loaded in the Indian Ocean, and a complete exchange (100% empty-refill) occurred in these tanks in the western central Pacific Ocean at the Guam Fuel Pier, ~16 days prior to sampling. The tanks were not depth-stratified, and environmental conditions were: salinity, 33.2-33.9 ppt; temperature, 15.2-16.6°C; DO, 7.3-7.6 mg · L⁻¹.

**Voyage 19** (Roll-on/Roll-off Carrier, sampled 3 May 2004) –
Two non-paired tanks were sampled. Tanks 6P and 7C contained tap water in varying amounts and had been topped off with open Indian and Pacific Ocean waters 26 and 104 days prior to sampling, respectively. The tanks were slightly depth-stratified, and environmental conditions were: salinity, 33.3-33.5 ppt (tank 6P) vs. 9.3 ppt (tank 7C); temperature, 12.9-18.1°C; DO, 3.7-3.8 mg · L⁻¹ (tank 6P) vs. 8.6-10.2 mg · L⁻¹ (tank 7C).

**Voyage 20** (Roll-on/Roll-off Carrier, sampled 7 May 2004) –
Two unpaired tanks were sampled. Records indicated that tanks 3U and 7C contained waters from the Persian Gulf (Figure 9D) and were topped off with waters from the open Pacific Ocean 2 and 5 days prior to sampling. The original waters were ballasted within 30 n.m. of the coast of
Oman. The tanks were not depth-stratified, and environmental conditions were: salinity, 29.0-32.8 ppt; temperature, 16.6-17.6°C; DO, 4.1-7.2 mg · L⁻¹.

**Voyage 21** (Roll-on/Roll-off Carrier, sampled 20 May 2004) –
A single wing tank (2CS) was available for sampling. The water had originated in the Indian Ocean. Exchange of unknown extent occurred 101 days before sampling, in the eastern central Atlantic Ocean ~400 n.m. west of Gibraltar. DO concentrations were lower at depth, and conditions were: salinity, 36.7-36.9 ppt; temperature, 22.2-23.7°C; DO, 4.8-6.3 mg · L⁻¹.

**Voyage 22** (Roll-on/Roll-off Carrier, sampled 2 June 2004) –
Two paired tanks were sampled. Coast Guard ballasting records indicated that a complete exchange (100% empty-refill) occurred in the open Atlantic Ocean 13 days before sampling. The tanks were not depth-stratified, and environmental conditions were: salinity, 31.4-37.2 ppt; temperature, 24.6-25.4°C; DO, 6.3-6.7 mg · L⁻¹.

**Voyage 23** (Roll-on/Roll-off Carrier, sampled 4 June 2004) –
A single forepeak tank was available for sampling. The ballast water had originated from the eastern central Pacific Ocean, and underwent a complete exchange (100% empty-refill) in the western Atlantic Ocean (46 n.m. east of Corpus Christi, Texas; Figure 9A) 30 days before sampling on the U.S. West Coast. The tank was not depth-stratified, and environmental conditions were: salinity, 34.3-34.4 ppt; temperature, 18.2-18.3°C; DO, 5.0-6.5 mg · L⁻¹.

**Voyage 24** (Roll-on/Roll-off Carrier, sampled 8 June 2004) –
Three tanks were sampled, including two paired tanks (6P/S) and a third unpaired tank (7S). All three tanks contained water from the open Indian Ocean, with 7S also having some water from the open Pacific Ocean. Tank 8S was ballasted 80 n.m. from the coast of Oman. Ancillary records indicated that water from tanks 7S and 8S may have mixed. DO differed with depth, and conditions were: salinity, 33.3-35.3 ppt; temperature, 14.2-17.3°C; DO, 2.1-8.8 mg · L⁻¹.

**Voyage 25** (Roll-on/Roll-off Carrier, sampled in June 2004 –
Two unpaired tanks were sampled. Ballasting logs were not available.

**Voyage 26** (Roll-on/Roll-off Carrier, sampled 29 June 2004) –
Two unpaired tanks were sampled. Tank #3 was completely exchanged (100% empty-refill) in Puget Sound 4 days prior to sampling. Tank 6S was subjected to a 3-fold exchange (300% empty-refill) in the open Atlantic Ocean 29 days prior to sampling. The tanks were not depth-stratified, and environmental conditions were: salinity, 31.0-37.0 ppt; temperature, 15.4-16.7°C; DO 2.7-4.9 mg · L⁻¹.

**Voyage 27** (Fleet Oiler, sampled 8-9 July 2004) –
Two sets of paired tanks were sampled in order to further characterize within-ship variation in microbiota. The four tanks had undergone empty-refill exchange to an unknown extent. Tank pair 2P/S was ballasted with waters from the northeast Atlantic Ocean (coastal Sicily and Portugal) 15 days before sampling (Figure 9C). Tanks 6S/P were ballasted 3 times (300% empty-
refill) in the northwestern Atlantic Ocean 8 days before sampling. The tanks were slightly depth-stratified, and were similar in salinity and temperature but differed in DO concentrations, as follows: salinity, 35.4-37.1 ppt; temperature, 26.1-27.0°C; DO 3.3-5.2 mg · L⁻¹ (tanks 2P/S) vs. 5.2-6.0 mg · L⁻¹ (tanks 8P/S).

**Voyage 28** (Lighter, sampled 21 July 2004) –
Two paired tanks were sampled, each in duplicate, from this U.S. Army lighter. The source region was the northeast Atlantic Ocean (unspecified location(s)). The targeted tanks underwent a complete exchange (100% empty-refill) 11 days before sampling.

4b. **Physical and Chemical Data**

The mean salinity of ballast water in tanks with Atlantic, Pacific, and Indian Ocean water sources were comparable, but tanks with Atlantic Ocean water sources were significantly warmer (Table 8, Figure 10). The pH was within expected ranges, and dissolved oxygen (DO) was also sufficient to maintain the biota (regional means 5.52 – 6.02 mg DO · L⁻¹), except for several tanks that were at somewhat lower levels of ~4 mg DO · L⁻¹ (Table 8, Figure 11). Most tanks showed little depth stratification, indicating they were well mixed at the time of sampling. Figure 12, from the first vessel sampled, exemplifies depth profiles for the vessels.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Atlantic</th>
<th>Pacific</th>
<th>Indian</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (°C)</td>
<td>21.4±4.8 (CV 22%, n=32)</td>
<td>17.0 ± 3.8 (CV 22%, n=21)</td>
<td>17.4 ± 3.8 (CV 21%, n=7)</td>
</tr>
<tr>
<td>Salinity (ppt)</td>
<td>36.7±1.6 (CV 4%, n=29)</td>
<td>34.1±3.0 (CV 9%, n=19)</td>
<td>31.3±3.0 (CV 31%, n=7)</td>
</tr>
<tr>
<td>pH</td>
<td>8.21±0.22 (CV 8%, n = 12)</td>
<td>8.13±0.11 (CV 2%, n=15)</td>
<td>8.12±0.03 (CV 1%, n=2)</td>
</tr>
<tr>
<td>DO (mg L⁻¹)</td>
<td>6.84 ± 0.71 (CV 5%, n=30)</td>
<td>7.63±0.58 (CV 22%, n=19)</td>
<td>7.66±0.63 (CV 21%, n=7)</td>
</tr>
</tbody>
</table>

**Figure 10.** Mean temperature and salinity in each tank, considered by major source region (note that U = unidentified major source). Numbers on the X-axis indicate the voyage number (prefix) and tank identifiers (suffix), and correspond to the designations in the previous section (Tables 4, 6, and 7). Within each major source region, tanks are arranged by age in ascending order from left to right.
TN, NO₃⁻, TP, and TOC concentrations were low to moderate relative to typical concentrations in coastal waters (e.g. Day et al. 1989) (Table 9). Using log-transformed data in ANOVA, no significant relationships were found between TN, NO₃⁻, TP, or TOC and water age, water source, dissolved oxygen, or other factors ($r^2 \text{all } < 0.05; P < 0.18$).

**Table 9.** Nutrient concentrations and TN:TP ratios in the ballast waters (means ± 1 SE; data available for 56 of 62 tanks). TKN, TN, and TP data are rounded to the nearest 5 µg·L⁻¹.

<table>
<thead>
<tr>
<th>Source</th>
<th>TKN (µg·L⁻¹)</th>
<th>NO₃⁻ + NO₂⁻ (µg·L⁻¹)</th>
<th>TN (µg·L⁻¹)</th>
<th>TP (µg·L⁻¹)</th>
<th>TN:TP</th>
<th>TOC (mg·L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tanks With Coastal Water Sources (n = 20)</td>
<td>105 ± 15</td>
<td>56 ± 17</td>
<td>165 ± 20</td>
<td>35 ± 5</td>
<td>7.7 ± 2.1</td>
<td>14.24 ± 2.52</td>
</tr>
<tr>
<td>Tanks With Ocean (Non-Coastal) Water Sources (n = 36)</td>
<td>135 ± 25</td>
<td>20 ± 7</td>
<td>155 ± 30</td>
<td>80 ± 40</td>
<td>5.9 ± 1.5</td>
<td>8.86 ± 1.77</td>
</tr>
<tr>
<td>Tanks Considered Collectively (n = 56)</td>
<td>125 ± 20</td>
<td>33 ± 8</td>
<td>155 ± 20</td>
<td>64 ± 26</td>
<td>6.4 ± 1.2</td>
<td>10.61 ± 1.46</td>
</tr>
</tbody>
</table>

**Figure 11.** Mean pH and dissolved oxygen in each tank, considered by major source region. Data are presented as in Figure 10.

**Figure 12.** Example from voyage #1 of depth profiles of temperature, pH, salinity, dissolved oxygen, turbidity, and chlorophyll a in the ballast tanks.
4c. Phytoplankton

4c.1. Taxa Composition, Including Cultures

A total of 100 phytoplankton species were identified from the ballast tanks examined, including 19 potentially harmful taxa (Table 10, Figure 13; Phytoplankton Atlas and references therein, Appendix 2). Phytoplankton species included 58 diatoms, 31 dinoflagellates, 1 raphidophyte flagellate, 4 other ochrophyte (golden) flagellates, 2 cryptophyte flagellates, 2 cyanobacteria, and 2 colonial green algae. Nearly all of the species found are cosmopolitan. Of the 62 tanks sampled in this study, ~30% (18) yielded culturable phytoplankton used in taxa identifications (Table 11). A total of 74 cultures were grown, including 52 taxa. About half of the tanks with culturable phytoplankton had a coastal water source, including the four that yielded most cultures (16-2S – coastal Washington state; 18-4P – Guam Fuel Pier; 20-3U, Persian Gulf; 26-3U, Puget Sound). The latter two tanks also contained the “newest” water (held less than 5 days). Tanks with ballast water age more than 33 days did not produce culturable phytoplankton.

4c.2. Phytoplankton Abundance

The viable phytoplankton assemblages in ballast tank waters were dominated by chain-forming diatoms (Phylum Ochrophyta, Class Bacillariophyceae) and dinoflagellates (Phylum Dinophyta), both in tanks with versus without coastal water sources (Figure 14). Phytoplankton abundance was highly variable, spanning four orders of magnitude, with median values substantially less than means (Figure 15). Highest cell densities were from tanks containing coastal water that that had recently been added. Considering median values, viable phytoplankton comprised ~50% of the total cells.

Median phytoplankton abundance in ballast waters from the Atlantic and Pacific Oceans was comparable (8.2 x 10^3 cells m^-3 and 15.0 x 10^3 cells m^-3, respectively), whereas median density in ballast waters from the Indian Ocean was lower (0.6 x 10^3 cells m^-3). Tanks with Indian Ocean water were fewer in number and did not include coastal sources. In addition, the greater distance between the Indian Ocean and U.S. sampling ports resulted in a longer average time interval between the last ballast water additions and sampling efforts.

Pair-wise Student’s t tests were performed to compare phytoplankton abundances from the three source regions, excluding tanks with coastal source waters, to assess whether there were regional differences independent of age and coastal variables. Phytoplankton abundances in tanks with Atlantic versus Indian Ocean source waters were significantly different (P = 0.02), but other pairings had comparable densities. ANOVA indicated that phytoplankton densities between tanks with coastal water sources versus the other tanks were significantly different (P = 0.016). Phytoplankton densities (viable and total) were regressed against several potential explanatory variables including vessel, ballast water age, and 11 physical/chemical factors. Several different data filtering scenarios were applied across variable pairings, such as removal of tanks based upon sampling method, coastal status, or exchange status. Lower phytoplankton abundance occurred in older ballast water, but there was high variability in phytoplankton abundance within water held less than 30 days (Figure 16). The only other statistically significant relationship discerned was between the abundance of centric diatoms and nitrate concentrations (r = 0.73).
Table 10. Phytoplankton taxa found in ballast water samples, also indicating potential harmful traits. Taxa are marine/estuarine unless otherwise indicated (note: tem – temperate, trop – tropical, subtrop – subtropical, frw – freshwater, ? – distribution uncertain). Species indicated as capable of producing toxin have nontoxic as well as toxic strains.

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Distribution</th>
<th>Harmful Traits</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Diatoms (Phylum Ochrophyta)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Actinocyclus curvatulus</td>
<td>Arctic</td>
<td></td>
</tr>
<tr>
<td>Actinoptychus senarius</td>
<td>Cosmopolitan</td>
<td></td>
</tr>
<tr>
<td>Asterionella formosa</td>
<td>Cosmopolitan (frw)</td>
<td></td>
</tr>
<tr>
<td>Asterionellopsis glacialis</td>
<td>Cosmopolitan</td>
<td></td>
</tr>
<tr>
<td>Asteromphalus marylandica</td>
<td>Cosmopolitan (tem, trop)</td>
<td></td>
</tr>
<tr>
<td>Asteromphalus hookeri</td>
<td>Cosmopolitan (cold)</td>
<td></td>
</tr>
<tr>
<td>Asteromphalus roperianus</td>
<td>Cosmopolitan (tem, trop)</td>
<td></td>
</tr>
<tr>
<td>Attheya septentrionalis</td>
<td>Cosmopolitan n. hemisphere (cold)</td>
<td></td>
</tr>
<tr>
<td>Bacteriastrum furcatum</td>
<td>Cosmopolitan (tem-trop)</td>
<td></td>
</tr>
<tr>
<td>Bacteriastrum hyalinum</td>
<td>Cosmopolitan (tem)</td>
<td></td>
</tr>
<tr>
<td>Bellerochea horologica</td>
<td>Cosmopolitan (trop-subtrop)</td>
<td></td>
</tr>
<tr>
<td>Chaetoceros curviset</td>
<td>Cosmopolitan (tem-subtrop)</td>
<td></td>
</tr>
<tr>
<td>Chaetoceros decipiens</td>
<td>Cosmopolitan (Polar to tem)</td>
<td></td>
</tr>
<tr>
<td>Chaetoceros didymus</td>
<td>Cosmopolitan (tem-subtrop)</td>
<td></td>
</tr>
<tr>
<td>Chaetoceros lorenzianus</td>
<td>Cosmopolitan (tem-subtrop)</td>
<td></td>
</tr>
<tr>
<td>Chaetoceros similis</td>
<td>Cosmopolitan (Arctic to tem)</td>
<td></td>
</tr>
<tr>
<td>Chaetoceros socialis</td>
<td>Cosmopolitan (Polar-subtrop)</td>
<td>Can cause fish death via gill irritation, mucus over-production.</td>
</tr>
<tr>
<td>Chaetoceros tetrastichon</td>
<td>Cosmopolitan (trop-tem)</td>
<td></td>
</tr>
<tr>
<td>Corethron zodiacus</td>
<td>Cosmopolitan except Arctic</td>
<td></td>
</tr>
<tr>
<td>Cylindrotheca closterium</td>
<td>Cosmopolitan</td>
<td></td>
</tr>
<tr>
<td>Ditylum brightwellii</td>
<td>Cosmopolitan except Polar regions</td>
<td></td>
</tr>
<tr>
<td>Eucampia zodiakus</td>
<td>Cosmopolitan except Polar regions</td>
<td></td>
</tr>
<tr>
<td>Helicocystis tamensis</td>
<td>Cosmopolitan (tem-trop)</td>
<td></td>
</tr>
<tr>
<td>Hemiaulus hauckii</td>
<td>Cosmopolitan (tem-trop)</td>
<td></td>
</tr>
<tr>
<td>Hemiaulus indicus</td>
<td>Cosmopolitan</td>
<td></td>
</tr>
<tr>
<td>Hemiaulus membranaceus</td>
<td>Cosmopolitan (tem-trop)</td>
<td></td>
</tr>
<tr>
<td>Hemiaulus sinensis</td>
<td>Cosmopolitan (tem-trop)</td>
<td></td>
</tr>
<tr>
<td>Lauderia annulata</td>
<td>Cosmopolitan (tem-trop)</td>
<td></td>
</tr>
<tr>
<td>Leptocylindrus danicus</td>
<td>Cosmopolitan except Antarctic</td>
<td></td>
</tr>
<tr>
<td>Leptocylindrus minimus</td>
<td>Cosmopolitan except Antarctic</td>
<td>Linked to fish death (gill irritation, mucus over-production).</td>
</tr>
<tr>
<td>Nitzschia acicularis</td>
<td>Cosmopolitan estuarine / frw (tem-subtrop)</td>
<td></td>
</tr>
<tr>
<td>Nitzschia americana</td>
<td>Cosmopolitan (tem-trop)</td>
<td></td>
</tr>
<tr>
<td>Nitzschia kolaczeckii</td>
<td>Cosmopolitan (?) (trop-subtrop)</td>
<td></td>
</tr>
<tr>
<td>Nitzschia laevis</td>
<td>Cosmopolitan</td>
<td></td>
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</tbody>
</table>
Table 10, cont’d.

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Distribution</th>
<th>Harmful Traits</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Diatoms (Phylum Ochrophyta)</strong> (cont’d.)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Nitzschia longissima</em></td>
<td>Cosmopolitan (tem)</td>
<td>-----</td>
</tr>
<tr>
<td><em>Nitzschia sinuata</em></td>
<td>Cosmopolitan frw</td>
<td>-----</td>
</tr>
<tr>
<td><em>Odontella aurita</em></td>
<td>Cosmopolitan (Polar-tem)</td>
<td>-----</td>
</tr>
<tr>
<td><em>Odontella mobilensis</em></td>
<td>Cosmopolitan</td>
<td>-----</td>
</tr>
<tr>
<td><em>Odontella sinensis</em></td>
<td>Cosmopolitan (tem)</td>
<td>-----</td>
</tr>
<tr>
<td><em>Proboscia alata</em></td>
<td>Cosmopolitan(?) (tem)</td>
<td>-----</td>
</tr>
<tr>
<td><em>Pseudo-nitzschia lineola</em></td>
<td>Cosmopolitan</td>
<td>-----</td>
</tr>
<tr>
<td><em>Pseudo-nitzschia multiseries</em></td>
<td>Cosmopolitan (tem-trop)</td>
<td>Can produce toxin that causes disease in humans, marine mammals, waterfowl, etc.</td>
</tr>
<tr>
<td><em>Pseudo-nitzschia seriata</em></td>
<td>Cosmopolitan (Polar-tem)</td>
<td>-----</td>
</tr>
<tr>
<td><em>Rhizosolenia hebetata</em></td>
<td>Cosmopolitan</td>
<td>-----</td>
</tr>
<tr>
<td><em>Rhizosolenia imbricata</em></td>
<td>Cosmopolitan except Polar regions</td>
<td>-----</td>
</tr>
<tr>
<td><em>Rhizosolenia pungens</em></td>
<td>Cosmopolitan (tem-subtrop)</td>
<td>-----</td>
</tr>
<tr>
<td><em>Rhizosolenia setigera</em></td>
<td>Cosmopolitan (tem-subtrop)</td>
<td>-----</td>
</tr>
<tr>
<td><em>Skeletonema costatum</em></td>
<td>Cosmopolitan except Polar regions</td>
<td>-----</td>
</tr>
<tr>
<td><em>Skeletonema potamos</em></td>
<td>Cosmopolitan estuarine (frw)</td>
<td>-----</td>
</tr>
<tr>
<td><em>Thalassionema bacillare</em></td>
<td>Cosmopolitan</td>
<td>-----</td>
</tr>
<tr>
<td><em>Thalassionema nitzschioides</em></td>
<td>Cosmopolitan except Arctic</td>
<td>-----</td>
</tr>
<tr>
<td><em>Thalassiosira eccentrica</em></td>
<td>Cosmopolitan except Polar regions</td>
<td>-----</td>
</tr>
<tr>
<td><em>Thalassiosira nordenskioeldii</em></td>
<td>Cosmopolitan n. hemisphere (Arctic-tem)</td>
<td>-----</td>
</tr>
<tr>
<td><strong>Dinoflagellates (Phylum Dinophyta)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Akashiwo sanguinea</em></td>
<td>Cosmopolitan (tem-trop)</td>
<td>Can produce reactive oxygen substances; also, blooms can cause hypoxia, fish kills.</td>
</tr>
<tr>
<td><em>Balechina coerulea</em></td>
<td>Cosmopolitan (trop-tem)</td>
<td>-----</td>
</tr>
<tr>
<td><em>Ceratium candelabrum</em></td>
<td>Cosmopolitan (warm tem-trop)</td>
<td>-----</td>
</tr>
<tr>
<td><em>Ceratium furca</em></td>
<td>Cosmopolitan (cold tem-trop)</td>
<td>-----</td>
</tr>
<tr>
<td><em>Ceratium fusus</em></td>
<td>Cosmopolitan (cold tem-trop)</td>
<td>-----</td>
</tr>
<tr>
<td><em>Ceratium lunula</em></td>
<td>Cosmopolitan (warm tem-trop)</td>
<td>-----</td>
</tr>
<tr>
<td><em>Ceratium macroceros</em></td>
<td>Cosmopolitan (cold tem-trop)</td>
<td>-----</td>
</tr>
<tr>
<td><em>Ceratium tripos</em></td>
<td>Cosmopolitan (cold tem-trop)</td>
<td>Blooms can cause hypoxia, fish kills.</td>
</tr>
<tr>
<td><em>Dinophysis acuminata</em></td>
<td>Cosmopolitan (polar-tem)</td>
<td>Can produce okadaic acid and derivative toxins, which cause disease in humans and shellfish.</td>
</tr>
<tr>
<td><em>Dinophysis caudata</em></td>
<td>Cosmopolitan (tem-trop)</td>
<td>Can produce toxins that cause disease in humans and shellfish.</td>
</tr>
<tr>
<td><em>Gambierdiscus toxicus</em></td>
<td>Cosmopolitan (trop-subtrop)</td>
<td>Can produce toxins that cause disease in humans; also linked to disease in fish, sea turtles.</td>
</tr>
<tr>
<td><em>Gonyaulax spinifera</em></td>
<td>Cosmopolitan (tem-subtrop)</td>
<td>Blooms can cause hypoxia, fish kills.</td>
</tr>
<tr>
<td>Taxon</td>
<td>Distribution</td>
<td>Harmful Traits</td>
</tr>
<tr>
<td>--------------------------------------</td>
<td>-----------------------------------</td>
<td>-------------------------------------------------------------------------------</td>
</tr>
<tr>
<td><em>Heterocapsa rotundata</em></td>
<td>Cosmopolitan (tem)</td>
<td>-----</td>
</tr>
<tr>
<td><em>Heterocapsa triquetra</em></td>
<td>Cosmopolitan (tem)</td>
<td>-----</td>
</tr>
<tr>
<td><em>Karlodinium veneficum</em></td>
<td>Cosmopolitan</td>
<td>At high densities ($&gt; 10^4$ cells·mL$^{-1}$), can kill fish and other aquatic life with toxin.</td>
</tr>
<tr>
<td><em>Lingulodinium polyedrum</em></td>
<td>Cosmopolitan (tem-trop)</td>
<td>Can produce saxitoxins and derivatives, which can cause disease and death in humans, shellfish, finfish.</td>
</tr>
<tr>
<td><em>Ornithocercus magnificus</em></td>
<td>Cosmopolitan (warm tem-trop)</td>
<td>-----</td>
</tr>
<tr>
<td><em>Oxyrrhis marina</em></td>
<td>Cosmopolitan</td>
<td>-----</td>
</tr>
<tr>
<td><em>Peridiniella danica</em></td>
<td>Cosmopolitan (Polar-tem)</td>
<td>-----</td>
</tr>
<tr>
<td><em>Peridinium aciculiferum</em></td>
<td>Cosmopolitan (frw-estuarine tem)</td>
<td>Can produce bioactive substances that harm beneficial phytoplankton.</td>
</tr>
<tr>
<td><em>Phalacroma rotundatum</em></td>
<td>Cosmopolitan</td>
<td>Can produce toxins that cause disease in humans and shellfish.</td>
</tr>
<tr>
<td><em>Podolampas palmipes</em></td>
<td>Cosmopolitan (warm tem-trop)</td>
<td>-----</td>
</tr>
<tr>
<td><em>Prorocentrum micans</em></td>
<td>Cosmopolitan (cold tem-trop)</td>
<td>-----</td>
</tr>
<tr>
<td><em>Prorocentrum minimum</em></td>
<td>Cosmopolitan estuarine/marine</td>
<td>Can produce heptaoxin that causes venerupin shellfish poisoning.</td>
</tr>
<tr>
<td><em>Protoperidinium brevipes</em></td>
<td>Cosmopolitan (cold)</td>
<td>-----</td>
</tr>
<tr>
<td><em>Protoperidinium conicum</em></td>
<td>Cosmopolitan (tem-trop)</td>
<td>-----</td>
</tr>
<tr>
<td><em>Protoperidinium depressum</em></td>
<td>Cosmopolitan (tem-trop)</td>
<td>Can produce toxins linked to fish kills.</td>
</tr>
<tr>
<td><em>Protoperidinium pallidum</em></td>
<td>Cosmopolitan (cold-warm tem)</td>
<td>-----</td>
</tr>
<tr>
<td><em>Protoperidinium pellucidum</em></td>
<td>Cosmopolitan (tem-trop)</td>
<td>Can produce toxins linked to fish kills.</td>
</tr>
<tr>
<td><em>Protoperidinium pentagonum</em></td>
<td>Cosmopolitan (tem-trop)</td>
<td>-----</td>
</tr>
<tr>
<td><em>Scrippsiella trochoidea</em></td>
<td>Cosmopolitan</td>
<td>-----</td>
</tr>
<tr>
<td><strong>Cryptophyte Flagellates (Phylum Cryptophyta)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Chroomonas minuta</em></td>
<td>Cosmopolitan estuarine/marine</td>
<td>-----</td>
</tr>
<tr>
<td><em>Cryptomonas erosa</em></td>
<td>Cosmopolitan estuarine/marine</td>
<td>-----</td>
</tr>
<tr>
<td><strong>Cyanobacteria</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Jaaginema geminatum</em></td>
<td>Cosmopolitan estuarine (frw)</td>
<td>Blooms can cause hypoxia, fish kills (frw).</td>
</tr>
<tr>
<td><em>Microcystis aeruginosa</em></td>
<td>Cosmopolitan estuarine (oligohaline), frw</td>
<td>Can produce toxins that cause disease and death of humans, waterfowl, wildlife, and fish. Blooms can also cause hypoxia, fish kills.</td>
</tr>
<tr>
<td><strong>Golden Flagellates (Phylum Ochrophyta)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Apedinella spinifera</em></td>
<td>Cosmopolitan</td>
<td>-----</td>
</tr>
<tr>
<td><em>Dictyocha fibula</em></td>
<td>Cosmopolitan</td>
<td>-----</td>
</tr>
</tbody>
</table>

38
<table>
<thead>
<tr>
<th>Taxon</th>
<th>Distribution</th>
<th>Harmful Traits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Golden Flagellates (Phylum Ochrophyta) (cont’d.)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Dictyocha speculum</em></td>
<td>Cosmopolitan (Polar-tem)</td>
<td>Can cause fish death (gill irritation, mucus over-production).</td>
</tr>
<tr>
<td><em>Pseudopedinella pyriforme</em></td>
<td>Cosmopolitan</td>
<td>-----</td>
</tr>
<tr>
<td>Flagellates – Other Ochrophytes (Class Raphidiophyceae)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Heterosigma akashiwo</em></td>
<td>Cosmopolitan</td>
<td>Can produce toxins that cause disease in humans and fish, and death of fish.</td>
</tr>
<tr>
<td>Green Algae (Phylum Chlorophyta)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Scenedesmus quadricauda</em></td>
<td>Cosmopolitan (freshwater)</td>
<td>-----</td>
</tr>
<tr>
<td><em>Westella botryoides</em></td>
<td>Cosmopolitan (freshwater)</td>
<td>-----</td>
</tr>
</tbody>
</table>
Figure 13. Sample page from the Phytoplankton Atlas prepared for this project, showing micrographs, taxonomic information including former names, potential impacts, known distribution, source regions of origin for the ballast tanks sampled, and references consulted for the taxonomic information (asterisk indicates the original naming reference). See Appendix 2 for the complete Atlas.
Table 11. Phytoplankton taxa that were cultured from ballast water (toxic, potentially toxic; mech., can cause harm via mechanical damage to fish gills, leading to mucus over-production and suffocation; noxious, harmful by causing hypoxia/anoxia or other adverse impacts).

<table>
<thead>
<tr>
<th>Voyage – Tank</th>
<th>Taxon</th>
<th>Algal Group</th>
<th>Harmful</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 – 8P</td>
<td><em>Heterocapsa rotundata</em></td>
<td>Dinoflagellates</td>
<td>-----</td>
</tr>
<tr>
<td>2 – 6P</td>
<td><em>Chaetoceros</em> sp.</td>
<td>Diatoms (centric)</td>
<td>-----</td>
</tr>
<tr>
<td>6 – 8S</td>
<td><em>Thalassiosira eccentrica Prorocentrum micans</em></td>
<td>Diatoms (centric)</td>
<td>Dinoflagellates</td>
</tr>
<tr>
<td>11 – 2P</td>
<td><em>Fragilariopsis</em> sp.</td>
<td>Diatoms (pennate)</td>
<td>-----</td>
</tr>
<tr>
<td>13 – 2S</td>
<td><em>Chaetoceros curvisetus</em></td>
<td>Diatoms (centric)</td>
<td>-----</td>
</tr>
<tr>
<td>14 – 6P</td>
<td><em>Amphora</em> sp.1</td>
<td>Diatoms (pennate)</td>
<td>-----</td>
</tr>
<tr>
<td></td>
<td><em>Amphora</em> sp.2</td>
<td>Diatoms (pennate)</td>
<td>-----</td>
</tr>
<tr>
<td>16 – 2S</td>
<td><em>Actinocyclus curvatulus Chaetoceros</em> sp. Ditylum brightwellii Ditylum sp. Nitzschia sp. Prorocentrum minimum Skeletonema costatum Thalassionema nitzschioides*</td>
<td>Diatoms (centric)</td>
<td>-----</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dinoflagellates</td>
<td>-----</td>
</tr>
<tr>
<td>18 – 4P</td>
<td><em>Ceratium tripos Chaetoceros didymus Chaetoceros socialis Heterosigma akashiwo Lingulodinium polyedrum Prorocentrum sp. Pseudo-nitzschia sp.</em></td>
<td>Diatoms (centric)</td>
<td>-----</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Raphidophyte flagellate</td>
<td>Toxic</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dinoflagellates</td>
<td>Toxic</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Diatoms (pennate)</td>
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<tr>
<td></td>
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<td>Diatoms (pennate)</td>
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<td>Diatoms (pennate)</td>
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<td></td>
<td></td>
<td>Diatoms (pennate)</td>
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<tr>
<td>20 – 3U</td>
<td><em>Chaetoceros</em> sp. Coscinodiscus sp. Cylindrotheca closterium Karlodinium sp. Nitzschia kolascecki Nitzschia laevis Prorocentrum sp. Prorocentrum sp. Prorocentrum sp. 1 Pseudo-nitzschia sp. 1 Pseudo-nitzschia sp. 2 Scripsiella trochoidea*</td>
<td>Diatoms (centric)</td>
<td>-----</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Diatoms (centric)</td>
<td>-----</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Diatoms (pennate)</td>
<td>-----</td>
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<td>Diatoms (pennate)</td>
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<td></td>
<td>Diatoms (pennate)</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>Diatoms (pennate)</td>
<td>-----</td>
</tr>
<tr>
<td>26 – 3U</td>
<td><em>Actinocyclus</em> sp. <em>Chaetoceros</em> sp. <em>Heterocapsa</em> sp. <em>Nitzschia americana</em></td>
<td>Diatoms (centric)</td>
<td>-----</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Diatoms (pennate)</td>
<td>-----</td>
</tr>
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<td>Diatoms (pennate)</td>
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</tr>
<tr>
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<td></td>
<td>Diatoms (pennate)</td>
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Table 11, cont’d.

<table>
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<th>Voyage – Tank</th>
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<th>Algal Group</th>
<th>Harmful</th>
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</tr>
<tr>
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<td><em>Thalassiosira nordenskioeldii</em></td>
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</tr>
<tr>
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</tr>
<tr>
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</tr>
<tr>
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<td>Diatoms (centric)</td>
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</tr>
<tr>
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<td><em>Navicula sp.</em></td>
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</tr>
<tr>
<td></td>
<td>Golden alga (Class</td>
<td>Ochrophyte</td>
<td>----</td>
</tr>
<tr>
<td></td>
<td>Pelagophyceae)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>27 – 8P</td>
<td><em>Chaetoceros decipiens</em></td>
<td>Diatoms (centric)</td>
<td>----</td>
</tr>
<tr>
<td></td>
<td><em>Prorocentrum minimum</em></td>
<td>Dinoflagellates</td>
<td>Toxic</td>
</tr>
<tr>
<td></td>
<td><em>Thalassionema bacillaris</em></td>
<td>Diatoms (pennate)</td>
<td>----</td>
</tr>
<tr>
<td></td>
<td>Golden alga (Class</td>
<td>Ochrophyte</td>
<td>----</td>
</tr>
<tr>
<td></td>
<td>Pelagophyceae)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>27 – 8S</td>
<td><em>Coscinodiscus sp.</em></td>
<td>Diatoms (centric)</td>
<td>----</td>
</tr>
<tr>
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<td>Dinoflagellates</td>
<td>----</td>
</tr>
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<td></td>
<td><em>Prorocentrum micans</em></td>
<td>Dinoflagellates</td>
<td>----</td>
</tr>
<tr>
<td></td>
<td><em>Prorocentrum minimum</em></td>
<td>Dinoflagellates</td>
<td>Toxic</td>
</tr>
<tr>
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<td><em>Amphora spp.1-4</em></td>
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<td>----</td>
</tr>
<tr>
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<td>Diatoms (pennate)</td>
<td>----</td>
</tr>
<tr>
<td></td>
<td><em>Nanofrustulum sp.</em></td>
<td>Diatoms (pennate)</td>
<td>----</td>
</tr>
</tbody>
</table>

**Figure 14.** Relative abundance of viable phytoplankton as major groupings.
Figure 15. Total and viable phytoplankton abundance for each ballast tank, quantitatively collected from plankton net tows (20-µm mesh) and whole-water samples (means ± 1 SD). The data are grouped by major source region (U = unidentified major source) and arranged by ascending age proceeding from left to right. The age (in days) of the ballast water (time held in tanks), based upon the most recent documented ballasting event prior to sampling, is shown over each bar.

While mean phytoplankton abundances were comparable in the summer and fall seasons, and much higher than in winter-spring (Figure 17), median abundance was significantly higher in summer (~2.1 x 10^4 cells m^{-3}) than in the other seasons (~3.1 x 10^3 cells m^{-3}). It should be noted that relatively few tanks (n = 7) were evaluated during the fall season, and included a voyage with coastal water that substantially elevated the seasonal mean. Exclusion of tanks with coastal source water from consideration lowered the overall abundances, especially the fall abundance. Although there were large differences between phytoplankton abundance in summer versus the other seasons, the high variance within each season masked statistically significant differences between phytoplankton abundance in summer versus other seasons.
As expected, mean total phytoplankton abundance was ~4-fold higher in ballast water with coastal sources (7.74 x 10^4 cells m^-3) than in ballast water from the open-ocean (1.97 x 10^4 cells m^-3) (P = 0.026). Median values approximated this same ratio (3.5-fold difference). Mean viable phytoplankton were ~5.5-fold higher (median, 3-fold higher) in ballast water with coastal sources (P = 0.022). Ballast tanks containing coastal source waters had significantly higher pennate diatoms and dinoflagellates, both as total cells and as viable cells, than tanks with open-ocean sources (P = 0.008 to 0.05). Ballast tanks within the coastal source group were stratified into two major age classes (1-14 days and >15 days) to further explore the influence of age on phytoplankton abundance, and similar results were obtained. Overall, the data indicate that coastal status influenced phytoplankton abundance in the ballast tanks, regardless of water age.

4c.3. Phytoplankton Diversity

Abundance and diversity can be viewed as indicators of risk for undesirable species introductions; thus, conditions and practices that promote higher abundance and greater diversity would be undesirable in terms of managing risk of bioinvasive species introductions (McCarthy and Crowder 2000). Phytoplankton diversity was assessed using several indices. Species richness among the ballast tanks spanned from 5-48, with much lower richness of harmful taxa (Figure 18). Highest species richness occurred in tank 18-4P, which received water from the Guam fuel pier area 16 days prior to sampling. Although ballast records indicated that the tanks were comparably managed as “pairs,” species richness for paired tanks sometimes differed markedly. In general (except for voyages 4, 12, 24-7S, and 18-4P), within each source region the species richness decreased with ballast water age. In addition, and as noted for phytoplankton abundance, phytoplankton species richness of ballast water from the Indian Ocean was lower than that from Atlantic or Pacific Ocean sources.

The Shannon-Weiner biodiversity index incorporates both the sample richness and the evenness of the abundance distribution. The latter feature led to a difference in the relative ranking of voyages 6 and 27, and tanks 16-2A and 20-7C (Figure 19A), which were dominated by centric diatoms. Application of this index also revealed a relative increase in phytoplankton diversity from tanks with Indian Ocean source water. Although the tanks were more evenly distributed, the relative mortality (evidenced by the decreases in viable phytoplankton cells)
suggests that the evenness of the species distribution was disrupted through attrition. Lack of a seasonal influence on phytoplankton diversity was supported by MDS representation of the similarity matrix for the phytoplankton assemblages (Figure 20), and by ANOSIM analyses (including some nested analyses that compared seasonal groupings within the ballast water age classes).

Newer diversity indices have incorporated another, potentially important component of diversity, taxonomic relatedness, which is a measure of taxonomically distinct or unique quality (Clark and Warwick 1998). Assessment of this “Taxonomic Distinctness” trait is accomplished through use of a Linnaean or phylogenetic tree to enable measurement of the distance over the hierarchy between every potential species pairing within a sample (Figure 19B). This index yields a mean and variance for the relatedness characteristic of the sample. As an example
Figure 19. Phytoplankton diversity indices for each ballast tank, considering total and viable assemblages quantitatively collected from 20-µm mesh net tows, augmented with data from the 5-µm mesh net tows: (A) Shannon-Weiner indices; (B) Taxonomic Distinctness indices for total phytoplankton assemblages; indices for viable phytoplankton are also included, for comparison to the Shannon-Weiner indices, for a subset of tanks that spans the range of ballast water age. Data are presented as in Figure 15.

Figure 20. Multi-dimensional scaling (MDS) patterns in a Phytoplankton Similarity Matrix by seasonal groupings, showing no significant effect of season on phytoplankton diversity (all ballast tanks considered collectively).
comparing traditional diversity indices with diversity measures that include relatedness, consider a sample that has 5 species within the same genus, versus another sample that has 5 species within 5 different families. The Shannon-Weiner (traditional) diversity index would assess the two samples as having the same “statistical” level of diversity. The more sensitive Linnaean diversity index would instead assess the two samples as having different levels of “true” diversity, with the second sample having higher diversity and, therefore, greater associated potential risk. Overall, phytoplankton species diversity was low to moderate relative to, for example, diversity in natural estuarine systems (e.g. Shannon-Weiner indices < 3; Clark and Warwick 1994, U.S. EPA 2002). Diversity was higher in tanks with coastal water (P = 0.035) (Figure 21), considering water of all ages as well as water that had been held in tanks for less than 15 days, and regardless of the metric (diversity index) used.

4d. Microzooplankton

Microzooplankton are ecologically important because they include the primary grazers of phytoplankton and bacteria. Most taxa are not considered harmful. A proportion of the 20-80 µm size range considered in this study consisted of larval or egg stages that could not be identified to species. The overall average abundance of microzooplankton across all ballast tanks assessed was ~470 ± 950 organisms · m⁻³ (CV=2) (median, 150 organisms · m⁻³) (Figure 22). Abundance was highly variable, with the range spanned three orders of magnitude, from ~4 to 4,290 organisms · m⁻³; such high variability has been reported in other studies (e.g. Murphy et al. 2002).

Microzooplankton were considered based upon the following groups: amoebae, ciliates (tintinnids, others), cladocerans, copepod nauplii, foraminifers, molluscan larvae, radiolarians, rotifers, turbellarians (flatworms), invertebrate eggs, and other invertebrate larvae. Assemblages were dominated by marine ciliates (mean, ~340 organisms · m⁻³, found in 84% of the tanks), especially tintinnids (Table 12, Figures 23-25), which are considered to be ubiquitous and cosmopolitan in distribution as cold- or warm-water taxa. Nematodes (mean ~20 organisms · m⁻³; in 60% of the tanks) and copepod nauplii (mean, 79 organisms · m⁻³; in 47% of the tanks) were sub-dominant. Various other early instar or larval forms of molluscs, marine worms, copepods, cladocerans, and other microcrustaceans were also found, and invertebrate eggs occurred in 23%
of the tanks. Zooplankton cultures consisted mostly of ciliates, especially within the genera *Aspidisca, Euplotes,* and *Oxytricha.* At least one ciliate was cultured from 55% of the ballast tanks.

Zooplankton density was grouped based on ten classification variables relating to vessel type, service group, exchange status, ocean basin, location and season and were compared via ANOVA, without significant results. Total zooplankton and group densities were also regressed against several potential explanatory variables including vessel and ballast age (e.g. Figure 26), and eleven other physical and chemical variables. Several different filtering scenarios were applied across these variable pairings including removal of tanks based on sampling method, coastal status, and exchange status. Densities were analyzed with and without transformation (log x+1), and no significant correlations were discerned. Principal Components Analysis (PCA) was also conducted, considering the data from all tanks and including zooplankton density, ballast age, vessel age, salinity, temperature, TN, NO3-, TP, and TOC. Various combinations of these potential independent variables were applied with zooplankton density, and different selection factors were applied excluding samples based on exchange status variables. The combinations were run with and without transformation of zooplankton density. The combined variance explained by the first 2 principal components rarely exceeded 50%, indicating that the selected variables did not strongly influence zooplankton density.

Limited analyses of microzooplankton species diversity were possible because of the prevalence of young life history stages that could be identified only to general groupings. Based on the available data, microzooplankton species richness ranged from 1 to 14 (Figure 27). Median species richness ranged from 3 (Indian Ocean) to 5-6 (Atlantic and Pacific source regions, respectively). There was no discernable pattern in species richness based on ballast water age or coastal status.

Figure 22. Overall total viable microzooplankton abundance (size class, 20-80 µm) in each ballast tank (means ± 1 SD), considered by major source region and status (coastal, open-ocean, uncertain). Data are given as in Figure 15.
Table 12. Microzooplankton taxa (size class, 20-80 µm) in the ballast tanks, also indicating maximum dimension (in units of µm) and information about known general distribution.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Larval Group</th>
<th>Functional Group</th>
<th>Minimum Dimension</th>
<th>Distribution</th>
<th>Latitude</th>
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<td>Amoebaes</td>
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<td>Amoeba</td>
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<td>Foraminiferans</td>
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<td></td>
<td></td>
</tr>
<tr>
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<td>Foraminiferans</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Globigerina sp.</td>
<td>NA</td>
<td>Foraminiferans</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Arachnocyclus circumincta</td>
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<td>Radiolarians</td>
<td>10</td>
<td>Cosmopolitan</td>
<td>Cold</td>
</tr>
<tr>
<td>Cladococcus cercophcos</td>
<td>NA</td>
<td>Radiolarians</td>
<td>50</td>
<td>Cosmopolitan</td>
<td>Cold</td>
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<td>Radiolarians</td>
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<td></td>
<td></td>
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<tr>
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<td>Other Ciliates</td>
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<td>Neritic</td>
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<td>Paracordella caudata</td>
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<td>10</td>
<td>?</td>
<td>Cold</td>
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<tr>
<td>Pleychoxia sp.</td>
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</tr>
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<td>Undella claparedai</td>
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<td>?</td>
<td>Warm</td>
</tr>
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<td>Tintinnid Ciliates</td>
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<td>?</td>
<td>Warm</td>
</tr>
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<td>Kystonella sp.</td>
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<td>Tintinnid Ciliates</td>
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<td>Copepods</td>
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<td>Crustacean Larvae</td>
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<td>Crustacean Larvae</td>
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<td>Unidentified species</td>
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<td>Invertebrate Eggs</td>
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<td>Unidentified species</td>
<td>Y</td>
<td>Invertebrate Larvae</td>
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<td>Rotifers</td>
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<td>N</td>
<td>Rotifers</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Synchaeta – species 1</td>
<td>N</td>
<td>Rotifers</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>
**Figure 23.** Microzooplankton assemblage composition (means, all ballast tanks considered collectively).

**Figure 24.** Relative abundance: mean percentage of tanks that contained the indicated microzooplankton groupings.

**Figure 25.** Mean relative abundance of microzooplankton groups, considering all tanks (total) versus excluding tanks with coastal source waters. A similar pattern in relative abundance of functional groups was discerned whether tanks with coastal waters were included or excluded from analysis, suggesting that coastal ballast water sources did not significantly influence the zooplankton assemblage structure.

**Figure 26.** Scatter plots of water age versus log-transformed densities of microzooplankton (correlation coefficient $r = -0.099$).
4e. Bacteria

Bacterial abundance in ballast tank water, determined from flow-cytometric methods, was surprisingly consistent across this study among ballast tanks and seasons, and varied within one order of magnitude (Figures 28, 29). The overall mean across all tanks examined was $3.13 \pm 1.27 \times 10^{11}$ cells $\cdot$ m$^{-3}$ (median, $2.79 \times 10^{11}$ cells $\cdot$ m$^{-3}$). Means by region were $2.7 \times 10^{11}$ cells $\cdot$ m$^{-3}$, $3.2 \times 10^{11}$ cells $\cdot$ m$^{-3}$, and $3.5 \times 10^{11}$ cells $\cdot$ m$^{-3}$ for the Atlantic, Indian, and Pacific Oceans, respectively. Means for ballast tanks with coastal and non-coastal waters were $3.13 \pm 1.27 \times 10^{11}$ cells $\cdot$ m$^{-3}$ (median, $3.22 \times 10^{11}$ cells $\cdot$ m$^{-3}$) and $2.96 \pm 1.41 \times 10^{11}$ cells $\cdot$ m$^{-3}$ (median, $2.66 \times 10^{11}$ cells $\cdot$ m$^{-3}$), respectively. *Vibrio* spp. were detected in 14 (23%) of the 62 tanks sampled (Figure 30), and densities ranged from ~200 CFU $\cdot$ 100 mL$^{-1}$ to TNTC (too numerous to count; practical estimate of

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**Figure 27.** Microzooplankton species richness for each ballast tank, considered by source region, water age, and status (coastal, open-ocean, uncertain). Data are given as in Figure 15.

**Figure 28.** Total bacterial densities by major source region, age, and status (coastal, open-ocean, uncertain). Data are given as in Figure 15.
ANOVA indicated that bacterial density differed by source region (significantly different in tanks containing Atlantic versus Pacific Ocean waters; \( P = 0.01 \)), but was unrelated to vessel type, service group, exchange status, location, or season. There were no statistically significant correlations between log-transformed bacterial densities or *Vibrio* spp. densities and minimum ballast water age (Figure 31) or physical/chemical variables. Principal Components Analysis, considering all ballast tanks, included bacterial density, ballast age, vessel age, salinity, temperature, TN, NO\(_3^–\), TP, and TOC. The first two principal components explained only 41% of the total variability; the dominant eigen vectors were TN for PC1 and bacterial density and minimum ballast water age for PC2.

Figure 29. Bacterial abundance by season, considering all ballast tanks collectively (means ± 1 SD).

Figure 30. Occurrence and densities of *Vibrio* spp. in each ballast tank by source region, age, and status (coastal, open-ocean, uncertain). The data were obtained using TCBS plating, which is selective for *Vibrio* but does not permit speciation. Red numbers represent the age of the water based upon the most recent ballasting activity. The line (at 1 CFU · 100 mL\(^{-1}\)) indicates the regulatory treatment standards threshold of the IMO Convention (2004) and the U.S. Ballast Water Management Act (2005) for *Vibrio cholerae*. Data are given as in Figure 15.

ANOVA indicated that bacterial density differed by source region (significantly different in tanks containing Atlantic versus Pacific Ocean waters; \( P = 0.01 \)), but was unrelated to vessel type, service group, exchange status, location, or season. There were no statistically significant correlations between log-transformed bacterial densities or *Vibrio* spp. densities and minimum ballast water age (Figure 31) or physical/chemical variables. Principal Components Analysis, considering all ballast tanks, included bacterial density, ballast age, vessel age, salinity, temperature, TN, NO\(_3^–\), TP, and TOC. The first two principal components explained only 41% of the total variability; the dominant eigen vectors were TN for PC1 and bacterial density and minimum ballast water age for PC2.
Of the 11 pathogenic eubacterial taxa screened, 4 (*Listeria monocytogenes*, *Escherichia coli*, *Mycobacterium* spp., *Pseudomonas aeruginosa*) were detected from analysis of all ballast tanks (Table 13). Approximately 48% of the ballast tanks contained at least 1 of these pathogens. Toxigenic *Vibrio cholerae* strains were not detected in any of the tanks.

### 4f. Comparative Analyses of Biota

#### 4f.1. Abundances of Phytoplankton, Microzooplankton, and Bacteria

The abundances of phytoplankton, microzooplankton, and bacteria in the ballast tank waters generally were consistent with marine trophic structure (Day et al. 1989) (Figure 32). Similarity matrix analyses (rank-sum procedures, Kendall, Spearman, and weighted Spearman) revealed no comparable patterns among the phytoplankton, microzooplankton, and bacterial assemblages (e.g. Figure 33). The low correlations ($r \leq 0.197$) confirmed the lack of pattern match considering all tanks, tank subgroupings, seasonality, and other factors.

#### 4f.2. Variance in Biota Abundance Within vs. Across Ships

Our experimental design (duplicate samples taken from each of two or more ballast tanks per ship for most vessels) allowed us to examine variability in abundances of bacteria, phytoplankton, and microzooplankton within and among ships, for ships that used different ballasting practices. We compared all tanks collectively and two subsets of data: paired tanks (with apparently similar ballasting history: 13 ships as 1-4, 7, 9, 10, 12, 14, 15, 18, 22, 28; n = 26) and unpaired tanks (vessels with at least two ballast tanks of differing management histories: 10 ships as 6 (with 8 tanks), 11 (with 2 tanks), 16 (with 2 tanks), 17 (with 4 tanks), 19 (with 2 tanks), 20 (with 2 tanks), 24 (with 3 tanks), 25 (with 2 tanks), 26 (with 2 tanks), and 27 (with 4 tanks); n = 31). Restricted maximum likelihood estimates of variance in biota abundances among ships, between or among ballast tanks within ships, and between duplicate samples per tank were calculated using PROC VARCOMP and SAS MIXED (SAS Institute, Inc. 1999).

Considering all ships and tanks, variance across tanks within ships was the dominant component (67-92% of the total) (Table 14). For ships with paired tanks of similar ballasting history, the largest source of variation was among ships. Variation in bacterial abundance between paired tanks was much lower than variation in abundance among ships; variation in phytoplankton abundance between paired tanks was about two-thirds of that among ships; and
variation in microzooplankton abundance between paired tanks was about half of that among ships. Duplicate samples contributed little to the variation in the data. For ships with unpaired tanks of differing histories (both data subsets 1 and 2), the largest source of variation in biota abundances was between or among ballast tanks within ships.

### Table 13. Molecular screening for pathogenic eubacteria.

<table>
<thead>
<tr>
<th>Voyage</th>
<th># Positive Pathogens</th>
<th>Listeria monocytogenes</th>
<th>Mycobacterium spp.</th>
<th>Pseudomonas aeruginosa</th>
<th>Voyage</th>
<th># Positive Pathogens</th>
<th>Listeria monocytogenes</th>
<th>Mycobacterium spp.</th>
<th>Pseudomonas aeruginosa</th>
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</table>

P = Positive  NA = sample compromised or unavailable for analysis
Blank = Negative  Note: Grayed voyage/tank designators indicated tanks included at least some coastal waters.
Figure 32. Comparative densities of total and viable phytoplankton (as cells), microzooplankton (as individuals), and bacteria (as cells). Data are given as means ± 1 SD, considering tanks with versus without coastal source waters. Asterisks (*) indicate a statistically significant difference between organism abundances in coastal versus open-ocean ballast water sources.

Figure 33. Multi-dimensional scaling (MDS) ordination of (A) phytoplankton and (B) microzooplankton zooplankton assemblages in the ballast tanks (by corresponding voyage numbers), showing no similarity in abundance patterns.

Table 14. Restricted maximum likelihood (REML) variance components for phytoplankton, microzooplankton, and bacterial abundances, expressed as proportions of the overall variance within and across ships, for all tanks (complete data set) and for tank subsets 1-3. Note that the error term for each of the four dataset analyses was a very small proportion of the overall variance (< 0.01).

<table>
<thead>
<tr>
<th>Variance Component</th>
<th>Complete Data Set</th>
<th>Paired Subset 1</th>
<th>Unpaired Subset 1</th>
<th>Unpaired Subset 2</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Phyto</td>
<td>MZoo</td>
<td>Bact</td>
<td>Phyto</td>
</tr>
<tr>
<td>Across Ships</td>
<td>0.33</td>
<td>0.09</td>
<td>0.15</td>
<td>0.57</td>
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<tr>
<td>Within Ships</td>
<td>0.67</td>
<td>0.52</td>
<td>0.86</td>
<td>0.43</td>
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</table>

1 = Paired Subset = Subset of ships for which only paired (same ballasting history) tanks were sampled 1,2,3,4,10,12,14,15,16,22,28.
2 = Unpaired Subset 1 = Subset of ships for which more than a single set of paired tanks were sampled 6,11,17,24,27.
3 = Unpaired Subset 2 = Subset of ships for which multiple pairs or a single set of unpaired tanks was sampled 6,11,15,17,20,24,25,26,27.
The variance in microbiota abundances for paired and unpaired tanks were also characterized using Principal Components Analysis (PCA) (Figure 34). Ballast water age, temperature, salinity, dissolved oxygen (DO) concentrations, NO$_3^-$ concentrations, and TOC concentrations were included in each PCA analysis, after log-transforming all data except ballast water age. The paired-tank ordinations (Figure 36A,C,E) were tightly clustered in comparison to the unpaired-tank ordinations. The percentage of the variance in the dataset that was explained by PC1 and PC2 was moderate, with means of 58% and 62% for the paired-tank and unpaired-tank datasets, respectively. For the paired-tanks dataset, the dominant variables for PC1 were salinity, DO, and TOC, and the dominant variables for PC2 were ballast water age and temperature. For the unpaired-tanks dataset, the dominant variables for PC1 were temperature and DO, and the dominant variables for PC2 were TOC and NO$_3^-$. Although PCA indicated strong similarity between paired tanks in phytoplankton abundance and controlling factors, as mentioned, species diversity sometimes differed markedly between paired tanks. In their analysis of macrozooplankton, Holm et al. (2005) reported that mean abundances were not significantly correlated between paired tanks. Thus, significant differences in biota abundances even in paired tanks may arise, likely related to environmental factors that can diverge in one tank versus another over time. In general, our data on abundances of bacteria, phytoplankton, and microzooplankton support the interpretations elegantly stated by Holm et al. (2005):

“These results have important implications for understanding how ballasting practices, in the absence of management or treatment, may affect the likelihood of transferring aquatic nonindigenous species. Fleet oilers and amphibious vessels utilize different subsets of ballast tanks at different times and places depending on operational requirements, producing ballast loads with variable or heterogeneous histories. For such vessels, concentrations of microbiota (including invasive microorganism) at the scale of the ship cannot be estimated by collecting samples from single tanks. Multiple ballast tanks must be sampled, with the number depending on the magnitude of variation in the tank histories. This conclusion also holds, but to a lesser extent, for ships carrying relatively homogeneous (in terms of history) ballast loads. For data from vessels with ballast tanks paired by age, there remained substantial variation between the tanks in microbiota abundances. Inspection of the mean abundances within these tanks suggests that paired tanks will often present abundances of similar magnitude. In this case a single ballast tank could serve as a reasonable predictor of microbiota abundances in all of a ship’s tanks of similar history. Such correspondence in concentrations between paired tanks, however, may not be universal. These data also suggest that there is little to be gained from taking replicate samples from individual tanks. Effort should instead be expended in sampling additional ballast tanks or additional ships, as these are the sources of most variation in microbiota abundances.”

4f.3. Considerations Based on Proposed / Established Standards for Ballast Water

The viable phytoplankton and microzooplankton analyzed in this study were categorized by their maximum cell dimension into two size classes, in order to interpret the data according to proposed clean ballast discharge standards. The standards considered were those proposed by the U.S. Senate (Senate Bill S.363; Ballast Water Management Act of 2005), or by the International Maritime Organization (IMO; Regulation D-2, 2004) (Table 15). For these ships, exceedences were negligible or very low in the small size category (10-50 µm) (Table 15; Figures 35, 36). In the larger (>50 µm) size category, however, viable phytoplankton in 47% of the ballast tanks and microzooplankton in 31-39% of the tanks exceeded the proposed standards.
Figure 34. Principal components analyses of biota abundance considering paired tanks with similar ballasting history (A, phytoplankton; C, microzooplankton; E, bacteria) versus “unpaired” tanks with different ballasting history (B, phytoplankton; D, microzooplankton; F, bacteria). For (A,C,E), paired tanks were from voyages 1,2,3,4,7,9,10,12,14,15,18,22, and 28; voyage numbers are shown, with the second member of each pair in red. For (B,D,F), the following tanks were included: voyages 11,16,19,20,25,26 each with two tanks; voyage 24 with three tanks; voyages 17 and 27 each with 4 tanks; and voyage 6 with 8 tanks. In (B), the lower right cluster includes six tanks from voyage 6, four tanks from voyage 17, two tanks from voyage 27, and one each from voyages 16, 20, and 26. In (D), most unpaired tanks formed a cluster with respect to microzooplankton abundance. In (F), the upper left cluster includes four tanks from voyage 6, two from voyage 24, one from voyage 26, and four from voyage 27; the lower left cluster includes four tanks from voyage 6, two from voyage 11, four from voyage 17, and one each from voyages 18,19, and 20.
4g. Heat Treatment Experiments

Six phytoplankton and 3 microzooplankton taxa were evaluated for thermal tolerance, including 3 dinoflagellates, 2 diatoms, 1 raphidophyte, 1 ciliate, 1 rotifer, and 1 copepod (Table 16). The pennate diatom *Pseudo-nitzschia* sp. was most sensitive to elevated temperature, with a complete kill achieved within 2 hours at 32°C. The rotifer *Brachionus plicatilis* was the most heat-tolerant, and a temperature of 41°C was needed to effect a complete kill; surprisingly for
known to have a

this organism, 40°C was the lowest temperature that produced complete mortality within 24 hours. The toxigenic raphidophyte *Heterosigma akashiwo* and ciliates within the genus *Euplotes* were also fairly heat-tolerant, with 24-hour kill temperatures of 36°C and 37°C, respectively (e.g. Figure 37). Of the 9 taxa tested, 5 phytoplankton taxa and the calanoid copepod *Acartia tonsa* exhibited complete mortality within 24 hours at 34°C or less. The dinoflagellate *Prorocentrum micans* was also highly sensitive to heat treatment, with complete mortality observed in 8 hours at 34°C based upon failure of re-culture attempts.

### Table 16. Heat treatment effects on selected phytoplankton and microzooplankton species.

<table>
<thead>
<tr>
<th>Test Organism</th>
<th>Major Grouping</th>
<th>Temperature Tested with Most Rapid Kill</th>
<th>Lowest Temperature with Complete Kill in 24 Hours</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Phytoplankton</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Coscinodiscus</em> sp.</td>
<td>Centric Diatom</td>
<td>35°C – 8 hours</td>
<td>34°C</td>
</tr>
<tr>
<td><em>Heterosigma akashiwo</em></td>
<td>Raphidophyte</td>
<td>36°C – 12 hours</td>
<td>36°C</td>
</tr>
<tr>
<td>Peridinoid species</td>
<td>Dinoflagellate</td>
<td>34°C – 4 hours</td>
<td>33°C</td>
</tr>
<tr>
<td><em>Prorocentrum micans</em></td>
<td>Dinoflagellate</td>
<td>34°C – 2 hours</td>
<td>33°C</td>
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<tr>
<td><em>Prorocentrum triestinum</em></td>
<td>Dinoflagellate</td>
<td>38°C – 6 hours</td>
<td>34°C</td>
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<tr>
<td><em>Pseudo-nitzschia</em> sp.</td>
<td>Pennate Diatom</td>
<td>32°C – 2 hours</td>
<td>32°C</td>
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<tr>
<td><strong>Microzooplankton</strong></td>
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<tr>
<td><em>Acartia tonsa</em></td>
<td>Calanoid Copepod</td>
<td>34°C – 4 hours</td>
<td>34°C</td>
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<tr>
<td><em>Brachionus plicatilis</em></td>
<td>Rotifers</td>
<td>41°C – 2 hours</td>
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<tr>
<td><em>Euplotes</em> spp.</td>
<td>Marine Ciliates</td>
<td>37°C – 8 hours</td>
<td>37°C</td>
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</table>

### 5. Summary and Conclusions

Bioinvasive species continue to present a significant threat to marine environments and to public health, energy, and local economies worldwide (Rayl 1999, Pimentel et al. 2000). Ballast water exchange is presently regarded as the most important mechanism for transfer of aquatic nonindigenous species. Although many aquatic microflora and microfauna are known to have a
cosmopolitan distribution, ballast water exchange practices can alter the abundances of harmful species and set up conditions where previously rare populations proliferate (e.g. Forbes and Hallegraeff 1998, Hallegraeff 1998).

DoD vessels have been documented to transport and introduce nonindigenous aquatic species (Coles et al. 1997, 1999). In the first study of microflora and microfauna transported in ballast tanks of DoD ships, Ruiz et al. (1999a) reported that the most abundant organisms in the ballast tanks of 18 U.S. Navy oilers and amphibious vessels entering Chesapeake Bay were copepods (macrozooplankton) and diatoms, among taxa ranging from protozoa to fish. The authors reported that abundances of macrofauna in the ballast water of the Navy vessels was ~ten-fold lower than macrofauna abundance in commercial vessels that were also sampled after entering the Bay. The present study contributes additional information by characterizing the environmental conditions and the phytoplankton, microzooplankton, and bacteria in 62 ballast tanks on 28 DoD vessels (11 fleet oilers, 15 roll-on/roll-off carriers, 1 container ship, and 1 lighter) from 13 different harbors on the U.S. East and West Coasts. A companion study by Holm et al. (2005) characterized the macrozooplankton, as well. In other project efforts, from 18 laboratory bench-scale experiments we assessed impacts of heat treatments on selected phytoplankton and zooplankton species, toward evaluating the efficacy of using heat to reduce the abundance of invasive microbiota in ballast water of DoD ships. We also designed an Atlas of phytoplankton species that were identified from the ballast tanks, and constructed a regional database for data organization, storage, and export for statistical analyses, and for future use in risk assessment.

In addition to depth profiles of environmental conditions (temperature, salinity, pH, dissolved oxygen, turbidity) in each tank, 280 analyses were conducted for total Kjeldahl nitrogen, nitrate, total phosphorus, total suspended solids, and chlorophyll \(a\) concentrations (as an overall index of phytoplankton biomass); 185 preserved phytoplankton samples and 122 preserved microzooplankton samples were analyzed for taxonomic composition; 372 phytoplankton microcultures were prepared from fresh (unpreserved) subsamples to assist in species identifications; 183 samples were assessed for bacterial abundance; 62 samples were screened for 11 bacterial pathogens using molecular techniques; and 62 samples were plated in triplicate for detection of selected pathogenic \textit{Vibrio} species.

A high percentage (94%) of the tanks had adequate records to determine the source locales and age of the ballast water. Most tanks (~90%) had undergone some form of ballast

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**Figure 37.** Heat treatment profile for the toxigenic raphidophyte flagellate, \textit{Heterosigma akashiwo}. Re-culture attempts and flow-cytometric enumeration demonstrated attrition of this species. Viability was maintained for 24 hours at 34°C, but the threshold or response curve was steep, with a marked decrease in viable cells at 36°C.
exchange, and contained at least a portion of ballast water from an open-ocean source by the
time ships entered a U.S. port. For 37% of the tanks, however, the extent of exchange could not
be determined from the available records, and for 10% of the tanks it was not possible to assess
whether any treatment or management practice had been applied.

The tanks were well mixed, with little evidence of depth stratification in most, adequate
dissolved oxygen for biota, negligible turbidity, and low to moderate nutrient concentrations. No
significant relationships were detected between physical/chemical parameters and
phytoplankton, microzooplankton, or bacterial abundances, except for a statistically significant
positive relationship between centric diatom densities and nitrate concentrations.

A total of 100 phytoplankton species were identified from the ballast tanks, including 19
potentially harmful taxa defined as capable of causing disease or death of humans or beneficial
aquatic life. Nearly all species, including the harmful taxa, are cosmopolitan, and all have
previously been reported from U.S. coastal waters. The phytoplankton assemblages were
dominated by diatoms and dinoflagellates. Viable cells comprised about half of the total.
Abundances of both phytoplankton and microzooplankton assemblages were highly variable.
Microzooplankton were dominated by tintinnid ciliates with cosmopolitan distribution, with
subdominant nematodes and copepod nauplii; many organisms were egg or larval stages that
could not be identified to species.

Bacterial abundances were surprisingly consistent among ballast tanks. Abundance was
significantly different in ballast tanks with Atlantic versus Pacific Ocean water, but was
unrelated to vessel type, exchange status, age of water, environmental conditions measured, or
other factors. At least 1 of 4 pathogenic eubacteria (Listeria monocytogenes, Escherichia coli,
Mycobacterium spp., Pseudomonas aeruginosa) was detected in 48% of the ballast tanks.
Toxigenic strains of Vibrio cholerae were not detected in any tanks of these DoD vessels,
differing from Ruiz et al.’s (1999a) findings of various densities of Vibrio cholerae serotypes in
the ballast waters of commercial vessels.

Overall, there were no similarities in abundance patterns among phytoplankton,
microzooplankton, and bacterial assemblages. Abundance of phytoplankton, but not of
microzooplankton or bacteria, was higher in tanks with coastal water sources. Despite a general
pattern of reduction in abundance of all three microbiota groups with ballast water age,
regression analyses indicated that age accounted for negligible variation in abundance. Thus,
microbiota abundances are more strongly influenced by other factors, possibly including
spatial/temporal variations in microbiota during ballasting, differential effects of ballast water
management, and variation in sources of stress factors among ballast tanks and ships. Evaluation
of sources of variability in microbiota abundances showed that for ships with tanks of similar
ballasting history, the largest source of variation was among ships. In contrast, for ships with
tanks of differing ballasting histories, and for all ships/tanks considered collectively, the largest
source of variation in biota abundances was between/among ballast tanks within ships. Duplicate
samples per tank contributed little variation to the data.

The findings from this study do not enable assessment of the densities of harmful species
that ensure a successful invasion or outbreak in receiving estuaries and coastal waters where
ballast tanks are unloaded. Many site-specific factors acting in concert – climatic conditions,
season, light regime, the available suite of nutrient supplies, the presence of potential predators,
mixing characteristics, water column depth, bottom sediment characteristics, and the presence and abundance of potential competitor microbiota – control whether a given harmful species can successfully establish and thrive in an area where it is introduced (e.g. Smith et al. 1999). It should perhaps be re-emphasized that all of the harmful taxa found in the ballast waters examined in this study have already been reported from U.S. coasts. In addition, records indicate that a very high proportion (~90%) of the tanks assessed on DoD ships were subjected to ballast water exchange practices, indicating that DoD ships are well managed in comparison to commercial ships (e.g. Ruiz et al. 1999a,b) in minimizing the risk for introduction of harmful microbiota.

6. Recommendations

Comparison of size class data for viable phytoplankton and microzooplankton with proposed clean ballast discharge standards indicated that the DoD vessels would have been in compliance for the small size category (organism maximum dimension, 10-50 µm). However, in the larger (>50 µm) size category, viable phytoplankton in 47% of the ballast tanks, and microzooplankton in 31-39% of the ballast tanks, exceeded the proposed standards. Thus, development, evaluation, and adoption of treatment technologies or alternative management strategies, along with open ocean ballast water exchange, will be necessary to enable DoD vessels to comply with proposed standards for ensuring safe and environmentally sound operations. It should be noted that in the heat treatment experiment of this study, except for a toxigenic raphidophyte flagellate, all phytoplankton taxa tested were killed at 34°C or less. Previous research (Hallegraeff 1998) suggests that this temperature can be attained in ballast tanks, at least for some ship designs, by using waste engine heat. A disadvantage of heat treatment would be the elevated corrosivity of warm seawater and the hull stress related to multiple heating and cooling cycles. However, if an effective stand-alone or combination treatment system could be made feasible that includes waste heat, its environmental and economic benefits may outweigh added vessel maintenance costs.

Based on the present study, reliable estimates of microbiota abundances at a vessel scale will require sampling multiple ballast tanks selected to encompass the variation in the tank histories. Efforts to obtain replicate samples from individual tanks should be minimized in favor of sampling additional tanks or additional ships. For ships with tanks containing ballast water of similar history, a single tank can often serve as a reasonable predictor of microbiota for all tanks. Nevertheless, multiple tanks should be sampled if possible because significant differences in biota abundances even in paired tanks can occur, likely related to environmental factors that diverge in one tank versus another over time.

Although various types of ships were sampled in this study, unfortunately amphibious vessels of the U.S. Navy were not available because of their operational commitments. Naval amphibious vessels carry a substantial proportion of the ballast water transported by DoD vessels that are regulated under the UNDS program (Uniform National Discharge Standards 1999). Different types of vessels and their ballast tanks can strongly influence the transport of viable harmful algae and other microbiota (e.g. Dickman and Zhang 1999). Characterization of the microbiota in ballast tanks on naval amphibious vessels is recommended, when operations allow. In addition, we were not able to determine whether ballast water exchange affects coastal microbiota assemblages, because comparisons to test for effects of exchange would require
samples from tanks holding coastal water that had not been exchanged. In the vessels available for study, no samples met this criterion with certainty. We recommend additional research to assess effects of ballast water exchange on coastal microbiota, when ships holding coastal water that has not been exchanged become available for comparison with tanks containing exchanged water.

The data from this study suggest that operations of DoD vessels may present a low risk of transfer of pathogenic bacteria such as toxigenic strains of *Vibrio cholerae*. Given that our assessment was limited by funding constraints, further research encompassing a broader suite of microbial pathogens would be desirable. Another shortcoming of this study and the companion effort of Holm et al. (2005) is that it did not include consideration of biota potentially transported on ship hulls and in seachests. Holm et al. (2000) determined that 64% of inspections of the Navy Sea Systems Command’s vessels operating beyond 12 n.m. from U.S. coasts had reported some level of fouling. In supporting work, Holm et al. (2003) found that ~26% of naval vessels undergoing hull cleaning had sustained heavy fouling on some portion of the hull. Thus, we also recommend additional research to assess the importance of ship fouling for introducing aquatic nonindigenous species.

We found no indication that harmful species can be selectively controlled through ballast water management practices – thus, practices that reduce microbiota abundances in general, such as open-ocean exchange in combination with treatment measures, will also minimize the risk of introduction of harmful taxa. Toward that goal, DoD vessels should be encouraged to conduct ballast water exchange as far from shore as possible, as also recommended by Ruiz et al. (1999b) and Holm et al. (2005). The U.S. Navy’s current ballast exchange policy requires that ships be at least 12 n.m. offshore for ballast water exchange, but this distance is still relatively close to shore. Regulations for commercial vessels (see Regulatory Drivers, 33 CFR Part 151) instead require ballast water exchange to be conducted in waters more than 200 n.m. from shore or 2,000 meters in depth. Moving farther offshore for ballast water exchange would help DoD to ensure that coastal organisms are not present in ship intake water (e.g. Locke et al. 1991), and would minimize survival of (oceanic) species subsequently deposited near shore.

Finally, although records indicate that a very high proportion (~90%) of the tanks assessed on DoD ships were subjected to ballast water exchange practices, indicating that DoD ships are well managed in comparison to commercial ships, it was not possible to determine the extent of ballast water exchange because of incomplete ship logs. U.S. Navy ships are required by regulation to maintain detailed logs (e.g. NENRP 1994). DoD vessels should be encouraged to maintain detailed records of ballast water management not only in order to improve compliance, but also to strengthen understanding about the origins and controlling influences on microbiota assemblages in DoD ballast waters.

7. References


Carlton, J.T., D.M. Reid, and H. van Leeuwen (1995) *The Role of Shipping in the Introduction of Nonindigenous Aquatic Organisms to the Coastal Waters of the United States (Other Than the Great Lakes) and an Analysis of Control Options*. Report Number CG-D-11-95. U.S. Coast Guard Research and Development Center, Groton, CT, USA.


APPENDIX I
NCSU Sample Kit Preparation Procedure for Ballast Water Samples

1.0 General:

A typical ballast sampling kit for this project consists of 2 coolers. The larger of the two will house the preserved samples and the smaller of the two holds the unpreserved samples and the nutrient/analytical samples. This will constitute the samples from two ballast tanks from a single ship, each sampled in triplicate. The following procedure describes types of bottles, preservation, the necessary labeling and the packing.

2.0 Supplies Listing – Supplies required to provide one kit, consisting of the sample bottles and packing materials to sample two ballast tanks, each in triplicate, for one vessel and to ship these samples back to the NCSU laboratory.

<table>
<thead>
<tr>
<th>Coolers</th>
</tr>
</thead>
<tbody>
<tr>
<td>1, 24 × 14 × 12 or closest dimensions possible</td>
</tr>
<tr>
<td>1, 14 × 10 × 12 or closest dimensions possible</td>
</tr>
</tbody>
</table>

Having the bottles fit as snugly as possible is desirable from a shipping stability standpoint.

<table>
<thead>
<tr>
<th>Bottles</th>
</tr>
</thead>
<tbody>
<tr>
<td>10, 1000 ml Nalgene Clear (6 of which must be acid-washed)</td>
</tr>
<tr>
<td>6, For use as kit bottle type 11</td>
</tr>
<tr>
<td>2, For use as kit bottle type 4C</td>
</tr>
<tr>
<td>2, For use as kit bottle type 6</td>
</tr>
<tr>
<td>2, 500 ml Nalgene Clear –</td>
</tr>
<tr>
<td>2, For use as kit bottle type 3C</td>
</tr>
<tr>
<td>8, 250 ml Nalgene Clear</td>
</tr>
<tr>
<td>2, For use as kit bottle type 9</td>
</tr>
<tr>
<td>6, For use as kit bottle type 2</td>
</tr>
<tr>
<td>6, 250 ml Nalgene Brown</td>
</tr>
<tr>
<td>6, For use as kit bottle type 1</td>
</tr>
<tr>
<td>8, 60 ml Nalgene Clear (2 of which must be acid washed)</td>
</tr>
<tr>
<td>6, For use as kit bottle type 10</td>
</tr>
<tr>
<td>2, For use as kit bottle type 8</td>
</tr>
<tr>
<td>2, 60 ml Amber Glass (acid washed)</td>
</tr>
<tr>
<td>2, For use as kit bottle type 7</td>
</tr>
<tr>
<td>2, 40 ml Amber Glass Vials (acid washed)</td>
</tr>
<tr>
<td>2, For use as kit bottle type 5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Graduated cylinders</td>
</tr>
<tr>
<td>1 set of bottle labels</td>
</tr>
<tr>
<td>Cooler ID labels for the bottles</td>
</tr>
<tr>
<td>Foam Inserts for stabilizing the package</td>
</tr>
<tr>
<td>1, TOC vial storage block</td>
</tr>
<tr>
<td>2-4 Coolie Packs</td>
</tr>
<tr>
<td>2, Completed Shipping Labels</td>
</tr>
<tr>
<td>Packing Tape</td>
</tr>
</tbody>
</table>

Page 1 of 4
3.0 **Bottle Preparation and Distribution** - Refer to the tables below for preparation and distribution to the two coolers, which constitute a kit.

3.1 **Cooler 1** - Two of the kit bottle types listed below are volume-critical, meaning marks must be made on the bottle for specific volume levels so that field personnel can achieve the proper sample to preservative ratios during sampling without the complication of taking actual measurement containers on board. These are bottle types 2 and 10. See supplies above and cooler tables below.

3.1.1 For sample kit bottle type number 2, take one of the six, 250 ml Nalgene bottles designated for this use type and fill it with 120 ml of DI water. Mark that line with a sharpie and write “Formalin Fill Line” next to it. Then add 80 more ml of DI water and apply the label designated for this bottle type so that the top of the label is aligned with the water mark. Be sure to shake out all of the water. Repeat with the remaining 5, type 2 bottles.

3.1.2 For sample kit bottle type number 10, fill each of the 6, 60 ml Nalgene bottles with 50 ml of DI and apply the top of the bottle label so it is aligned with the watermark. Thoroughly shake the excess water out of each.

3.1.3 Apply the applicable labels to bottle types 1 and 11.

3.1.4 Apply one of the round label stickers to the top of each bottle and clearly write the bottle type number appropriate to that bottle within the circular label.

3.1.5 This constitutes the contents of cooler 1 (bigger cooler). Place these 24 bottles into this cooler in an organized fashion and secure for shipment. Place 2 coolie packs into the cooler and cover all contents with the foam sheet for shipping stability.

### Cooler Number 1 – Preserved samples

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Container Size</th>
<th>Container Material</th>
<th>Bottle Prep</th>
<th>Sample volume</th>
<th>Sample:Preservative</th>
<th>Number of Bottles / Samples</th>
<th>Kit Sample Type #</th>
<th>Volume Critical?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phytoplankton</td>
<td>250 ml</td>
<td>Brown - Nalgene</td>
<td>Soap/DI</td>
<td>200 ml</td>
<td>Lugols – 12 drops</td>
<td>6</td>
<td>1</td>
<td>N</td>
</tr>
<tr>
<td>Zooplankton</td>
<td>250 ml</td>
<td>Nalgene</td>
<td>Soap/DI</td>
<td>80 ml</td>
<td>120 ml 10% Form.</td>
<td>6</td>
<td>2</td>
<td>Y</td>
</tr>
<tr>
<td>Flow Cytometry - Bacteria</td>
<td>60 ml</td>
<td>Nalgene</td>
<td>Soap/DI</td>
<td>50 ml</td>
<td>1ml, 50% Glut.</td>
<td>6</td>
<td>10</td>
<td>Y</td>
</tr>
<tr>
<td>Small Phyto &amp; 16S</td>
<td>1000 ml</td>
<td>Nalgene</td>
<td>Acid Strip</td>
<td>600 ml</td>
<td>Lugols – 2 drops</td>
<td>6</td>
<td>11</td>
<td>N</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td><strong>24</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
3.2 *Cooler 2* – Other than the preservative for the TOC vials, none of these containers receive preservative and none are volume-critical.

3.2.1 Apply the appropriate label from the label sheet to each bottle

3.2.2 Apply one of the round label stickers to the top of each bottle and clearly write the bottle type number appropriate to that bottle within the circular label.

3.2.3 This constitutes the contents of cooler 2 (smaller cooler).

3.2.4 Place these 14 bottles into this cooler in an organized fashion and secure for shipment. Use one of the foam blocks for the TOC vials. Place 2 coolie packs into the cooler and cover all contents with the foam sheet for shipping stability.

### Cooler Number 2 – Preserved samples

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Container Size</th>
<th>Container Material</th>
<th>Sample Volume</th>
<th>Bottle Prep</th>
<th>Preserv.</th>
<th>Number of Bottles / Samples</th>
<th>Kit Sample Type #</th>
<th>Volume Critical?</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;20-um phytoplankton growout comp</td>
<td>500 ml</td>
<td>Nalgene</td>
<td>300 ml</td>
<td>Soap / DI</td>
<td>None</td>
<td>2</td>
<td>3C</td>
<td>N</td>
</tr>
<tr>
<td>Nut Composite</td>
<td>1000 ml</td>
<td>Nalgene</td>
<td>Full</td>
<td>Acid Strip</td>
<td>None</td>
<td>2</td>
<td>4C</td>
<td>N</td>
</tr>
<tr>
<td>TOC</td>
<td>40 ml</td>
<td>Amber-Glass</td>
<td>Full</td>
<td>Combust</td>
<td>2 drops H3PO4</td>
<td>2</td>
<td>5</td>
<td>N</td>
</tr>
<tr>
<td>TSS/Chla</td>
<td>1000 ml</td>
<td>Nalgene</td>
<td>Full</td>
<td>Soap / DI</td>
<td>None</td>
<td>2</td>
<td>6</td>
<td>N</td>
</tr>
<tr>
<td>Nutrient – TP</td>
<td>60 ml</td>
<td>Amber-Glass</td>
<td>40 ml</td>
<td>Acid Strip</td>
<td>None</td>
<td>2</td>
<td>7</td>
<td>N</td>
</tr>
<tr>
<td>Nutrient – TN</td>
<td>60 ml</td>
<td>Nalgene</td>
<td>50 ml</td>
<td>Acid Strip</td>
<td>None</td>
<td>2</td>
<td>8</td>
<td>N</td>
</tr>
<tr>
<td>Phytoplankton growout &lt;20-um</td>
<td>250 ml</td>
<td>Nalgene</td>
<td>150 ml</td>
<td>Soap / DI</td>
<td>None</td>
<td>2</td>
<td>9</td>
<td>N</td>
</tr>
<tr>
<td><strong>Totals</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td><strong>14</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
NCSU Sample Kit Preparation Procedure for Ballast Water Samples

4.0 Packaging & Shipment

4.1 Using the information below, have the shipping administrator generate Fed Ex or UPS labels using the following information. Unless there is a known urgency, an inexpensive shipping route should be used.

<table>
<thead>
<tr>
<th>NCSU Vessel Kit – NCSU Cooler #1</th>
<th>NCSU Vessel Kit – NCSU Cooler #2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dimensions: 24 × 14 × 12</td>
<td>Dimensions: 14 × 10 × 12</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Item</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cooler &amp; Bottles</td>
<td>12 lb</td>
</tr>
<tr>
<td>Samples</td>
<td>15 lb</td>
</tr>
<tr>
<td>Ice / packs</td>
<td>5.5 lb</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>33 lb</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Item</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cooler &amp; Bottles</td>
<td>7.1 lb</td>
</tr>
<tr>
<td>Samples</td>
<td>4.4 lb</td>
</tr>
<tr>
<td>Ice / packs</td>
<td>5.5 lb</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>17 lb</strong></td>
</tr>
</tbody>
</table>

4.2 The coolers will be sent to one of the following places depending upon need. As a policy, as coolers are returned from the field with samples, a new kit should be prepped and sent back to replenish the sender’s stock as soon as possible. The intention is to keep each coastal operation stocked with three kits at all times (with capacity to sample six ballast tanks in triplicate).

Attention: **Eric Holm**

Naval Surface Warfare Center - Carderock Div.
Code 641, Bldg. 60, Room 334
9500 MacArthur Blvd.
West Bethesda, MD
20817-5700
(301) 227-4948

Attention: **Jeff Grovhough**

Space & Naval Warfare Systems Center
Code 2362, Marine Environmental Quality Branch - San Diego
53475 Strothe Road (Bayside, Bldg 111, Rm 259)
San Diego, CA
92152-6310
Ph: (619)553-5475  FAX: (619)553-6305
Procedures for the Collection of Water Quality Data From Ballast Tanks
Using the Hydrolab Surveyor 4 Data Sonde

Edition:  C

Effective Date:  September, 2002

Approval:

Interim Revisions:

<table>
<thead>
<tr>
<th>Number</th>
<th>Section</th>
<th>Pages</th>
<th>Description</th>
<th>Initial/Date</th>
</tr>
</thead>
<tbody>
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</tr>
</tbody>
</table>
1.0 **Scope**
This SOP covers the procedures relating to the collection of *in-situ* water quality data, from ballast tanks.

2.0 **Objective**
2.1 To help maintain production of high-quality data through use of standardized and well documented procedures.
2.2 To insure sample integrity from collection through storage.
2.3 To maintain good record keeping.
2.4 To provide a reference for all personnel and trainees in particular.

3.0 **In-situ Water Quality Data Collection with the Hydrolab Data Surveryor 4 with SCUFA Chlorophyll-a Fluorescence Detector**

3.1 **Attachment**
Attach the 9-pin end of the Hydrolab cable to the back of the Surveyor and the 5-pin end to the Hydrolab. When attaching the Hydrolab end, be sure the rubber bump on the side of the plug lines up exactly with the large pin on the Hydrolab. The pins will bend easily if not properly alligned.

3.2 **The Keys**
3.2.1 The Surveyor4 front panel has eleven keys. These keys can be divided into the following sets: the top keys, the rectangular function keys, the triangular cursor keys, and the Hydrolab key.
3.2.2 The top right-hand key turns the Surveyor on and off. The top left-hand key turns the screen backlight on and off.
3.2.3 The four rectangular function keys correspond to the four rectangles at the bottom of the screen and allow setup, calibration, and other selections.
3.2.4 The four triangular cursor keys allow the cursor to be moved through the Surveyor menus, submenus, and virtual keyboards. If the cursor button is held down, the cursor will move in a fast-repeat mode.
3.2.5 The Hydrolab button is used to access manufacturing and programming information.

3.3 **The Screen**
The screen displays real-time readings, graphs, or submenus (such as setup or calibration). The backlight feature lets you use the instrument in low-light or dark conditions. It can be turned on and off with the top left-hand key.
3.4 **The History Line**

The dark gray line at the bottom of the screen is the “history line.” It shows how the Surveyor is connected and allows the operator to follow the path selected, through the menu and submenu structure.

3.5 **The Function and Arrow Keys**

The four function keys are the blue rectangular soft keys on the front panel. They activate four corresponding rectangular “screen keys” shown on the bottom of the Surveyor screen. The illustration below matches the function keys to their corresponding screen keys.

3.5.1 **Menu Navigation Example – See Below**

Pressing the upper left function key will move the operator to the Setup/Cal option. The screen will now read:
3.5.2 Press Setup again to choose between the Setup options. The screen will now read:

![Setup Options]

3.5.3 Use the arrow keys to highlight the specific option of interest and press select to access it.

3.5.4 Press the Go Back key if there is a need to select a different option.

3.6 **File Setup**

3.6.1 A separate file is created in the Surveyor at the beginning of each day the Hydrolab is used.

3.6.2 Use the function keys to enter the file menu. The pathway is: Files-> Surveyor4. Select Create from the menu.
3.6.3 Choose the Manual option. The next screen that appears is the Log: Filename screen. The default file name is Surveyor4. Use the arrow, select, and backspace keys to enter the new file name using the characters in the text box. Generally, use the ship’s name as the file name for routine sampling trips. Press done.

3.6.4 The next screen will list the parameters that will be collected each time the log button is pressed. The Surveyor4 automatically applies the last group of selected parameters as the default group for the file currently being created. This is the proper set of parameters. Do not change this order. Press done.

3.6.5 The “File Created…” message will appear when setup is complete.

3.7 **Annotation**

3.7.1 The annotation function allows the user to manually enter information, such as ballast tank number or location on the ship, into a file.

3.7.2 Open the file menu. The pathway is: Files-> Surveyor4.

3.7.3 Highlight “Annotate” and press select. This will open the list of files stored in the data logger. Use the arrow keys to highlight the file to be annotated and press select.

3.7.4 Use the arrow keys and the select button to enter the annotation using the characters in the text box. Press done.

3.7.5 The annotation is complete.

3.8 **Logging**

3.8.1 To log data into the Surveyor memory, simply press the “Store” function key.

3.8.2 After the “store” key is pressed, the list of files will appear. Highlight the file the data is to be stored in and press select.

3.8.3 The file list will disappear when the data is logged.

3.9 **Site Deployment**

3.9.1 Carefully unscrew the storage cup and install the weighted sensor guard.

3.9.2 Once the system is assembled and a file is created the Hydrolab is ready to be deployed. Begin by annotating the file with the ballast tank number.

3.9.3 Lower the Hydrolab into the water so that the sensors are just below the surface. Slightly raise or lower the Hydrolab until the depth reading is approximately 0.1 meters.

3.9.4 Allow the readings to stabilize and log the results.

3.9.5 Lower the Hydrolab to ~0.5 meters, allow the readings to stabilize, and log the results.
3.9.6 Repeat the previous step in half-meter increments if the standing water depth is shallow, and at one-meter increments if the depth is greater (e.g. > 6 or 7 meters) until the bottom is reached. Be certain to allow the readings to completely stabilize at each depth.

3.9.7 Upon completion of the cast, carefully replace the storage cup and power down the unit.
### Sampling Supplies

(Assumes an event = 1 vessel @ 2 ballast tanks per vessel and @ 3 reps per tank)

<table>
<thead>
<tr>
<th>Vessel-Based Sample Bottles</th>
<th>Number</th>
<th>K, P, S</th>
</tr>
</thead>
<tbody>
<tr>
<td>60 ml Opaque Nalgene</td>
<td>8</td>
<td>K</td>
</tr>
<tr>
<td>60 ml Amber glass</td>
<td>2</td>
<td>K</td>
</tr>
<tr>
<td>40 ml Amber glass Vial</td>
<td>2</td>
<td>K</td>
</tr>
<tr>
<td>250 ml Nalgene widemouth</td>
<td>6</td>
<td>K</td>
</tr>
<tr>
<td>250 ml Brown Nalgene wide</td>
<td>6</td>
<td>K</td>
</tr>
<tr>
<td>500 ml Brown Nalgene wide</td>
<td>2</td>
<td>K</td>
</tr>
<tr>
<td>1000 ml Nalgene widemouth</td>
<td>6</td>
<td>K</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>32</strong></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Compositing Bottles</th>
<th>Number</th>
<th>K, P, S</th>
</tr>
</thead>
<tbody>
<tr>
<td>250 ml Nalgene</td>
<td>2</td>
<td>K</td>
</tr>
<tr>
<td>500 ml Nalgene (grow composite)</td>
<td>2</td>
<td>K</td>
</tr>
<tr>
<td>1000 ml Nalgene (nutrient comp)</td>
<td>2</td>
<td>K</td>
</tr>
<tr>
<td><strong>Rinsing and Concentrating</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Large squirt bottle (for raw water)</td>
<td>1</td>
<td>P</td>
</tr>
<tr>
<td>Large squirt bottle (for 20-um filtrate)</td>
<td>1</td>
<td>P</td>
</tr>
<tr>
<td>Collection bucket (for raw water)</td>
<td>1</td>
<td>P</td>
</tr>
<tr>
<td>Collection bucket (for 20-um filtrate)</td>
<td>1</td>
<td>P</td>
</tr>
<tr>
<td>2 or 4-liter Nalgene pitchers</td>
<td>2</td>
<td>P</td>
</tr>
<tr>
<td>Collection funnels</td>
<td>4</td>
<td>P</td>
</tr>
<tr>
<td>8”, 20-um sieves</td>
<td>4</td>
<td>P</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Preservatives &amp; Chemicals</th>
<th>Number</th>
<th>K, P, S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lugols dropper bottle</td>
<td>1</td>
<td>P</td>
</tr>
<tr>
<td>Rose Bengal dropper bottle</td>
<td>1</td>
<td>P</td>
</tr>
<tr>
<td>200 ml glass bottle for Lugols</td>
<td>1</td>
<td>P</td>
</tr>
<tr>
<td>200 ml glass bottle of glutaraldehyde</td>
<td>1</td>
<td>P</td>
</tr>
<tr>
<td>Gluteraldehyde dropper</td>
<td>1</td>
<td>P</td>
</tr>
<tr>
<td>1000 ml 10% buff. formalin</td>
<td>1</td>
<td>P</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sampling Equip</th>
<th>Number</th>
<th>K, P, S</th>
</tr>
</thead>
<tbody>
<tr>
<td>20-um, 30 x 100 cm net</td>
<td>2/6¹</td>
<td>P</td>
</tr>
<tr>
<td>1 liter cod end jar assembly</td>
<td>2/6¹</td>
<td>P</td>
</tr>
<tr>
<td>35 meter, 3/8” rope with stainless snap shackle and winding board</td>
<td>2/4¹</td>
<td>P</td>
</tr>
<tr>
<td>Lab-line sampler</td>
<td>1/4¹</td>
<td>P</td>
</tr>
<tr>
<td>Hydrolab</td>
<td>1/2¹</td>
<td>K</td>
</tr>
<tr>
<td>Metronome</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>Watch or timer</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>Depth meter / measurement</td>
<td>S</td>
<td></td>
</tr>
</tbody>
</table>

1= X/Y indicates that X will be required for a 2-tank sampling event on a given ship. Y indicates that this will likely be the total number that will be provided for the project (e.g. the minimum number required to supply both coastal operations

<table>
<thead>
<tr>
<th>Misc.</th>
<th>Number</th>
<th>K, P, S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tops for cod end jars</td>
<td>1</td>
<td>P</td>
</tr>
<tr>
<td>Large cable ties</td>
<td>4</td>
<td>P</td>
</tr>
<tr>
<td>Flathead screwdriver</td>
<td>1</td>
<td>S</td>
</tr>
<tr>
<td>Extra 30 meter lines with winding boards and snap shackles</td>
<td>1</td>
<td>P</td>
</tr>
<tr>
<td>Extra bottles of each type</td>
<td>6</td>
<td>P</td>
</tr>
<tr>
<td>Extra net weights</td>
<td>2</td>
<td>P</td>
</tr>
<tr>
<td>Flashlights</td>
<td>1</td>
<td>S</td>
</tr>
</tbody>
</table>

K = Included in a ship kit and returned to NCSU after each event

P = Project Stock Item. Necessary to sample a vessel, but will be a long-term stock item provided to NSWC teams at project incept.

S = Sampling Equipment expected to be available as part of NSWC sampling efforts
Preservation Instructions

Preservatives:
- Acidic Lugols: Each coastal operation will be provided with 2, 100 ml amber dropper bottles of Lugols.
- 10% Buffered Formalin: Each coastal operation will be provided with 4, 4-liter jugs of 10% buffered formalin. Three of these jugs have been adjusted to a salinity of 20 psu. The fourth is at 0 psu in the event that low salinities are encountered.
- Rose Bengal: Each coastal operation will be provided with 1, 100 ml amber dropper bottle.
- 50% Glutaraldehyde: Each coastal operation will be provided with a 100 ml container of Glutaraldehyde.

Phytoplankton:
For the phytoplankton sample that is in excess of 20-um (net sample), add 12 drops of Lugols. The phytoplankton and 16S RNA sample, which will be collected as a whole water sample from the labline sampler will be lightly preserved with lugols – only two drops. **The preservative can be added to the sample bottle prior to departure to conduct the sampling.**

Zooplankton
The zooplankton net sample will be first stained with Rose Bengal and then fixed with 10% buffered formalin. Each of the sample bottles will have volume lines. The black sharpie line on the bottle will be the sample line (80 ml.). After adding the sample add three drops of Rose Bengal and let it sit for 5 minutes. The add 120 ml of 10% buffered Formalin. The top of the label is this formalin fill line. This will make for a total volume of 200 ml. **The preservative can be added to the sample bottle prior to departure to conduct the sampling.**

Bacteria
Using the pipets in the bag add 1 ml of the 50% Glutaraldehyde to each 60 ml bottle just prior to departure for sampling. **This material is toxic and noxious and care should be taken with its use. This should be conducted under a hood or in a well ventilated area. Use eye protection and gloves. An MSDS is included.** When sampling, fill the bottle to the top of the label with sample from the labline sampler as indicated in the graphics below.
Samples >20-um - The Lugols and formaldehyde preserved samples will be subjected to size fractionation and further concentration at the NCSU laboratory for distribution and analysis. A bottle that is designated as “volume-critical” will be marked with a fill level. Bottles not marked “volume critical” should be filled to approximate the volumes listed in the tables and on the labels. The 500 ml. of net concentrate that remains from each tow is available to the NSWC/SERC should they wish to pass this sample through an 80-µm sieve. If sufficient, the sampling effort could be consolidated to provide the NSWC with the samples they need. This is an option.

<table>
<thead>
<tr>
<th>Ident.</th>
<th>Container</th>
<th>Vol. / Rep</th>
<th>Chemical Preserv.</th>
<th>Sample : Preservative</th>
<th># Per Rep</th>
<th># Per Tank</th>
<th># Per Ship</th>
<th>Kit Sample Type #</th>
<th>Cooler</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phytoplankton</td>
<td>250 ml Nalg.</td>
<td>~200 ml</td>
<td>Acid Lugols</td>
<td>200ml: 12 drops of Lugols</td>
<td>1</td>
<td>3</td>
<td>6</td>
<td>1</td>
<td>NCSU-1</td>
</tr>
<tr>
<td>Zooplankton</td>
<td>250 ml Nalg.</td>
<td>200 ml</td>
<td>10% Form</td>
<td>80ml : 3 drops of Rose Bengal, 120 ml form</td>
<td>1</td>
<td>3</td>
<td>6</td>
<td>2</td>
<td>NCSU-1</td>
</tr>
<tr>
<td>Growout Comp</td>
<td>500 ml Nalg.</td>
<td>~300 ml</td>
<td>None</td>
<td>None</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>3C</td>
<td>NCSU-2</td>
</tr>
</tbody>
</table>

Two tows per replicate if water depth is 2.5 meter or less.

Net ring diam: 30 cm
Overall length: 100 cm
Theoretical Vol: 70.7 L / meter
Tow Rate: 0.5 meter / Sec
Marked at 750 ml

Ident. Container Vol. / Rep Chemical Preserv. Sample : Preservative # Per Rep # Per Tank # Per Ship Kit Sample Type # Cooler
Phytoplankton | 250 ml Nalg. | ~200 ml | Acid Lugols | 200ml: 12 drops of Lugols | 1 | 3 | 6 | 1 | NCSU-1 |
Zooplankton | 250 ml Nalg. | 200 ml | 10% Form | 80ml : 3 drops of Rose Bengal, 120 ml form | 1 | 3 | 6 | 2 | NCSU-1 |
Growout Comp | 500 ml Nalg. | ~300 ml | None | None | 1 | 1 | 2 | 3C | NCSU-2 |
NCSU Ballast Sampling Schematic Continued:

1. < 20-µm = phytoplankton, cysts, and bacteria for fractionation separation back at the laboratory
2. Nutrients, Chl, solids, and TOC

The solids, nutrients, and pigments will be collected and composited in a 1-liter container for mixing and distribution to the bottles listed below.

<table>
<thead>
<tr>
<th>Identification</th>
<th>Container</th>
<th>Vol. / Rep</th>
<th>Chem Pres.</th>
<th>Kit Bottle Type #</th>
<th>Cooler</th>
<th># Per Rep</th>
<th># Per Tank</th>
<th># Per Ship</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOC</td>
<td>40 ml amber glass</td>
<td>Full</td>
<td>None</td>
<td>5</td>
<td>NCSU-2</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>TSS/Chla</td>
<td>500 ml Nalgene</td>
<td>Full</td>
<td>None</td>
<td>6</td>
<td>NCSU-2</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>TP</td>
<td>60 ml amber glass</td>
<td>~40 ml</td>
<td>None</td>
<td>7</td>
<td>NCSU-2</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>TN</td>
<td>60 ml Nalgene</td>
<td>~50 ml</td>
<td>None</td>
<td>8</td>
<td>NCSU-2</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Phytoplankton growout</td>
<td>250 ml Nalgene</td>
<td>~150 ml</td>
<td>None</td>
<td>9</td>
<td>NCSU-2</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Flow Cytometry</td>
<td>60 ml Nalgene</td>
<td>50 ml</td>
<td>1ml Glut.</td>
<td>10</td>
<td>NCSU-1</td>
<td>1</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Phytoplankton count &amp; 16S RNA</td>
<td>1000 ml Nalgene</td>
<td>~600 ml</td>
<td>Acid Lugols</td>
<td>11</td>
<td>NCSU-1</td>
<td>1</td>
<td>3</td>
<td>6</td>
</tr>
</tbody>
</table>
Two samplers will be provided in the kit for each coastal operation and a third will be provided as backup for opportunistic sampling. One sampler can be used for all three replicates for a given tank, as is the case with the nets, but must be disinfected in between sampling trips with bleach. The units are completely autoclavable in the event there is access to an autoclave. The whole unit should be rinsed thoroughly after bleaching, including the foam near the cap. Copies of the units instructions will be included in the kit.

<table>
<thead>
<tr>
<th>Depth (Meters)</th>
<th>Number of Vertical Stations</th>
<th>~Time Interval at each Vertical Station</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>1 min</td>
</tr>
<tr>
<td>1.5</td>
<td>2</td>
<td>30 sec</td>
</tr>
<tr>
<td>2.0</td>
<td>2</td>
<td>30 sec</td>
</tr>
<tr>
<td>2.5</td>
<td>3</td>
<td>20 sec</td>
</tr>
<tr>
<td>3.0</td>
<td>3</td>
<td>20 sec</td>
</tr>
<tr>
<td>3.5</td>
<td>4</td>
<td>15 sec</td>
</tr>
<tr>
<td>4.0</td>
<td>4</td>
<td>15 sec</td>
</tr>
<tr>
<td>4.5</td>
<td>5</td>
<td>12 sec</td>
</tr>
<tr>
<td>5.0</td>
<td>5</td>
<td>12 sec</td>
</tr>
<tr>
<td>5.5</td>
<td>6</td>
<td>10 sec</td>
</tr>
<tr>
<td>6.0</td>
<td>6</td>
<td>10 sec</td>
</tr>
<tr>
<td>6.5</td>
<td>7</td>
<td>8 sec</td>
</tr>
<tr>
<td>7.0</td>
<td>7</td>
<td>8 sec</td>
</tr>
<tr>
<td>7.5</td>
<td>8</td>
<td>7 sec</td>
</tr>
<tr>
<td>8.0</td>
<td>8</td>
<td>7 sec</td>
</tr>
<tr>
<td>8.5</td>
<td>9</td>
<td>6 sec</td>
</tr>
<tr>
<td>9.0</td>
<td>9</td>
<td>6 sec</td>
</tr>
<tr>
<td>9.5</td>
<td>10</td>
<td>5 sec</td>
</tr>
<tr>
<td>10.0</td>
<td>10</td>
<td>5 sec</td>
</tr>
</tbody>
</table>

The intent is to obtain a sample that is as representative as possible of the water column. Maintaining the Labline sampler at the vertical stations and time periods indicated will produce a total volume between 1400 and 1800 ml. This will provide more than enough volume for the samples indicated above. The biological samples will be sieved and concentrated at the NCSU laboratory. Time periods are approximate, but avoid varying too much from the chart. A little more or a little less water will enter the canister based on pressure (depth), but the differences are manageable. For instance, during testing, the volume obtained at 1 meter versus the volume obtained 7 meters for a 60-second time period was approximately, 1400 ml and 1550 ml, respectively. If any diversion from the above table were to occur, shorter time periods would be desirable over longer periods, because complete filling of the canister is to be avoided. The fill volume is ~2000 ml. and a volume at or below that is desirable because one can be sure that the canister did not fill completely before reaching the surface.
**Cooler and Bottle Distribution—Constitutes a Vessel Kit**
(Assumes 1 vessel @ 2 ballast tanks per vessel and @ 3 reps per tank)

**Shipment sequence:** NCSU Vessel Kit Cooler NCSU-1 (Field site to NCSU)

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Container Size</th>
<th>Container Material</th>
<th>Sample volume</th>
<th>Sample:Preservative</th>
<th>Number of Bottles / Samples</th>
<th>Kit Sample Type #</th>
<th>Volume Critical?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phytoplankton</td>
<td>250 ml</td>
<td>Brown - Nalgene</td>
<td>200 ml</td>
<td>Lugols – 12 drops</td>
<td>6</td>
<td>1</td>
<td>N</td>
</tr>
<tr>
<td>Zooplankton</td>
<td>250 ml</td>
<td>Nalgene</td>
<td>80 ml</td>
<td>120 ml 10% Form.</td>
<td>6</td>
<td>2</td>
<td>Y</td>
</tr>
<tr>
<td>Flow Cytometry - Bacteria</td>
<td>60 ml</td>
<td>Nalgene</td>
<td>50 ml</td>
<td>1ml, 50% Glut.</td>
<td>6</td>
<td>10</td>
<td>Y</td>
</tr>
<tr>
<td>Small Phyto &amp; 16S</td>
<td>1000 ml</td>
<td>Nalgene</td>
<td>600 ml</td>
<td>Lugols – 2 drops</td>
<td>6</td>
<td>11</td>
<td>N</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td><strong>24</strong></td>
<td></td>
</tr>
</tbody>
</table>

A bottle that is designated, as “volume-critical” will be marked with a fill level. Bottles not marked “volume-critical” should be filled to approximate the volumes listed in the tables and on the labels. The TOC sample below needs to be filled completely to produce an inverted meniscus before cap closure precluding any air bubbles.

**Shipment sequence:** NCSU Vessel Kit Cooler NCSU-2 (Field site to NCSU)

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Container Size</th>
<th>Container Material</th>
<th>Sample volume</th>
<th>Preserv.</th>
<th>Number of Bottles / Samples</th>
<th>Kit Sample Type #</th>
<th>Volume Critical?</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;20-um phytoplankton growout composite</td>
<td>500 ml</td>
<td>Nalgene</td>
<td>300 ml</td>
<td>None</td>
<td>2 / 2</td>
<td>3C</td>
<td>N</td>
</tr>
<tr>
<td>Nutrient Composite Bottle</td>
<td>1000 ml</td>
<td>Nalgene</td>
<td>750 ml</td>
<td>None</td>
<td>2 / 0</td>
<td>4C</td>
<td>N</td>
</tr>
<tr>
<td>TOC</td>
<td>40 ml</td>
<td>Amber-Glass</td>
<td>Full</td>
<td>2 drops H₂PO₄</td>
<td>2 / 2</td>
<td>5</td>
<td>N</td>
</tr>
<tr>
<td>TSS/Chla</td>
<td>500 ml</td>
<td>Nalgene</td>
<td>Full</td>
<td>None</td>
<td>2 / 2</td>
<td>6</td>
<td>N</td>
</tr>
<tr>
<td>Nutrient – TP</td>
<td>60 ml</td>
<td>Amber-Glass</td>
<td>40 ml</td>
<td>None</td>
<td>2 / 2</td>
<td>7</td>
<td>N</td>
</tr>
<tr>
<td>Nutrient – TN</td>
<td>60 ml</td>
<td>Nalgene</td>
<td>50 ml</td>
<td>None</td>
<td>2 / 2</td>
<td>8</td>
<td>N</td>
</tr>
<tr>
<td>Phytoplankton growout &lt;20-um</td>
<td>125 ml</td>
<td>Nalgene</td>
<td>100 ml</td>
<td>None</td>
<td>2 / 2</td>
<td>9</td>
<td>N</td>
</tr>
<tr>
<td><strong>Totals</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td><strong>14</strong></td>
<td></td>
</tr>
</tbody>
</table>
NCSU Sample Processing Procedure for Ballast Water Samples

1.0 General:

A typical ballast sampling kit for this project will consist of two coolers. The larger of the two will hold the preserved samples and the smaller of the two will house the unpreserved samples and the nutrient/analytical samples. This will constitute the samples from two ballast tanks from a single ship, each sampled in triplicate. The following procedure describes in detail the various size fractionation and concentration steps necessary to provide the various analytical aliquots. In addition, this process will produce several samples that will require molecular analysis by Dr. Oldach at UMD and samples to be sent to our zooplankton subcontractor.

Contents of Kit Cooler Number 1 – Preserved Samples

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Container Size</th>
<th>Container Material</th>
<th>Bottle Prep</th>
<th>Sample volume</th>
<th>Sample:Preservative</th>
<th>Number of Bottles / Samples</th>
<th>Kit Sample Type #</th>
<th>Volume Critical?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phytoplankton</td>
<td>250 ml</td>
<td>Brown - Nalgene</td>
<td>Soap/DI</td>
<td>200 ml</td>
<td>Lugols – 12 drops</td>
<td>6</td>
<td>1</td>
<td>N</td>
</tr>
<tr>
<td>Zooplankton</td>
<td>250 ml</td>
<td>Nalgene</td>
<td>Soap/DI</td>
<td>80 ml</td>
<td>120 ml 10% Form.</td>
<td>6</td>
<td>2</td>
<td>Y</td>
</tr>
<tr>
<td>Flow Cytometry - Bacteria</td>
<td>60 ml</td>
<td>Nalgene</td>
<td>Soap/DI</td>
<td>50 ml</td>
<td>1 ml, 50% Glut.</td>
<td>6</td>
<td>10</td>
<td>Y</td>
</tr>
<tr>
<td>Small Phyto &amp; 16S</td>
<td>1000 ml</td>
<td>Nalgene</td>
<td>Acid Strip</td>
<td>600 ml</td>
<td>Lugols – 2 drops</td>
<td>6</td>
<td>11</td>
<td>N</td>
</tr>
<tr>
<td>TOTAL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>24</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Contents of Kit Cooler Number 2

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Container Size</th>
<th>Container Material</th>
<th>Sample volume</th>
<th>Bottle Prep</th>
<th>Preserv.</th>
<th>Number of Bottles / Samples</th>
<th>Kit Sample Type #</th>
<th>Volume Critical?</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;20-um phytoplankton growout comp</td>
<td>500 ml</td>
<td>Nalgene</td>
<td>300 ml</td>
<td>Soap / DI</td>
<td>None</td>
<td>2</td>
<td>3C</td>
<td>N</td>
</tr>
<tr>
<td>Used for nut. comp on board, then for raw tank water for use as culture media</td>
<td>1000 ml</td>
<td>Nalgene</td>
<td>Full</td>
<td>Acid Strip</td>
<td>None</td>
<td>2</td>
<td>4C</td>
<td>N</td>
</tr>
<tr>
<td>TOC</td>
<td>40 ml</td>
<td>Amber-Glass</td>
<td>Full</td>
<td>Combust</td>
<td>2 drops H₃PO₄</td>
<td>2</td>
<td>5</td>
<td>N</td>
</tr>
<tr>
<td>TSS/Chla</td>
<td>1000 ml</td>
<td>Nalgene</td>
<td>Full</td>
<td>Soap / DI</td>
<td>None</td>
<td>2</td>
<td>6</td>
<td>N</td>
</tr>
<tr>
<td>Nutrient – TP</td>
<td>60 ml</td>
<td>Amber-Glass</td>
<td>40 ml</td>
<td>Acid Strip</td>
<td>None</td>
<td>2</td>
<td>7</td>
<td>N</td>
</tr>
<tr>
<td>Nutrient – TN</td>
<td>60 ml</td>
<td>Nalgene</td>
<td>50 ml</td>
<td>Acid Strip</td>
<td>None</td>
<td>2</td>
<td>8</td>
<td>N</td>
</tr>
<tr>
<td>Phytoplankton growout &lt;20-um</td>
<td>250 ml</td>
<td>Nalgene</td>
<td>150 ml</td>
<td>Soap / DI</td>
<td>None</td>
<td>2</td>
<td>9</td>
<td>N</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>14</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
## NCSU Sample Processing Procedure for Ballast Water Samples

### 2.0 Supplies:

<table>
<thead>
<tr>
<th>4” Sieves</th>
<th>Glassware</th>
</tr>
</thead>
<tbody>
<tr>
<td>2, 350-um designated for use with Lugols&lt;br&gt;2, 350-um designated for use with unpreserved sample&lt;br&gt;2, 80-um designated for use with Formalin&lt;br&gt;2, 80-um designated for use with unpreserved sample&lt;br&gt;2, 20-um designated for use with Lugols&lt;br&gt;2, 20-um designated for use with unpreserved sample&lt;br&gt;2, 5-um designated for use with Lugols</td>
<td>2, 250 ml glass beakers for use with 350-µm Lugols filtrate&lt;br&gt;2, 250 ml glass beakers for use with formaldehyde&lt;br&gt;2, 250 ml glass beakers for use with Lugols composite for UMD&lt;br&gt;2, 250 ml grad cylinder for use with Lugols&lt;br&gt;2, 500 ml grad cylinder for use with Lugols &lt;20-µm&lt;br&gt;2, 100 ml grad cylinders for Lugols</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Bottles</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>2, 250 ml Nalgene for Zooplankton growout&lt;br&gt;2, 250 ml Nalgene for 20-80 Phytoplankton growout&lt;br&gt;2, 125 ml Nalgene for 16S RNA composite to UMD&lt;br&gt;6, 125 ml Nalgene for &lt;20-µm phyto for eventual composite with 20-350-µm&lt;br&gt;6, 60 ml Brown Nalgene for phyto ID above 350-µm&lt;br&gt;6, 60 ml Brown Nalgene for phyto ID 20-350-µm&lt;br&gt;6, 60 ml Nalgene for 5-20-µm flow cytometry sample&lt;br&gt;6, 60 ml Nalgene for 5-µm filtrate of flow cytometry sample&lt;br&gt;2, 60 ml Nalgene for preserve of UMD samples</td>
<td>2, plastic funnels for use with unpreserved&lt;br&gt;2, plastic funnels for use with Lugols&lt;br&gt;2, 0.45-µm filtered seawater squirt bottles for rinsing&lt;br&gt;2, syringes for Flow cytometry sample filtration&lt;br&gt;6, 5-µm acrodisks</td>
</tr>
</tbody>
</table>
NCSU Sample Processing Procedure for Ballast Water Samples

3.0 Procedure:

3.1 Receipt:

Open the coolers and obtain the chain of custody and sampling data document and sign the samples into the lab. File the document. Due to the use of preservatives, perform this procedure under a hood.

3.2 Handling of Live Growout Samples:

3.2.1 As in section 1.0 there will be two composite samples designated for grow-out, one from each ballast tank. These were composited from the three, replicate 20-µm net tows completed for each ballast tank and have the kit bottle type number 3C. Each of these is to be handled as follows:

**Figure 1. Growout Samples - (20-350 micron size range). Net Concentrated Tank Composite.**

<table>
<thead>
<tr>
<th>Identification</th>
<th>Container</th>
<th>Vol. / Rep</th>
<th>Chemical Preservative</th>
<th># Per Rep</th>
<th># Per Tank</th>
<th># Per Ship</th>
<th>Destination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zoop grow-out</td>
<td>250 ml Nalgene</td>
<td>150 ml</td>
<td>None</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>NCSU</td>
</tr>
<tr>
<td>20-80 Phyto growout</td>
<td>250 ml Nalgene</td>
<td>150 ml</td>
<td>None</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>NCSU</td>
</tr>
</tbody>
</table>

Note: Bottle 4C in the kit will be in cooler 2 as well and is used to composite whole water samples for nutrient samples from the labline casts (see below), but after it serves as a nutrient composite it will be filled with raw tank water for filtration in the laboratory for the purpose of providing extra culture media (e.g. base water from the source).
3.2.2 Phytoplankton Grow-out Sample – Net-Concentrated Sample

Pour ~ 150 ml of the unpreserved sample from the 3C bottle over the 20-80-µm sieve designated for use with unpreserved sample and capture the filtrate in a plastic funnel and a labeled 250 ml Nalgene container. This fraction is now ready for transfer to the culturist. It will be enriched and allowed to grow in the incubator for six weeks with weekly examinations with documentation of the species observed. This sample is non-quantitative. See Figure 1.

3.2.3 Zooplankton Grow-out Sample – Net-Concentrated Sample

Pour ~ 150 ml of the unpreserved sample from the 3C bottle over the 20-350-µm sieve designated for use with unpreserved sample and capture the filtrate in a plastic funnel and a labeled 250 ml Nalgene container. This fraction is now ready for transfer to the zooplankton culturist. It will be enriched and allowed to grow for six weeks with weekly examinations with documentation of the species observed. This sample is non-quantitative. See Figure 1.

3.3 Handling of Net-Concentrated Phytoplankton and Zooplankton Samples:

3.3.1 As in section 1.0 6 samples will be designated for phytoplankton ID and enumeration and 6 samples will be designated for zooplankton ID and enumeration, a kit bottle type numbers 1 and 2, respectively. These are preserved samples and are quantitative in nature, so any sieving and reconstitution below will be volume-critical. Three of the six from each preservation type will be from tank 1 and the remaining 3 replicates will be from tank 2. Use only the sieves designated for the preservative in use. In other words, sieves labeled for use with Lugols can only be used with Lugols etc. These samples will be found in cooler 1.

3.3.2 Quantitative Phytoplankton Samples (20-um and above)

Two primary size fractions from these samples will be obtained through use of Lugols designated sieves and reconstitution to known volumes with artificial seawater. These will be 20-350-µm and >350-µm (large dinoflagellates and diatoms). See figure 2

3.3.2.1. Take the first Lugols replicate (kit bottle type # 1) from Tank 1 in cooler #1 and pour into a 250 ml graduated cylinder to get an exact volume and record the volume on the data form.

3.3.2.2. Pour the contents over a 350-µm, Lugols designated sieve and catch filtrate in a 250 ml beaker with light rinsing using a squirt bottle containing ASW (15 psu).
**NCSU Sample Processing Procedure for Ballast Water Samples**

3.3.2.3. Rinse the material collected on the 350-um sieve into a 100 ml grad cylinder using a funnel and reconstitute to 50 ml. Verify on the data form that the final volume was 50 ml. and transfer to a labeled 60 ml brown Nalgene bottle. Add 6 drops of Lugols.

3.3.2.4. Pour the 200 ml of 350-um filtrate in the 250 ml beaker over the 20-um Lugols sieve.

3.3.2.5. Rinse the material collected on the 20-um sieve into a 100 ml grad cylinder using a funnel and reconstitute to 50 ml. Verify on the data form that the final volume was 50 ml. and transfer to a labeled 60 ml brown Nalgene bottle. Add 6 drops of Lugols.

3.3.2.6. Repeat for the remaining two replicates of Tank 1 and then move onto the bottles from Tank 2.

3.3.2.7. Rinse the sieves, grad cylinders, beakers and funnels under the DI spigot in between replicates.

3.3.2.8. **Use new sieves, grad cylinders, beakers, and funnels between tanks.**

3.3.2.9. Store preserved samples in walk-in cooler.

---

**Figure 2. - Phytoplankton Identification and Enumeration Samples – Net-Concentrated > 20-µm**

<table>
<thead>
<tr>
<th>Identification</th>
<th>Container</th>
<th>Vol. / Rep</th>
<th>Chemical Preservative</th>
<th># Per Rep</th>
<th># Per Tank</th>
<th># Per Ship</th>
<th>Destination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Large dinos &amp; diat</td>
<td>60 ml Brown Nalg.</td>
<td>50 ml</td>
<td>Lugols</td>
<td>1</td>
<td>3</td>
<td>6</td>
<td>NCSU</td>
</tr>
<tr>
<td>20-350-µm phyto</td>
<td>60 ml Brown Nalg.</td>
<td>50 ml</td>
<td>Lugols</td>
<td>1</td>
<td>3</td>
<td>6</td>
<td>NCSU</td>
</tr>
</tbody>
</table>

This fraction will be combined with the <5-µm phytoplankton fraction and therefore an independent concentration factor will be computed for the organisms <20-µm and those between 20-µm and 350-µm.
3.3.3 Quantitative Zooplankton Samples (Small Zooplankton ≤ 80 µm)

3.3.3.1 The volumes for these samples were measured on-board using the label and marks, so determination of volume prior to sieving is unnecessary unless the liquid level is not at the designated mark. If the liquid level is not at the designated mark, measure the volume with a grad cylinder and record.

3.3.3.2 Take the first formalin replicate (kit bottle type # 2) from Tank 1 in cooler #1 and pour over a formalin designated 80-um sieve.

3.3.3.3 Collect the filtrate in a 250 ml beaker. See Figure 3.

3.3.3.4 Rinse the 250 ml Nalgene with DI and shake remaining water out.

3.3.3.5 Transfer the filtrate in the beaker back to the rinsed Nalgene bottle and apply the new label.

3.3.3.6 Repeat for the remaining two replicates for Tank 1 and then proceed to Tank 2.

3.3.3.7 Rinse the sieve, beaker and funnel under the DI spigot in between replicates.

3.3.3.8 Use a new sieve, beaker, and funnel between tanks.

3.3.3.9 Place tape over the cap seems on each bottle. These samples can then be placed in the cooler for shipment to the zooplankton contractor.

Figure 3. – Small Zooplankton Samples for Identification and Enumeration – Net-Concentrated ≤ 80-um

<table>
<thead>
<tr>
<th>Identification</th>
<th>Container</th>
<th>Vol. / Rep</th>
<th>Chemical Preservative</th>
<th># Per Rep</th>
<th># Per Tank</th>
<th># Per Ship</th>
<th>Destination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small Zoo</td>
<td>250 ml Nalgene</td>
<td>200 ml</td>
<td>RB &amp; formalin</td>
<td>1</td>
<td>3</td>
<td>6</td>
<td>NCSU</td>
</tr>
</tbody>
</table>
3.3.4 Small Phytoplankton, Bacteria, and Analytical Samples/Nutrients (Raw Water from Labline Casts <20-µm)

See Figure 4

3.3.4.1 These samples are vertical composites of unconcentrated raw water samples obtained from the labline casts.

3.3.4.2 From page 1, the physical and chemical analytical samples will be found in cooler number 2 and represent kit bottle types 5-8. These can immediately be handled and stored as per standard CAAE procedures. See figure 4.

3.3.4.3 The single grow-out sample (kit bottle type # 9) in cooler 2 from each tank is immediately ready for transfer to the CAAE culturist.

3.3.4.4 Repeat steps 3.3.4.2 and 3.3.4.3 for Tank 2.

3.3.4.5 Sample bottle kit type # 10 -- Flow Cytometry Sample should have been carefully measured on-deck using the label as a mark. If this is the case, then proceed to 3.3.4.6; otherwise, measure volume with grad cylinder.

3.3.4.6 Take each flow cytometry sample (kit bottle type # 10) and filter approximately half the volume using an acrodisk into a second, 60 ml labeled Nalgene. This step will likely be eliminated later, but it is being employed now to explore the utility of this fractionation for flow cytometric analysis. Store bottles in refrigerator.

3.3.4.7 Get the first replicate of the 1-liter bottles (kit bottle type # 11) from cooler #1. This is the sample that is weekly preserved with acidic Lugols. Two bottles will be produced. Bottle A - Take 50 ml. from each of the three replicates and composite them into a 250 ml beaker. Transfer about 100 ml. of this composite to a 125 ml Nalgene for transport to UMD. Bottle B - Then transfer 50 ml. to a 60 ml, Brown Nalgene and preserve with 6 drops of Lugols. This is experimental for Oldach and will likely be eliminated later as part of the routine.

3.3.4.8 Take the remainder of replicate 1 of tank 1 and measure out 500 mls in a graduated cylinder.

3.3.4.9 Denote the volume on the form

3.3.4.10 Pour this over the 5-µm sieve, concentrate the sample and reconstitute with ASW up to 50 ml. in a 100 ml grad cylinder.

3.3.4.11 Transfer to a labeled, 60 ml Nalgene.

3.3.4.12 Repeat for the remaining two replicates of Tank 1 and then move onto the bottles from Tank 2.

3.3.4.13 Each of these enumeration replicates will be composited in a 1:1 ratio with the 20-350-µm phytoplanktion enumeration aliquot in section 3.2.2 and Figure 2. This will mean that organisms in this 0-350-µm composite that are below 20 and those that are above 20 will have independent concentration factors.

3.3.4.14 Add 6 drops of Lugols to each bottle and immediately mix.

3.3.4.15 Rinse the sieves, grad cylinders, beakers and funnels under the DI spigot in between replicates.

3.3.4.16 Use new sieves, grad cylinders, beakers, and funnels between tanks.

3.3.4.17 Store preserved samples in walk-in cooler.
### NCSU Sample Processing Procedure for Ballast Water Samples

**Figure 4. Small Phyto, Bacteria and Nutrients.** Samples <20-µm. Samples are raw and unconcentrated. All chemical analyses, phytoplankton counts and flow cytometry will be performed at NCSU. 16S ribosomal bacteria screens will go to UMD.

<table>
<thead>
<tr>
<th>Identification</th>
<th>Kit Bottle #</th>
<th>Container</th>
<th>Vol. / Rep</th>
<th>Chem Pres.</th>
<th># Per Rep</th>
<th># Per Tank</th>
<th># Per Ship</th>
<th>Destination</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOC</td>
<td>5</td>
<td>40 ml amber glass</td>
<td>40 ml</td>
<td>None</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>NCSU</td>
</tr>
<tr>
<td>TSS/Chla</td>
<td>6</td>
<td>500 ml Nalgene</td>
<td>~400 ml</td>
<td>None</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>NCSU</td>
</tr>
<tr>
<td>TP</td>
<td>7</td>
<td>60 ml amber glass</td>
<td>40 ml</td>
<td>None</td>
<td>1</td>
<td>3</td>
<td>6</td>
<td>NCSU</td>
</tr>
<tr>
<td>TN</td>
<td>8</td>
<td>60 ml Nalgene</td>
<td>50 ml</td>
<td>None</td>
<td>1</td>
<td>3</td>
<td>6</td>
<td>NCSU</td>
</tr>
<tr>
<td>Phytoplankton grow-out (&lt;20-µm)</td>
<td>9</td>
<td>125 ml Nalgene</td>
<td>100 ml</td>
<td>None</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>NCSU</td>
</tr>
<tr>
<td>Flow Cytometry</td>
<td>10</td>
<td>60 ml Nalgene</td>
<td>50 ml</td>
<td>1 ml Glut</td>
<td>1</td>
<td>3</td>
<td>6</td>
<td>NCSU</td>
</tr>
<tr>
<td>Phytoplankton count &amp; 16S RNA</td>
<td>11</td>
<td>1000 ml Nalgene</td>
<td>600 ml</td>
<td>2 drops Lugols</td>
<td>1</td>
<td>3</td>
<td>6</td>
<td>NCSU/UMD</td>
</tr>
</tbody>
</table>

**Standard Storage, Distribution and Analysis**

<table>
<thead>
<tr>
<th>Identification</th>
<th>Container</th>
<th>Vol. / Rep</th>
<th>Chemical Preservative</th>
<th># Per Rep</th>
<th># Per Tank</th>
<th># Per Ship</th>
<th>Destination</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 - 20-µm Phyto ID &amp; Enumeration</td>
<td>125 ml Nalgene</td>
<td>50 ml</td>
<td>5 drops Acid Lugols</td>
<td>1</td>
<td>2</td>
<td>6</td>
<td>NCSU</td>
</tr>
</tbody>
</table>

Note, each of these replicates will be composited with the corresponding 20-350-µm phytoplankton replicate in Fig. 2.
## NCSU Sample Processing Procedure for Ballast Water Samples

### 3.4 Shipments to Subcontractors

**Shipments from NCSU to UMD**

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Container Size</th>
<th>Container Type</th>
<th>Sample Volume</th>
<th>Preservative</th>
<th>Number of Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>16S RNA Screen</td>
<td>125 ml</td>
<td>Opaque -Nalgene</td>
<td>50 ml</td>
<td>Weak Acid Lugols</td>
<td>2</td>
</tr>
<tr>
<td>16S RNA Screen</td>
<td>60 ml</td>
<td>Opaque -Nalgene</td>
<td>50 ml</td>
<td>Strong Acid Lugols</td>
<td>2</td>
</tr>
</tbody>
</table>
Actinocyclus curvatulus
Janisch in A. Schmidt

Identification/Taxonomic Information

Diatoms

<table>
<thead>
<tr>
<th>Kingdom</th>
<th>Protista</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phylum</td>
<td>Ochrophyta</td>
</tr>
<tr>
<td>Class</td>
<td>Coscinodiscophyceae</td>
</tr>
<tr>
<td>Order</td>
<td>Coscinodisccales</td>
</tr>
<tr>
<td>Family</td>
<td>Hemidiscaceae</td>
</tr>
</tbody>
</table>

Harmful/Nuisance  Pathogen

<table>
<thead>
<tr>
<th>Toxic</th>
<th>Human</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>N</td>
</tr>
</tbody>
</table>

Nuisance  Other

<table>
<thead>
<tr>
<th>Nuisance</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>N</td>
</tr>
</tbody>
</table>

Impact  Ecologically beneficial

Microscopy

NCSU CAAE
www.ncsu.edu/wq/aboutCAAE/

Former Name(s)
Actinocyclus subocellatus  
Coscinodiscus curvatulus var subocellatus

Distribution
Arctic marine

Source Region(s)
~ 250 nautical miles off northern California coast

References


**Actinoptychus senarius**

(Ehrenberg) Ehrenberg

**Identification/Taxonomic Information**

**Diatoms**

Kingdom Protista
Phylum Ochrophyta
Class Coscinodiscophyceae
Order Coscinodiscales
Family Heliopeltaceae

**Harmful/Nuisance**

Toxic N  Human N
Nuisance N  Other N
Impact Ecologically beneficial

**Microscopy**

The Astrobiology Institute - Marine Biological Laboratory, Woods Hole, MA
microscope.mbl.edu/baypaul/microscope/general/page_01.htm

**Former Name(s)**

Actinocyclus senarius
Actinoptychus undulatus

**Distribution**

Cosmopolitan marine

**Source Region(s)**

Undetermined

**References**


Asterionella formosa

Hassall

Identification/Taxonomic Information

Diatoms

Kingdom  Protista
Phylum    Ochrophyta
Class     Fragilariophyceae
Order     Fragilariales
Family    Fragilariaceae

Harmful/Nuisance  Pathogen
Toxic N Human N
Nuisance N Other N
Impact Ecologically beneficial

Microscopy

Photo by Jason Oyadomari, Michigan Technological University
www.bio.mtu.edu/~jkoyadom/algae_webpage/ALGAL_PAGES/b

Former Name(s)

Distribution
Cosmopolitan freshwater

Source Region(s)
Small proportion of Indian Ocean, majority Mid-Atlantic
Southeast Pacific, east Pacific, with Persian Gulf
Pacific Ocean
Open Pacific ~ 400 nautical miles, east of Mariana Islands
Open Pacific >300 nautical miles north of Midway Island
Open Atlantic
Mediterranean highly diluted with Gulf Stream waters
Mediterranean with Gulf Stream dilution, some coastal Carolina
Mediterranean waters diluted with mid and eastern Atlantic waters
Exchange occurred at Fuel Pier in Guam
100 - 200 nautical miles off Louisiana coast
~ 250 nautical miles off northern California coast

References


Identification/Taxonomic Information

**Diatoms**

**Kingdom**: Protista  
**Phylum**: Ochrophyta  
**Class**: Bacillariophyceae  
**Order**: Fragilariales  
**Family**: Fragilariaceae

**Harmful/Nuisance** | **Pathogen**
--- | ---
Toxic | N | Human | N  
Nuisance | N | Other | N  
Impact | Ecologically beneficial

Microscopy

NCSU CAAE

www.ncsu.edu/wq/aboutCAAE/

Former Name(s)

Asterionella japonica  
Asterionellopsis japonica

Distribution

Cosmopolitan marine

Source Region(s)

Undetermined

References

Identification/Taxonomic Information

Diatoms

Kingdom: Protista
Phylum: Ochrophyta
Class: Coscinodiscophyceae
Order: Asterolamprales
Family: Asterolampraceae

Harmful/Nuisance Pathogen
Toxic: N Human: N
Nuisance: N Other: N
Impact: Ecologically beneficial

Distribution
Cosmopolitan marine, temperate to tropical

Source Region(s)
Pacific Ocean
Open Atlantic 400 nautical miles west of Gibraltar
Open Atlantic
Atlantic 450 nautical miles west of Canaries

References
**Identification/Taxonomic Information**

**Diatoms**

Kingdom: Protista  
Phylum: Ochrophyta  
Class: Coscinodiscophyceae  
Order: Asterolamprales  
Family: Asterolampraceae

**Harmful/Nuisance Pathogen**

Toxic: N  
Nuisance: N  
Human: N  
Other: N  
Impact: Ecologically beneficial

**Distribution**

Cosmopolitan marine, cold coastal/marine waters

**Source Region(s)**

Atlantic, 200 nautical miles off Virginia

**References**

Asteromphalus roperianus
(Grevelle) Ralfs in Pritchard

Identification/Taxonomic Information

Diatoms

Kingdom  Protista
Phylum      Ochrophyta
Class       Coscinodiscophyceae
Order       Asterolamprales
Family      Asterolampraceae

Harmful/Nuisance  Pathogen
Toxic        N         Human      N
Nuisance     N         Other      N
Impact       Ecologically beneficial

Distribution
Cosmopolitan marine, temperate to tropical

Source Region(s)
400 nautical miles west of Africa with northwest track to open Atlantic

References
**Identification/Taxonomic Information**

**Diatoms**

- **Kingdom**: Protista
- **Phylum**: Ochrophyta
- **Class**: Coscinodiscophyceae
- **Order**: Chaetocerotales
- **Family**: Attheyaceae

**Harmful/Nuisance**
- **Toxic**: N
- **Nuisance**: N

**Impact**: Ecologically beneficial

**References**


**Microscopy**

IOW - Institut für Ostseeforschung Warnemuende
www.io.warnemuende.de/research/de_galerie.html

**Former Name(s)**

- Chaetoceros septentrionalis
- Gonioceros septentrionalis

**Source Region(s)**

Atlantic 450 nautical miles west of Canaries

**Distribution**

Cosmopolitan northern hemisphere, marine coastal, Arctic to temperate
Identification/Taxonomic Information

Diatoms

Kingdom Protista
Phylum Ochrophyta
Class Coscinodiscophyceae
Order Chaetocerotales
Family Chaetocerotaceae

Harmful/Nuisance Pathogen
Toxic N Human N
Nuisance N Other N
Impact Ecologically beneficial

Microscopy

Hasle & Syvertsen (1997)

Former Name(s)

Distribution
Cosmopolitan marine, tropical to subtropical (rare, temperate)

Source Region(s)
Northern Indian Ocean (~50%); Some recent open Pacific

References
**Identification/Taxonomic Information**

**Diatoms**

- **Kingdom**: Protista
- **Phylum**: Ochrophyta
- **Class**: Coscinodiscophyceae
- **Order**: Chaetocerotales
- **Family**: Chaetocerotaceae

**Harmful/Nuisance** | **Pathogen**
--- | ---
Toxic | N
Nuisance | N
Impact | Ecologically beneficial

**Microscopy**

Smithsonian Environmental Research Center - Phytoplankon Ecology Laboratory
serc5.si.edu/algae/

**Former Name(s)**


**Distribution**

Cosmopolitan marine, temperate to tropical

**Source Region(s)**

Atlantic 450 nautical miles west of Canaries

**References**

Identification/Taxonomic Information

Diatoms

Kingdom: Protista
Phylum: Ochrophyta
Class: Coscinodiscophyceae
Order: Chaetocerotales
Family: Chaetocerotaceae

Harmful/Nuisance  Pathogen
Toxic   N     Human   N
Nuisance N     Other  N
Impact: Ecologically beneficial

Distribution

Cosmopolitan estuarine / marine, temperate to tropical (distribution poorly known)

Source Region(s)

Northern Indian Ocean (~50%); Some recent open Pacific

References


Identification/Taxonomic Information

Diatoms

Kingdom  Protista
Phylum     Ochrophyta
Class      Coscinodiscophyceae
Order      Chaetocerotales
Family     Chaetocerotaceae

Harmful/Nuisance Pathogen
Toxic      N  Human   N
Nuisance   N  Other   N
Impact     Ecologically beneficial

Microscopy

The Astrobiology Institute - Marine Biological Laboratory, Woods Hole, MA
microscope.mbl.edu/baypaul/microscope/general/page_01.htm

Former Name(s)

Distribution
Cosmopolitan marine, temperate

Source Region(s)
Open Atlantic
Exchange occurred at Fuel Pier in Guam
Atlantic, 200 nautical miles off Virginia

References
**Identification/Taxonomic Information**

**Diatoms**

- **Kingdom**: Protista
- **Phylum**: Ochrophyta
- **Class**: Coscinodiscophyceae
- **Order**: Hemiaulales
- **Family**: Bellerocheaceae

**Harmful/Nuisance**

- **Toxic**: N
- **Nuisance**: N

**Pathogen**

- **Human**: N
- **Other**: N

**Impact**

- Ecologically beneficial

**Distribution**

Cosmopolitan marine, temperate to subtropical

**Source Region(s)**

Northern Indian Ocean (~50%); Some recent open Pacific

**References**


**Microscopy**


**Former Name(s)**

-
Identification/Taxonomic Information

**Diatoms**

- **Kingdom**: Protista
- **Phylum**: Ochrophyta
- **Class**: Coscinodiscophyceae
- **Order**: Chaetocerotales
- **Family**: Chaetocerotaceae

**Harmful/Nuisance**

- **Toxic**: N
- **Nuisance**: N
- **Impact**: Ecologically beneficial

**Pathogen**

- **Human**: N
- **Other**: N

Microscopy

Smithsonian Environmental Research Center - Phytoplankon Ecology Laboratory
serc5.si.edu/algae/

Former Name(s)

Distribution
Cosmopolitan marine, tropical to subtropical

Source Region(s)
Undetermined

References

**Identification/Taxonomic Information**

**Diatoms**

<table>
<thead>
<tr>
<th>Kingdom</th>
<th>Protista</th>
</tr>
</thead>
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<tr>
<td>Phylum</td>
<td>Ochrophyta</td>
</tr>
<tr>
<td>Class</td>
<td>Coscinodiscophyceae</td>
</tr>
<tr>
<td>Order</td>
<td>Chaetocerotales</td>
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<tr>
<td>Family</td>
<td>Chaetocerotaceae</td>
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</table>

**Harmful/Nuisance**

<table>
<thead>
<tr>
<th>Toxic</th>
<th>Nuisance</th>
<th>Pathogen</th>
<th>Impact</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>Y</td>
<td>N</td>
<td>Can cause fish death (gill irritation, mucus overproduction)</td>
</tr>
</tbody>
</table>

**Microscopy**


**Former Name(s)**

<table>
<thead>
<tr>
<th>Source Region(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Southeast Pacific, east Pacific, with Persian Gulf</td>
</tr>
<tr>
<td>Open Atlantic 400 nautical miles west of Gibraltar</td>
</tr>
<tr>
<td>Open Atlantic</td>
</tr>
<tr>
<td>Indian Ocean with Tacoma Tap Water</td>
</tr>
<tr>
<td>Hawaiian Pacific, with Persian Gulf</td>
</tr>
<tr>
<td>Exchange occurred at Fuel Pier in Guam</td>
</tr>
<tr>
<td>Atlantic, 200 nautical miles off Virginia</td>
</tr>
<tr>
<td>Atlantic 450 nautical miles west of Canaries</td>
</tr>
</tbody>
</table>

**References**

Chaetoceros criophilus

Castracane

Identification/Taxonomic Information

Diatoms

Kingdom: Protista
Phylum: Ochrophyta
Class: Coscinodiscophyceae
Order: Chaetocerotales
Family: Chaetocerotaceae

Harmful/Nuisance: Toxic - N, Nuisance - N
Pathogen: Toxic - Human - N, Nuisance - Other - N
Impact: Ecologically beneficial

Microscopy

Hasle & Syvertsen (1997)

Former Name(s)

Distribution

Marine, southern hemisphere cold waters

Source Region(s)

Atlantic 450 nautical miles west of Canaries

References


Identification/Taxonomic Information

Diatoms

Kingdom: Protista
Phylum: Ochrophyta
Class: Coscinodiscophyceae
Order: Chaetocerotales
Family: Chaetocerotaceae

Harmful/Nuisance Pathogen
Toxic: N
Nuisance: N
Impact: Ecologically beneficial

Microscopy

Göteborg University, Dept. of Marine Ecology, Marine Botany
www.marbot.gu.se/SSS/SSShome.htm

Former Name(s)

References


Distribution

Cosmopolitan, temperate to subtropical

Source Region(s)

Mid-Atlantic
**Identication/Taxonomic Information**

**Diatoms**

**Kingdom**  
Protista

**Phylum**  
Ochrophyta

**Class**  
Coscinodiscophyceae

**Order**  
Chaetocerotales

**Family**  
Chaetocerotaceae

**Harmful/Nuisance**  
Pathogen

<table>
<thead>
<tr>
<th>Toxic</th>
<th>Human</th>
<th>Nuisance</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
</tbody>
</table>

**Impact**  
Ecologically beneficial

**Microscopy**

The Astrobiology Institute - Marine Biological Laboratory,  
Woods Hole, MA  
microscope.mbl.edu/baypaul/microscope/general/page_01.htm

**Former Name(s)**

**Distribution**

Cosmopolitan marine, polar to temperate

**Source Region(s)**

Puget Sound water, partial Panama  
Pacific Ocean  
Open Atlantic 400 nautical miles west of Gibraltar  
Open Atlantic  
Northern Indian Ocean (~50%); Some recent open Pacific  
Hawaiian Pacific, with Persian Gulf  
Exchange occurred at Fuel Pier in Guam  
Atlantic, 200 nautical miles off Virginia  
Atlantic, 200 nautical miles off Gibraltar plus Mediterranean water  
Atlantic 450 nautical miles west of Canaries

**References**

**Identification/Taxonomic Information**

**Diatoms**

- **Kingdom**: Protista
- **Phylum**: Ochrophyta
- **Class**: Coscinodiscophyceae
- **Order**: Chaetoceratales
- **Family**: Chaetocerotaceae

**Harmful/Nuisance**

- **Toxic**: N
- **Nuisance**: N
- **Impact**: Ecologically beneficial

**Pathogen**

- **Human**: N
- **Other**: N

**Microscopy**

20.0 μm

**Distribution**

Cosmopolitan marine, temperate to subtropical

**Source Region(s)**

- Exchange occurred at Fuel Pier in Guam
- Atlantic 450 nautical miles west of Canaries

**References**

**Identification/Taxonomic Information**

**Diatoms**

**Kingdom** Protista  
**Phylum** Ochrophyta  
**Class** Coscinodiscophyceae  
**Order** Chaetocerotales  
**Family** Chaetocerotaceae  

**Harmful/Nuisance**  
**Pathogen**  
**Toxic** N  
**Human** N  
**Nuisance** N  
**Other** N  
**Impact** Ecologically beneficial

**Distribution**

Cosmopolitan marine, temperate to subtropical

**Source Region(s)**

Puget Sound water, partial Panama  
Atlantic 450 nautical miles west of Canaries

**References**

Chaetoceros similis

Cleve

**Identification/Taxonomic Information**

**Diatoms**

**Kingdom** Protista  
**Phylum** Ochrophyta  
**Class** Coscinodiscophyceae  
**Order** Chaetocerotales  
**Family** Chaetocerotaceae

**Harmful/Nuisance**  
**Toxic** N  
**Nuisance** N  
**Impact** Ecologically beneficial

**Microscopy**

Göteborg University, Dept. of Marine Ecology, Marine Botany
www.marbot.gu.se/SSS/SSShome.htm

**Former Name(s)**

**Distribution**
Cosmopolitan northern hemisphere marine, Arctic to temperate

**Source Region(s)**
Open Atlantic
Exchange occurred at Fuel Pier in Guam

**References**

**Identification/Taxonomic Information**

**Diatoms**

**Kingdom** Protista  
**Phylum** Ochrophyta  
**Class** Coscinodiscophyceae  
**Order** Chaetocerotales  
**Family** Chaetocerotaceae

<table>
<thead>
<tr>
<th>Toxic</th>
<th>Nuisance</th>
<th>Pathogen</th>
<th>Impact</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>Y</td>
<td>Human</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Other</td>
<td>Y</td>
</tr>
</tbody>
</table>

**Impact** Can cause fish death (gill irritation, mucus overproduction)

**Microscopy**

Exchange occurred at Fuel Pier in Guam  
Central Pacific between Hawaii and Midway  
100 - 200 nautical miles off southern Mexican coast

**References**


**Identification/Taxonomic Information**

**Diatoms**

<table>
<thead>
<tr>
<th>Kingdom</th>
<th>Protista</th>
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</thead>
<tbody>
<tr>
<td>Phylum</td>
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<tr>
<td>Class</td>
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<tr>
<td>Order</td>
<td>Chaetoceratales</td>
</tr>
<tr>
<td>Family</td>
<td>Chaetocerotaceae</td>
</tr>
</tbody>
</table>

**Harmful/Nuisance**

- Toxic: N
- Nuisance: N
- Impact: Ecologically beneficial

**Microscopy**

Hasle & Syvertsen (1997)

**Former Name(s)**

- Hasle & Syvertsen (1997)

**Distribution**

Cosmopolitan marine, tropical to temperate waters

**Source Region(s)**

- 400 nautical miles west of Africa with northwest track to open Atlantic

**References**

Corethron criophilum

Castracane

**Identification/Taxonomic Information**

**Diatoms**

<table>
<thead>
<tr>
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<th>Protista</th>
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<tbody>
<tr>
<td>Phylum</td>
<td>Ochrophyta</td>
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<td>Class</td>
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<tr>
<td>Order</td>
<td>Corethrales</td>
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<td>Family</td>
<td>Corethraceae</td>
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**Harmful/Nuisance**

<table>
<thead>
<tr>
<th>Toxic</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuisance</td>
<td>Y</td>
</tr>
<tr>
<td>Impact</td>
<td>Can cause fish death (gill irritation, mucus overproduction)</td>
</tr>
</tbody>
</table>

**Microscopy**

© Australian Antarctic Division 2002 Kingston Tasmania; Image by Harvey Marchant


**Former Name(s)**

<table>
<thead>
<tr>
<th>Source Region(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Undetermined</td>
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</tbody>
</table>

**Distribution**

Cosmopolitan marine, north temperate to Antarctic

**References**


**Identification/Taxonomic Information**

**Diatoms**

Kingdom: Protista  
Phylum: Ochrophyta  
Class: Bacillariophyceae  
Order: Bacillariales  
Family: Bacillariaceae  

<table>
<thead>
<tr>
<th>Harmful/Nuisance</th>
<th>Pathogen</th>
<th>Toxic</th>
<th>Nuisance</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>Human</td>
<td>N</td>
<td>Other</td>
</tr>
</tbody>
</table>

Impact: Ecologically beneficial

**Distribution**

Cosmopolitan marine

**Source Region(s)**

Puget Sound water, partial Panama  
Open Pacific >300 nautical miles north of Midway Island  
Open Atlantic 400 nautical miles west of Gibraltar  
Open Atlantic  
Mediterranean with significant coastal Carolina water  
Mediterranean highly diluted with Gulf Stream waters  
Mediterranean with Gulf Stream dilution, some coastal Carolina  
Mediterranean waters diluted with mid and eastern Atlantic waters  
Exchange occurred at Fuel Pier in Guam  
Central Pacific >200 nautical miles north of Wake Island  
Atlantic 350 nautical miles south of Bermuda  
50-100 nautical miles west of Hawaii  
400 nautical miles west of Africa with northwest track to open Atlantic  
~ 250 nautical miles off northern California coast  
Southeast Pacific, east Pacific, with Persian Gulf

**References**

Identification/Taxonomic Information

**Diatoms**

**Kingdom** Protista  
**Phylum** Ochrophyta  
**Class** Coscinodiscophyceae  
**Order** Lithodesmiales  
**Family** Lithodesmiaceae

**Harmful/Nuisance**  
**Toxic** N  
**Nuisance** N

**Pathogen**  
**Human** N  
**Other** N

**Impact** Ecologically beneficial

Microscopy

Distribution

Cosmopolitan marine, except for polar regions

**Source Region(s)**

- Small proportion of Indian Ocean, majority Mid-Atlantic
- Puget Sound water, partial Panama
- Open Pacific ~ 400 nautical miles, east of Mariana Islands
- Open Pacific >300 nautical miles north of Midway Island
- Open Atlantic
- Mediterranean with significant coastal Carolina water
- Central Pacific >200 nautical miles north of Wake Island
- Central Indian Ocean
- 100 - 200 nautical miles off Louisiana coast
- ~ 250 nautical miles off northern California coast

References

**Eucampia zodiacus**

**Identification/Taxonomic Information**

- **Kingdom**: Protista
- **Phylum**: Ochrophyta
- **Class**: Coscinodiscophyceae
- **Order**: Biddulphiales
- **Family**: Biddulphiaceae

**Harmful/Nuisance**

- **Toxic**: N
- **Nuisance**: N
- **Human**: N
- **Other**: N
- **Impact**: Ecologically beneficial

**Distribution**

Cosmopolitan marine, except polar waters

**Source Region(s)**

- Puget Sound water, partial Panama
- Open Atlantic
- Hawaiian Pacific, with Persian Gulf
- Exchange occurred at Fuel Pier in Guam
- Atlantic, 200 nautical miles off Virginia

**References**

**Identification/Taxonomic Information**

**Diatoms**

**Kingdom** Protista  
**Phylum** Ochrophyta  
**Class** Bacillariophyceae  
**Order** Hemiaulales  
**Family** Streptothecaceae

**Harmful/Nuisance** Pathogen

<table>
<thead>
<tr>
<th>Toxic</th>
<th>Human</th>
<th>Other</th>
<th>Impact</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>N</td>
<td>N</td>
<td>Ecologically beneficial</td>
</tr>
</tbody>
</table>

**Microscopy**

Smithsonian Environmental Research Center - Phytoplankon  
Ecology Laboratory  
serc5.si.edu/algae/

**Former Name(s)**

Streptotheca tamensis  
Streptotheca thamensis

**Distribution**

Cosmopolitan marine / estuarine, temperate to tropical

**Source Region(s)**

Undetermined  
100 - 200 nautical miles off Louisiana coast

**References**


**Identification/Taxonomic Information**

**Diatoms**

**Kingdom** Protista  
**Phylum** Ochrophyta  
**Class** Coscinodiscophyceae  
**Order** Hemiaulales  
**Family** Hemiaulaceae

**Harmful/Nuisance**  
**Toxic** N  
**Nuisance** N  
**Impact** Ecologically beneficial

**Pathogen**  
**Human** N  
**Other** N

**Distribution**  
Cosmopolitan marine, temperate to tropical

**Source Region(s)**
- Northern Indian Ocean (~50%); Some recent open Pacific  
- Hawaiian Pacific, with Persian Gulf  
- Exchange occurred at Fuel Pier in Guam  
- Atlantic, 200 nautical miles off Virginia  
- Atlantic, 200 nautical miles off Gibraltar plus Mediterranean water  
- Atlantic 450 nautical miles west of Canaries  
- 400 nautical miles west of Africa with northwest track to open Atlantic

**References**

**Microscopy**

Smithsonian Environmental Research Center - Phytoplankon Ecology Laboratory
serc5.si.edu/algae/

**Former Name(s)**
**Identification/Taxonomic Information**

Diatoms

**Kingdom**  
Protista

**Phylum**  
Ochrophyta

**Class**  
Coscinodiscophyceae

**Order**  
Hemiaulales

**Family**  
Hemiaulaceae

**Harmful/Nuisance**  
N

**Pathogen**  
Human N

**Toxic**  
N

**Nuisance**  
N

**Impact**  
Ecologically beneficial

**Distribution**

Indian Ocean, Sea of Java

**Source Region(s)**

Southeast Pacific, east Pacific, with Persian Gulf

Hawaiian Pacific, with Persian Gulf

**References**


**Hemiaulus membranaceus**

*Identification/Taxonomic Information*

**Diatoms**

- **Kingdom**: Protista
- **Phylum**: Ochrophyta
- **Class**: Coscinodiscophyceae
- **Order**: Hemiaulales
- **Family**: Hemiaulaceae

<table>
<thead>
<tr>
<th>Harmful/Nuisance</th>
<th>Pathogen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toxic</td>
<td>Human</td>
</tr>
<tr>
<td>N</td>
<td>N</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Nuisance</th>
<th>Other</th>
<th>Impact</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>N</td>
<td>Ecologically beneficial</td>
</tr>
</tbody>
</table>

**Microscopy**

Smithsonian Environmental Research Center - Phytoplankon Ecology Laboratory
[serc5.si.edu/algae/](serc5.si.edu/algae/)

**Former Name(s)**

**Distribution**

Cosmopolitan marine, temperate to tropical

**Source Region(s)**

- Northern Indian Ocean – 800 nautical miles west Sri Lanka
- Northern Indian Ocean (~50%); Some recent open Pacific
- Mid-Atlantic

**References**

Hemiaulus sinensis

Identification/Taxonomic Information

Diatoms

<table>
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<th>Protista</th>
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<tr>
<td>Order</td>
<td>Hemiaulales</td>
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<tr>
<td>Family</td>
<td>Hemiaulaceae</td>
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</tbody>
</table>

Harmful/Nuisance       Pathogen

Toxic    N    Human    N

Nuisance  N    Other    N

Impact    Ecologically beneficial

Microscopy

Smithsonian Environmental Research Center - Phytoplankon Ecology Laboratory
serc5.si.edu/algae/

Former Name(s)

Distribution

Cosmopolitan marine, temperate to tropical

Source Region(s)

Atlantic, 200 nautical miles off Virginia

References


**Identification/Taxonomic Information**

**Diatoms**

- **Kingdom**: Protista
- **Phylum**: Ochrophyta
- **Class**: Coscinodiscophyceae
- **Order**: Thalassiosirales
- **Family**: Lauderiacae

**Harmful/Nuisance**

- **Toxic**: N
- **Nuisance**: N

**Impact**

Ecologically beneficial

**Distribution**

Cosmopolitan marine / estuarine, temperate to tropical

**Source Region(s)**

Puget Sound water, partial Panama

**References**


**Identification/Taxonomic Information**

**Diatoms**

**Kingdom** Protista  
**Phylum** Ochrophyta  
**Class** Coscinodiscophyceae  
**Order** Leptocylindrales  
**Family** Leptocylindraceae

**Harmful/Nuisance**  
**Toxic** N  
**Nuisance** N

**Pathogen**  
**Human** N  
**Other** N

**Impact** Ecologically beneficial

**Distribution**

Cosmopolitan marine, except for Antarctic

**Source Region(s)**

Puget Sound water, partial Panama  
Open Pacific >300 nautical miles north of Midway Island  
Mediterranean with significant coastal Carolina water  
Mediterranean highly diluted with Gulf Stream waters  
Mediterranean with Gulf Stream dilution, some coastal Carolina  
Mediterranean waters diluted with mid and eastern Atlantic waters  
Gulf of Mexico coastal Texas with 100 nautical miles on shelf  
Atlantic, 200 nautical miles off Virginia  
Atlantic, 200 nautical miles off Gibraltar plus Mediterranean water  
Atlantic 350 nautical miles south of Bermuda  
100 - 200 nautical miles off southern Mexican coast  
100 - 200 nautical miles off Louisiana coast  
~ 250 nautical miles off northern California coast

**References**

Leptocylindrus minimus
Gran

Identification/Taxonomic Information

Diatoms

Kingdom       Protista
Phylum        Ochrophyta
Class         Coscinodiscophyceae
Order         Leptocylindrales
Family        Leptocylindraceae

Harmful/Nuisance  Pathogen
Toxic          N  Human    N
Nuisance       Y  Other    Y
Impact         Linked to fish death (gill irritation, mucus overproduction)

Microscopy

The Astrobiology Institute - Marine Biological Laboratory, Woods Hole, MA
microscope.mbl.edu/baypaul/microscope/general/page_01.htm

Distribution
Cosmopolitan marine, except for Antarctic

Source Region(s)
Mediterranean waters diluted with mid and eastern Atlantic waters

References
Nitzschia acicularis
(Kützing) W. Smith

**Identification/Taxonomic Information**

Diatoms

- **Kingdom**: Protista
- **Phylum**: Ochrophyta
- **Class**: Bacillariophyceae
- **Order**: Bacillariales
- **Family**: Bacillariaceae

**Harmful/Nuisance**

- **Toxic**: N
- **Nuisance**: N
- **Impact**: Ecologically beneficial frw

**Microscopy**

Smithsonian Environmental Research Center - Phytoplankon Ecology Laboratory
serc5.si.edu/algae/

**Former Name(s)**

**Distribution**

Cosmopolitan freshwater / estuarine, temperate to subtropical

**Source Region(s)**

50-100 nautical miles west of Hawaii

**References**

- ITIS Standard Report page
**Nitzschia americana**

Hasle

**Identification/Taxonomic Information**

**Diatoms**

**Kingdom** Protista  
**Phylum** Ochrophyta  
**Class** Bacillariophyceae  
**Order** Bacillariales  
**Family** Bacillariaceae

**Harmful/Nuisance**  
**Toxic** N  
**Nuisance** N

**Pathogen**  
**Human** N  
**Other** N

**Impact** Ecologically beneficial

**Distribution**

Cosmopolitan marine, temperate to tropical

**Source Region(s)**

Puget Sound water, partial Panama

**References**

Nitzschia kolaczeckii
Grunow

Identification/Taxonomic Information

Diatoms
Kingdom Protista
Phylum Ochrophyta
Class Bacillariophyceae
Order Bacillariales
Family Bacillariaceae

Harmful/Nuisance Pathogen
Toxic N Human N
Nuisance N Other N
Impact Ecologically beneficial

Distribution
Cosmopolitan marine (distribution poorly known; subtropical to tropical)

Source Region(s)
Southeast Pacific, east Pacific, with Persian Gulf

References
Identification/Taxonomic Information

Diatoms

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<tr>
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<td>Bacillariales</td>
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<td>Bacillariaceae</td>
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Harmful/Nuisance Pathogen

<table>
<thead>
<tr>
<th>Toxic</th>
<th>Human</th>
<th>Nuisance</th>
<th>Other</th>
<th>Impact</th>
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<tbody>
<tr>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>Ecologically beneficial</td>
</tr>
</tbody>
</table>

Distribution

Cosmopolitan marine / estuarine

Source Region(s)

Southeast Pacific, east Pacific, with Persian Gulf

References


Identification/Taxonomic Information

Diatoms

Kingdom Protista
Phylum Ochrophyta
Class Bacillariophyceae
Order Bacillariales
Family Bacillariaceae

Harmful/Nuisance Pathogen
Toxic N Human N
Nuisance N Other N
Impact Ecologically beneficial

Distribution
Cosmopolitan marine / estuarine, temperate

Source Region(s)
Small proportion of Indian Ocean, majority Mid-Atlantic
Open Pacific >300 nautical miles north of Midway Island
Mediterranean with Gulf Stream dilution, some coastal Carolina
Mediterranean waters diluted with mid and eastern Atlantic waters
Central Pacific >200 nautical miles north of Wake Island
Atlantic, 200 nautical miles off Gibraltar plus Mediterranean water
~ 250 nautical miles off northern California coast

References
Identification/Taxonomic Information

**Diatoms**

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**Harmful/Nuisance**

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<tr>
<td>Nuisance</td>
<td>N</td>
<td>Other</td>
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**Impact**

Ecologically beneficial

**Distribution**

Cosmopolitan freshwater

**Source Region(s)**

Undetermined

**References**


- Diatom Image Database


**Identification/Taxonomic Information**

**Diatoms**
- **Kingdom**: Protista
- **Phylum**: Ochrophyta
- **Class**: Bacillariophyceae
- **Order**: Centrales
- **Family**: Eupodiscaceae

**Ecologically beneficial**

**Microscopy**

National Institute for Environmental Studies - Japan

www.nies.go.jp/biology/mcc/images/PCD4211/0635L.jpg

**Former Name(s)**

*Bidulphia aurita*

**Distribution**

Cosmopolitan marine / estuarine, benthic, polar to temperate

**Source Region(s)**

Open Atlantic

**References**

Odontella mobilensis (Bailey) Grunow

**Identification/Taxonomic Information**

Diatoms

- **Kingdom:** Protista
- **Phylum:** Ochrophyta
- **Class:** Bacillariophyceae
- **Order:** Centrales
- **Family:** Eupodiscaceae

**Harmful/Nuisance**

- Toxic: N
- Nuisance: N

**Pathogen**

- Human: N
- Other: N

**Impact**

Ecologically beneficial

**Distribution**

Cosmopolitan marine / estuarine

**Source Region(s)**

Open Atlantic

**References**

Odontella sinensis
(Greville) Grunow

Identification/Taxonomic Information

Diatoms

Kingdom: Protista
Phylum: Ochrophyta
Class: Bacillariophyceae
Order: Centrales
Family: Eupodiscaceae

Harmful/Nuisance Pathogen
Toxic: N Human: N
Nuisance: N Other: N
Impact: Ecologically beneficial

Distribution
Cosmopolitan marine, temperate

Source Region(s)
Atlantic 450 nautical miles west of Canaries

References

Microscopy

The Astrobiology Institute - Marine Biological Laboratory, Woods Hole, MA
microscope.mbl.edu/baypaul/microscope/general/page_01.htm

Former Name(s)
Bidulphia sinensis
**Proboscia alata**  
(Brightwell) Sundström

**Identification/Taxonomic Information**

**Diatoms**

**Kingdom** Protista  
**Phylum** Ochrophyta  
**Class** Coscinodiscophyceae  
**Order** Rhizosoleniales  
**Family** Rhizosoleniaceae

**Harmful/Nuisance**  
**Toxic** N  
**Nuisance** N

**Impact** Ecologically beneficial

**Microscopy**

The Astrobiology Institute - Marine Biological Laboratory, Woods Hole, MA  
microscope.mbl.edu/baypaul/microscope/general/page_01.htm

**Former Name(s)**  
**Rhizosolenia alata**

**Distribution**
Cosmopolitan marine, temperate (distribution poorly known)

**Source Region(s)**
Southeast Pacific, east Pacific, with Persian Gulf  
Open Atlantic 400 nautical miles west of Gibraltar  
Open Atlantic  
Northern Indian Ocean (~50%) Some recent open Pacific  
Mid-Atlantic  
Exchange occurred at Fuel Pier in Guam  
Atlantic, 200 nautical miles off Virginia  
Atlantic, 200 nautical miles off Gibraltar plus Mediterranean water  
Atlantic 450 nautical miles west of Canaries

**References**
Identification/Taxonomic Information

Diatoms

Kingdom: Protista
Phylum: Ochrophyta
Class: Bacillariophyceae
Order: Bacillariales
Family: Bacillariaceae

Harmful/Nuisance Pathogen
Toxic: N  Human: N
Nuisance: N  Other: N
Impact: Ecologically beneficial

Microscopy

Hasle & Syvertsen (1997)

Former Name(s)

Nitzschia barkleyi
Nitzschia lineola
Pseudo-nitzschia barkleyi

Distribution

Cosmopolitan marine / estuarine

Source Region(s)

100 - 200 nautical miles off southern Mexican coast

References


**Identification/Taxonomic Information**

**Diatoms**

*Kingdom*  Protista  
*Phylum*  Ochrophyta  
*Class*  Bacillariophyceae  
*Order*  Bacillariaceae  
*Family*  Bacillariaceae

**Harmful/Nuisance**  
Toxic: N  
Nuisance: Y

**Pathogen**  
Human: N  
Other: N

**Impact**  
Ecologically beneficial

**Distribution**
Cosmopolitan marine, temperate to tropical

**Source Region(s)**
- Mediterranean with Gulf Stream dilution, some coastal Carolina
- 100 - 200 nautical miles off Louisiana coast
- 100 - 200 nautical miles off southern Mexican coast
- Atlantic 350 nautical miles south of Bermuda
- Atlantic 450 nautical miles west of Canaries
- Atlantic, 200 nautical miles off Gibraltar plus Mediterranean water
- Atlantic, 200 nautical miles off Virginia
- Central Indian Ocean
- Central Pacific >200 nautical miles north of Wake Island
- Exchange occurred at Fuel Pier in Guam
- ~ 250 nautical miles off northern California coast
- Mediterranean waters diluted with mid and eastern Atlantic waters
- Small proportion of Indian Ocean, majority Mid-Atlantic
- Mediterranean highly diluted with Gulf Stream waters
- Mediterranean with significant coastal Carolina water
- Northern Indian Ocean (~50%) Some recent open Pacific
- Open Atlantic
- Open Atlantic 400 nautical miles west of Gibraltar
- Open Pacific >300 nautical miles north of Midway Island
- Open Pacific ~ 400 nautical miles, east of Mariana Islands
- Puget Sound water, partial Panama
- Southeast Pacific, east Pacific, with Persian Gulf
- Gulf of Mexico coastal Texas with 100 nautical miles on shelf

**References**

Pseudo-nitzschia pungens

(Grunow ex Cleve) Hasle


**Identification/Taxonomic Information**

**Diatoms**

*Kingdom*  
Protista

*Phylum*  
Ochrophyta

*Class*  
Bacillariophyceae

*Order*  
Bacillariales

*Family*  
Bacillariaceae

**Harmful/Nuisance Pathogen**

*Toxic*  
Y  
*Human*  
Y

*Nuisance*  
Y  
*Other*  
Y

**Impact**

Produces the toxin domoic acid, which can cause disease and death of humans, marine mammals, and waterfowl and ASP (amnesic shellfish poisoning) in humans

**Distribution**

Cosmopolitan marine, polar to temperate

**Source Region(s)**

Exchange occurred at Fuel Pier in Guam

**References**


© 2000 Leibniz-Institut für Ostseeforschung Warnemünde  
www.io-warnemuende.de/research/de_galerie.html
Rhizosolenia hebetata
Bailey

**Identification/Taxonomic Information**

**Diatoms**
- **Kingdom**: Protista
- **Phylum**: Ochrophyta
- **Class**: Coscinodiscophyceae
- **Order**: Rhizosoleniales
- **Family**: Rhizosoleniaceae

**Harmful/Nuisance**
- **Toxic**: N
- **Nuisance**: N
- **Impact**: Ecologically beneficial

**Pathogen**
- **Human**: N
- **Other**: N

**Distribution**
Cosmopolitan marine / coastal

**Source Region(s)**
- Southeast Pacific, east Pacific, with Persian Gulf
- Puget Sound water, partial Panama
- Pacific Ocean
- Open Atlantic 400 nautical miles west of Gibraltar
- Open Atlantic
- Northern Indian Ocean – 800 nautical miles west Sri Lanka
- Northern Indian Ocean (~50%) Some recent open Pacific
- Hawaiian Pacific, with Persian Gulf
- Gulf of Mexico coastal Texas with 100 nautical miles on shelf
- Exchange occurred at Fuel Pier in Guam
- Atlantic, 200 nautical miles off Virginia
- Atlantic, 200 nautical miles off Gibraltar plus Mediterranean water
- Atlantic 450 nautical miles west of Canaries

**References**
Identification/Taxonomic Information

Diatoms

Kingdom          Protista
Phylum           Ochrophyta
Class            Coscinodiscophyceae
Order            Rhizosoleniales
Family           Rhizosoleniaceae

Harmful/Nuisance Pathogen
Toxic            N                Human    N
Nuisance         N                Other    N
Impact           Ecologically beneficial

Microscopy

The Astrobiology Institute - Marine Biological Laboratory, Woods Hole, MA
microscope.mbl.edu/baypaul/microscope/general/page_01.htm

Former Name(s)
Rhizosolenia shrubsolei

Distribution
Cosmopolitan marine coastal / estuarine, except for polar regions

Source Region(s)
Pacific Ocean
Open Atlantic 400 nautical miles west of Gibraltar
Northern Indian Ocean – 800 nautical miles west Sri Lanka
Indian Ocean with Tacoma Tap Water
Exchange occurred at Fuel Pier in Guam
Atlantic, 200 nautical miles off Virginia

References
Identification/Taxonomic Information

Diatoms

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</table>

Harmful/Nuisance | Pathogen
---|---
Toxic | Human | N
Nuisance | Other | N
Impact | Ecologically beneficial

Microscopy

Göteborg University, Dept. of Marine Ecology, Marine Botany
www.marbot.gu.se/SSS/SSShome.htm

Former Name(s)

Source Region(s)

Cosmopolitan marine coastal / estuarine, temperate to subtropical

Undetermined

References

Identification/Taxonomic Information

Diatoms

Kingdom Protista
Phylum Ochrophyta
Class Coscinodiscophyceae
Order Rhizosoleniales
Family Rhizosoleniaceae

Harmful/Nuisance Pathogen
Toxic N Human N
Nuisance N Other N
Impact Ecologically beneficial

Distribution
Cosmopolitan marine coastal / estuarine, temperate to subtropical (absent from polar regions?)

Source Region(s)
Puget Sound water, partial Panama
Open Atlantic
Northern Indian Ocean (~50%); Some recent open Pacific
Atlantic, 200 nautical miles off Virginia

References
Identification/Taxonomic Information

Diatoms

Kingdom: Protista
Phylum: Ochrophyta
Class: Coscinodiscophycea
Order: Thalassiosirales
Family: Skeletonemaceae

Harmful/Nuisance Pathogen
Toxic: N  Human: N
Nuisance: N  Other: N
Impact: Ecologically beneficial

Microscopy

Göteborg University, Dept. of Marine Ecology, Marine Botany
www.marbot.gu.se/SSS/SSShome.htm

Former Name(s)


Skeletonema potamos

(Cl. Webber) Hasle

Identification/Taxonomic Information

Diatoms

Kingdom Protista
Phylum Ochrophyta
Class Coscinodiscophyceae
Order Thalassiosirales
Family Skeletonemaceae

Harmful/Nuisance Pathogen
Toxic N Human N
Nuisance N Other N
Impact Ecologically beneficial

Microscopy

www.epa.gov

Former Name(s)
Microsiphona potamos

Distribution
Cosmopolitan estuarine (freshwater)

Source Region(s)
Mediterranean with significant coastal Carolina water

References

**Identification/Taxonomic Information**

**Diatoms**

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**Harmful/Nuisance**

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<th>Other</th>
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<tbody>
<tr>
<td>N</td>
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**Impact**

Ecologically beneficial

**Distribution**

Cosmopolitan marine / estuarine

**Source Region(s)**

Atlantic, 200 nautical miles off Virginia

**References**

Identification/Taxonomic Information

Diatoms

Kingdom Protista
Phylum Ochrophyta
Class Fragilariophyceae
Order Thalassionematales
Family Thalassionemataceae

Harmful/Nuisance Pathogen
Toxic N Human N
Nuisance N Other N
Impact Ecologically beneficial

Microscopy

NCSU CAAE
www.ncsu.edu/wq/aboutCAAE/

Former Name(s)
Synedra nitzschioides
Thalassiothrix nitzschioides

Distribution
Cosmopolitan except for Arctic

Source Region(s)
Puget Sound water, partial Panama
Open Pacific ~ 400 nautical miles, east of Mariana Islands
Open Atlantic
Northern Indian Ocean (~50%) Some recent open Pacific
Mediterranean with significant coastal Carolina water
100 - 200 nautical miles off southern Mexican coast
~ 250 nautical miles off northern California coast

References
Identification/Taxonomic Information

**Diatoms**

**Kingdom**  Protista  
**Phylum**  Ochrophyta  
**Class**  Coscinodiscophyceae  
**Order**  Thalassiosirales  
**Family**  Thalassiosiraceae

**Harmful/Nuisance**  
**Toxic**  N  
**Nuisance**  N  
**Impact**  Ecologically beneficial

**Microscopy**

NCSU CAAE

www.ncsu.edu/wq/aboutCAAE/

**Former Name(s)**

Coscinodiscus eccentricus

**Distribution**

Cosmopolitan marine / coastal, except for polar regions

**Source Region(s)**

Mediterranean with significant coastal Carolina water

**References**

**Identification/Taxonomic Information**

**Diatoms**

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**Harmful/Nuisance**

<table>
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<th>Human</th>
<th>N</th>
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</table>

**Nuisance**

| N | Other | N |

**Impact**

Ecologically beneficial

**Distribution**

Cosmopolitan northern hemisphere marine, Arctic to temperate

**Source Region(s)**

Puget Sound water, partial Panama

Pacific Ocean

Mediterranean with significant coastal Carolina water

Exchange occurred at Fuel Pier in Guam

~ 250 nautical miles off northern California coast

**References**

Akashiwo sanguinea
(Hirasaka) G. Hansen et Moestrup

Identification/Taxonomic Information

Dinoflagellates

Kingdom Protista
Phylum Dinophyta
Class Dinophyceae
Order Gymnodiniales
Family Gymnodiniaeace

Harmful/Nuisance  Pathogen
Toxic  N  Human  N
Nuisance  Y  Other  Y
Impact  Can form nuisance blooms that are sometimes linked to fish kills

Distribution
Cosmopolitan estuarine / marine coastal, temperate to tropical

Source Region(s)
Undetermined

References
- Daugbjerg, N., G. Hansen, J. Larsen, & Ø. Moestrup 2000. Phylogeny of some of the major genera of dinoflagellates based on ultrastructure and partial LSU rDNA sequence data, including the erection of three new genera of unarmoured dinoflagellates. Phycologia
**Balechina coerulea**

*(Dogiel) F.J.R. Taylor*

**Identification/Taxonomic Information**

- **Kingdom**: Protista
- **Phylum**: Dinophyta
- **Class**: Dinophyceae
- **Order**: Ptychodiscales
- **Family**: Ptychodiscaceae

**Harmful/Nuisance**

- **Toxic**: N
- **Nuisance**: N
- **Ecologically beneficial**: N
- **Human**: N
- **Other**: N

**Impact**

Ecologically beneficial

**Microscopy**

![Microscopy Image](Steidinger & Tangen (1997)

**Former Name(s)**

*Gymnodinium coeruleum*

**Distribution**

Cosmopolitan marine, temperate to tropical (mostly warm waters)

**Source Region(s)**

Atlantic, 200 nautical miles off Virginia

**References**

Identification/Taxonomic Information

Dinoflagellates

Kingdom  Protista
Phylum    Dinophyta
Class     Dinophyceae
Order     Gonyaulacales
Family    Ceratiaceae

Harmful/Nuisance  Pathogen
Toxic        N  Human  N
Nuisance    N  Other  N
Impact      Ecologically beneficial

Distribution
Cosmopolitan marine, warm temperate to tropical

Source Region(s)
Atlantic, 200 nautical miles off Gibraltar plus Mediterranean water

References
- Stein, F. 1883. Die Organismus der Flagellaten nach eigenen Forschungen in systematischer Reihenfolge bearbeitet. Der Organismus
der Infusionstiere. II. Hälfte, Die naturgeschichte der arthrodelen Flagellaten. Einleitung und Erklärung der Abbildungen, pp
Ceratium furca
(Ehrenberg) Claparède et Lachman

Identification/Taxonomic Information

Dinoflagellates

Kingdom Protista
Phylum Dinophyta
Class Dinophyceae
Order Gonyaulacales
Family Ceratiaceae

Harmful/Nuisance Pathogen
Toxic N Human N
Nuisance N Other N
Impact Ecologically beneficial

Distribution
Cosmopolitan marine / estuarine, cold temperate to tropical

Source Region(s)
Hawaiian Pacific, with Persian Gulf
100 - 200 nautical miles off southern Mexican coast
400 nautical miles west of Africa with northwest track to open Atlantic
Atlantic 350 nautical miles south of Bermuda
Atlantic 450 nautical miles west of Canaries
Atlantic, 200 nautical miles off Gibraltar plus Mediterranean water
Central Indian Ocean
~ 250 nautical miles off northern California coast
Exchange occurred at Fuel Pier in Guam
Southeast Pacific, east Pacific, with Persian Gulf
Indian Ocean with Tacoma Tap Water
Mediterranean waters diluted with mid and eastern Atlantic waters
Mediterranean with Gulf Stream dilution, some coastal Carolina
Mediterranean highly diluted with Gulf Stream waters
Mediterranean with significant coastal Carolina water
Mid-Atlantic
Open Pacific >300 nautical miles north of Midway Island
Central Pacific >200 nautical miles north of Wake Island

References

**Ceratium fusus**

(Ehrenberg) Dujardin

**Identification/Taxonomic Information**

**Dinoflagellates**

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**Harmful/Nuisance Pathogen**

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<table>
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<tr>
<th>Nuisance</th>
<th>Other</th>
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</thead>
<tbody>
<tr>
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<td>N</td>
</tr>
</tbody>
</table>

**Impact**

Ecologically beneficial

**Distribution**

Cosmopolitan marine / estuarine, cold temperate to tropical

**Source Region(s)**

Central Pacific between Hawaii and Midway
100 - 200 nautical miles off southern Mexican coast
400 nautical miles west of Africa with northwest track to open Atlantic
50-100 nautical miles west of Hawaii
Atlantic 350 nautical miles south of Bermuda
Atlantic 450 nautical miles west of Canaries
Atlantic, 200 nautical miles off Gibraltar plus Mediterranean water
100 - 200 nautical miles off Louisiana coast
Central Pacific >200 nautical miles north of Wake Island
Southeast Pacific, east Pacific, with Persian Gulf
Exchange occurred at Fuel Pier in Guam
Mediterranean waters diluted with mid and eastern Atlantic waters
Mediterranean with Gulf Stream dilution, some coastal Carolina
Mediterranean highly diluted with Gulf Stream waters
Mediterranean with significant coastal Carolina water
Mid-Atlantic
Puget Sound water, partial Panama
Central Indian Ocean

**References**

Ceratium lunula
(Schimper) Jørgensen

Identification/Taxonomic Information

Dinoflagellates

Kingdom: Protista
Phylum: Dinophyta
Class: Dinophyceae
Order: Gonyaulacales
Family: Ceratiaceae

Harmful/Nuisance Pathogen
Toxic: N
Human: N
Nuisance: N
Other: N
Impact: Ecologically beneficial

Microscopy

Steidinger & Tangen (1997)

Former Name(s)
Ceratium tripus lunula

Distribution
Cosmopolitan marine / estuarine warm temperate to tropical

Source Region(s)
Undetermined

References

**Identification/Taxonomic Information**

*Dinoflagellates*

**Kingdom** Protista  
**Phylum** Dinophyta  
**Class** Dinophyceae  
**Order** Gonyaulacales  
**Family** Ceratiaceae  

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<td>Human</td>
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<td>Other</td>
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<tr>
<td>Impact</td>
<td>Ecologically beneficial</td>
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**Distribution**  
Cosmopolitan marine, cold temperate to tropical

**Source Region(s)**  
Southeast Pacific, east Pacific, with Persian Gulf  
Mid-Atlantic  
Mediterranean with significant coastal Carolina water  
Mediterranean with Gulf Stream dilution, some coastal Carolina  
Hawaiian Pacific, with Persian Gulf  
Exchange occurred at Fuel Pier in Guam  
Central Pacific >200 nautical miles north of Wake Island  
Atlantic, 200 nautical miles off Virginia  
Atlantic 450 nautical miles west of Canaries  
Atlantic 350 nautical miles south of Bermuda  
400 nautical miles west of Africa with northwest track to open Atlantic  
100 - 200 nautical miles off southern Mexican coast

**References**

Ceratium tripos

(O.F. Müller) Nitzsch

Identification/Taxonomic Information

Dinoflagellates

Kingdom Protista
Phylum Dinophyta
Class Dinophyceae
Order Gonyaulacales
Family Ceratiaceae

Harmful/Nuisance Pathogen
Toxic N Human N
Nuisance Y Other Y
Impact Blooms have caused anoxia/hypoxia, fish kills

Distribution
Cosmopolitan marine, cold temperate to tropical

Source Region(s)
Mediterranean waters diluted with mid and eastern Atlantic waters
100 - 200 nautical miles off southern Mexican coast
400 nautical miles west of Africa with northwest track to open Atlantic
Atlantic 350 nautical miles south of Bermuda
Atlantic 450 nautical miles west of Canaries
Atlantic, 200 nautical miles off Gibraltar plus Mediterranean water
Central Indian Ocean
Central Pacific >200 nautical miles north of Wake Island
Central Pacific between Hawaii and Midway
100 - 200 nautical miles off Louisiana coast
Hawaiian Pacific, with Persian Gulf
Southeast Pacific, east Pacific, with Persian Gulf
Mediterranean with Gulf Stream dilution, some coastal Carolina
Mediterranean highly diluted with Gulf Stream waters
Mediterranean with significant coastal Carolina water
Mid-Atlantic
Northern Indian Ocean (~50%); Some recent open Pacific
Open Atlantic 400 nautical miles west of Gibraltar
Open Pacific >300 nautical miles north of Midway Island
Open Pacific ~ 400 nautical miles, east of Mariana Islands
Puget Sound water, partial Panama
Exchange occurred at Fuel Pier in Guam

References
Ceratium tripos

(O.F. Müller) Nitzsch

Dinophysis acuminata

Claparède et Lachmann

Identification/Taxonomic Information

**Kingdom** Protista

**Phylum** Dinophyta

**Class** Dinophyceae

**Order** Dinophysiales

**Family** Dinophysiaceae

Harmful/Nuisance  Pathogen

Toxic  Y  Human  Y

Nuisance  Y  Other  Y

Impact  Can be ecologically harmful; produces various toxins and linked to human illness via seafood consumption

Microscopy

NCSU CAAE

www.ncsu.edu/wq/aboutCAAE/

Former Name(s)

Dinophysis lachmannii

Dinophysis skagii

Distribution

Cosmopolitan marine, polar to temperate

Source Region(s)

Mediterranean with Gulf Stream dilution, some coastal Carolina

400 nautical miles west of Africa with northwest track to open Atlantic

Puget Sound water, partial Panama

Open Atlantic

Exchange occurred at Fuel Pier in Guam

References


### Identification/Taxonomic Information

**Dinoflagellates**

- **Kingdom**: Protista
- **Phylum**: Dinophyta
- **Class**: Dinophyceae
- **Order**: Dinophysiales
- **Family**: Dinophysiae

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<th>Harmful/Nuisance</th>
<th>Pathogen</th>
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<tr>
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<td>Human</td>
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<tr>
<td>Nuisance</td>
<td>Other</td>
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**Impact**: Can be ecologically harmful; produces various toxins and linked to human illness via seafood consumption.

### Distribution

Cosmopolitan marine / estuarine, temperate to tropical, rarely in cold waters.

### Source Region(s)

Southeast Pacific, east Pacific, with Persian Gulf.

100 - 200 nautical miles off southern Mexican coast.

### References

**Gambierdiscus toxicus**

Adachi et Fukuyo

**Identification/Taxonomic Information**

Dinoflagellates

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<td>Goniodomataceae</td>
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Harmful/Nuisance Pathogen

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<tr>
<th>Toxic</th>
<th>Y</th>
<th>Human</th>
<th>Y</th>
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</thead>
<tbody>
<tr>
<td>Nuisance</td>
<td>Y</td>
<td>Other</td>
<td>Y</td>
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</table>

Impact: Can be ecologically harmful; produces various toxins and linked to human illness via seafood consumption.

**Distribution**

Cosmopolitan marine benthic, tropical to subtropical

**Source Region(s)**

Exchange occurred at Fuel Pier in Guam

**References**

Gonyaulax spinifera
(Claparède et Lachmann) Diesing

Identification/Taxonomic Information

Dinoflagellates

Kingdom  Protista
Phylum    Dinophyta
Class     Dinophyceae
Order     Gonyaulacales
Family    Gonyaulacaceae

Harmful/Nuisance  Pathogen
Toxic    N       Human N
Nuisance Y       Other Y
Impact   Blooms have caused anoxia/hypoxia, fish kills

Distribution
Cosmopolitan marine / estuarine, temperate to subtropical

Source Region(s)
Southeast Pacific, east Pacific, with Persian Gulf
Exchange occurred at Fuel Pier in Guam
400 nautical miles west of Africa with northwest track to open Atlantic

References

Microscopy

Göteborg University, Dept. of Marine Ecology, Marine Botany
www.marbot.gu.se/SSS/SSShome.htm

Former Name(s)
Peridinium spiniferum
**Identification/Taxonomic Information**

**Kingdom**  Protista  
**Phylum**  Dinophyta  
**Class**  Dinophyceae  
**Order**  Peridiniales  
**Family**  Peridiniaceae

**Harmful/Nuisance**  |  **Pathogen**  
--- | ---  
Toxic  |  Human  
Nuisance  |  Other

**Impact**  
Ecologically beneficial, but also blooms in response to nutrient pollution

**Distribution**  
Cosmopolitan estuarine / marine, temperate

**Source Region(s)**  
Southeast Pacific, east Pacific, with Persian Gulf  
Mediterranean waters diluted with mid and eastern Atlantic waters  
Central Pacific >200 nautical miles north of Wake Island  
Atlantic, 200 nautical miles off Virginia  
Atlantic, 200 nautical miles off Gibraltar plus Mediterranean water  
400 nautical miles west of Africa with northwest track to open Atlantic  
100 - 200 nautical miles off Louisiana coast

**References**  
- Daugbjerg, N., G. Hansen, J. Larsen, & Ø. Moestrup 2000. Phylogeny of some of the major genera of dinoflagellates based on ultrastructure and partial LSU rDNA sequence data, including the erection of three new genera of unarmoured dinoflagellates. Phycologia
Identification/Taxonomic Information

Heterocapsa triquetra
(Ehrenberg) Stein

Dinoflagellates

Kingdom  Protista
Phylum    Dinophyta
Class     Dinophyceae
Order     Peridiniales
Family    Peridiniaceae

Harmful/Nuisance  Pathogen
Toxic      N  Human      N
Nuisance   Y  Other     Y
Impact     Ecologically beneficial, but also blooms in response to nutrient pollution

Distribution
Cosmopolitan estuarine / marine, temperate

Source Region(s)
400 nautical miles west of Africa with northwest track to open Atlantic

References

Stein, F. 1883. Die Organismus der Flagellaten nach eigenen Forschungen in systematischer Reihenfolge bearbeitet. Der Organismus der Infusionstiere. II. Hälfte, Die naturgeschichte der arthrodelen Flagellaten. Einleitung und Erklärung der Abbildungen, pp

**Identification/Taxonomic Information**

**Kingdom** Protista  
**Phylum** Dinophyta  
**Class** Dinophyceae  
**Order** Gymnodiniales  
**Family** Gymnodiniaceae

**Harmful/Nuisance**  
Toxic: N  
Nuisance: Y  
Impact: At high cell densities, can be toxic to fish

**Pathogen**  
Toxic: N  
Nuisance: Y

**Distribution**  
Cosmopolitan marine

**Source Region(s)**  
- Exchange occurred at Fuel Pier in Guam  
- 100 - 200 nautical miles off Louisiana coast  
- 400 nautical miles west of Africa with northwest track to open Atlantic  
- Atlantic 350 nautical miles south of Bermuda  
- Atlantic 450 nautical miles west of Canaries  
- Atlantic, 200 nautical miles off Gibraltar plus Mediterranean water  
- Atlantic, 200 nautical miles off Virginia  
- ~ 250 nautical miles off northern California coast  
- Central Pacific >200 nautical miles north of Wake Island  
- Southeast Pacific, east Pacific, with Persian Gulf  
- Gulf of Mexico coastal Texas with 100 nautical miles on shelf  
- Mediterranean waters diluted with mid and eastern Atlantic waters  
- Mediterranean with Gulf Stream dilution, some coastal Carolina  
- Mediterranean highly diluted with Gulf Stream waters  
- Mediterranean with significant coastal Carolina water  
- Mid-Atlantic  
- Open Pacific >300 nautical miles north of Midway Island  
- Central Indian Ocean

**References**  
Daugbjerg, N., G. Hansen, J. Larsen, & Ø. Moestrup 2000. Phylogeny of some of the major genera of dinoflagellates based on ultrastructure and partial LSU rDNA sequence data, including the erection of three new genera of unarmoured dinoflagellates. Phycology
**Lingulodinium polyedrum**  
(Stein) Dodge

**Identification/Taxonomic Information**

**Dinoflagellates**

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<td>Gonyaulacales</td>
</tr>
<tr>
<td>Family</td>
<td>Gonyaulacaceae</td>
</tr>
</tbody>
</table>

**Harmful/Nuisance**  
- Toxic: Y  
- Nuisance: Y

**Pathogen**  
- Human: Y  
- Other: Y

**Impact**  
Can be toxic to fish, shellfish, & humans (illness and death); toxic strains produce saxitoxins

**Distribution**
Cosmopolitan marine, temperate to tropical

**Source Region(s)**
Exchange occurred at Fuel Pier in Guam

**References**
**Identification/Taxonomic Information**

**Dinoflagellates**

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</table>

**Harmful/Nuisance**

- Toxic: N
- Nuisance: N

**Impact**

Ecologically beneficial

**Microscopy**

NCSU CAAE

www.ncsu.edu/wq/aboutCAAE/

**Former Name(s)**

Stein, F. 1883. Die Organismus der Flagellaten nach eigenen Forschungen in systematischer Reihenfolge bearbeitet. Der Organismus der Infusionstiere. II. Hälfte, Die naturgeschichte der arthrodelen Flagellaten. Einleitung und Erklärung der Abbildungen, pp


**Source Region(s)**

- Open Pacific ~ 400 nautical miles, east of Mariana Islands
- Central Pacific between Hawaii and Midway
- 400 nautical miles west of Africa with northwest track to open Atlantic
- ~ 250 nautical miles off northern California coast

**Distribution**

Cosmopolitan marine, warm temperate to tropical

**References**

- Stein, F. 1883. Die Organismus der Flagellaten nach eigenen Forschungen in systematischer Reihenfolge bearbeitet. Der Organismus der Infusionstiere. II. Hälfte, Die naturgeschichte der arthrodelen Flagellaten. Einleitung und Erklärung der Abbildungen, pp
**Oxyrrhis marina**

Dujardin

**Identification/Taxonomic Information**

- **Kingdom**: Protista
- **Phylum**: Dinophyta
- **Class**: Dinophyceae
- **Order**: Peridiniales
- **Family**: Pronoctilucaceae

**Harmful/Nuisance**

- **Toxic**: N
- **Nuisance**: N
- **Impact**: Ecologically beneficial

**Distribution**

Cosmopolitan marine / estuarine

**Source Region(s)**

- Mediterranean with significant coastal Carolina water
- Atlantic, 200 nautical miles off Virginia
- 100 - 200 nautical miles off Louisiana coast

**References**

Peridiniella danica
(Paulsen) Okolodkov et Dodge

Identification/Taxonomic Information

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<td>Family</td>
<td>Gonyaulacaceae</td>
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Harmful/Nuisance  Pathogen
Toxic           N    Human  N
Nuisance        Y    Other  N
Impact          Ecologically beneficial

Distribution
Cosmopolitan marine / estuarine, polar to temperate

Source Region(s)
Mediterranean with Gulf Stream dilution, some coastal Carolina

References

Microscopy

Smithsonian Environmental Research Center - Phytoplankon Ecology Laboratory
serc5.si.edu/algae/

Former Name(s)
Glenodinium danicum
**Peridinium aciculiferum**
(Lemmerman) Lindeman

**Identification/Taxonomic Information**

**Dinoflagellates**

**Kingdom** Protista  
**Phylum** Dinophyta  
**Class** Dinophyceae  
**Order** Peridiniales  
**Family** Peridiniaceae

**Harmful/Nuisance**  
Toxic: N  
Nuisance: Y  
Impact: Allelopathic to other algae in freshwaters

**Pathogen**  
Toxic: N  
Nuisance: Y

**Microscopy**

Photo by Dr. Susan Carty, Heidelberg College, OH

www.aves.net/algaeweb/dinopics.htm

**Former Name(s)**

Glendinium aciculiferum  
Peridinium stagnale  
Peridinium umbonatum var. aciculiferum

**Distribution**

Cosmopolitan freshwater / estuarine, temperate

**Source Region(s)**

Open Pacific >300 nautical miles north of Midway Island  
Mediterranean with significant coastal Carolina water  
Mediterranean with Gulf Stream dilution, some coastal Carolina  
Central Pacific >200 nautical miles north of Wake Island  
Central Indian Ocean  
Atlantic 450 nautical miles west of Canaries  
400 nautical miles west of Africa with northwest track to open Atlantic

**References**

Phalacroma rotundatum

(Claparède et Lachmann) Kofoid & Michener

**Identification/Taxonomic Information**

**Dinoflagellates**

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**Harmful/Nuisance**

<table>
<thead>
<tr>
<th>Toxic</th>
<th>Y</th>
<th>Human</th>
<th>Y</th>
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<tbody>
<tr>
<td>Nuisance</td>
<td>Y</td>
<td>Other</td>
<td>Y</td>
</tr>
</tbody>
</table>

**Impact**

Produces toxins that can cause shellfish and human disease

**Distribution**

Cosmopolitan marine

**Source Region(s)**

Exchange occurred at Fuel Pier in Guam

**References**


**Microscopy**

Göteborg University, Dept. of Marine Ecology, Marine Botany

www.marbot.gu.se/SSS/SSShome.htm

**Former Name(s)**

*Dinophysis rotandata*

*Prodinophysis rotundatum*
**Identification/Taxonomic Information**

**Podolampas palmipes**

**Stein**

**Dinoflagellates**

- **Kingdom**: Protista
- **Phylum**: Dinophyta
- **Class**: Dinophyceae
- **Order**: Peridiniales
- **Family**: Podolampaceae

**Ecologically beneficial**

**Impact**

- **Toxic**: N
- **Nuisance**: N
- **Pathogen**: N
- **Harmful/Nuisance**: N

**Distribution**

Cosmopolitan marine, warm temperate to tropical

**Source Region(s)**

Atlantic 450 nautical miles west of Canaries

**References**

- Stein, F. 1883. Die Organismus der Flagellaten nach eigenen Forschungen in systematischer Reihenfolge bearbeitet. Der Organismus der Infusionstiere. II. Hälfte, Die naturgeschichte der arthrodelen Flagellaten. Einleitung und Erklärung der Abbildungen, pp [340x393]
**Prorocentrum micans**

Ehrenberg

**Taxonomic Information**

- **Kingdom**: Protista
- **Phylum**: Dinophyta
- **Class**: Dinophyceae
- **Order**: Prorocentrales
- **Family**: Prorocentraceae

**Identification**

<table>
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<tr>
<th>Toxic</th>
<th>Nuisance</th>
<th>Pathogen</th>
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<tbody>
<tr>
<td>Y</td>
<td>Y</td>
<td>N</td>
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</table>

**Impact**

**Distribution**

Cosmopolitan, cold temperate to tropical

**Source Region(s)**

- Puget Sound water, partial Panama
- Open Ocean >300 nautical miles north of Midway Island
- Mediterranean with significant coastal Carolina water
- Mediterranean with Gulf Stream dilution, some coastal Carolina
- Hawaiian Pacific, with Persian Gulf
- Gulf of Mexico coastal Texas with 100 nautical miles on shelf
- Central Pacific >200 nautical miles north of Wake Island
- Central Indian Ocean
- Atlantic, 200 nautical miles off Virginia
- Atlantic, 200 nautical miles off Gibraltar plus Mediterranean water
- 400 nautical miles west of Africa with northwest track to open Atlantic

**References**

Prorocentrum minimum
(Pavillard) Schiller

Identification/Taxonomic Information

Dinoflagellates

Kingdom: Protista
Phylum: Dinophyta
Class: Dinophyceae
Order: Prorocentrales
Family: Prorocentraceae

Harmful/Nuisance: Toxic Y, Nuisance Y
Pathogen: Human Y, Other Y

Impact: Can be toxic to shellfish; blooms in response to nutrient pollution

Microscopy

NCSU CAAE

References


Source Region(s)

Mediterranean with significant coastal Carolina water
400 nautical miles west of Africa with northwest track to open Atlantic
50-100 nautical miles west of Hawaii
Atlantic, 200 nautical miles off Virginia
Exchange occurred at Fuel Pier in Guam
Mediterranean waters diluted with mid and eastern Atlantic waters
~ 250 nautical miles off northern California coast
Mediterranean highly diluted with Gulf Stream waters
Southeast Pacific, east Pacific, with Persian Gulf
Northern Indian Ocean – 800 nautical miles west Sri Lanka
Open Atlantic
Open Atlantic 400 nautical miles west of Gibraltar
Open Pacific >300 nautical miles north of Midway Island
Open Pacific ~ 400 nautical miles, east of Mariana Islands
Puget Sound water, partial Panama
Mediterranean with Gulf Stream dilution, some coastal Carolina

Distribution

Cosmopolitan marine, cold temperate to tropical

www.ncsu.edu/wq/aboutCAAE/
Protoperidinium brevipes
(Paulsen) Balech

Identification/Taxonomic Information

**Protista**

**Dinophyta**

**Dinophyceae**

**Peridiniales**

**Protoperidiniaceae**

**Ecologically beneficial**

**Microscopy**

**NOAA**


**Former Name(s)**

*Peridinium brevipes*

Distribution

Cosmopolitan coastal marine, cold waters

Source Region(s)

400 nautical miles west of Africa with northwest track to open Atlantic

References


Identification/Taxonomic Information

**Protoperidinium conicum**

(Gran) Balech

**Dinoflagellates**

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**Identification/Taxonomic Information**

**Harmful/Nuisance**  **Pathogen**

Toxic: N  Human: N

Nuisance: N  Other: N

Impact: Ecologically beneficial

**Distribution**

Cosmopolitan marine, temperate to tropical

**Source Region(s)**

Puget Sound water, partial Panama

Open Atlantic

**References**


Protoperidinium depressum
(Bailey) Balech

Identification/Taxonomic Information

Dinoflagellates

Kingdom Protista
Phylum Dinophyta
Class Dinophyceae
Order Peridiniales
Family Protoperidiniaceae

Harmful/Nuisance Pathogen
Toxic Y Human N
Nuisance Y Other Y
Impact May produce toxins; linked with fish kills

Microscopy

The Astrobiology Institute - Marine Biological Laboratory,
Woods Hole, MA
microscope.mbl.edu/baypaul/microscope/general/page_01.htm

Former Name(s)
Peridinium depressum

Distribution
Cosmopolitan marine, temperate to tropical

Source Region(s)
Puget Sound water, partial Panama
Open Atlantic
Exchange occurred at Fuel Pier in Guam
100 - 200 nautical miles off southern Mexican coast

References

Protoperidinium pallidum

(Ostenfeld) Balech

Identification/Taxonomic Information

Dinoflagellates

Kingdom Protista
Phylum Dinophyta
Class Dinophyceae
Order Peridiniales
Family Protoperidiniaceae

Harmful/Nuisance Pathogen
Toxic N Human N
Nuisance N Other N
Impact Ecologically beneficial

Distribution
Cosmopolitan marine, cold temperate to warm temperate

Source Region(s)
Puget Sound water, partial Panama
Open Atlantic

References


Microscopy

Göteborg University, Dept. of Marine Ecology, Marine Botany
www.marbot.gu.se/SSS/SSShome.htm

Former Name(s)

Peridinium pallidum

Page 89 of 102
**Protoperidinium pellucidum**

**Identification/Taxonomic Information**

**Dinoflagellates**

**Kingdom** Protista  
**Phylum** Dinophyta  
**Class** Dinophyceae  
**Order** Peridiniales  
**Family** Protoperidiniaceae  

**Harmful/Nuisance**  
**Toxic** Y  
**Nuisance** Y  
**Impact** May produce toxins; linked with fish kills

**Microscopy**

![Microscope Image](image_url)

**Distribution**

Cosmopolitan marine, temperate to tropical

**Source Region(s)**

Puget Sound water, partial Panama  
Open Atlantic  
Atlantic, 200 nautical miles off Virginia  
Atlantic, 200 nautical miles off Gibraltar plus Mediterranean water  
100 - 200 nautical miles off southern Mexican coast

**References**


**Protoperidinium pentagonum**
(Gran) Balech

**Identification/Taxonomic Information**

**Dinoflagellates**

- **Kingdom**: Protista
- **Phylum**: Dinophyta
- **Class**: Dinophyceae
- **Order**: Peridiniales
- **Family**: Protoperidiniaceae

**Harmful/Nuisance**

- **Toxic**: N
- **Nuisance**: N

**Ecologically beneficial**

**Microscopy**

University of Port Elizabeth

www.upe.ac.za/botany/dinos/images/

**Former Name(s)**

- Peridinium pentagonum
- Peridinium sinuosum

**Source Region(s)**

Atlantic 450 nautical miles west of Canaries

**Distribution**

Cosmopolitan marine coastal / estuarine, temperate to tropical

**References**

Scrippsiella trochoidea
(Stein) Loeblich III

Identification/Taxonomic Information

Dinoflagellates

Kingdom: Protista
Phylum: Dinophyta
Class: Dinophyceae
Order: Gonyaulacales
Family: Calciodinellaceae

Harmful/Nuisance Pathogen
Toxic: N
Human: N
Nuisance: N
Other: N
Impact: Ecologically beneficial

Distribution
Cosmopolitan marine coastal / estuarine

Source Region(s)
Southeast Pacific, east Pacific, with Persian Gulf
Puget Sound water, partial Panama
Open Pacific >300 nautical miles north of Midway Island
Open Atlantic 400 nautical miles west of Gibraltar
Open Atlantic
Mediterranean highly diluted with Gulf Stream waters
Mediterranean with Gulf Stream dilution, some coastal Carolina
Mediterranean waters diluted with mid and eastern Atlantic waters
Gulf of Mexico coastal Texas with 100 nautical miles on shelf
Atlantic, 200 nautical miles off Virginia
50-100 nautical miles west of Hawaii
400 nautical miles west of Africa with northwest track to open Atlantic
100 - 200 nautical miles off southern Mexican coast
Exchange occurred at Fuel Pier in Guam
Atlantic 450 nautical miles west of Canaries

References

Chroomonas minuta
(Skuja) Santore

Identification/Taxonomic Information

Other Flagellates
Cryptomonads

Kingdom: Protista
Phylum: Cryptophyta
Class: Cryptophyceae
Order: Cryptomonadales
Family: Cryptomonadaceae

Harmful/Nuisance Pathogen
Toxic: N  Human: N
Nuisance: N  Other: N
Impact: Ecologically beneficial

Microscopy

NCSU CAAE
www.ncsu.edu/wq/aboutCAAE/

Former Name(s)
Rhodomonas minuta var nannoplanktonica

Distribution
Cosmopolitan, estuarine / marine to freshwater

Source Region(s)
Open Pacific >300 nautical miles north of Midway Island

References
Cryptomonas erosa

Ehrenberg

Identification/Taxonomic Information

Other Flagellates

Cryptomonads

Kingdom: Protista
Phylum: Cryptophyta
Class: Cryptophyceae
Order: Cryptomonadales
Family: Cryptomonadaceae

Impact: Ecologically beneficial

Microscopy

References

**Identification/Taxonomic Information**

**Chlorophytes**

- **Kingdom**: Protista
- **Phylum**: Chlorophyta
- **Class**: Chlorophyceae
- **Order**: Chlorococcales
- **Family**: Scenedesmaceae

**Harmful/Nuisance Pathogen**

- **Toxic**: N
- **Human**: N
- **Nuisance**: N
- **Other**: N
- **Impact**: Ecologically beneficial in freshwaters, but can form nuisance blooms in response to nutrients, especially in aquaculture facilities

**Distribution**

Cosmopolitan; freshwaters (occasionally oligohaline)

**Source Region(s)**

- Mediterranean highly diluted with Gulf Stream waters
- Mediterranean with Gulf Stream dilution, some coastal Carolina

**References**


**Microscopy**

National Institute for Environmental Studies - Japan

www.nies.go.jp/biology/mcc/images/PCD4212/0060L.jpg
Westella botryoides
(W. West) de Wildeman

Identification/Taxonomic Information

Chlorophytes

<table>
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<tr>
<th>Kingdom</th>
<th>Protista</th>
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<tbody>
<tr>
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</tr>
<tr>
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<tr>
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Harmful/Nuisance Pathogen

<table>
<thead>
<tr>
<th>Toxic</th>
<th>Human</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuisance</td>
<td>Other</td>
<td>N</td>
</tr>
<tr>
<td>Impact</td>
<td>Ecologically beneficial</td>
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</tr>
</tbody>
</table>

Microscopy

www.glerl.noaa.gov

Former Name(s)

Tetracoccus botryoides

Distribution

Cosmopolitan freshwater

Source Region(s)

Mediterranean with significant coastal Carolina water
Central Pacific >200 nautical miles north of Wake Island
400 nautical miles west of Africa with northwest track to open Atlantic

References

Identification/Taxonomic Information

Cyanobacteria

**Kingdom**  Monera  
**Phylum**  Cyanophyta (Cyanobacteria)  
**Class**  Cyanophyceae  
**Order**  Nostocales  
**Family**  Oscillatoriaceae

*Harmful/Nuisance Pathogen*

**Toxic**  N  **Human**  N  
**Nuisance**  Y  **Other**  N  
**Impact**  Can form nuisance blooms in freshwaters

Microscopy

NCSU CAAE

www.ncsu.edu/wq/aboutCAAE/

Former Name(s)

Oscillatoria geminata

Distribution

Cosmopolitan freshwater (estuarine)

Source Region(s)

Open Pacific >300 nautical miles north of Midway Island

References

**Identification/Taxonomic Information**

Other Flagellates

**Chrysophytes**

- **Kingdom**: Protista
- **Phylum**: Ochrophyta
- **Class**: Chrysophyceae
- **Order**: Pedinелаles
- **Family**: Pedinellaceae

**Harmful/Nuisance**

- **Toxic**: N
- **Nuisance**: N

**Pathogen**

- **Human**: N
- **Other**: N

**Impact**

Ecologically beneficial

**Distribution**

Cosmopolitan estuarine / coastal

**Source Region(s)**

- Mediterranean with significant coastal Carolina water
- Mediterranean highly diluted with Gulf Stream waters
- Mediterranean with Gulf Stream dilution, some coastal Carolina

**References**


**Microscopy**

Göteborg University, Dept. of Marine Ecology, Marine Botany

www.marbot.gu.se/SSS/SSShome.htm

**Former Name(s)**

- Apedinella radians
- Meringosphaera radians
**Identification/Taxonomic Information**

**Other Flagellates**

**Chrysophytes**

- **Kingdom**: Protista
- **Phylum**: Ochrophyta
- **Class**: Chrysophyceae
- **Order**: Pedinellales
- **Family**: Pedinellaceae

**Harmful/Nuisance**

- **Toxic**: N
- **Nuisance**: N
- **Impact**: Ecologically beneficial

**Pathogen**

- **Human**: N
- **Other**: N

**Distribution**

Cosmopolitan estuarine / marine

**Source Region(s)**

Mediterranean with significant coastal Carolina water

**References**

Dictyocha fibula

Identification/Taxonomic Information

Other Flagellates
Silicoflagellates

Kingdom Protista
Phylum Ochrophyta
Class Dictyophyceae
Order Dictyoales
Family Dictyochaceae

Harmful/Nuisance Pathogen
Toxic N Human N
Nuisance N Other N
Impact Ecologically beneficial

Microscopy

Smithsonian Environmental Research Center - Phytoplankon Ecology Laboratory
serc5.si.edu/algae/

Former Name(s)

References


Distribution

Cosmopolitan marine

Source Region(s)

Puget Sound water, partial Panama
Open Atlantic 400 nautical miles west of Gibraltar
Mediterranean with significant coastal Carolina water
Exchange occurred at Fuel Pier in Guam
Atlantic 450 nautical miles west of Canaries
Atlantic 350 nautical miles south of Bermuda
400 nautical miles west of Africa with northwest track to open Atlantic
**Dictyocha speculum**

Ehrenberg

**Identification/Taxonomic Information**

**Other Flagellates**

**Silicoflagellates**

<table>
<thead>
<tr>
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<th>Protista</th>
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<tbody>
<tr>
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</tr>
<tr>
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**Harmful/Nuisance**

<table>
<thead>
<tr>
<th>Toxic</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuisance</td>
<td>Y</td>
</tr>
<tr>
<td>Impact</td>
<td>Can cause fish death (gill irritation, mucus overproduction)</td>
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**Pathogen**

<table>
<thead>
<tr>
<th>Human</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Other</td>
<td>Y</td>
</tr>
</tbody>
</table>

**Microscopy**

NCSU CAAE

www.ncsu.edu/wq/aboutCAAE/

**Former Name(s)**

Distephanus speculum

**Distribution**

Cosmopolitan marine, polar to temperate

**Source Region(s)**

Puget Sound water, partial Panama

Open Atlantic

**References**


Heterosigma akashiwo

(Hada) Hada

Identification/Taxonomic Information

Other Flagellates

Raphidophytes

Kingdom Protista
Phylum Ochrophyta
Class Raphidophyceae
Order Chattonellales
Family Chattonellaceae

Harmful/Nuisance Pathogen
Toxic Y Human Y
Nuisance Y Other Y
Impact Produces toxins that can cause disease and death in fish and disease in humans; blooms in response to nutrient pollution

Microscopy

NCSU CAAE

www.ncsu.edu/wq/aboutCAAE/

Former Name(s)

Entomosigma akashiwo

Distribution

Cosmopolitan marine / estuarine

Source Region(s)

Exchange occurred at Fuel Pier in Guam

References


Appendix 3

Database Structure, Contents, and Documentation
The database is built using Microsoft Access 2000 (Access). This version can be converted to any of the more recent releases at SERDP’s convenience. [Please note – Access 2000 is not backward-compatible.] Access is ODBC compliant and easily connected to any other ODBC compliant database or Microsoft Excel (Excel) for further analysis.

The database consists of a series of flat tables with minimal relationships due to the ad hoc nature of the majority of work being done with the database, and the framework provides flexibility in accommodating various inquiries. The database contains raw data, validation queries and resulting (100+) tables, numerous queries and resulting tables used during the receipt and analysis phases of this project, several macros, many reports, and four code modules of Visual Basic for Applications (VBA).

The following pages provide examples of two tables, one macro, and one code module of VBA. All of this documentation is available through Access standard features. We have included an electronic copy of the database in its present form, accompanying this Draft Report. At SERDP’s direction, if desirable we can reduce the database to only the raw and finished tables and the functions that constitute the basic system. Depending upon counsel as to what final delivery form would be of most utility to SERDP, we can provide:

- The database stripped of all ad hoc components;
- The database as an executable – this will allow connection to any other ODBC compliant database but little, if any, work directly within the database other then those functions already defined within the database; and/or
- All raw and finished tables extracted as Excel spreadsheets for entry into any analysis tool of SERDP’s choice.

The Phytoplankton Atlas (Appendix 2) is supported in a separate database, which is linked to the finished table portion of the primary database. The primary database is also linked to a database constructed from the data downloads available from ITIS (the Integrated Taxonomic Information System (ITIS), a web-based multinational taxonomic database; see Section 3.b.3 of this Draft Report, p.12). These tables were used as a beginning framework for phytoplankton taxonomy. The database also is linked to additional auxiliary databases that were used to support extraction of SAS and other analytical efforts.
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## Columns

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## Table Indices

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Fields:
- Ascending
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**View:**
- **Datasheet**

**Data Mode:**
- **Edit**

**TransferSpread sheet**
- **Transfer Type:** **Export**

**Spreadsheet Type:** **Microsoft Excel 8-9**
- **Table Name:** **50 - Tracking Extract**
- **File Name:** **Tracking.xls**
- **Has Field Names:** **Yes**
- **Range:**

**StopMacro**
Code

1  Attribute VB_Name = "Utilities"
2  Option Compare Database
3
4  Dim dbBallast As Database
5
6  Dim TblWork As DAO.Recordset
7
8  Function Spliter1(ina As String) As String
9
10  Dim Holder As Integer
11
12  Holder = InStr(ina, " ")
13  Spliter1 = Mid(ina, 3, (Holder - 3))
14  End Function
15
16  Function Spliter2(ina As String) As Long
17
18  Dim Holder As Integer
19  Dim I As Integer
20  Dim k As String
21
22  I = Len(ina)
23  Holder = InStr(ina, " ")
24  k = Mid(ina, (Holder + 1), (I - Holder))
25  Spliter2 = CLng(k)
26  End Function
27
28  Function Rounder(Dec As Double) As Double
29
30  Dim Holder As Long
31
32  Holder = Dec * 100000
33  Rounder = Holder / 100000
34  End Function
35
36  Function Split_Type(ina As String) As String
37
38  Dim Holder As Integer
39  Dim J As Integer
40
41  If InStr(ina, "T-") Then
42  J = 3
43  Else
44  J = 1
45  End If
46
47  Holder = InStr(J, ina, " ")
Module: Utilities

Function Split_Type(ina As String) As String

    Dim Holder As Integer
    Dim I As Integer
    Dim k As String
    Dim Holder_1 As Integer

    I = Len(ina)
    Holder = InStr(ina, " ")
    k = Mid(ina, (Holder + 1), (I - Holder))
    Split_Type = Mid(k, Holder_1, (I - Holder_1))

Function Split_Number(ina As String) As Long

    Dim Holder As Integer
    Dim l As Integer
    Dim k As String

    l = Len(ina)
    Holder = InStr(ina, " ")
    k = Mid(ina, (Holder + 1), (l - Holder))
    Split_Number = CLng(k)

Public Function Calc_Dec_Coord(Deg As Double, Min As Double, NSEW As String) As Double

    Dim Hold As Double

    Hold = Deg + (Min / 60)
    If NSEW = "S" Or NSEW = "W" Then
        Hold = Hold * -1
    End If
    Hold = Rounder(Hold)
    Calc_Dec_Coord = Hold

Function Fixer(Bar As String) As String

    Dim Holder As String
    Dim num_part As Long

    Holder = Mid(Bar, 2)
    num_part = CLng(Holder)
    num_part = num_part - 1
    Fixer = "B" & Format(num_part, "######")

Function Load_Receipt_Select(ev As Long)

    Set TblWork = CurrentDb().OpenRecordset("02 - Receipt Relabel Select")
    TblWork.AddNew
    TblWork!Event Number = ev
    TblWork.Update
    TblWork.Close

End Function
Function Load_Culture_Select(ev As Long)
Set TblWork = CurrentDb().OpenRecordset("02 - Culture Relabel Select")
TblWork.AddNew
TblWork![Event Number] = ev
TblWork.Update
TblWork.Close
End Function

Function Load_Kit_Select(Kit As Long)
Set TblWork = CurrentDb().OpenRecordset("01 - Kit Relabel Select")
TblWork.AddNew
TblWork![Kit Number] = Kit
TblWork.Update
TblWork.Close
End Function

Function Strip_Comma(ina As String) As String
If Not IsNull(ina) Then
If Mid(ina, 1, 1) = "," Then
Strip_Comma = Mid(ina, 2)
Else
Strip_Comma = ina
End If
End If
End Function