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Global redistribution of bacterioplankton and virioplankton communities

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Introduction

Nonindigenous aquatic species have the potential to effect great ecological and economic change (e.g., Carlton et al. 1990; Mills et al. 1993), and microbial constituents can represent risks to human health (e.g., McCarthy and Khambaty 1994; Hallegraeff 1998). A primary vector for global transport of nonindigenous aquatic species is ballast water discharged from ships (Carlton 1985; Ruiz et al. 1997), and the United States annually receives more than 79 million metric tons of ballast water of foreign origin (Carlton et al. 1995). When ships take on water in one port and discharge it at another, the ballast water can contain a diverse mix of plankton, nekton, and benthos (e.g., Carlton and Geller 1993; Lavoie et al. 1999).

Research on ballast-water introductions has focused largely on metazoans, yet microorganisms are indisputably the most abundant of aquatic organisms. For example, naturally occurring bacteria and viruses in coastal waters are found in concentrations on the order of 10^6–10^{11} l^{-1} (e.g., Ducklow and Shiah 1993; Wommack and Colwell 2000). Given such high densities and that many microorganisms have high reproductive rates, broad tolerances to physical conditions, and the capability to form resting stages, microorganisms likely are frequent invaders of coastal ecosystems (Ruiz et al. 2000b).

In an example of probable microbial transport via ballast water, toxigenic *Vibrio cholerae* O1, the bacterium that causes human cholera, was found in oysters and intestinal contents of fish in Mobile Bay, Alabama in 1991 (DePaola et al. 1992). The strain of *V cholerae* was indistinguishable from the strain responsible for a cholera epidemic in Latin America at that time. When the ballast waters of ships leaving Latin American countries and arriving in Mobile Bay were later tested for the cholera bacterium, they contained the epidemic-causing strain (McCarthy et al. 1992). This scenario suggests that ballast water was a vector for introduction of the epidemic-causing strain to the Gulf Coast of the United States. The United States Coast Guard subsequently published the International Maritime Organization's Ballast Water Control Guidelines in the US Federal Register and recommended that mariners take steps to reduce the spread of pathogens in ballast water (Federal Register 1991).

The only method now in widespread use to control the spread of nonindigenous aquatic species by ships is open-ocean exchange of ballast water. In this procedure, a ship that has taken on ballast water in a coastal port discharges that water in the open ocean and replaces it with oceanic water. In turn, the oceanic water is released at the next port of call. By reducing the density of coastal organisms and replacing them with oceanic species, the invasion success rate of the discharged organisms is theoretically lowered. Hydrographic differences between the oceanic water and that at the receiving port, where the exchanged water is discharged, is in theory great enough to kill oceanic species (Smith et al. 1999). There are several problems with this exchange procedure; foremost is the danger posed to vessel and crew in rough seas or if the procedure is performed

improperly. Furthermore, many ships undertake only partial exchange (J.T. Carlton, personal communication); even when exchange is undertaken, it is not always completely effective (Zhang and Dickman 1999), and the sediment in the bottom of ships' tanks and holds may not be completely removed during the exchange (Williams et al. 1988). Finally, with respect to microorganisms and especially their resting stages, changes in salinity may affect them little, if at all.

Studies of microorganisms in ballast water have been limited to date and have focused mainly on *V. cholerae* (e.g., McCarthy and Khambaty 1994), dinoflagellates (e.g., Hallegraeff 1993, 1998), and other protists (e.g., Galil and Hülsmann 1997; Pierce et al. 1997). Even less well characterized are the more abundant, naturally occurring components of the microbial community (i.e., bacterioplankton and virioplankton) and the effects of open-ocean exchange on these microbial constituents (Table 1). In this paper, we present the abundance of bacteria, viruses, and phytoplankton pigments, as well as bacterial activity, in ballast water collected at the endpoint of trans-oceanic voyages.

Materials and methods

Sample collection

Twenty-five bulk carriers originating in foreign ports and arriving in Chesapeake Bay (Norfolk or Newport News, Virginia and Baltimore, Maryland) were boarded from January 1996 to February 2000 and sampled for one or more microbial characteristics. After boarding each vessel, the Chief Mate or another officer was interviewed to determine the source of water in all ballast-water tanks or ballasted cargo holds, the volume of ballast water in each, and whether or not coastal ballast water had been exchanged in the open ocean (exchanged or unexchanged) or if the tanks or holds had been empty upon departure from the last port of call then filled in the open ocean (oceanic). In one (or, in a single instance, two) ballast-water tank per ship, two independent, replicate water samples were collected from the surface. Often, two independent, replicate samples were collected from the bottom of the tank as well. In some cases, water at the surface was collected by hand into a sterile bottle. In other cases, a Niskin bottle was used to collect water samples from the surface and bottom of the tank. In two instances, water samples contained suspended sediments. In one case, sediment at the bottom of a drained hold was collected by hand, centrifuged, and the expressed pore water was analyzed. Immediately following collection, temperature and salinity were measured in subsamples. Samples were transferred to sterile bottles, protected from light, and transported to the laboratory.

Bacteria enumeration

Samples were fixed in formaldehyde solution (final concentration 1% or 2.7%) and stored in the dark at 4 °C for up to 7 days until they were filtered onto 0.2 μm black polycarbonate filters (Osmonics Inc.; Livermore, California) and stained with the nucleic acid stain DAPI (4′6-diamidino-2-phenylindole) (final concentration of 1 $\mu g\,ml^{-1}$) (Sigma Chemical Company; St. Louis, Missouri) (Porter and Feig 1980). Filters were stored in the dark at −85 °C for up to 60 days until the bacteria were counted using an Olympus BX50 System Microscope with a BX-FLA epifluorescence attachment.

Thymidine incorporation

Bacterial growth was approximated by the incorporation of [methyl-^{3}H]-thymidine (specific activity 79 $Ci\,mmol^{-1}$, Amersham Pharmacia Biotech Inc.; Piscataway, New Jersey) into bacterial DNA (Fuhrman and Azam 1982). Ballast-water samples of 20–40 ml were amended with ^{3}H-thymidine (final concentration 10 nM), then incubated in containers filled with ballast water collected from the corresponding depths. Adding formaldehyde solution (final concentration 0.5%) to the samples terminated the incubations. Control samples killed with formaldehyde solution were used to determine abiotic and other effects. Samples were kept at 4 °C until they were filtered onto 0.2 μm cellulose-nitrate membrane filters (Millipore Corporation; Bedford, Massachusetts). The filters were washed three times with 5% ice-cold trichloroacetic acid and three times with 80% ice-cold ethanol. The filters were dissolved in ethyl acetate, scintillation cocktail (ScintiVerse, Fisher Scientific Inc.; Pittsburgh, Pennsylvania) was added, and radioactivity was determined with a TRI-CARB 2300TR scintillation counter (Packard Instrument Company, Inc.; Meriden Connecticut).

Table 1. Reports of microorganisms in ships' ballast waters.

Group	Reference
Viruses[a]	Ruiz et al. (2000b); this study
Bacteria	Galil and Hülsmann (1997); Ruiz et al. (2000b); this study
Cyanobacteria	Gollasch et al. (1998); Smith et al. (1999); Gollasch et al. (2000); McCarthy and Crowder (2000)
Purple Bacteria	
Gamma Group	
Vibrio cholerae	McCarthy et al. (1992); McCarthy and Khambaty (1994); Ruiz et al. (2000b)
Eukarya (protists only)[b,c]	
Protozoa	
Ciliophora	Carlton (1985); Carlton and Geller (1993); Kelly (1993); Subba Rao et al. (1994); Yoshida et al. (1996); Chu et al. (1997); Galil and Hülsmann (1997); Pierce et al. (1997); Macdonald (1998); Smith et al. (1999); Lavoie et al. (1999); Rigby et al. (1999)
Dinozoa	Hallegraeff and Bolch (1991, 1992); Carlton and Geller (1993); Kelly (1993); Subba Rao et al. (1994); Gosselin et al. (1995); Yoshida et al. (1996); Chu et al. (1997); Galil and Hülsmann (1997); Gollasch et al. (1998); Macdonald (1998); Lavoie et al. (1999); Rigby et al. (1999); Smith et al. (1999); Zhang and Dickman (1999); Gollasch et al. (2000); Hamer et al. (2000); McCarthy and Crowder (2000)
Euglenozoa	Kelly (1993); Subba Rao et al. (1994); Galil and Hülsmann (1997)
Foraminifera	Carlton and Geller (1993); Chu et al. (1997); Galil and Hülsmann (1997); Gollasch et al. (1998); Macdonald (1998); Lavoie et al. (1999); Smith et al. (1999); McGann et al. (2000)
Heliozoa	Galil and Hülsmann (1997)
Radiozoa	Carlton and Geller (1993); Chu et al. (1997); Smith et al. (1999)
Rhizopoda	Galil and Hülsmann (1997)
Chromista	
Bicosoecae	Galil and Hülsmann (1997)
Chrysophyta	Chu et al. (1997); Gollasch et al. (2000)
Cryptomonada	Galil and Hülsmann (1997)
Diatomae	Hallegraeff and Bolch (1992); Carlton and Geller (1993); Kelly (1993); Subba Rao et al. (1994); Yoshida et al. (1996); Gollasch et al. (1998); Macdonald (1998); Lavoie et al. (1999); Rigby et al. (1999); Smith et al. (1999); Zhang and Dickman (1999); Gollasch et al. (2000); McCarthy and Crowder (2000)
Haptomonada	Galil and Hülsmann (1997)
Labyrinthomorpha	Galil and Hülsmann (1997)
Raphidophyta	Yoshida et al. (1996)
Plantae	
Chlorophyta	Carlton and Geller (1993); Gollasch et al. (1998); Macdonald (1998); McCarthy and Crowder (2000)
Prasinophyta	Kelly (1993)
Rhodophyta	Carlton and Geller (1993); Smith et al. (1999)
Animalia	
Choanozoa	Galil and Hülsmann (1997)

[a]Category includes virus-like particles.
[b]Following classification scheme of Corliss (1999).
[c]Several genera listed by Galil and Hülsmann (1997) were not clearly assignable to the protistan phyla above.

Virus-like particle enumeration

Virus-like particles (VLPs) were counted using the method of Hennes and Suttle (1995). Upon return to the laboratory, unfixed samples were diluted with 0.1 μm filtered distilled, deionized water. Next, diluted samples were filtered onto 0.02 μm-pore size Anodisc filters (Whatman International Ltd.; Maidstone, UK) and stained in the dark for two days at room temperature with a cyanide-based working solution of the nucleic acid stain Yo-Pro™-1 iodide (491/509) (Quinolinium,4-[(3-methyl-2(3H)-benzoxazolylidene)methyl]-1-[3-(trimethylammonio)-propyl]-,diiodide) (Molecular Probes, Inc.; Eugene, Oregon). Filters were rinsed twice with 0.02 μm filtered distilled, deionized water, placed on microscope slides, and stored in the dark at −85 °C until the VLPs were counted. Filters were randomly chosen (in groups of two), thawed in the dark at room temperature for about 5 min, and VLPs were counted using epifluorescent microscopy (see above). For each set of filters prepared, two control filters were prepared using

only 0.02 μm filtered distilled, deionized water and their average VLP count was subtracted from values determined in field samples.

Chlorophyll a and phaeopigment determination

Chlorophyll *a* (chl *a*) samples were collected by filtering 200–500 ml of seawater onto 47 mm-diameter glass fiber filters (Whatman International Ltd.; Maidstone, UK) at a vacuum pressure of 100 mm Hg. Filters were wrapped in foil and stored at −85 °C until the chl *a* on the filters was extracted in acetone and measured fluorometrically (Parsons et al. 1992). Phaeopigment concentration (phaeophytin and phaeophorbide) was quantified by acidifying the chl *a* samples with 5% hydrochloric acid, and again determining the sample's fluorescence.

Results

Bacteria density

Bacteria concentrations ranged 263-fold, from 0.057 to 15 × 10^9 cells l^{-1} (grand mean = 1.8; SD = 3.2; n = 18). The large variability was due in part to very high bacteria counts in samples from a ship arriving from the Netherlands in July and one from Wales in August; both of these ships had exchanged ballast water in the open ocean. All other samples had bacteria values less than 2 × 10^9 cells l^{-1}.

There was no consistent pattern when concentrations of bacteria from surface and bottom samples were compared (n = 5 surface-bottom pairs). In ballast water from any one country, the concentration of bacteria was greater in months of warmer water, in four of five cases in which pairwise (or more extensive) comparisons could be made among samples of the same water type (e.g., unexchanged or exchanged) arriving in different seasons.

Thymidine incorporation

Mean thymidine incorporation varied by a factor of 200, from 0.065 to 13 pmol l^{-1} h^{-1} (grand mean = 1.9; SD = 3.3; n = 16). The highest value occurred in the oceanic sample containing sediments. Of the nine tanks in which both surface and bottom samples were collected, the surface samples yielded higher thymidine incorporation in six cases.

Ships arriving from the Netherlands provided data on temporal variation of unexchanged ballast water, indicating that thymidine incorporation was greater in samples from ships arriving in May rather than December or March. Ships arriving from Wales with exchanged ballast water did not support the pattern of higher values recorded in samples collected during warmer months; thymidine values were higher in samples from the ship arriving in January than from the ship arriving in April.

Virus-like particle abundance

Densities of VLPs varied 183-fold, from 0.35 to 64 × 10^9 l^{-1} (grand mean = 14.1; SD = 16.3; n = 12). In three of four tanks (two unexchanged, two exchanged) for which the comparison could be made, bottom samples had higher VLP values than surface samples. Ships with exchanged water and arriving from Wales and the Netherlands during winter or summer had greater VLP concentrations in samples collected during the summer. In contrast, ships with unexchanged water arriving from Italy and ships with oceanic water had higher VLP counts in samples collected in winter. Those were a sample of sediment pore water and a sample containing sediments, respectively.

Phytoplankton pigments

Chlorophyll *a* concentrations were very low, ranging 10-fold from 0.003–0.030 μg l^{-1} (grand mean = 0.016; SD = 0.013; n = 7). Phaeopigment values, although also low, were up to 100 times greater than the chl *a* values and ranged from 0.01–0.30 μg l^{-1} (grand mean = 0.10; SD = 0.11; n = 7). When both surface and bottom samples were collected in a tank or hold, bottom chl *a* values were higher than surface values in three of four comparisons, and the same was true of phaeopigment values.

Hydrographic characteristics

Temperature in ballast-water tanks and holds ranged from 8–26 °C (grand mean = 18.4; SD = 5.5; n = 26), and salinity ranged from 1 to 41 ppt (grand mean = 32.3; SD = 10.6; n = 26). When all data for each microbial metric (bacteria density, thymidine incorporation, VLP abundance, chl *a* concentration, and phaeopigment concentration) were correlated with

temperature, salinity, and water age, no significant relationships emerged from any of the 15 comparisons ($p > 0.05$), with the exception of a negative correlation between bacteria abundance and salinity ($p = 0.011$, $r = -0.541$; Spearman's coefficient of rank correlation, SPSS Inc.; Chicago, Illinois).

Discussion

These data serve to characterize broadly the microorganism communities delivered in ballast water, underscoring the magnitude of this transfer and the high level of variation among vessels. Both attributes have important consequences for invasion ecology and management. First, the concentrations of bacteria and viruses found in ballast water indicate that invasions by microorganisms may be relatively common, compared to invertebrates, but remain virtually unexplored (e.g., Ruiz et al. 2000a,b). Second, the source(s) of variation in microorganisms among vessels are poorly resolved at the present time, resulting from the interactive effects of ballast-water history (e.g., source region, vessel history, ballast-water management, and volume of exchange), season, voyage duration, and voyage conditions. Therefore, we are reluctant to use the present data to address questions of exchange efficacy on the microbial community.

This study is the second appraisal of microorganisms arriving to Chesapeake Bay in ballast water of ships from foreign ports, and the mean concentrations of bacteria and VLPs were two-fold greater than in the previous report (Ruiz et al. 2000b). It is important to recognize that although there is overlap in the data used in both studies (7 ships common to bacteria abundances in both studies, $n = 11$ for Ruiz et al., $n = 18$ in this study; 6 ships common to VLP abundances in both studies, $n = 7$ in Ruiz et al., $n = 12$ in this study), such a comparison does not control for ballast-water history, exchange status, season, or other factors we know to affect community characteristics. Instead, these two studies provide initial data on this type of microbial transfer. Increased sampling effort and controlled experiments are now necessary to test the effects of source, season, and management (ballast-water exchange) on the arriving community.

Although numbers of bacteria and virus-like particles in the present study were sometimes high, as much as $15 \times 10^9\,l^{-1}$ and $64 \times 10^9\,l^{-1}$, respectively, their mean abundance generally was lower than values occurring normally in Chesapeake Bay, $1.7–12 \times 10^9$ bacteria l^{-1} (Choi 2000) and $3–140 \times 10^9$ VLPs l^{-1} (Wommack et al. 1992; Drake et al. 1998). In fact, these means lie within the range of densities of bacteria (Carlson et al. 1996) and viruses (Proctor 1997) recorded in open-ocean surface waters. Furthermore, thymidine incorporation was no greater and chl *a* concentrations were much less than values observed in the lower Chesapeake Bay, 0.23–95 pmol thymidine $l^{-1}\,h^{-1}$ and 0.92–34 μg chl *a* l^{-1} (Choi 2000). In summary, the ballast water on the ships we sampled was not enriched with bacteria and viruses, was no higher in bacterial productivity, and was impoverished in phytoplankton abundance relative to the Chesapeake Bay water into which it was discharged. Nonetheless, if one extrapolates from the present study and that of Ruiz et al. (2000b), the number of foreign microorganisms delivered annually to Chesapeake Bay via ballast-water discharge is profound.

The low values of chl *a* in ballast-water samples are not surprising, given tanks' and holds' characteristic absence of light (or its near absence, in the case of vented holds). We interpret the high ratio of phaeopigments to chl *a* as indicating the death of much of the phytoplankton, leaving more degradation products than living cells. The higher values of chl *a* and phaeopigments in samples collected in bottom samples may reflect viable phytoplankton cells and phytodetritus resuspended during sampling or by the ship's movement.

Although our data provide some of the first end-of-voyage values for bacterial abundance, thymidine incorporation, and abundance of virus-like particles (see also Ruiz et al. 2000b), the range of values for each measure was highly variable (263-, 200- and 183-fold, respectively). In cases where comparisons could be made, there was evidence supporting a seasonal effect, i.e., bacterial abundance, thymidine incorporation, and VLP concentrations were usually greater in warm seasons than in cold ones (when comparisons including samples containing sediments or samples of sediment pore water were excluded). However, further data are required to adequately control for the factors of port (source water) and season.

Given the diversity in bacterio- and virioplankton communities (DeLong et al. 1993; Wommack et al. 1999), it is likely that microorganisms in exchanged ballast water differ from those in unexchanged ballast water. The present research begins to characterize

microbial concentrations in ballast water, but it does not estimate species richness or composition. This latter information, however, may greatly influence the assessment of ballast-water exchange efficiency, as well as the risk of invasion. Furthermore, potential for invasion of coastal waters by nonindigenous species (in exchanged or unexchanged water) cannot fully be predicted without knowing more about the identity, abundance, frequency of occurrence, and physiological state of the microbial community. Such an understanding is clearly the next step in the study of invasion biology of microorganisms, and it is necessary for making well-informed management decisions.

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