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SHIPPING STUDY II
Biological Invasions by Nonindigenous Species in United States Waters: Quantifying the Role of Ballast Water and Sediments
Parts I and II

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16. Abstract

This study examines the roles of ballast water and ballast sediments from foreign ports as methods for the transport and release of nonindigenous species in the United States coastal and aquatic ecosystems. It specifically assesses the types of vessels arriving to ports in Chesapeake Bay and the amount of ballast water discharged. It compares the physical and chemical characterization of arriving ballast water and site discharge, as well as the biodiversity of the ballast water and sediments arriving in Chesapeake Bay aboard vessels from world ports. The Chesapeake Bay was chosen as the site to be investigated since prior studies indicated that it was receiving more ballast waters from foreign ports than any other harbor region on the Atlantic coast of the United States.

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METRIC CONVERSION FACTORS

Approximate Conversions to Metric Measures

Symbol	When You Know	Multiply By	To Find	Symbol
		LENGTH		
in	inches	*2.5	centimeters	cm
ft	feet	30	centimeters	cm
yd	yards	0.9	meters	m
mi	miles	1.6	kilometers	km
		AREA		
in^2	square inches	6.5	square centimeters	cm^2
ft^2	square feet	0.09	square meters	m^2
yd^2	square yards	0.8	square meters	m^2
mi^2	square miles	2.6	square kilometers	km^2
	acres	0.4	hectares	ha
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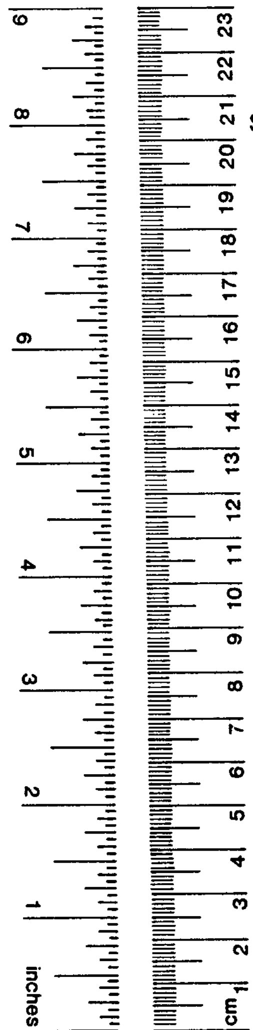
oz	ounces	28	grams	g
lb	pounds	0.45	kilograms	kg
	short tons (2000 lb)	0.9	tonnes	t
		VOLUME		
tsp	teaspoons	5	milliliters	ml
tbsp	tablespoons	15	milliliters	ml
fl oz	fluid ounces	30	milliliters	ml
c	cups	0.24	liters	l
pt	pints	0.47	liters	l
qt	quarts	0.95	liters	l
gal	gallons	3.8	liters	l
ft^3	cubic feet	0.03	cubic meters	m^3
yd^3	cubic yards	0.76	cubic meters	m^3
		TEMPERATURE (EXACT)		
°F	Fahrenheit temperature	5/9 (after subtracting 32)	Celsius temperature	°C

*1 in = 2.54 (exactly).



Approximate Conversions from Metric Measures

Symbol	When You Know	Multiply By	To Find	Symbol
		LENGTH		
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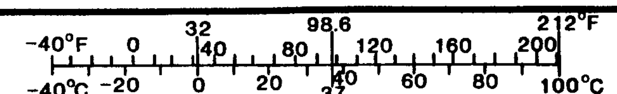
cm	centimeters	0.4	inches	in
m	meters	3.3	feet	ft
m	meters	1.1	yards	yd
km	kilometers	0.6	miles	mi
		AREA		
cm^2	square centimeters	0.16	square inches	in^2
m^2	square meters	1.2	square yards	yd^2
km^2	square kilometers	0.4	square miles	mi^2
ha	hectares (10,000 m^2)	2.5	acres	
		MASS (WEIGHT)		
g	grams	0.035	ounces	oz
kg	kilograms	2.2	pounds	lb
t	tonnes (1000 kg)	1.1	short tons	
		VOLUME		
ml	milliliters	0.03	fluid ounces	fl oz
l	liters	0.125	cups	c
l	liters	2.1	pints	pt
l	liters	1.06	quarts	qt
l	liters	0.26	gallons	gal
m^3	cubic meters	35	cubic feet	ft^3
m^3	cubic meters	1.3	cubic yards	yd^3
		TEMPERATURE (EXACT)		
°C	Celsius temperature	9/5 (then add 32)	Fahrenheit temperature	°F



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EXECUTIVE SUMMARY

The transport of ballast water in ships is now recognized as the primary vector for the movement of aquatic organisms within and between oceans. Ballast water is used to to maintain stability during a voyage and is actively pumped or gravitated into dedicated tanks and cargo holds at one port and released at other ports when receiving or delivering cargo. The volumes being transported and released are immense. In 1991 alone, large commercial vessels transported and released approximately 79 metric tons (the equivalent of 2.4 million gallons/hour) of ballast water from foreign ports into U.S. waters. Because water is usually ballasted in bays and estuaries rich in plankton and nekton, these ships carry a diverse assemblage of organisms in their cargo holds and ballast tanks. It has been estimated that on any one day more than 3,000 species of freshwater, brackish, and marine organisms may be in motion in ballast water 'conveyor belts' around the world. Ballast water continues in the 1990's as the major mechanism for the global transport of aquatic nuisance species.

These conditions mandate comprehensive studies of ballast water as a transport mechanism, in order to facilitate quarantine measures to reduce new exotic species invasions into coastal and aquatic habitats. This study attempts to expand our understanding of the ballast-mediated transport of exotic organisms into United States waters. It examines the roles of ballast water and ballast sediments from foreign ports as vectors for the transport and release of nonindigenous species into U.S. coastal and aquatic ecosystems. It provides a quantitative and qualitative assessment of the abundance and diversity of living organisms found in ballast water and sediment, as a function of tank type, ballast water source region, season of arrival, and physical and chemical characteristics of the ballast water. It examines the factors that affect the survivorship of organisms during transit, including the role of long-distance and/or long-term voyages. It examines the transport of ballast to Chesapeake Bay from global hot spots known to be sites of previous invasions or of blooms of nuisance species.

The results of the data demonstrate that the largest estuary in the United States, the Chesapeake Bay is being inoculated on a massive and frequent basis by a diverse assemblage of live organisms transported from around the world in ships' ballast water. Despite a conservative method of identification, this study found 278 species of protist, animals, and plant taxa in 70 vessels sampled for ballast water and 4 taxa in 5 vessels sampled for ballast sediments. All major taxonomic groups, developmental stages, and reproductive modes were represented. Organisms came from freshwater, brackish water, open-ocean, and coastal high-salinity habitats.

Densities of organisms in the ballast water were extraordinarily variable from ship to ship and reflect the stochastic nature of ballast transport. Analysis of a subset of the samples showed densities ranging from 0 to 18,000 organisms per cubic meter of ballast water (excluding viruses and bacteria). If one extrapolates to include the total amount of ballast water on board, a bulker deballasting in Chesapeake could release up to 1 billion organisms.

The study found the densities of organisms in ballast water to decrease as the age of the water increased. The transit time for most bulkers arriving to Chesapeake Bay was sufficiently short as to allow survival of planktonic organisms entrained in the ballast water.

One critical factor influencing the survival of the biological inoculum after deballasting is the compatibility of physical conditions between donor and recipient ports. Certainly, a port will be at greater risk of a ballast-mediated invasion if the temperature and salinity of its water are similar to those of the donor ports. The study suggests that the risk of ballast-mediated invasion in United States coastal waters remains extraordinarily high. At present, it is not clear whether repeated inoculations are needed over time or whether a single vessel, densely packed with organisms is sufficient to establish a population. If the latter is the case, then no port receiving water from an exogenous source is immune. There is circumstantial evidence that both mechanisms may be in operation. Many marine invasive species, apparently distributed by ballast water (or by hull fouling), are found in ports and harbors in much of the world, suggesting their constant (multiple inoculation) and extensive (massive inoculation) transport. Other invasions appear in only one site, suggesting rare and inconsistent transport.

The results of this study support prior recommendations to use mid-ocean saltwater exchange for freshwater ballast as a cost-effective method to reduce the risk of ballast-mediated invasions. At the least, vessels coming from known hot spots or with water similar in salinity to that of the recipient port should be requested to attempt open-ocean exchange. In the long-term, technological innovations are needed either to prevent intake of aquatic organisms into ballast tanks, or eliminate them once they are on board.

TECHNICAL SUMMARY

The transport of ballast water in ships is now recognized as the primary vector for the movement of aquatic organisms within and between oceans. Used to maintain stability and for other purposes during a voyage, ballast water is actively pumped or gravitated into tanks and cargo holds at one port and released (to varying degrees) at other ports when receiving cargo. The volumes of ballast water being transported and released are immense. In 1991 alone, large commercial vessels released approximately 79 million metric tons (the equivalent of 2.4 million gallons/hour) of ballast water from foreign ports into U.S. waters . It has been estimated that on any one day more than 3,000 species of freshwater, brackish, and marine organisms may be in motion in ballast water 'conveyor belts' around the world.

These considerations mandate comprehensive studies of ballast water as a transport mechanism, in order to facilitate quarantine measures to reduce new exotic species invasions into coastal and aquatic habitats. The work reported herein contributes to such studies.

(1)
The "Nonindigenous Aquatic Nuisance Prevention and Control Act of 1990" (Public Law 101-646, 16 USC 4701 et seq.) established the National Ballast Water Control Program. Under this Program, a Shipping Study was conducted that examined the degree to which shipping may be a pathway of transmission of aquatic nuisance species into United States waters and possible alternatives for controlling the introduction of such species (Carlton, Reid, and van Leeuwen 1995, or Shipping Study I). The Study assumed the working name of the National Biological Invasions Shipping Study (NABISS 1).

(2)
The present work was conducted under the aegis of the Shipping Study and is referred to as NABISS 2 (or Shipping Study II). SSII examines the roles of ballast water and ballast sediments from foreign ports as vectors for the transport and release of nonindigenous species into U.S. coastal and aquatic ecosystems. It specifically addresses the following topics:

- *Vessel Diversity and Ballast Water Discharges*
  Assessment of the types of vessels arriving to ports in Chesapeake Bay and the amounts of ballast water discharged, particularly by bulk cargo vessels (bulkers), with additional consideration of other vessel types (roll-on roll-off vessels).

- *Physical -Chemical Characterization of Arriving Ballast versus Port of Discharge*
  Comparison of selected water parameters in arriving ballast water and the site (port) of water discharge.

- *Biodiversity of Ballast Water and Sediments*
  Quantitative and qualitative assessment of the abundance and diversity of living organisms found in ballast water and ballast sediments arriving in Chesapeake Bay aboard vessels from world ports, as a function of tank type, ballast water source region, season of arrival, and physical and chemical characteristics of the ballast water.

- *Effectiveness of Ballast Exchange*
  Examination, relative to vessel availability, of the biota in ballast water that had or had not been exchanged with oceanic water.

- *Transport of Ballast from Global Hot Spots to Chesapeake Bay*
  Examination of European or Mediterranean ballast arriving in Chesapeake Bay from ports known to be sites of previous invasions or of blooms of nuisance species.

- *The Role of Long-Distance and/or Longer-Term Voyages in Ballast Biota Survival*
  Investigation of the survival of plankton in ballast water relative to water age.

(3)
Shipping Study 1 identified the Chesapeake Bay system as receiving the most ballast water from foreign ports of any harbor region on the Atlantic coast of the United States. For this reason, in August 1993 a ballast water field and laboratory unit was established at the Smithsonian Environmental Research Center (SERC) in Edgewater, Maryland. Edgewater is located 45 minutes from the Port of Baltimore and the anchorage of Annapolis, and 5 hours from the Port of Hampton Roads (Norfolk).

(4)
Between August 1993 and August 1994, foreign commercial vessels arriving in the Port of Baltimore, Maryland in upper Chesapeake Bay and in the Port of Hampton Roads, Virginia in lower Chesapeake Bay were surveyed and sampled for their ballast water. Vessel traffic statistics were derived from weekly vessel forecasts and monthly and annual summaries of the Baltimore Maritime Exchange (BME) for the Port of Baltimore, and the Hampton Roads Maritime Association (HRMA) for the Norfolk/Hampton Roads/Newport News complex (hereafter, Norfolk). Baltimore traffic data span the period from January to December 1994. The Norfolk load and discharge figures were summarized directly from the HRMA data for the 12 months between September 1993 and August 1994. Vessel load and discharge information for both ports was then directly comparable from January to August 1994. Typically, vessels coming to load cargo are in a ballast condition and discharge some or all of their water.

(5)
The vast majority of foreign ballast water arriving to Chesapeake Bay is transported by general cargo carriers, bulk carriers, and colliers (hereafter collectively referred to as

bulkers) coming to load grain and coal. As a consequence, most efforts were focused toward sampling these ships. Most bulkers were sampled in Baltimore due to proximity to the laboratory. Some car carriers ["Roll on - Roll off" vessels (RoRos)] were also sampled. Vessels were chosen at random with respect to last port of call. To simplify taxonomic identifications of ballast plankton assemblages, vessels were targeted that contained ballast water from single water sources (preferably from a foreign port of call or from a mid-ocean exchange). In practice, because cargo holds and ballast tanks were often topped up (pressed) during the voyage (e.g., to compensate for water lost by overflow), the ballast water sampled was of mixed origin. Ship's particulars (country of registry, gross registered tonnage (GRT), and ballast water capacity); last and next ports of call; the amount of ballast water on board; amount(s), date(s) and location(s) of ballasted, exchanged, and pressed water; and date and location of deballasting, were recorded.

(6)
Ballast water was sampled for planktonic organisms using replicated vertical tows of a plankton net . To sample smaller members of the dinoflagellate and ciliate community whole water samples were collected by bucket near the water's surface. To test for the presence of organisms (particularly dinoflagellate cysts) associated with ballast sediments, sediment samples were collected from cargo holds and ballast tanks of 10 vessels in Baltimore, of which 4 were in drydock and 6 were in port. Net tow samples were analyzed for the presence of live plankton. After completion of the live analysis, most organisms in a sample were preserved and stored together in 75% ethanol. Samples from a subset of vessels were analyzed after preservation to generate exact organism counts for statistical analyses. When sufficient numbers of a particular larval type were present in samples, individuals were removed and cultured to juvenile or adult stages when possible for the purpose of identification. In all tanks sampled, water temperature, salinity and dissolved oxygen content were also recorded.

(7)
Vessel statistics (e.g., GRT, ballast water capacity) were calculated separately for bulkers and RoRos. Because few bulkers were sampled from Norfolk, bulker data from Baltimore and Norfolk were combined for analyses. Geographic sources of the ballast water and last ports of call were classified by region according to the Food and Agriculture Organization (FAO) standardized division of the world's oceans. "Topped" cargo holds and ballast tanks are defined as those in which at least 50% of the original amount of water remained prior to new water being added; in most cases, less than 15% of the water in these holds and tanks had been added. "Exchanged" cargo holds and ballast tanks were defined as those in which greater than 50% of the original water was flushed before being replaced with ocean water. The maximum efficiency of ballast water exchange was calculated as a percentage of ballast water salinity to open ocean salinity [(salinity of ballast water/35 ppt) x 100].

(8)
The ports of Baltimore and Norfolk received similar numbers of commercial vessels

(2,101 and 2,304 respectively) between January and December 1994, but the frequency distribution of vessel types differed significantly between ports . Bulkers, which account for most of the ballast water released, comprised approximately 30% of the traffic in each port . In contrast, RoRo traffic was proportionately greater in Baltimore (25%) than in Norfolk (2%). Container ships constituted the majority of the traffic in both ports (39% for Baltimore, 52% for Norfolk).

(9)
Despite similar numbers of bulker arrivals , the amount of ballast water discharged in Baltimore was substantially less than that in Norfolk. Between January and August 1994, significantly fewer bulkers loaded cargo (i.e., discharged ballast water) in Baltimore (32%) than in Norfolk (75%). The majority of the bulkers arriving in Baltimore carried gypsum, wood pulp, ores, or sugar products and, thus, carried little ballast water . In contrast, most of the bulkers arriving in Norfolk were in ballast and awaiting coal.

(10)
Between August 1993 and August 1994 ballast water samples were obtained from 99 cargo holds and dedicated ballast tanks of 70 foreign commercial vessels. Eleven ballast tanks were sampled from 10 RoRos. Eighty-eight cargo holds and ballast tanks were sampled from 60 bulkers. Of these 88 tanks, 26 were cargo holds and 62 were ballast tanks (e.g., wing, wing bottom, double bottom, aft and fore peak tanks). All RoRos were sampled in Baltimore. Of the 10 RoRos sampled, repeated visits by 2 vessels accounted for 5 of the samples. Fifty-four bulkers were sampled in Baltimore and 6 bulkers in Norfolk. Of the 60 bulkers sampled, repeat traffic by 3 vessels accounted for 12 of the samples.

(11)
Eighty-eight percent of the bulkers reported deballasting some percentage of their ballast water in port. In contrast, none of the 10 RoRos deballasted in port. Nearly half (48%) of the bulkers sampled arrived with their ballast water unmodified (i.e., not topped up or exchanged during the voyage). Thirty-five percent of the bulkers pressed up their cargo holds or ballast tanks to replace water lost in transit to overflow ($\leq$ 15% of the existing ballast water amount added in all but one case). Approximately 1 in 6 bulkers attempted full exchange of one or more of their tanks . Of the 15 tanks exchanged on these bulkers, 4 (27%) were cargo holds and 11 (73%) were wing or wing bottom ballast tanks. The salinity of water in exchanged cargo holds and ballast tanks was often less than that of open-ocean water ($\approx$ 35 ppt). In only one-third of the holds or tanks was 95% or greater exchange achieved. The maximum mean percentage exchanged was $91.6 \pm 6.6\%$.

(12)
Bulkers arrived in Baltimore and Norfolk from 25 countries and 39 ports . The FAO region for last port of call for the majority of bulkers (n = 60) was the Northeast Atlantic (42%). A substantial percentage of bulkers, however, had a last port of call in the

Mediterranean/Black Sea (28%) or West Central Atlantic (20%) regions. For 56 of 60 vessels (93%), the FAO region for last port of call was the same as that of the ballast water source. For the remaining 7%, the last region of call and the source of the ballast water differed because cargo holds or ballast tanks were either filled or exchanged later in the voyage. In particular, a significant number of cargo holds and ballast tanks (15%) had water from the Northwest Atlantic.

(13)
Significant amounts of ballast water were discharged in Baltimore and Norfolk in 1993-94. The mean amount of ballast water on board bulkers discharging ballast water (n = 56) in Baltimore or Norfolk in this study was 31,457 MT. Multiplying this amount (which assumes 100% discharge) times the 187 bulkers that visited Baltimore to load cargo between January and December 1994, bulkers alone released an estimated 5,882,459 MT of ballast water into Baltimore harbor in 1994. This amount is equivalent to 1.52 billion gallons per year (4.17 million gallons per day). In contrast, the amount of ballast water discharged by bulkers (n = 484) in Norfolk over twelve months was over 2.5 times that received by Baltimore. An estimated 15,225,188 MT of ballast water from bulkers were released in Norfolk between September 1993 and August 1994 ($\approx$ 3.94 billion gallons per year or 10.8 million gallons per day). Thus, even for ports within a single estuary, the type of commodities being imported and exported greatly influences the amount of ballast water each received.

(14)
Physical characteristics (temperature, salinity, dissolved oxygen, age) of the ballast water were not constant among vessel types, regions of ballast water origin, or seasons. For example, ballast water from RoRos was significantly older (65 d), and less saline (21 ppt) than ballast water from bulkers (14 d and 28 ppt). In bulkers, ballast water in cargo holds and ballast tanks did not differ significantly in mean temperature, age, or dissolved oxygen. Mean salinity of the ballast water was greater in cargo holds (34 ppt) than in ballast tanks (26 ppt). When examined by region, however, this difference held true only for Mediterranean/Black Sea and reflected high salinities in the cargo hold of one repeat bulker. Dissolved oxygen was not limiting to life in either cargo holds (7.2 ± 1.4 mg/l) or ballast tanks (6.9 ± 2.2 mg/l).

(15)
Ballast water in the cargo hold was generally well-mixed; there was no evidence of vertical stratification of temperature, salinity, or dissolved oxygen (0 to 15 m depth). Water in all but one of the ballast tanks was also generally unstratified in the uppermost level of the ballast tank (3 to 5 m). On one occasion water in a ballast tank in a bulker that had sailed from St. Petersburg, Russia and that had attempted an exchange in the mid-Atlantic Ocean, ranged from 14 ppt at 7 m depth to 30 ppt at 13.5 m depth.

(16)
Mean ballast water temperature did not differ in bulkers arriving from the four most

common FAO regions for combined seasons. Mean ballast water salinity from the Mediterranean/Black Sea (35 ppt), however, was significantly higher than that of the Northeast Atlantic (26 ppt), Northwest Atlantic (26 ppt), or West Central Atlantic (22 ppt). The age of the ballast water arriving from these four regions also differed significantly. Mean age was greatest for ballast water arriving from the Mediterranean/Black Sea region (19 d). Ballast water arriving from the Northeast Atlantic region was of intermediate age (14 d). The shortest residence times for ballast water occurred in bulkers carrying water from the Northwest Atlantic (5 d) and the West Central Atlantic (7 d) regions.

(17)
Mean temperature of ballast water was significantly higher in summer (26°C) than in the other three seasons when averaged across all regions. In contrast, mean salinity and age of the ballast water showed no significant seasonal variation.

(18)
The temperature and salinity of ballast water discharged into Baltimore harbor varied greatly as a result of seasonal differences and the disparate source regions. Between August 1993 and August 1994, temperatures of ballast water discharged by bulkers into Baltimore harbor ranged between 11°C and 35°C. The majority of ballast water had temperatures between 16 and 20°C. The salinity range of ballast water discharged by bulkers into Baltimore harbor was extreme (0 to 45 ppt); however, peak discharge occurred at higher salinities (36 to 40 ppt). While temperature differences between ballast water and harbor water were slight (< 5°C) for over half of the ballast water released into Baltimore harbor, the remaining ballast water differed from the port receiving waters by 6°C to 20°C. Ballast and port water temperatures matched most consistently when ships arrived with ballast water from the Northwest Atlantic or in summer. Salinity differences between ballast water and harbor water were great. Over 80% of the ballast water discharged was at least 21 ppt higher in salinity than that of Baltimore harbor. Salinity differences between ballast water and harbor water were high regardless of source region of the ballast water or the season in which bulkers arrived. Overall, there were few instances where both the salinity and temperature of the discharged ballast water matched conditions in Baltimore harbor. Of 47 deballasting cargo holds and ballast tanks sampled in Baltimore harbor between August 1993 and August 1994, only 5 (10.6%) had ballast water temperatures and salinities differing by ≤ 5°C or ≤ 5 ppt respectively from that of the harbor water.

(19)
Ninety-one percent of the 70 vessels sampled in this survey contained live aquatic organisms. Living organisms were collected in all seasons. These organisms included representatives from 15 animal and 3 protist phyla, 2 plant divisions and cyanobacteria. A minimum of 282 distinctly different taxa were identified. This number is an extremely conservative estimate of the diversity reaching Chesapeake Bay. All major taxonomic groups, developmental stages, and reproductive modes were represented. Organisms came from freshwater, brackish water, open-ocean, and

coastal high-salinity habitats. The total diversity of organisms being brought to Chesapeake Bay is similar in magnitude to that reported in ballast water studies from 3 other regions: 367 species have been reported as arriving in Coos Bay, Oregon in 159 vessels from Japan. In 31 vessels sampled in Australia 67 taxa of zooplankton and fish were found. A minimum of 213 protist, animal, and plant taxa were found in 86 vessels arriving in the Great Lakes. The level of taxonomic resolution and emphasis for specific groups, however, varies significantly among these studies. Thus, subtracting ciliates, flatworms, diatoms, and dinoflagellates, as well as fish and unidentified small protistan or algal taxa, more likely captures uniform biases across all of the studies. These adjusted numbers and the data from Oregon, the Great Lakes, and the present studies, suggest that samples of > 70 ships should yield a minimum biota of > 100 species.

(20)
In the majority of samples, organisms were actively swimming and appeared healthy following their voyage. Evidence of their vigor was demonstrated indirectly by the success in rearing many of these organisms to later stages. The maximum age of ballast water containing a living organism was 41 d. Of the 6 vessels in which no life was found, 4 were RoRos whose ballast water was 132 to 730 d old; the other 2 were bulkers (one vessel had undergone mid-ocean exchange; the other was sampled in winter). Both bulkers had water that was 15 d old. Significantly, the absence of life in one ballast tank did not preclude its existence in other tanks on the same vessel. On 4 ships in which no living organisms were recorded from one tank, live organisms were detected in other tanks.

(21)
The biota in bulker cargo holds was diverse taxonomically and included protozoans (dinoflagellates, sarcodines, ciliates), plants (diatoms), invertebrates (crustaceans, annelids, molluscs, platyhelminthes, cnidarians, and chaetognaths), and vertebrates (fish). Invertebrate taxa included both meroplanktonic and holoplanktonic representatives. Crustaceans were found in all cargo holds and were abundant (> 100 organisms per net tow) in one-third of the cargo holds sampled. Other taxa prevalent in cargo holds included dinoflagellates (79% of holds), annelids (75%), diatoms (62%), molluscs (58%), and platyhelminthes (50%). Of the 10 most prevalent taxa, six (crustaceans, dinoflagellates, annelids, diatoms, molluscs, sarcodines) were abundant in at least some vessels. Reported here is the first known occurrence of live ctenophores (comb jellyfish) in ballast water. This finding lends support to the hypothesized role of ballast water in transporting the western Atlantic comb jelly *Mnemiopsis leidyi* to the Black Sea in the early 1980s.

(22)
More prevalent taxonomic groups were typically dominated (both in percent occurrence and abundance) by one or two major subclasses. For example, the occurrence of crustaceans in all cargo holds was due to the omnipresence of copepods. In terms of abundance, copepods were common or abundant in 96% of

the cargo holds. Barnacle larvae were found in 75% of the cargo holds, but were common in only 21% of the cargo holds. Representatives from at least 10 polychaete worm families were identified from cargo holds, but larvae of the family Spionidae were largely responsible for the prevalence of worms. Similarly, mollusks were represented chiefly by bivalve larvae.

(23)
Ninety-one percent of bulker ballast tanks sampled by quantitative plankton net tows contained living organisms. As with cargo hold samples, the biota was taxonomically diverse. Crustaceans (primarily copepods) were most prevalent, occurring in 70% of bulker ballast tanks. Seven of 10 most prevalent taxa in ballast tanks were also found in cargo holds, although the rank order of the taxa differed .

(24)
Qualitative samples yielded valuable information for non-planktonic and benthic taxa. For example, no representatives of the 6 fish families were captured in quantitative plankton net tows. Instead, fish were dip netted while they were swimming near the surface of the water, or they were captured after the cargo hold or ballast tank had been emptied. Using the latter technique, a number of living benthic organisms were collected, including shrimp (*Crangon crangon*), juvenile brachyuran crabs, nematodes, and polychaetes.

(25)
The benthic sediments of both ballasted cargo holds and ballast tanks contained living organisms. In the case of ballasted cargo holds, these sediments act as a temporary sink or habitat for a number of benthic organisms and/or their life history stages, such as dinoflagellate cysts, crabs, shrimp, and bottom-dwelling fish. These sediment "communities", however, are only as old as the ballast leg, because the cargo hold is cleaned out whenever cargo is to be loaded. Because ballast tanks often are not cleaned out for extended periods (e.g., months to years); the potential to build a stable benthic community is much greater than in a cargo hold. Generally, however, there was little access to these longer-term or semi-permanent ballast tank sediments. The potential importance of these communities is that they may act as a source of ciliates, dinoflagellates, and invertebrate larvae. Living organisms were encountered in the still-wet sediments of several ballast tanks that were accessed in dry dock. These included copepods, nematodes, foraminiferans, filamentous green algae, flatworms, and several species of encysted dinoflagellates. In one instance, a barge, stationed for a number of months off of the Pt. Loma sewage treatment plant near Los Angeles, was sampled in drydock in Baltimore, having ballasted in southern California three months earlier. The sediments in the tank were in layers 2.5 - 5 cm deep in places, very fine, slightly anoxic (blackish) with many rust articles and with a clear petroleum-based odor. A thin layer of water covered some of the mud, although the tank had been emptied of water about 5 days prior to sampling. In this sediment one live specimen of a female capitellid polychaete, with eggs along its tube walls, was found.

(26)
No fouling organisms were seen in the few ballast tanks sampled for sediments in dry dock; however, no concerted effort was made to search for such organisms. The majority of ballast tanks were sampled for planktonic organisms with nets, with no access to the tank bottoms many levels below (where permanent benthic communities or fouling organisms might survive in unpumpable water when the tank was deballasted).

(27)
Whole water samples contained an additional 48 species of ciliates and 8 species of dinoflagellates not collected in plankton net tows. No toxic dinoflagellates were identified in sediment or plankton samples, although the stochastic nature of ballast transport cautions against concluding that Chesapeake Bay is not being inoculated by these organisms.

(28)
Dinoflagellates were prevalent in whole water samples of ballast water and as cysts in ballast sediments (96% and 70% of the sampled tanks and cargo holds respectively). Dinoflagellate densities averaged 0.89 individuals per ml in whole water and 84.6 encysted individuals per gram dry weight in sediments.

(29)
Several lines of evidence indicate that mid-ocean exchange was effective in reducing the abundance and taxonomic richness of plankton in ballast water. Cargo holds and ballast tanks with original water had significantly higher mean densities of organisms (906 individuals/m^3) and greater mean number of taxa (14) than did exchanged cargo holds and ballast tanks (43 individuals/m^3 and 7 taxa, respectively). Some caution must be exercised in interpreting these data, because only one vessel had paired exchanged and unexchanged tanks. The remaining samples were taken from different ships. All vessels, however, originated in the Northeast Atlantic, and only vessels reported to have exchanged water in mid-ocean (i.e., no exchanges over the continental shelf) were used in the comparison. Furthermore, all vessels used had exchanged more than 90% of their water.

(30)
Comparison of densities of a signature coastal taxon (coastal species of spionid polychaetes) in exchanged and original ballast water of a bulker travelling from Belgium to Baltimore provides additional evidence that mid-ocean exchange was effective in removing coastal plankton. In this case, the mean density of spionid polychaetes in a 91% exchanged ballast tank was significantly lower than that in an unexchanged cargo hold. Similarly, the total density of organisms and the total number of taxa were lower in the exchanged than the unexchanged water. These data, while strongly suggestive, must also be interpreted with caution, because only one tank per exchange condition was sampled and the tank types differed. Samples

from replicate exchanged and unexchanged ballast tanks (or cargo holds) on multiple vessels are needed to strengthen statistical arguments concerning the effectiveness of mid-ocean exchange.

(31)
The continued presence of some coastal taxa (e.g., balanomorph cirripede nauplii and cyprids, bryozoan larvae, most spionid polychaetes) in ships that exchanged 91 to 100% of their ballast water in mid-ocean suggests that the procedure, while effective in reducing abundances, cannot eliminate all traces of the original biota.

(32)
Densities of organisms in the ballast water were extraordinarily variable from ship to ship and reflect the stochastic nature of ballast transport. Analysis of a subset of samples showed densities ranging from 0 to 18,000 organisms per cubic meter of ballast water (excluding bacteria and viruses). If one extrapolates to include the total amount of ballast water on board, a bulker deballasting in Chesapeake Bay could release up to 1 billion organisms.

(33)
For combined regions and seasons, plankton densities were significantly negatively correlated with the age , temperature, and salinity of ballast water. The present data suggest that densities of organisms in ballast water decrease as the age of the water (i.e., voyage duration) increases. In particular, ballast water less than 14 days old had higher densities of plankton than did 14 to 24 day old ballast water. Caution, however, must be exercised before concluding that older water is necessarily of 'lower risk'. First, given existing trade patterns,whether low abundances in 14-24 day old water were due to regional differences (most of these vessels were from a single, lower diversity region in the eastern Mediterranean) or whether they, in fact, represented age-dependent mortality could not be distinguished. In order to tease apart these alternative hypotheses, it will be necessary either to (1) monitor one or more additional trade routes from other regions that experience voyages of similar length, or (2) measure plankton survivorship on existing transoceanic routes directly. Second, living organisms were found in this study in vessels with water 33 days old (from Ulsan, Korea), and live copepods have been found in other studies in ballast water up to 95 days old. Finally, there is evidence that benthic and fouling communities exist in permanent ballast tanks suggesting that adult populations could generate larvae into the ballast water column for many weeks or months. The available data do not permit, at this time, setting a minimum or maximum "safe" time threshold for water age. Importantly, the data demonstrate that the transit time for most bulkers arriving to Chesapeake Bay from European and Caribbean ports is sufficiently short as to ensure survival of many planktonic organisms entrained in the ballast water.

(34)
One instance was found of a species transported to Chesapeake Bay that came from a

previously invaded region. More than 50 specimens of the fish *Alepes djedaba* Forskaal, known as the Jeddah Jack, were observed in a vessel from Israel. This species is a Lessepsian invader, that is, it moved from the Red Sea through the Suez Canal to the eastern Mediterranean, where it has become an important part of the commercial fishery. While this fish was unlikely to have survived the low salinities in Baltimore harbor or the colder winter waters of Chesapeake Bay, its path (Red Sea to Eastern Mediterranean to North America) is of interest in light of the recent invasion of the portunid crab *Charybdis helleri* into the greater Caribbean region and the Atlantic coast of Florida (Lemaitre, 1995). This carnivorous crab is also believed to have come from the Red Sea via the Suez Canal into the Mediterranean and then to the Americas. *Charybdis* could have significant impact on mollusc and decapod communities in newly invaded areas. Also encountered in ballast water from Europe were certain species that have previously been introduced from North America *to* Europe (e.g., the American barnacle *Balanus improvisus* and the American copepod *Acartia tonsa*) and species that may have been introduced earlier from Europe (e.g., the hydroid *Ectopleura dumortieri*, known on American Atlantic shores since the 1860s). Mussel (*Mytilus*) larvae were encountered in a number of samples from Europe, but whether these represented *Mytilus edulis* or the invasive species *Mytilus galloprovincialis* awaits molecular genetic analyses.

(35)
Organisms in ballasted cargo holds did well in transit, because bulker cargo holds function as well-mixed, physically constant, ocean-going lakes. They are centrally located in the vessel and contain a large water mass (average > 4 million gallons); consequently, they are usually well buffered from temperature changes caused by surrounding waters. Salinity remains constant unless the holds are exchanged or substantial amounts of water are pressed. If the voyage length does not exceed food resources, organisms may not experience environmental conditions dramatically different from those of their native habitat (with the exception of prolonged darkness and perhaps lack of exposure to some predators). Ballast tanks have significantly less capacity than cargo holds. Whether physical conditions are more variable in ballast tanks than in cargo holds is unknown. Ballast tanks are more exposed to ambient water temperatures, and there is potential for temperature and salinity stratification in wing bottom tanks when pressed, which may effect the viability of the biotic assemblage. Biological diversity was lower in ballast tanks than in cargo holds, but sampling bias could not be ruled out as a source of the difference. Dissolved oxygen content and densities of organisms were similar between cargo holds and ballast tanks, which suggests that conditions in the latter did not inhibit survival.

(36)
Comparison of the present findings to those from studies in Coos Bay, Oregon, suggests geographic differences in the abundances of organisms received by U.S. ports. For most taxa, Chesapeake Bay had a lower percentage of ballasted cargo holds arriving with abundant organisms (i.e., > 100 per tow) than did Coos Bay, Oregon. This difference may reflect the greater variability in the prevalence and

abundance of organisms arriving to Chesapeake Bay from multiple source regions. In contrast, Coos Bay received a more constant supply of organisms from a single source region. Comparisons of initial abundances and survivorship between these and other regions are needed to understand more fully the correlations among inoculation density, inoculation frequency and invasion success.

(37)
In a number of cases, larval or juvenile invertebrates were found in ballast samples that were less than the age of the ballast water itself, suggesting *in situ* generation of these individuals. Examples include larval hydromedusan jellyfish, polychaete worm larvae, ascidian (sea squirt) tadpole larvae, barnacle nauplii, and copepod nauplii. Explanations for the presence of these life history stages in the ballast samples include the following: (1) larvae could be produced from adult organisms in semi-permanent benthic communities or as fouling organisms in ballast tanks,(2) larvae could be produced from newly-ballasted adult organisms pumped or gravitated into cargo holds (either originally or by pressing up at a later date); this could especially occur if the vessel ballasted tychoplankton (small suspended benthic organisms) in shallow port waters,(3) larvae could be produced by adult fouling organisms in the ships' sea chests, (4) larvae could be produced by species with a very short generational time [e.g., the hydroid *Tubularia crocea* is reported to have settled on a ship's hull in Hawaii, and grown to reproductive maturity by the time the ship reached Panama 10 days later).

(38)
The transport of specific nuisance taxa from a recognized global hot spot to another port remains poorly documented, not only because of difficulties in taxonomic resolution, but also because of the stochastic nature of vessel traffic and sampling. Nevertheless, the potential for nuisance species to 'leap-frog' from one region to another is great as evidenced by the apparent spread of *Vibrio cholera* introduced from South America to Mobile Bay, Alabama and the dispersal of toxic dinoflagellates from Japanese to Australian and New Zealand waters. Dedicated route studies and experimental programs that focus on this phenomenon would be of a significant value.

(39)
A critical factor influencing the survival of the biological inoculum after deballasting is the compatibility of physical conditions between donor and recipient ports. Certainly, a port will be at greater risk of a ballast-mediated invasion if the temperature and salinity of its water are similar to those of the donor ports. The present data show a striking difference between the salinity of Baltimore harbor and that of the majority of ballast water it receives. Much of the ballast water arriving from the Mediterranean, Northeast Atlantic and West Central Atlantic regions was greater than 21 ppt . With the exception of very euryhaline species or those with resistant stages, most ballast water organisms should perish following their release into Baltimore's low salinity (3 to 8 ppt) water. In contrast, organisms deballasted in the higher salinity waters (20 to 28 ppt) in Norfolk should have a greater probability of survival. The latter conclusion assumes that the

salinity profile of the ballast water reaching Norfolk, from where there were few samples, is similar to that arriving in Baltimore. Differences in temperature between Baltimore harbor and deballasted water were less extreme than those for salinity. Not surprisingly, differences were least in summer, when temperatures in Chesapeake Bay most closely matched the warm water ports of the eastern Mediterranean and West Central Atlantic. Survivorship of deballasted organisms should be higher in summer. In contrast, organisms arriving from these ports to Chesapeake Bay between late fall and early spring would likely experience significant temperature-related physiological stress.

(40)
Despite calls from the International Maritime Organization for voluntary open-ocean exchange of ballast water, exchanges were reported in only 17% of the bulkers sampled in the present study. Furthermore, in these instances, ship's officers often overestimated the effectiveness of the exchange . Reasons for conducting an exchange included a desire to flush tanks of sediment-laden water taken on in port and the assumption that exchange was 'required' before entering U.S. coastal ports. Ship's officers indicated that exchange of cargo holds was more difficult than that of ballast tanks. In the present survey exchanges were reported more frequently for the latter.

(41)
Ballast exchange remains the primary means of ballast management in the mid-1990s. While undoubtedly acting to reduce significantly the numbers of original organisms, and while saltwater exchange for freshwater ballast has both a flushing and a biocidal effect , the fact that aboriginal taxa remain (due to inadequate exchange) argues that ballast exchange is not a complete solution. In addition, many vessels, for safety reasons, may not be able to undertake ballast exchange. These considerations are some of the primary motivations for seeking ballast management options other than, or in addition to, ballast exchange.

(42)
The large number of source regions contributing nonindigenous taxa into one estuary reflects one of the greatest difficulties in assessing invasion risk due to ballast water transport. Local and regional variations (e.g., tidal, hydrographic, physical-chemical), spatial variations (e.g., different regions within a harbor, proximity to a sewage outfall), and temporal variations (diurnal, lunar, seasonal, annual, decadal) could and do generate extensive variation in the composition and abundance of plankton carried out of a port by a departing ship. A second layer of temporal variation is then added upon these earlier parameters, because different vessels retain ballast water for different lengths of time, depending upon many factors, including length of voyage, cargo requirements, and weather conditions. Thus RoRos and container vessels frequently contain older water, in contrast to bulker cargo hold water, which is often no older than the length of the voyage from the last port of call. When these complexities are considered against the larger backdrop of many different global source regions the

scale of complexity becomes enormous.

(43)
The present study suggests that the risk of ballast-mediated invasion in United States coastal waters remains extraordinarily high. At present, little is known of the processes that mediate successful ballast invasions. It is not clear whether repeated inoculations are needed over time or whether a single vessel, densely packed with organisms is sufficient to establish a population. If the latter is the case, then no port receiving water from an exogenous source is immune. There is circumstantial evidence that both mechanisms may be in operation. Many marine invasive species, apparently distributed by ballast water (or by hull fouling), are found in ports and harbors in much of the world, suggesting their constant (multiple inoculation) and extensive (massive inoculation) transport. Other invasions appear in only one site, suggesting rare and inconsistent transport. Thus the common Japanese shore crab *Hemigrapsus sanguineus*, otherwise known only from western Pacific shores, has invaded the mid-Atlantic coast of North America (from an initial colonization site in New Jersey, near the mouth of Delaware Bay), rather than any site in the Pacific Ocean. That few vessels from the western Pacific discharge ballast at the entrance to Delaware Bay suggests the possibility that the inoculation may have been due to a single vessel release. Given the constant threat of a ballast-mediated introduction, control measures are critically needed.

(44)
The enormous variability in the composition and abundance of organisms encountered in ballast water of ships coming to Chesapeake Bay coupled with the extreme difficulty (if not impossibility) in identifying most organisms to species argue against the establishment of biomonitoring programs that assess whether incoming ships are safe to deballast in port. First, morphological identification of the myriad larvae from different parts of the world could not be accomplished in timely fashion. Although relatively rapid assays to detect the presence of some harmful organisms, such as *Vibrio cholera* may exist, these specific tests cannot guarantee that other (current or future) nuisance species are not present. Third, it is difficult, if not impossible, to sample all tanks for organisms. Ballast tanks and cargo holds on a single ship can have water from different sources. Furthermore, access to the lower levels of ballast tanks is often not possible, which means many organisms may be missed.

(45)
In the short term, the ballast micromanagement practices recommended in NABISS 1 combined with ballast exchange may be the best method for reducing the risk of ballast-mediated invasions. While these data suggest that mid-ocean exchange was effective in removing most (but not all) coastal plankton, more rigorous experiments are needed that compare matched exchanged and unexchanged tanks on the same ship. At the least, vessels coming from known hot spots or with water similar in salinity to that of the recipient port should be requested to attempt open-ocean exchange. In

the long-term, technological innovations are needed either to prevent intake of aquatic organisms into ballast tanks, or eliminate them once they are on board.

(46)
The data reported here expand considerably our understanding of the ballast-mediated transport of exotic organisms into United States waters. Previously published research has been limited primarily to the arrival of plankton in ballast water in the Pacific Northwest (Coos Bay, Oregon and Port Angeles, Washington) from a single source region (Japan)). In the present study, a picture is now provided of the diversity of ballast plankton arriving from multiple source regions into the largest estuary in the United States, the Chesapeake Bay. These data demonstrate conclusively that the Chesapeake Bay is being inoculated by a diverse assemblage of live organisms transported from around the world in ship's ballast water. Given that (1) the ports of Baltimore, Maryland and Norfolk, Virginia receive hundreds of bulkers each year; (2) the average bulker sampled had over 31,000 MT (> 8.1 million gallons) of ballast on board, and (3) 91% of the bulkers sampled contained living organisms, it is evident that these inoculations are occurring on massive and frequent basis.

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Chapter 1.

INTRODUCTION

The "Nonindigenous Aquatic Nuisance Prevention and Control Act of 1990" (Public Law 101-646, 16 USC 4701 et seq.) established the National Ballast Water Control Program. Under this Program, a Shipping Study was conducted that examined the degree to which shipping may be a pathway of transmission of aquatic nuisance species into United States waters and possible alternatives for controlling the introduction of such species (Carlton, Reid, and van Leeuwen 1995). The Study assumed the working name of the National Biological Invasions Shipping Study (NABISS).

The present work was conducted under the aegis of the Shipping Study. The report of Carlton, Reid,and van Leeuwen is referred to as "NABISS 1"; the present work is referred to as NABISS 2 (or Shipping Study II). In August 1993, through funding provided to Dr. James T. Carlton (Principal Investigator) by the United States Coast Guard (USCG) and facilitated by the National Sea Grant College Program/Connecticut Sea Grant, a ballast water field and laboratory unit was established with Dr. L. David Smith as Postdoctoral Research Associate (1993 to 1994), Linda McCann (1993 to February 1994) and Marjorie Wonham (1993 to 1995) as Research Associates, and Donald M. Reid, of NABISS 1, as a Visiting Investigator (August 1993 to January 1994).

NABISS 1 identified the Chesapeake Bay system as receiving the most ballast water from foreign ports of any harbor region on the Atlantic coast of the United States. For this reason, NABISS 2 was established at the Smithsonian Environmental Research Center (SERC) in Edgewater, Maryland, hosted in the laboratory of Dr. Gregory M. Ruiz. Edgewater is located 45 minutes from the Port of Baltimore and the anchorage of Annapolis, and 5 hours from the Port of Hampton Roads (Norfolk).

NABISS 2 examines the roles of ballast water and ballast sediments from foreign ports as vectors for the transport and release of nonindigenous species into United States coastal and aquatic ecosystems. It specifically addresses the following topics:

- ***Vessel Diversity and Ballast Water Discharges***
  Assessment of the types of vessels arriving to ports in Chesapeake Bay and the amounts of ballast water discharged, particularly by bulk cargo vessels (bulkers), with additional consideration of other vessel types (roll-on roll-off vessels).

- ***Physical and Chemical Characterization of Arriving Ballast Water versus the Port of Discharge***
  Comparison of selected water parameters in arriving ballast water and the site (port) of water discharge.

- ***Biodiversity of Ballast Water and Sediments***
  Quantitative and qualitative assessment of the abundance and diversity of living organisms found in ballast water and ballast sediments arriving in Chesapeake Bay aboard vessels from world ports, as a function of tank type, ballast water

source region, season of arrival, and physical and chemical characteristics of the ballast water.

- ***Effectiveness of Ballast Exchange***
  As feasible relative to vessel availability, examination of the biota in ballast water that had or had not been exchanged with oceanic water.

- ***Transport of Ballast from Global Hot Spots to Chesapeake Bay***
  Examination of European or Mediterranean ballast arriving in Chesapeake Bay from ports known to be sites of previous invasions or of blooms of nuisance species.

- ***The Role of Long-Distance and/or Longer-Term Voyages in Ballast Biota Survival***
  Investigation of the survival of plankton in ballast water relative to water age.

Vessels were sampled from August 1993 to August 1994, followed by sample analyses, laboratory culture studies, consultation with taxonomists, data analyses, and report preparation. This report was submitted in March 1996. Acronyms and abbreviations used in this report are listed in Appendix A.

Chapter 2.

METHODS

Study Sites

Between August 1993 and August 1994, we surveyed and sampled ballast water from foreign commercial vessels arriving in the Port of Baltimore, Maryland in upper Chesapeake Bay and in the Port of Hampton Roads, Virginia in lower Chesapeake Bay. We based our operations in the laboratory of Dr. Gregory Ruiz at the Smithsonian Environmental Research Center (SERC) in Edgewater, Maryland.

Vessel Traffic Information

Vessel traffic statistics were derived from weekly vessel forecasts and monthly and annual summaries of the Baltimore Maritime Exchange (BME) for the Port of Baltimore, and the Hampton Roads Maritime Association (HRMA) for the Norfolk/Hampton Roads/Newport News complex (hereafter, Norfolk). For Baltimore traffic, we estimated the load and discharge figures from our notes on individual vessels, and from interviews with the BME and the vessel agents. These data span the 12 month period from January to December 1994. The Norfolk load and discharge figures were summarized directly from the HRMA data for the 12 months between September 1993 and August 1994. Vessel load and discharge information for both ports, then, was directly comparable from January to August 1994. Typically, vessels coming to load cargo are in ballast condition and discharge some or all of their water.

Vessel Identification and Tracking

Our survey targeted commercial vessels arriving to Chesapeake Bay in ballast with foreign water. In the early stages of the project, the U.S. Coast Guard (USCG) Marine Safety Offices (MSO) in Baltimore, Maryland, and Norfolk, Virginia forwarded to us information compiled by their respective port maritime exchanges on daily and weekly vessel arrivals. This information included vessel names, their estimated date of arrival, last port of call, and the shipping agent. Through weekly telephone conversations with the shipping agents, we identified vessels likely to be arriving with foreign ballast water. Once an appropriate vessel had been identified we tracked its progress with the assistance of the shipping agent. The vessel's estimated time of arrival (ETA) was available during working hours from the agent and the cargo loading dock, and after hours from the dock only. In the latter stages of our study, we obtained memberships in both the BME and HRMA. Both organizations supplied weekly and daily faxed forecasts and monthly summaries directly to us, thereby streamlining the process of gathering information. The BME also provided updated ETAs upon request by telephone, 24 hours a day, 7 days a week, which greatly lessened the burden on the shipping agent. We also obtained samples of ballast sediment from empty ballast tanks. With the assistance of the USCG Inspection Office at Sparrows Point shipyard in Baltimore, we identified vessels scheduled for drydocking.

Early on, we recognized that the vast majority of foreign ballast water arriving to Chesapeake Bay was being transported by general cargo carriers, bulk carriers, and colliers (hereafter collectively referred to as bulkers) coming to load grain and coal. As a consequence, we focused most of our efforts toward sampling these ships. Because of SERC's proximity to Baltimore (0.75 h travel time vs. 5 h to Norfolk), most bulkers were sampled in Baltimore. During occasional lulls in bulker traffic, we also sampled ballast water from car carriers ["Roll on - Roll off" vessels (RoRos)]. Our goal was to sample 1 to 2 vessels per week over the course of the year ($\approx$ 50-100 vessels). Vessels were chosen at random with respect to last port of call. To simplify taxonomic identifications of ballast plankton assemblages, we targeted vessels that contained ballast water from single water sources (preferably from a foreign port of call or from a mid-ocean exchange). In practice, because cargo holds and ballast tanks were often topped up (pressed) during the voyage (e.g., to compensate for water lost by overflow), we routinely sampled ballast water of mixed origin.

On Board Interviews

In most instances, we met vessels at the loading docks in Baltimore or Norfolk shortly after their arrival. On several occasions, detachments from the USCG (Baltimore and Annapolis offices) ferried us by boat to ships at anchorage. Vessels arriving from foreign ports were boarded within one to two hours after berthing, after U. S. Customs and Immigration had departed, but before ballast tanks could be emptied. We first presented the Captain or Chief Mate with a letter of introduction describing our study (Appendix B), and then requested permission to sample ballast from one or more of their tanks. We next interviewed the officer to establish the ship's particulars (country of registry, gross register tonnage (GRT; 1 register ton = 1000 ft^3), and ballast water capacity); last and next ports of call; the amount of ballast water on board; amount(s), date(s) and location(s) of ballasted, exchanged, and pressed water; and date and location of deballasting (Appendix C).

COLLECTION OF SAMPLES

Tank Identification

Typically, one or two cargo holds on bulkers are reinforced to hold ballast water. Smaller ballast tanks (e.g., wing tanks, double bottom tanks, peak tanks) are distributed in various configurations throughout the ship (Carlton, Reid and van Leeuwen 1995). We sampled ballast water from one or two cargo holds or ballast tanks per ship. Cargo hold covers were usually already open, and the ballast water could be sampled easily without assistance from the crew. To sample ballast water from a ballast tank, crewmen were needed to unbolt and open the deck tank hatch covers. If ballast tanks were pressed, we allowed water to flood out for several minutes until the water level reached that of the tank opening before sampling. Because the water loss from pressed tanks affected the vessel's draft and trim, officers were sometimes reluctant to provide us access to these tanks. Deck tanks that had not been pressed above the hatch level were easily sampled. On a number of ships we were unable to take samples because the water level was inaccessible to net sampling.

Physical Variables

In all tanks sampled, we measured water temperature and salinity using a SCT meter (YSI model 33) and dissolved oxygen content using an oxygen meter (YSI model 57). Measurements were taken at the surface and at 1 m (ballast tanks) or 5 m (cargo holds) depth intervals. Temperature readings were corroborated in the field with a thermometer, and salinity readings in the laboratory with a refractometer. Temperature, salinity and dissolved oxygen content of the harbor water were recorded upon leaving the vessel.

Biological Samples

Plankton Net Tows. We sampled ballast water for planktonic organisms using replicate vertical tows of a plankton net (net length, 0.9 m; diameter of net opening, 0.30 m; mesh size, 80 μm; Sea Gear Corporation 90-30:3-80, Hialeah Gardens, FL). All plankton tows were filtered into 1 liter cod-end jars. Three to 5 quantitative samples were taken at evenly spaced intervals along the length of a cargo hold. For each sample, the plankton net was towed vertically at approximately 0.5 m s^{-1} through the entire water column (10 to 25 m depth). An additional single, but qualitative, tow was taken diagonally from the bottom to the surface along the entire side of the hold to sample for rare organisms. In ballast tanks, 2 quantitative samples were taken through the opened deck hatch. Each quantitative sample consisted of a single vertical tow from the bottom of the compartment to the water's surface. These were followed by a single qualitative tow, in which the plankton net was drawn through the water column repeatedly for a total distance of 10 m. Because most wing and peak ballast tanks were divided into compartments by vertical supports and horizontal shelves, in most cases, the net could not be lowered below the uppermost level ($\approx$ 3 m depth). To prevent mixing of plankton assemblages, different nets were used to take samples from different cargo holds or ballast tanks. Plankton samples were transported by vehicle from Baltimore or Norfolk to the laboratory at SERC in insulated coolers. Ice packs were used in summer to reduce metabolic activity of the plankton concentrate. Because of the length of the trip, samples from Norfolk were diluted by half after collection (in order to maximize organism survival) and aerated with battery-powered aquarium air pumps during transport.

Whole Water and Opportunistic Samples. To sample smaller members of the dinoflagellate and ciliate community that were not efficiently retained by our plankton net, we collected whole water samples by bucket near the water's surface. These samples were fixed immediately in Bouin's solution. Whenever isopods, amphipods, or fish were observed at the water surface, we collected them with aquarium dip nets. On several occasions, we returned to a vessel 6 to 8 h after sampling the ballast water for plankton in order to examine the deballasted cargo hold. Larger organisms (crabs, fish, shrimp) were collected in this manner.

Sediment Samples. To test for the presence of organisms (particularly dinoflagellate cysts) associated with ballast sediments, we collected sediment samples from cargo holds and ballast tanks of 10 vessels in Baltimore, of which 4 were in drydock and 6 were in port. The USCG and the vessel's port captain or a crewman generally accompanied us into the ballast tanks of drydocked vessels. In compliance with drydock regulations, ballast tanks were entered only after air quality had been approved by a marine chemist. Sediment was collected from the lowest level of the tank, where the most sediment had accumulated. Vessels arriving in port were sampled opportunistically for sediment. For several vessels, we returned after sampling the ballast water to collect sediment from the deballasted cargo holds.

BIOLOGICAL ANALYSES:

Containment Protocol

All procedures were conducted in accordance with the letter and spirit of the Containment Protocols established for research on nonindigenous aquatic species (Exotic Species Workgroup of the Chesapeake Bay Program 1992; Aquatic Nuisance Species Task Force 1992; Reid et al. 1993). Containment procedures are outlined in Appendix D.

Analysis of Live Plankton from Net Tows

Upon returning to SERC and within 1 to 24 h after collection, net tow samples were analyzed for the presence of live plankton. All samples were aerated individually while awaiting analysis. We concentrated each plankton sample using an 80 μm mesh sieve, and washed the contents into a glass dish. All organisms were categorized to the lowest taxonomic level possible and then by morphotype (e.g. calanoid copepod A; spionid polychaete B) using a Wild M8 dissecting scope (Appendix E). For quantitative live samples, the abundance of each morphotype was estimated on a logarithmic-scale (rare, < 10 individuals per sample; common, 10 to 100 per sample; or abundant, > 100 per sample). Qualitative tows were scanned for new organisms, or used to collect specimens for culturing and identification (see below).

After completion of the live analysis, most organisms in a sample were preserved and stored together in 75% ethanol. Unidentified, rare, unusual, or fragile (e.g., gelatinous forms) morphotypes, however, were vouchered and stored separately. Fragile specimens were fixed in 10% buffered formalin and preserved in 95% ethanol. More robust specimens were preserved directly in 95% ethanol. Whenever possible, vouchered specimens were sent to appropriate taxonomists for identification. Photographs were taken under a compound microscope of common organisms (e.g. copepod species); organisms that did not preserve well (e.g. heteropods); and organisms that were distinctive or unusual (e.g. some barnacle nauplii). When sufficient numbers (≈ 10-20) of an unidentified larval morphotype were present in samples, individuals were removed and cultured (see below) to juvenile or adult stages when possible for the purpose of identification.

Analysis of Preserved Plankton from Net Tows

Samples from a subset of vessels were analyzed after preservation to generate exact organism counts for statistical analyses (see below). We transferred samples from the preservative to water, and stained the organisms with Rose Bengal. Only clearly stained organisms were counted; those that did not take up the stain were considered to have been dead at the time of preservation and were not counted. In less dense samples (less than 500 organisms) all stained individuals were counted. For denser samples, abundances were estimated by counting and averaging replicate subsamples taken from a known volume. Specifically, the total volume of a sample was brought to 80 ml and organisms were suspended by stirring while six 1 ml or 4 ml subsamples were pipeted out. More fragile taxa (ciliates, rotifers, flatworms), noted during live analyses were rarely observed in the preserved samples. For these taxa, the minimum number recorded in the live analysis was used as a conservative estimate of abundance.

Analysis of Whole Water Samples

Whole water samples were analyzed by microfiltration in the laboratory of Dr. Wayne Coates at SERC and a species list was generated.

Sediment Analysis

Subsamples of collected sediment were examined under a dissecting scope for living organisms, and a taxonomic list was generated for all sediment samples. In addition, ciliates and dinoflagellates were examined in the lab of Dr. W. Coates.

Cultures

Larval and juvenile organisms from live plankton samples were cultured to facilitate identification. Individuals of a single morphotype were raised in 100 ml glass crystallizing dishes at a density of approximately 10 organisms per dish. For the first week, organisms were kept in 80 μm-filtered ballast tank water; afterwards, they were transferred to artificial seawater of comparable salinity. All dishes were stored in incubators at temperatures within 5°C of the ballast water temperature on a 14:10 h light:dark cycle. Herbivorous larvae were fed a 1:1 mixture of the prymnesiophyte *Isochrysis galbana* and the chlorophyte *Dunaliella tertiolecta*. Carnivorous larvae were provided with nauplii of the crustacean *Artemia* spp. or the rotifer *Brachionus plicatilis* as food. The culture water and food were changed daily for certain taxa (e.g., decapod zoea) and every third day for other groups or later developmental stages. Sample protocols for rearing barnacles, spionid polychaetes, and bivalves are described in Appendix F. Animals were counted and transferred to clean water 1 to 3 times a week, depending on the taxon. Adults and dead larvae or juveniles were preserved for later identification.

DATA ANALYSIS:

Vessel and Ballast Water Analyses

Vessel statistics (e.g., gross register tonnage, ballast water capacity) were calculated separately for bulkers and RoRos. Because few bulkers were sampled from Norfolk, bulker data from Baltimore and Norfolk were combined for analyses unless specified otherwise. Vessels expressed ballast water capacities in different units (e.g., long tons, LT; cubic meters, m^3; metric tons, MT). For our analyses, we converted all measurements to metric tons (1LT = 1.0162 MT; 1 m^3 of fresh water = 1 MT). We used the following approximations to convert cubic meters of saline ballast water to metric tons: for salinities (a) less than 10 ppt, multiply m^3 by 1; (b) between 10 to 20 ppt, multiply m^3 by 1.0125; (c) greater than 20 ppt, multiply m^3 by 1.025.

Geographic sources of the ballast water and last ports of call were classified by region according to the United Nations' Food and Agriculture Organization (FAO) standardized division of the world's oceans (Fig. 2-1). We defined "topped" cargo holds and ballast tanks as those in which at least 50% of the original amount of water remained prior to new water being added; in most cases, less than 15% of the water in these holds and tanks had been added. "Exchanged" cargo holds and ballast tanks were defined as those in which greater than 50% of the original water was flushed before being replaced with ocean water. The maximum efficiency of ballast water exchange was calculated as a percentage of ballast water salinity to open ocean salinity [(salinity of ballast water/35 ppt) x 100].

Biological Analyses

Live samples. From the live analysis data, we estimated the relative abundance (rare, common, abundant) and frequency with which a given taxon occurred on bulkers. Because tow volumes differed significantly between cargo holds and dedicated ballast tanks, data from these tank types were analyzed separately. The use of log-scale categories of abundance prevented us from averaging replicate samples within or between tanks on a vessel; consequently, we used the maximum abundance category for a given taxon from all samples collected on a ship. If larvae could not be subclassified within a taxon, they were pooled instead into a general category (e.g., polychaete larvae rather than capitellid larvae).

We were extremely conservative in estimating the number of species within a taxonomic group. By definition, a minimum of one species was present for any taxonomic level (phylum, class, order, family, genus) we observed. To this, we added the number of distinct lower taxonomic levels identified. For example, if no lower taxonomic level of bivalve was identified, the total number of bivalve species was 1. If

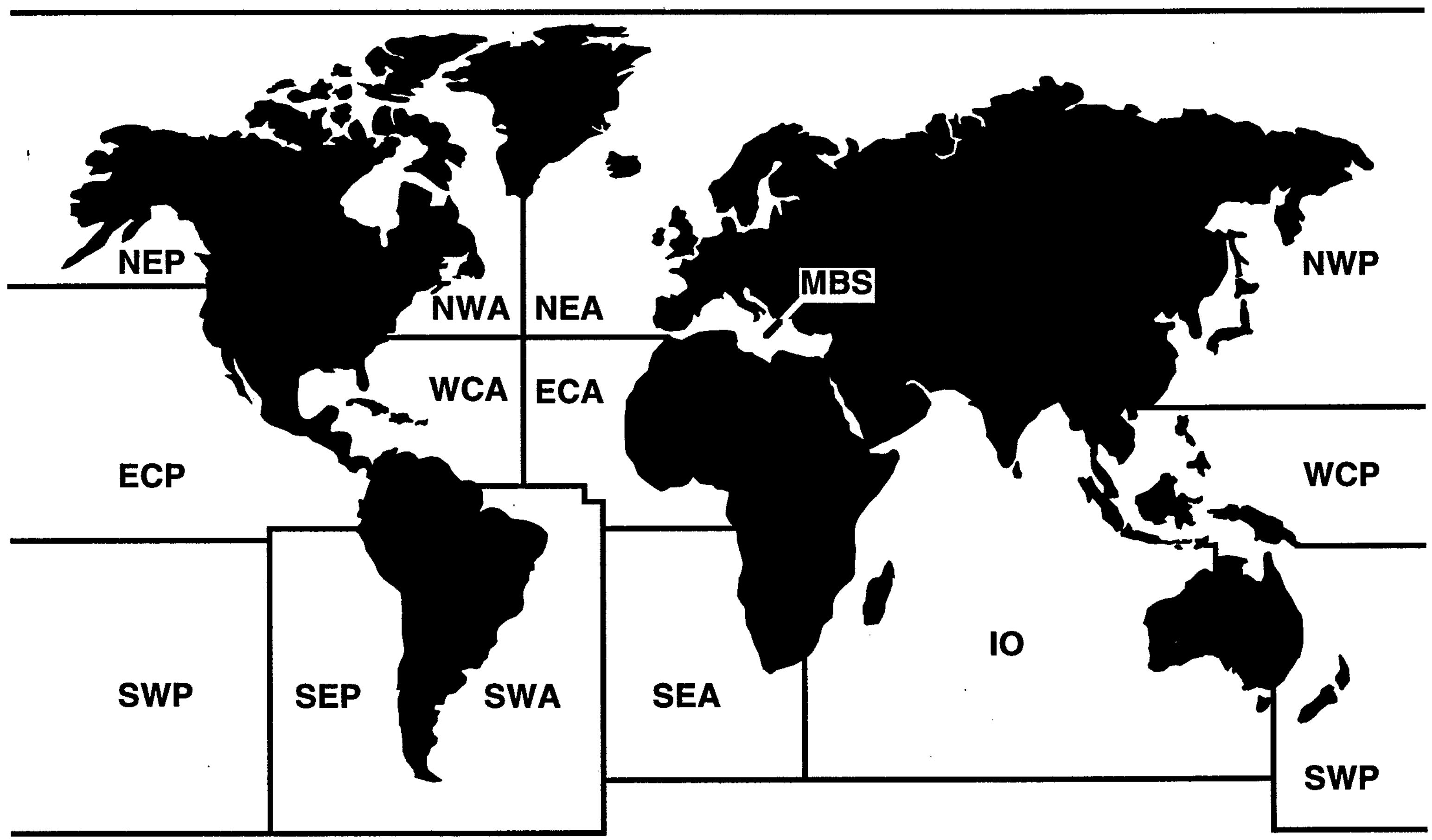

Figure 2-1. Waters of the world by United Nations' Food and Agriculture Organization (FAO) region. ECP, Eastern Central Pacific; IO, Indian Ocean; MBS, Mediterranean - Black Sea; NEA, Northeast Atlantic; NEP, Northeast Pacific; NWA, Northwest Atlantic; NWP, Northwest Pacific; SEA, Southeast Atlantic; SEP, Southeast Pacific; SWA, Southwest Atlantic; SWP, Southwest Pacific; WCA, West Central Atlantic; WCP, West Central Pacific.

we were able to identify 3 species of bivalve positively, then the total number of bivalve taxa was listed as 4 (the base number of 1 + 3 identified species). We kept this convention, even if multiple species were likely (e.g., bivalve specimens came from several ocean regions). Our data, then, provide a minimum estimate of taxonomic diversity in the ballast water assemblages arriving in Chesapeake Bay.

Estimates of taxonomic diversity also reflected the availability of qualified taxonomists and our success in culturing larvae. Bivalves, for example, are difficult to identify as larvae or juveniles, and poor survivorship during culture prevented us from raising most to an identifiable stage. In contrast, some decapods were identifiable as zoea and, those that were not, were relatively easy to rear to an identifiable stage.

Quantitative samples. Plankton densities were calculated by dividing the number of organisms collected in a plankton tow by the volume of ballast water filtered by plankton net (i.e., the tow volume, in m^3). Tow volume was calculated as tow depth multiplied by the area of the net opening [tow depth (m) x πr^2; where r = 0.15 m]. Plankton densities were $\log_{10}$-transformed prior to statistical analyses, then back-transformed for presentation. Ninety-five percent confidence limits are reported around the back-transformed means (Sokal & Rohlf 1981). Statistical comparisons of the number of taxa in ballast tanks were calculated using untransformed data.

Statistical Analyses

All the data were entered into spreadsheets on a microcomputer using Excel version 5.0 (1995). Figures were produced using Excel or DeltaGraph®Pro3.5 (1995) software. Simple descriptive statistics were generated with Excel; we used Statistical Analysis Systems software (SAS Institute 1985) for more complex parametric and non-parametric analyses. Where appropriate, group variances were tested to assure homogeneity (F_{max}-test; Sokal and Rohlf, 1981) and residuals were examined for normality. Those data that met parametric assumptions were analyzed using analysis of variance (ANOVA), analysis of covariance (ANCOVA), Student's t-tests, or, for frequency data, G-tests. If ANOVA models proved significant, unplanned multiple comparisons were used to distinguish group differences (Ryan's Q-test; Day and Quinn 1989). If *a priori* predictions could be made about the direction of change of a parameter, one-tailed statistics were used (Gaines and Rice 1990, Rice and Gaines 1994). For frequency data, among-treatment differences were compared using a simultaneous test procedure (STP test; Sokal and Rohlf 1981). If transformations (e.g., logarithmic) failed to correct for non-normal or heteroscedastic data, non-parametric tests (Kruskal-Wallis test, Wilcoxon 2-sample test, Spearmans Rank Correlation) were used. If Kruskal-Wallis models proved significant, unplanned multiple comparisons were used to distinguish group differences (Siegel and Castellan 1988). In all multiple comparisons, the experimentwise error rate (α) was 5 %.

Chapter 3.

RESULTS

DATA DRAWN FROM BALTIMORE MARITIME EXCHANGE AND HAMPTON ROADS MARITIME ASSOCIATION

Port Traffic Profile

The ports of Baltimore, Maryland and Norfolk, Virginia received similar numbers of commercial vessels (2,101 and 2,304 respectively) between January and December 1994, but the frequency distribution of vessel types differed significantly between ports (G-test, $p < 0.001$) (Fig. 3-1). Bulkers, which account for most of the ballast water released (Carlton, Reid and van Leeuwen 1995), comprised approximately 30% of the traffic in each port (Fig. 3-1). In contrast, RoRo traffic was proportionately greater in Baltimore (25%) than in Norfolk (2%). Container ships constituted the majority of the traffic in both ports (39% for Baltimore, 52% for Norfolk), but, based on interviews with ships' officers, released relatively little ballast water (Carlton, Reid and van Leeuwen 1995). Within each port, the relative frequency of vessel types arriving showed little seasonal variation (Appendix G).

Despite similar numbers of bulker arrivals (Appendix H), the amount of ballast water discharged in Baltimore was substantially less than that in Norfolk. Between January and August 1994, significantly fewer bulkers loaded cargo (i.e., discharged ballast water) in Baltimore (32%) than in Norfolk (75%) (G-test, $p < 0.001$) (Fig. 3-2). The majority of the bulkers arriving in Baltimore were laden with gypsum, wood pulp, ores, or sugar products and, thus, carried little ballast water (Carlton, Reid and van Leeuwen 1995). In contrast, most of the bulkers arriving in Norfolk were in ballast and awaiting coal (HMRA 1994). Seasonally, fewer bulkers were loading cargo in Baltimore in fall than in winter or spring (STP test, experimentwise alpha = 0.05) (Fig. 3-3). In Norfolk, the percentage of bulkers loading cargo was lower in the spring than in the summer (Fig. 3-3; see also Appendix H).

DATA DRAWN FROM NABISS BOARDINGS:

Vessels Sampled

Between 25 August, 1993 and 31 August, 1994, we obtained ballast water samples from 99 cargo holds and dedicated ballast tanks of 70 foreign commercial vessels. Eleven ballast tanks were sampled from 10 RoRos. Eighty-eight cargo holds and ballast tanks were sampled from 60 bulkers. Of these 88 tanks, 26 were cargo holds and 62 were ballast tanks (e.g., wing, wing bottom, double bottom, aft and fore peak tanks). In most cases, when multiple tanks were sampled on a ship, the water in each tank was from the same region and had identical temperature and salinity characteristics. In a few cases, the water sources differed (e.g., one tank had been exchanged, filled, or pressed en route) resulting in between-tank differences in

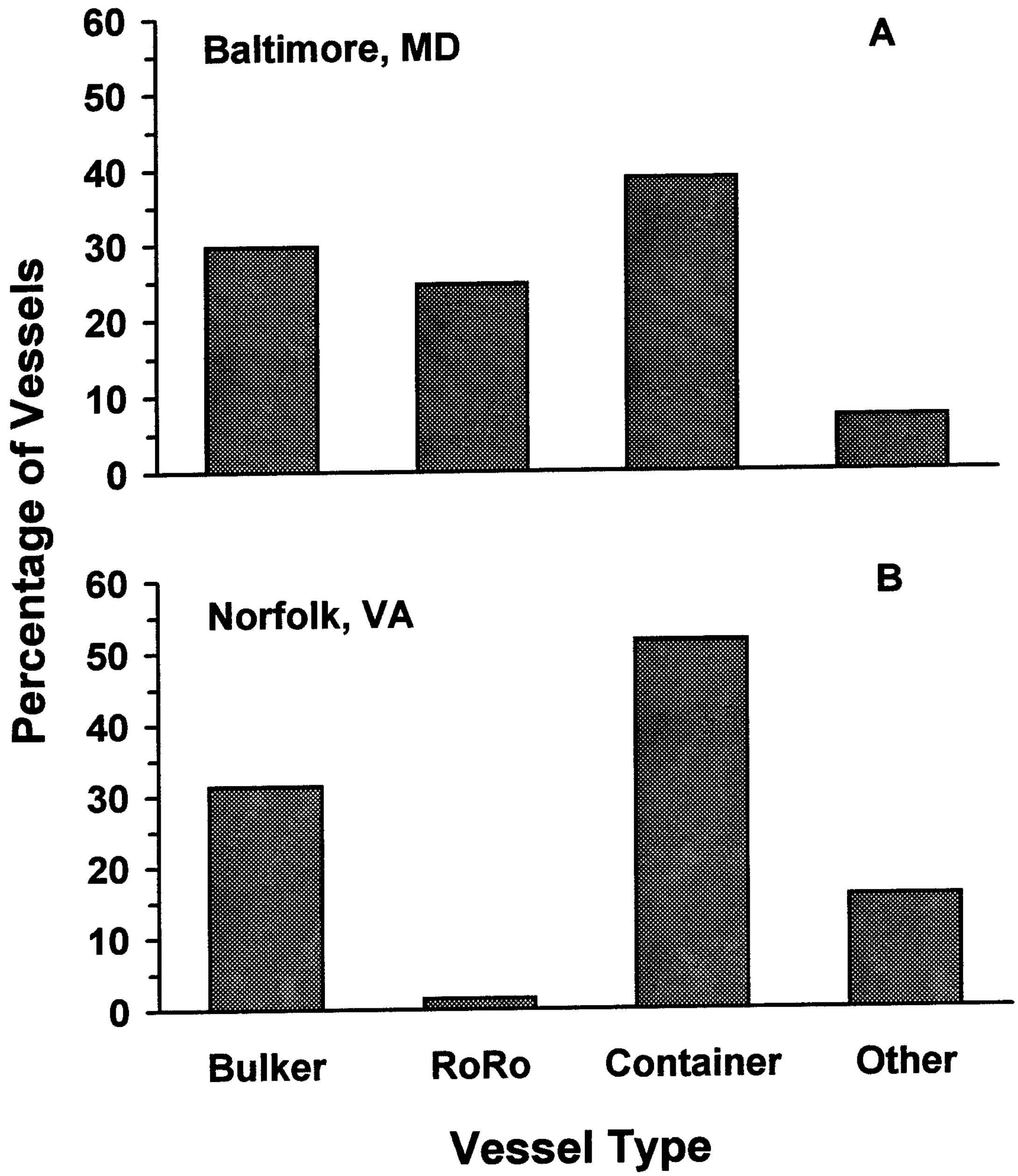

Figure 3-1. Percentage of all vessel types arriving between January and December 1994 in (A) Baltimore, Maryland (n = 2101) and (B) Norfolk, Virginia (n = 2304). Other includes tankers, tugs, barges, ice breakers, passenger ships, cable ships, and combinations. The distribution of vessel types differs significantly between ports (G-test, $p<0.001$).

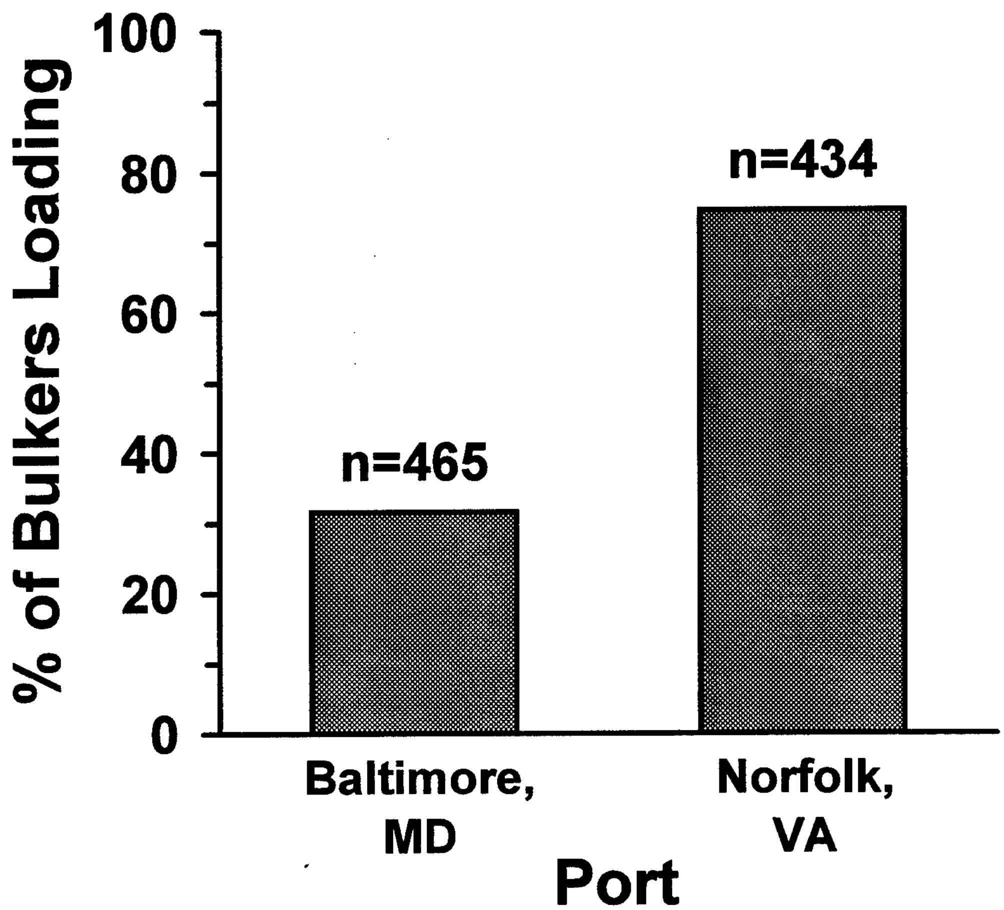

Figure 3-2. Percentage of bulkers arriving to load cargo in (i.e., discharging ballast water) Baltimore, Maryland and in Norfolk, Virginia between January and August 1994. n = number of vessels. Percentage of bulkers loading differs significantly between ports (G-test, $p<0.001$).

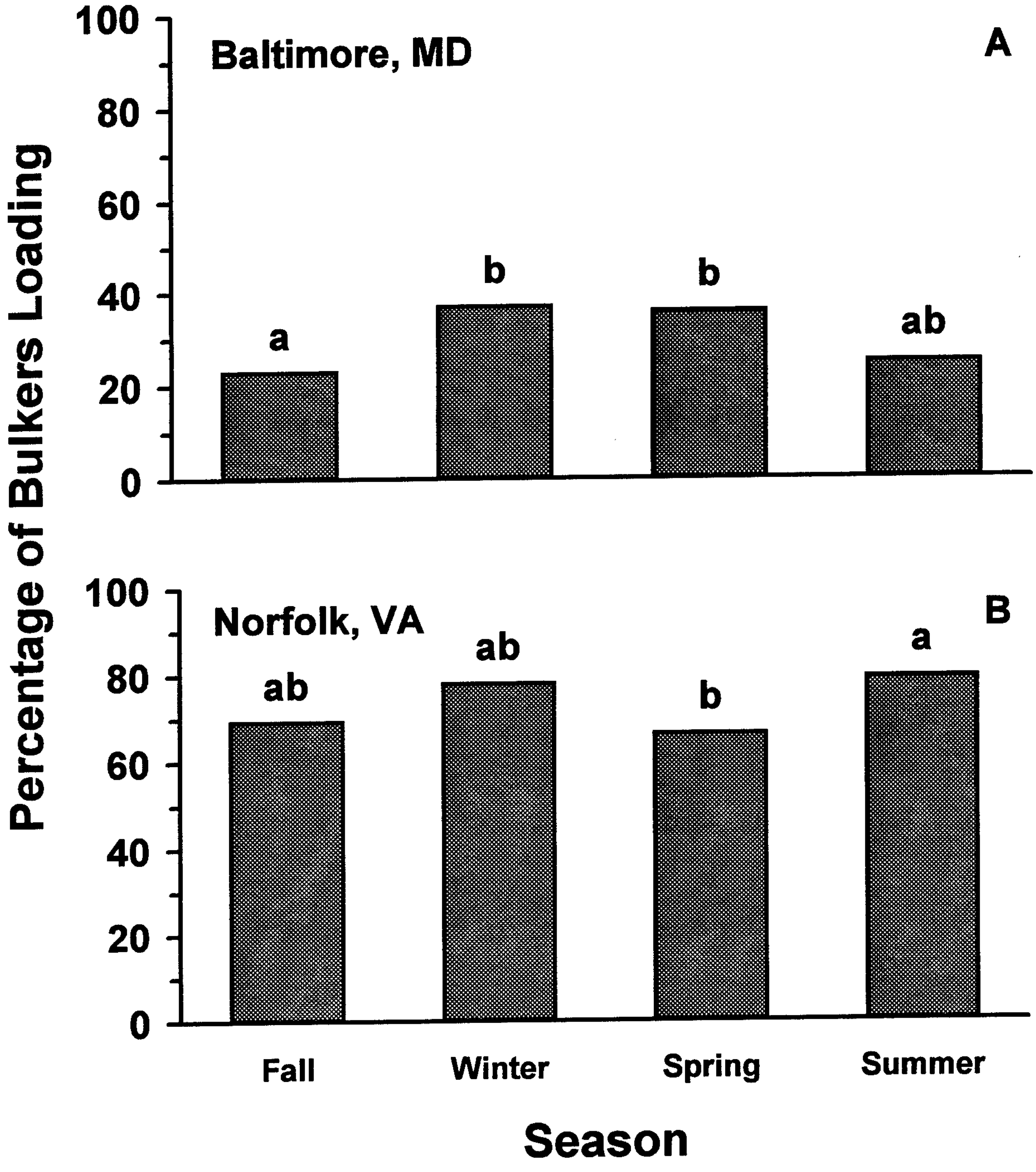

Figure 3-3. Percentage of bulkers arriving to load cargo, by season, in (A) Baltimore, Maryland between January and December 1994 (n = 624), and (B) Norfolk, Virginia between September 1993 and August 1994 (n = 660). Different letters above columns denote significant differences among seasons in the percentage of bulkers loading (thus, a column with 'a' differs significantly from a column with 'b', but a column with 'ab' does not differ from either column 'a' or 'b') (STP test, experimentwise $\alpha = 0.05$).

physical characteristics (arbitrarily defined as ≥ 3 ppt or 3°C). As a consequence, only 67 holds or tanks on bulkers were considered 'distinctly different'.

All RoRos were sampled in Baltimore. Of the 10 RoRos sampled, repeated visits by 2 vessels accounted for 5 of the samples. Fifty-four bulkers were sampled in Baltimore and 6 bulkers in Norfolk. Of the 60 bulkers sampled, repeat traffic by 3 vessels accounted for 12 of the samples.

Sediment samples were collected from deballasted cargo holds and ballast tanks of 10 vessels (8 bulkers, 2 barges) in Baltimore. Five of the vessels were in dry dock undergoing repairs; the remaining 5 bulkers (also sampled for ballast water) were in port to load cargo.

Vessel Tonnages and Ballast Water Amounts

Bulkers were larger and transported significantly greater volumes of ballast water than RoRos. For example, the mean gross register tonnage of bulkers was approximately twice that of RoRos (Wilcoxon test, $p = 0.002$) (Table 3-1). The mean ballast water capacity (42,834 MT) and the mean amount of ballast water on board (31,457 MT) bulkers were nearly 7 times those of RoRos (Wilcoxon tests, $p < 0.001$). Both vessel types carried, on average, 70% of their ballast water capacity (Table 3-1).

On bulkers, cargo holds had significantly greater mean tank <u>capacity</u> (16,808 MT) than either wing (2,170 MT) or peak (733 MT) tanks (Kruskal-Wallis test, chi-square = 54.7, df = 2, $p < 0.001$) (Table 3-2). Similarly, the mean <u>amount</u> of ballast water in cargo holds was significantly greater than that in wing or peak tanks (Kruskal-Wallis test, chi-square = 54.4, df = 2, $p < 0.001$) (Table 3-2). The amount of ballast water in a single cargo hold typically comprised 50% of the total ballast water carried on bulkers. Mean capacity of RoRo ballast tanks was less than that of the smallest bulker tanks (Table 3-2).

Ballasting Operations

Eighty-eight percent of the bulkers reported deballasting some percentage of their ballast water in port (Table 3-3). In contrast, <u>none</u> of the 10 RoRos deballasted in port. Nearly half (48%) of the bulkers we sampled arrived with their ballast water unmodified (i.e., not topped up or exchanged during the voyage) (Table 3-4). Thirty-five percent of the bulkers pressed up their cargo holds or ballast tanks to replace water lost in transit to overflow (≤ 15% of the existing ballast water amount added in all but one case). Approximately 1 in 6 bulkers attempted full exchange of one or more of their tanks (Table 3-4). Of the 15 tanks exchanged on these bulkers, 4 (27%) were cargo holds and 11 (73%) were wing or wing bottom ballast tanks. The salinity of water in exchanged cargo holds and ballast tanks was often less than that of open-ocean water (≈ 35 ppt). In only one-third of the holds or tanks was 95% or greater exchange achieved (Table 3-5). The maximum mean percentage exchanged (± 1 S.D.) was 91.6 ± 6.6%.

Table 3-1

Comparison of mean vessel tonnage and amounts of ballast water carried on bulkers and RoRos sampled in Baltimore, Maryland and Norfolk, Virginia between August 1993 and August 1994. Categories include gross register tonnage (GRT)[1], ballast water capacity (BWCAP, in MT), ballast water on board (BWOB, in MT); % of ballast capacity filled (% BWCAP). N, number of vessels; S. D., 1 standard deviation. Wilcoxon 2-sample test compares between vessel types for each category.

		Category			
Vessel Type		GRT	BWCAP	BWOB	% BWCAP
Bulker	Mean	49,200	42,834	31,457	71
	N	59	57	56	56
	S. D.	24,077	26,801	24,861	26
RoRo	Mean	24,616	6,428	4,368	70
	N	10	10	8	8
	S. D.	15,078	760	904	12
Wilcoxon test	Z	-2.94	-4.13	-3.22	-0.74
	p-value	0.002	<0.001	0.001	0.46

[1] 1 register ton = 1000 ft^3.

Table 3-2

Summary of mean ballast tank capacities and amounts of ballast water carried on bulkers and RoRos sampled in Baltimore, Maryland and Norfolk, Virginia between August 1993 and August 1994. Categories include ballast tank capacity (BWTCAP, in MT), ballast water in tank (BWIT, in MT), and % of tank filled with ballast (% BWTCAP) N, number of tanks; S.D., 1 standard deviation. Means with the same superscripted letter, read down the column in each category, do not differ significantly from each other (thus, 'a' differs significantly from 'b') (Kruskal-Wallis tests followed by multiple comparisons with experimentwise $\alpha = 0.05$).

			Category		
Vessel Type	Tank Type		BWTCAP	BWIT	% BWTCAP
Bulker	Cargo Hold	Mean	16,808 [a]	15,911 [a]	94 [a]
		N	25	25	25
		S. D.	2,177	3,646	15
	Wing	Mean	2,170 [b]	2,114 [b]	98 [a]
		N	51	51	51
		S. D.	1,962	1,932	11
	Peak	Mean	733 [b]	654 [b]	90 [a]
		N	7	7	7
		S. D.	689	610	19
RoRo	All tanks	Mean	576	460	69
		N	10	10	10
		S. D.	298	401	40

Table 3-3

Summary of vessel types[1] sampled and the number and percentage of vessels deballasting in Baltimore, Maryland and Norfolk, Virginia between August 1993 and August 1994.

Type	No. Sampled	No. Deballasting	% Deballasting
Bulker	60	53	88.3
RoRo	10	0	0.0
Total	70	53	75.7

[1] Vessel types: Bulkers consist of bulk carriers (n = 42), colliers (n = 8), general cargo carriers (n = 8), and oil/bulk/ore carriers (n = 2). RoRos are roll on/roll-off vessels.

Table 3-4

Summary by season[1] of the frequency of ballast water exchange in bulkers sampled in Baltimore, Maryland and Norfolk, Virginia between August 1993 and August 1994.

	Original[2]		Topped[3]		Exchanged[4]	
Season	No.	%	No.	%	No.	%
Fall	5	33	7	47	3	20
Winter	6	60	3	30	1	10
Spring	9	60	6	40	0	0
Summer	9	45	5	25	6	30
Total	29	48	21	35	10	17

[1] Fall, Sept. - Nov.; Winter, Dec. - Feb.; Spring, Mar. - May; Summer, June - Aug.

[2] Original: original water in ballast tank unmodified during transit.

[3] Topped: water added to fill cargo hold or ballast tank during voyage ($\leq$15% of ballast volume added in 20 cases and 43% in one case).

[4] Exchanged: original water in cargo hold or ballast tank flushed and replaced with ocean water during voyage. Maximum mean percentage exchanged $\pm$ 1 S. D. = 91.6 $\pm$ 6.6%.

Table 3-5

Measured salinities, percentages of exchange reported by vessel's officers, and estimated percentage of exchange. Estimated exchange is calculated as a percentage of open-ocean salinity (35 ppt). N = 10 bulkers arriving in Baltimore, Maryland, between August 1993 and August 1994.

BW Source	Measured Salinity (ppt) Ballast Tank	Measured Salinity (ppt) Cargo Hold	Reported % Exchange	Estimated % Exchange
Antwerp		32	100	91.4
Bilbao	34		100	97.1
Dunkirk		32	80	91.4
Ghent	35, 35		100	100, 100
Mediterranean	36		100	100
Rotterdam	28,32		100	78.6, 91.4
Rotterdam	35	30	100	100, 82.8
St. Petersburg	14, 26		100	40.0, 72.8
Wilhelmshaven	31	32	100	88.6, 91.4
Zeebrugge	32		100	91.4

Vessel and Ballast Water Origin: Geographic and Seasonal Patterns

Bulkers arrived in Baltimore and Norfolk from 25 countries and 39 ports (Appendix I). We sampled most bulkers in the summer (n = 20) and fewest in winter (n = 10) (Table 3-6). The FAO region for last port of call for the majority of bulkers (n = 60) was the Northeast Atlantic (42%) (Fig. 3-4). A substantial percentage of bulkers, however, had a last port of call in the Mediterranean/Black Sea (28%) or West Central Atlantic (20%) regions. For 56 of 60 vessels (93%), the FAO region for last port of call was the same as that of the ballast water source. For the remaining 7%, the last region of call and the source of the ballast water differed because cargo holds or ballast tanks were either filled or exchanged later in the voyage. In particular, a significant number of cargo holds and ballast tanks (15%) had water from the Northwest Atlantic (Fig. 3-5).

Bulkers from the Northeast Atlantic and Mediterranean/Black Sea regions were significantly larger (Kruskal-Wallis test, chi-square = 10.7, df = 2, $p = 0.005$), had greater ballast water capacity (Kruskal-Wallis test, chi-square = 14.6, df = 2, $p < 0.001$), and carried more ballast water on board (Kruskal-Wallis test, chi-square = 15.8, df = 2, $p < 0.001$) than did bulkers from the West Central Atlantic (Table 3-7, Appendix J). Most of the 1,761,593 MT of ballast water that was on board sampled vessels discharging in Baltimore and Norfolk was either of Mediterranean/Black Sea (47.1%) or Northeast Atlantic (37.0%) origin (Fig. 3-6).

There were no seasonal differences in gross register tonnage, ballast water capacity, or ballast water on board for ships coming from the three primary regions (Kruskal-Wallis tests, df = 3, $p > 0.55$) (Table 3-8, Appendix J).

Ballast Water Amounts Discharged by Bulkers

Our data, in combination with information from the BME and HRMA, indicate that significant amounts of ballast water were discharged in Baltimore and Norfolk in 1993-94. The mean amount of ballast water on board bulkers discharging ballast water (n = 56) in Baltimore or Norfolk in our study was 31,457 MT (Appendix J). Multiplying this amount (which assumes 100% discharge) times the 187 bulkers that visited Baltimore to load cargo between January and December 1994 (Appendix H), we estimate that bulkers, alone, released 5,882,459 MT of ballast water into Baltimore harbor in 1994. This amount is equivalent to 1.52 billion gallons per year (4.17 million gallons per day). In contrast, the amount of ballast water discharged by bulkers (n = 484; Appendix H) in Norfolk over twelve months was over 2.5 times that received by Baltimore. An estimated 15,225,188 MT of ballast water from bulkers were released in Norfolk between September 1993 and August 1994 (≈ 3.94 billion gallons per year or 10.8 million gallons per day).

Physical Characteristics of Ballast Water: Baltimore and Norfolk

Physical characteristics (temperature, salinity, dissolved oxygen, age) of the ballast water were not constant among vessel types, regions of ballast water origin, or

Table 3-6

Frequency of bulkers sampled in Baltimore, Maryland and Norfolk, Virginia between August 1993 and August 1994 by season[1] and by FAO region[2] of ballast water origin.

FAO Region	Season				Total
	Fall	Winter	Spring	Summer	
Mediterranean-Black Sea	7	2	3	5	17
North-East Atlantic	4	5	7	4	20
West-Central Atlantic	1	3	4	3	11
North-West Atlantic	1	0	1	5	7
Other[3]	2	0	0	3	5
Total	15	10	15	20	60

[1] Fall, Sept. - Nov.; Winter, Dec. - Feb.; Spring, Mar. - May; Summer, June - Aug.

[2] United Nations' Food and Agriculture Organization (FAO) standardized ocean regions of the world.

[3] Other includes ballast from East-Central Atlantic (n = 1), North-West Pacific (n = 1), East-Central Pacific (n = 1), and Indian Ocean (n = 2) regions.

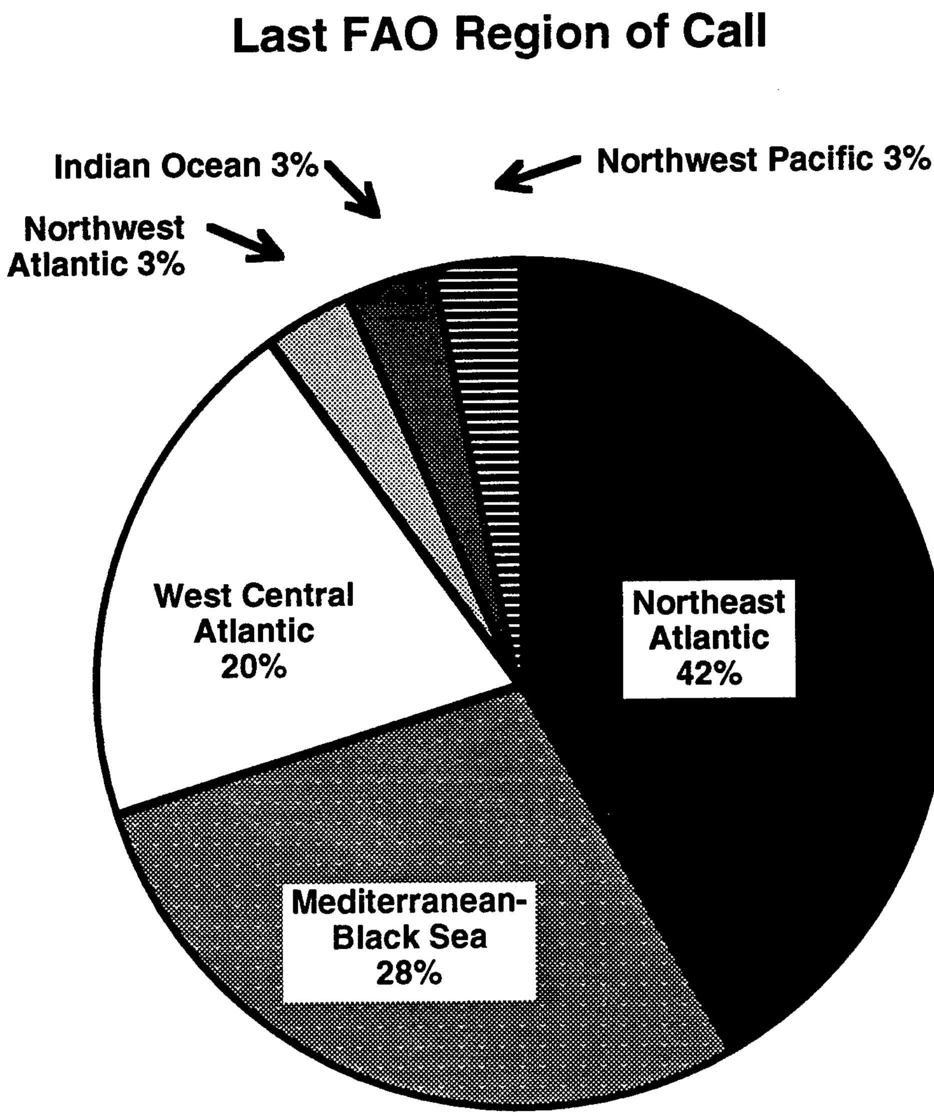

Figure 3-4. Frequency of bulkers sampled in Baltimore, Maryland and Norfolk, Virginia between August 1993 and August 1994 (n = 60 vessels) by last FAO region of call.

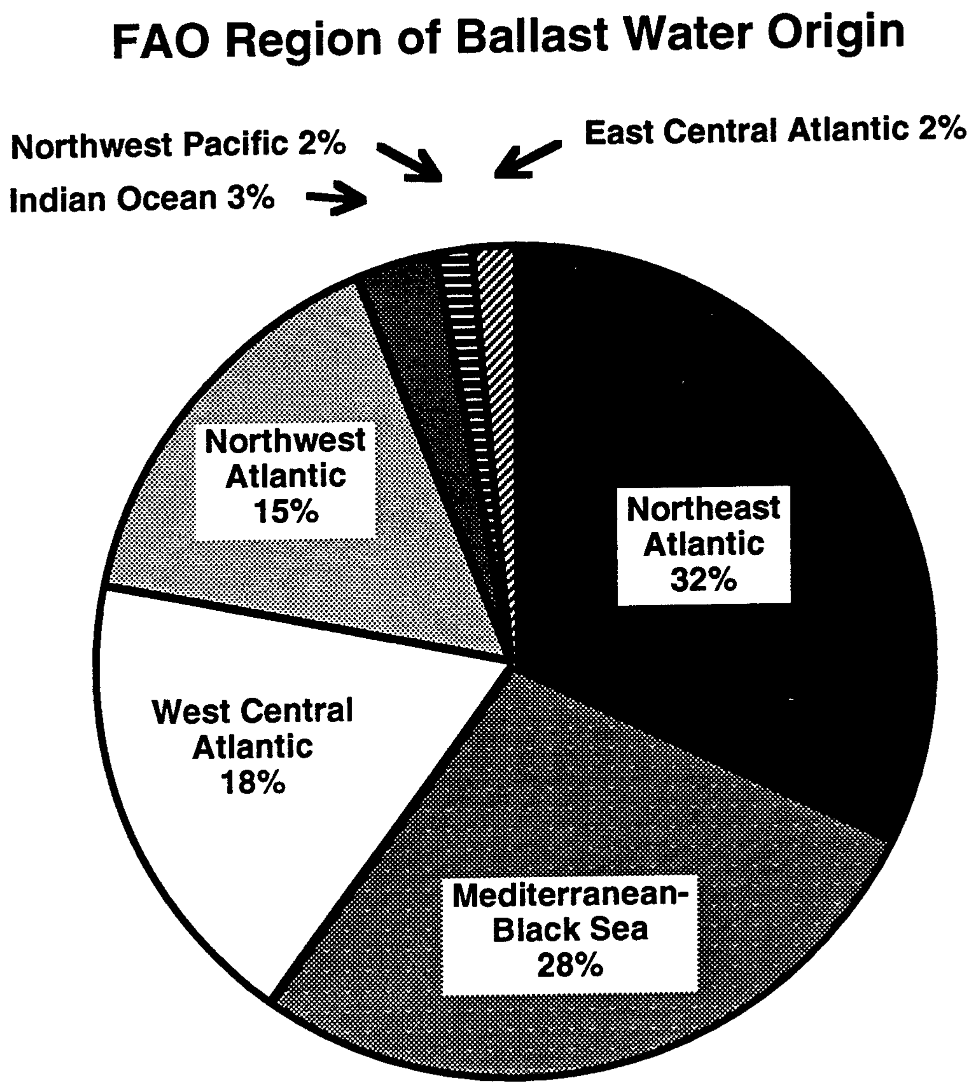

Figure 3-5. Frequency of ballast tanks and cargo holds on bulkers sampled in Baltimore, Maryland and Norfolk, Virginia between August 1993 and August 1994 (n = 65 tanks and holds) by FAO region of ballast water origin.

Table 3-7

Summary of mean vessel tonnage and amount of ballast water carried on bulkers sampled in Baltimore, Maryland and Norfolk, Virginia between August 1993 and August 1994 by the 3 most common FAO regions[1] of ballast water origin. Categories include gross register tonnage (GRT)[2], ballast water capacity (BWCAP, in MT), ballast water on board (BWOB, in MT); % of ballast capacity filled (% BWCAP). N, number of vessels; S.D., 1 standard deviation. Means with the same superscripted letter, read down the column in each category, do not differ significantly from each other (thus, 'a' differs significantly from 'b') (Kruskal-Wallis tests followed by multiple comparisons with experimentwise $\alpha = 0.05$).

		Category			
FAO Region		GRT	BWCAP	BWOB	% BWCAP
Northeast Atlantic	Mean	59,177 [a]	52,376 [a]	40,690 [a]	79 [a]
	N	19	17	16	16
	S. D.	24,588	25,490	24,045	21
Mediterranean-Black Sea	Mean	59,546 [a]	60,146 [a]	48,803 [a]	79 [a]
	N	17	17	17	17
	S. D.	16,213	22,968	21,488	14
West Central Atlantic	Mean	30,281 [b]	19,079 [b]	11,003 [b]	67 [a]
	N	11	11	11	11
	S. D.	20,615	13,552	8,459	24
Total	Mean	52,548	47,172	36,403	76
	N	47	45	44	44
	S. D.	23,983	27,251	24,956	20

[1] United Nations' Food and Agriculture Organization (FAO) standardized ocean regions of the world.

[2] 1 register ton = 1000 ft^3.

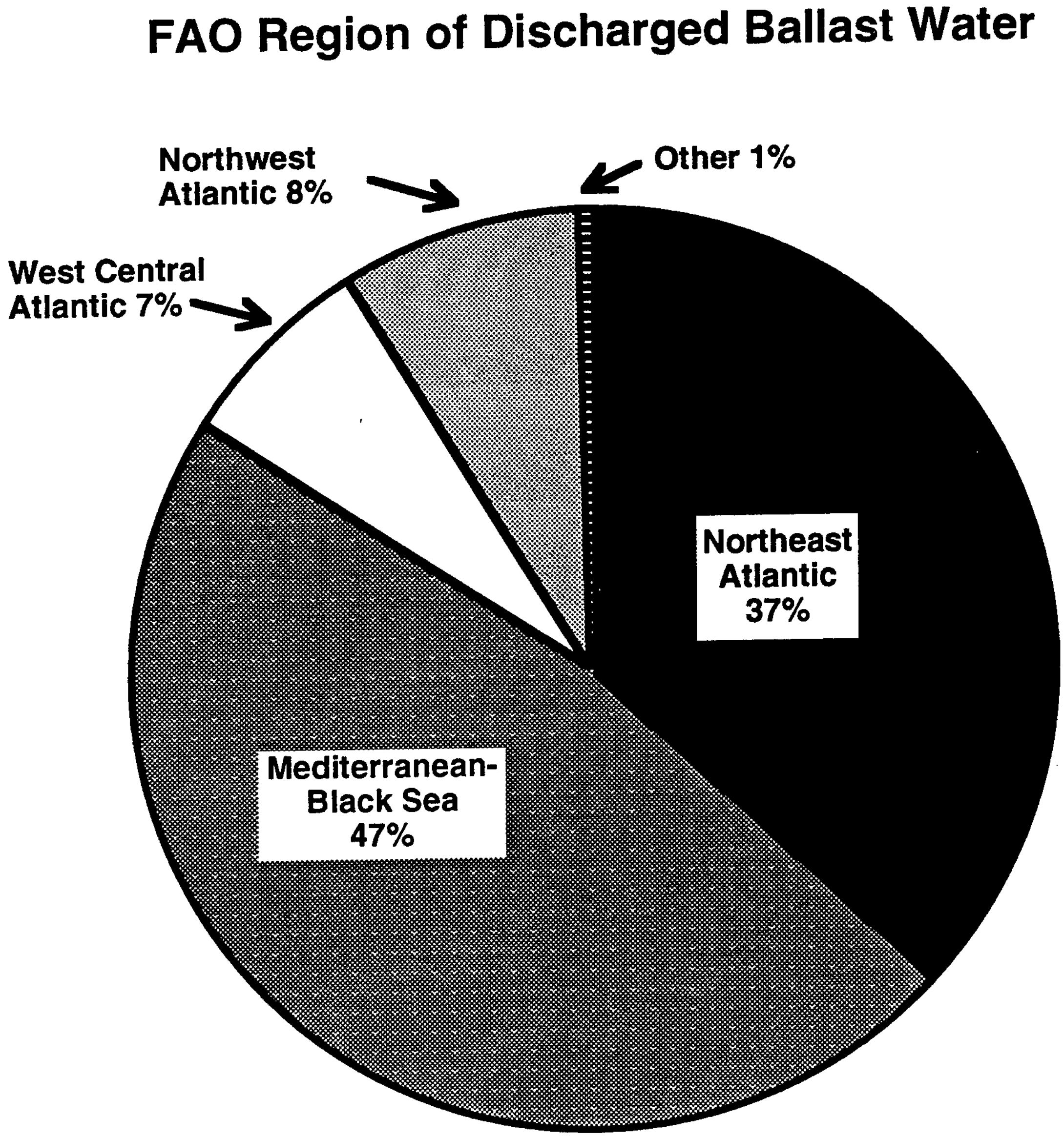

Figure 3-6. Relative amounts of ballast water discharged from bulkers sampled in Baltimore, Maryland and Norfolk, Virginia, between August 1993 and August 1994, by FAO region of ballast water origin. Total amount of ballast water = 1, 761, 593 MT.

Table 3-8

Summary of mean vessel tonnage and amounts of ballast water carried on bulkers sampled in Baltimore, Maryland and Norfolk, Virginia between August 1993 and August 1994 by season[1] for the 3 most common FAO regions[2]. Categories include gross register tonnage (GRT)[3], ballast water capacity (BWCAP, in MT), ballast water on board (BWOB, in MT), % of ballast capacity filled (% BWCAP). N, number of vessels; S. D., 1 standard deviation. Superscripted letters indicate no significant differences among seasons for a given category (Kruskal-Wallis tests followed by multiple comparisons with experimentwise $\alpha = 0.05$).

		Category			
Season		GRT	BWCAP	BWOB	% BWCAP
Fall	Mean	49,153 [a]	46,780 [a]	38,650 [a]	81 [a]
	N	11	11	11	11
	S. D.	21,882	26,538	23,271	13
Winter	Mean	50,463 [a]	42,746 [a]	29,236 [a]	64 [a]
	N	10	9	9	9
	S. D.	28,241	28,648	28,432	28
Spring	Mean	53,883 [a]	45,004 [a]	32,431 [a]	75 [a]
	N	14	13	12	12
	S. D.	23,752	28,439	24,950	19
Summer	Mean	55,898 [a]	53,200 [a]	43,690 [a]	83 [a]
	N	12	12	12	12
	S. D.	24,920	28,102	24,691	14
Total	Mean	52,548	47,172	36,403	76
	N	47	45	44	44
	S. D.	23,983	27,251	24,956	20

[1] Fall, Sept. - Nov.; Winter, Dec. - Feb.; Spring, Mar. - May; Summer, June - Aug.

[2] United Nations' Food and Agriculture Organization (FAO) standardized ocean regions of the world.

[3] 1 register ton = 1000 ft^3.

seasons. For example, ballast water from RoRos was significantly older (65 d), and less saline (21 ppt) than ballast water from bulkers (14 d, 28 ppt respectively) (Wilcoxon tests, $p < 0.01$) (Table 3-9). Temperature of the ballast water in RoRos and bulkers did not differ (Wilcoxon test, $p = 0.58$). In bulkers, ballast water in cargo holds and ballast tanks did not differ significantly in mean temperature, age, or dissolved oxygen (Wilcoxon tests, $p > 0.2$) (Table 3-10). Mean salinity of the ballast water was greater in cargo holds (34 ppt) than in ballast tanks tanks (26 ppt) (Wilcoxon test, $p = 0.002$). When examined by region; however, this difference held true only for Mediterranean/Black Sea and reflected high salinities in the cargo hold of one repeat bulker. Dissolved oxygen (mean ± 1 S.D.) was not limiting to life in either cargo holds (7.2 ± 1.4 mg/l) or ballast tanks (6.9 ± 2.2 mg/l) (Table 3-10). These values were only slightly below saturated values at the mean indicated salinities and temperatures (Beer 1983).

Ballast water in the cargo hold was well-mixed; there was no evidence of vertical stratification of temperature, salinity, or dissolved oxygen (0 to 15 m depth). Water in all but one of the ballast tanks was also unstratified; however, our probe rarely reached past the uppermost level of the ballast tank (usually 3 to 5 m). One one occasion, we were able to measure water from a bulker ballast tank to a depth of 14 m. This vessel, which sailed from St. Petersburg, Russia, attempted an exchange in the mid-Atlantic. Water salinity in the ballast tank ranged from 14 ppt at 7 m depth to 30 ppt at 13.5 m depth, indicating the potential for stratification in these subdivided tanks.

Mean ballast water temperature did not differ in bulkers arriving from the four most common FAO regions for combined seasons (Kruskal-Wallis test, chi-square = 6.4, df = 3, $p = 0.093$) (Table 3-11, Appendix K). Mean ballast water salinity from the Mediterranean/Black Sea (35 ppt), however, was significantly higher than that of the Northeast Atlantic (26 ppt), Northwest Atlantic (26 ppt), or West Central Atlantic (22 ppt) (Kruskal-Wallis test, chi-square = 18.4, df = 3, $p < 0.001$) (Table 3-11, Appendix K). The age of the ballast water arriving from these four regions also differed significantly (Kruskal-Wallis test, chi-square = 39.9, df = 3, $p < 0.001$) (Table 3-11, Appendix K). Mean age was greatest for ballast water arriving from the Mediterranean/Black Sea region (19 d). Ballast water arriving from the Northeast Atlantic region was of intermediate age (14 d). The shortest residence times for ballast water occurred in bulkers carrying water from the Northwest Atlantic (5 d) and the West Central Atlantic (7 d) regions.

Mean temperature of ballast water was significantly higher in summer (26°C) than in the other three seasons when averaged across all regions (Kruskal-Wallis test, chi-square = 34.8, df = 3, $p < 0.001$) (Table 12, Appendix K). In contrast, mean salinity and age of the ballast water showed no significant seasonal variation (Kruskal-Wallis tests, df = 3, $p > 0.29$) (Table 12, Appendix K).

Table 3-9

Comparison of mean temperature (°C), salinity (ppt), and age (days) of ballast water in bulkers and RoRos sampled in Baltimore, Maryland and Norfolk, Virginia between August 1993 and August 1994. N, number of ballast tanks and cargo holds; S. D., 1 standard deviation. Wilcoxon 2-sample test compares vessel types for each category.

		Ballast Water		
Vessel Type		Temp.	Salinity	Age
Bulker	Mean	22	28	14
	N	67	66	66
	S. D.	5	11	11
RoRo[1]	Mean	20	21	65
	N	10	10	10
	S. D.	6	11	70
Wilcoxon test	Z	-0.55	-2.72	2.62
	p-value	0.58	0.007	0.009

[1] One outlier RoRo containing ballast water 730 days old was excluded.

Table 3-10

Comparison of mean ballast water temperature (°C), salinity (ppt), age (days), and dissolved oxygen (D.O., in mg/l) in cargo holds and ballast tanks of bulkers sampled in Baltimore, Maryland and Norfolk, Virginia between August 1993 and August 1994 (n = number of ballast tanks and cargo holds). Wilcoxon 2-sample test compares tank types for each ballast water category.

		Ballast Water			
Tank type		Temp.	Salinity	Age	D. O.
Cargo Hold	Mean	21.0	34.2	14.0	7.2
	N	26	26	26	11
	S. D.	3.7	4.5	5.8	1.4
Ballast Tank	Mean	22.2	26.4	13.8	6.9
	N	59	59	61	22
	S. D.	5.2	11.6	11.6	2.2
Wilcoxon test	z	-1.0	3.1	1.2	1.0
	p-value	0.30	0.002	0.23	0.31

[1] Ballast tanks include wing, wing bottom, double bottom, fore and aft peak tanks.

Table 3-11

Summary of mean temperature (°C), salinity (ppt), and age (days) of ballast water in ballast tanks and cargo holds of bulkers sampled in Baltimore, Maryland and Norfolk, Virginia between August 1993 and August 1994 by 4 most common FAO regions[1] of ballast water origin. N, number of tanks and holds; S. D., 1 standard deviation. Means with the same superscripted letter, read down the column in each category, do not differ significantly from each other (thus, 'a', 'b', and 'c' all differ significantly from each other) (Kruskal-Wallis tests followed by multiple comparisons with experimentwise $\alpha = 0.05$).

FAO Region		Temp.	Salinity	Age
Mediterranean-	Mean	22 [a]	35 [a]	19 [a]
Black Sea	N	18	18	18
	S. D.	4	8	3
Northeast	Mean	20 [a]	26 [b]	14 [b]
Atlantic	N	22	21	21
	S. D.	4	11	7
Northwest	Mean	24 [a]	26 [b]	5 [c]
Atlantic	N	10	10	10
	S. D.	4	12	4
West Central	Mean	23 [a]	22 [b]	7 [c]
Atlantic	N	12	12	12
	S. D.	5	14	3
Total	Mean	22	28	12
	N	62	61	61
	S. D.	5	12	7

[1] United Nations' Food and Agriculture Organization (FAO) standardized ocean regions of the world.

Table 3-12

Summary of mean temperature (°C), salinity (ppt), and age (days) of ballast water in ballast tanks and cargo holds of bulkers sampled in Baltimore, Maryland and Norfolk, Virginia between August 1993 and August 1994 by season[1]. N, number of tanks and holds; S. D., 1 standard deviation. Means with the same superscripted letter, read down the column in each category do not differ significantly from each other (thus, 'a' differs significantly from 'b') (Kruskal-Wallis tests followed by multiple comparisons with experimentwise $\alpha = 0.05$).

Season		Temp.	Salinity	Age
Fall	Mean	21^{a}	32^{a}	16^{a}
	N	17	17	17
	S. D.	3	6	10
Winter	Mean	17^{a}	25^{a}	14^{a}
	N	11	2	12
	S. D.	3	14	10
Spring	Mean	20^{a}	25^{a}	13^{a}
	N	15	15	15
	S. D.	4	12	6
Summer	Mean	26^{b}	28^{a}	14^{a}
	N	23	23	22
	S. D.	4	12	15
Total	Mean	22	28	14
	N	67	66	66
	S. D.	5	11	28

[1] Fall, Sept. - Nov.; Winter, Dec. - Feb.; Spring, Mar. - May; Summer, June - Aug.

Comparisons between Ballast Water and Baltimore Harbor Water: Temperature and Salinity

The temperature and salinity of ballast water discharged into Baltimore harbor varied greatly as a result of seasonal differences and the disparate source regions. Between August 1993 and August 1994, temperatures of ballast water discharged by bulkers into Baltimore harbor ranged between 11°C and 35°C (Fig. 3-7A). The majority of ballast water had temperatures between 16 and 20°C. The salinity range of ballast water discharged by bulkers into Baltimore harbor was extreme (0 to 45 ppt); however, peak discharge occurred at higher salinities (36 to 40 ppt) (Fig. 3-7B).

While temperature differences between ballast water and harbor water were slight (< 5°C) for over half of the ballast water released into Baltimore harbor (Fig. 3-8A), the remaining ballast water differed from the port receiving waters by 6°C to 20°C. Ballast and port water temperatures matched most consistently when ships arrived with ballast water from the Northwest Atlantic (Fig. 3-9A, see also Appendix L) or in summer (Fig. 3-10A, see also Appendix L).

Salinity differences between ballast water and harbor water were great (Fig. 3-8B). Over 80% of the ballast water discharged was at least 21 ppt higher in salinity than that of Baltimore harbor. Salinity differences between ballast water and harbor water were high regardless of source region of the ballast water (Fig. 3-9B, see also Appendix L) or the season in which bulkers arrived (Fig. 3-10B, Appendix L).

Overall, there were few instances where both the salinity <u>and</u> temperature of the discharged ballast water matched conditions in Baltimore harbor. Of 47 deballasting cargo holds and ballast tanks sampled in Baltimore harbor between August 1993 and August 1994, only 5 (10.6%) had ballast water temperatures and salinities differing by ≤ 5°C or ≤ 5 ppt respectively from that of the harbor water (Fig. 3-11).

Biological Information: Live Analyses

Ninety-one percent of the 70 vessels sampled in our survey contained live aquatic organisms. Living organisms were collected in all seasons and from 7 of 8 FAO regions (nothing was found in the lone vessel from East Central Atlantic). These organisms included representatives from 15 animal and 3 protist phyla, 2 plant divisions and cyanobacteria (Tables 3-13, 3-14). A minimum of 282 distinctly different taxa were identified (Tables 3-13, 3-14). The number of total taxa was calculated as follows: 192 taxa were identified in plankton samples from ballasted cargo holds and ballast tanks in bulk cargo vessels. For 29 groups of zooplankton or phytoplankton sampled by plankton net (Cirripedia, Harpacticoida, Calanoida, Cyclopoida, Poecilostomatoida, Brachyura, Anomura, Caridea, Mysidacea, Isopoda, Amphipoda, Ostracoda, Branchiopoda, Nereidae, Phyllodocidae, Spionidae, Platyhelminthes, Bivalvia, Heteropoda, Hydrozoa, Chaetognatha, Ctenophora, Echinoidea, Rotifera, Nematoda, Dinoflagellida, Tintinnida, Other Ciliata, and Diatomacea), we arbitrarily added "1" taxon when we knew we had at least one additional unidentified member of

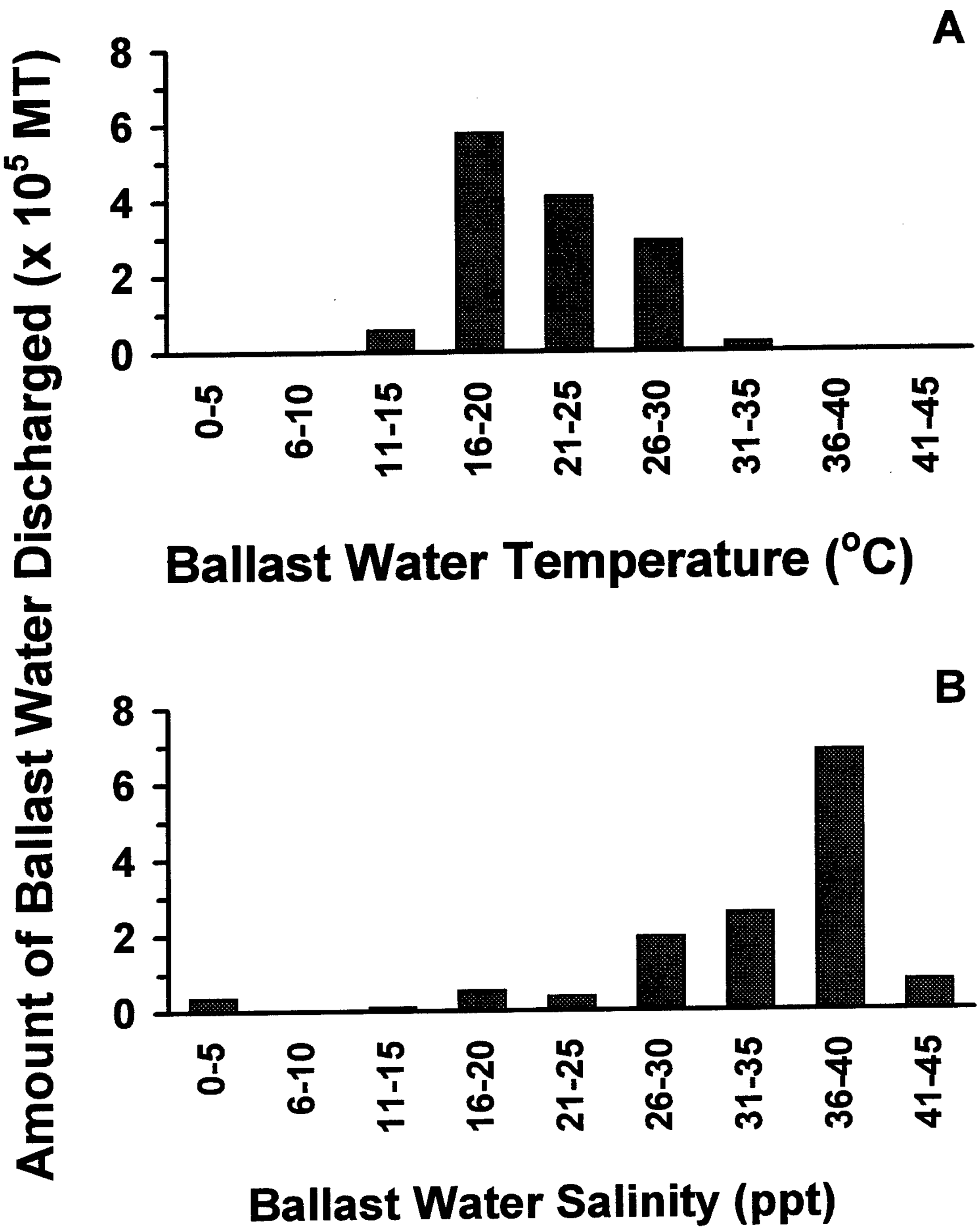

Figure 3-7. Distribution of the amount of ballast water discharged (x 10^5 MT) by bulkers sampled in Baltimore, Maryland as a function of ballast water (A) temperature (n = 54 vessels) and (B) salinity (n = 53 vessels) between August 1993 and August 1994.

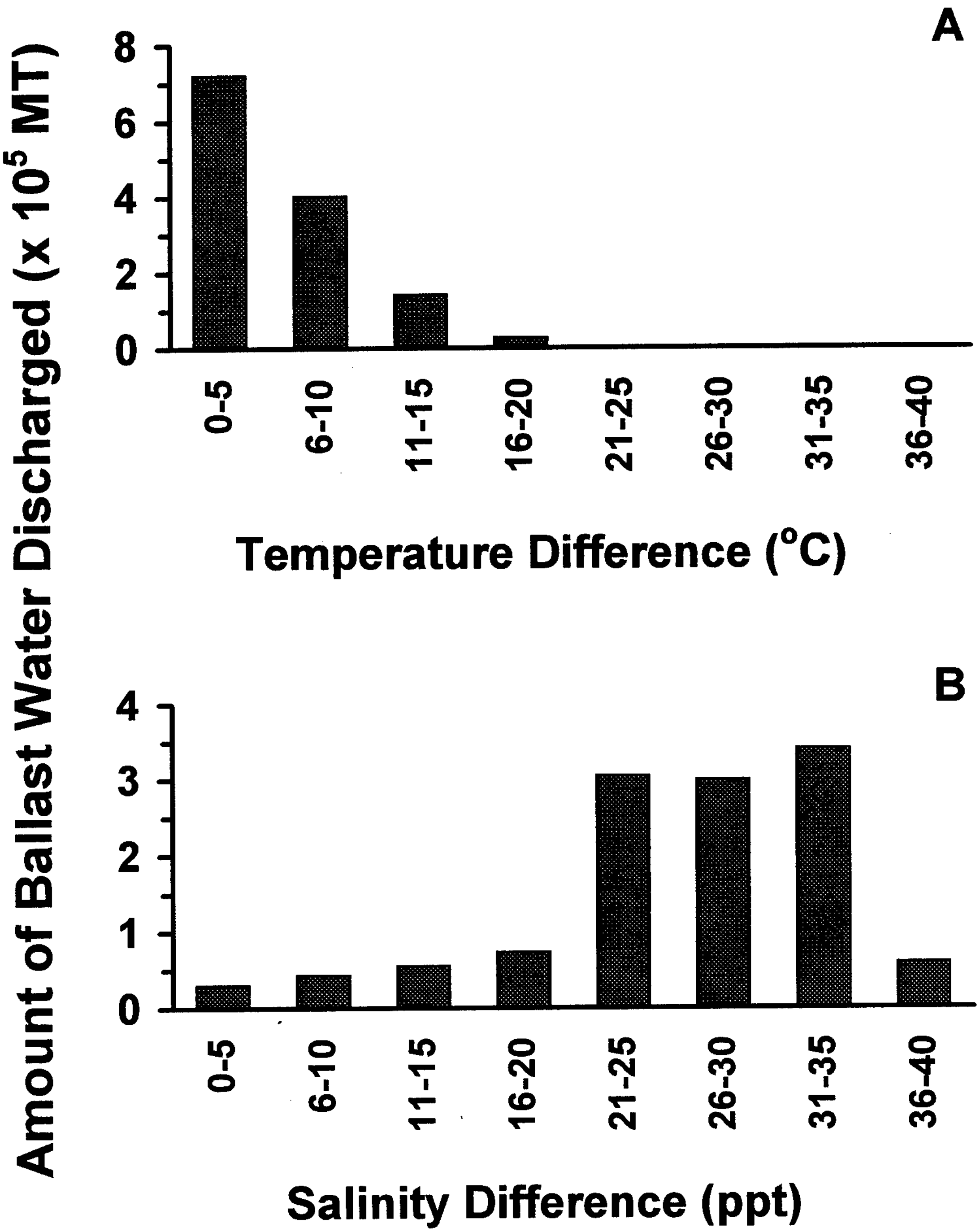

Figure 3-8. Distributions of the amount of ballast water discharged (x 10^5 MT) by bulkers sampled in Baltimore, Maryland as a function of differences in (A) temperature (n = 51 vessels) and (B) salinity (n = 47 vessels) between ballast and port water (August 1993 to August 1994).

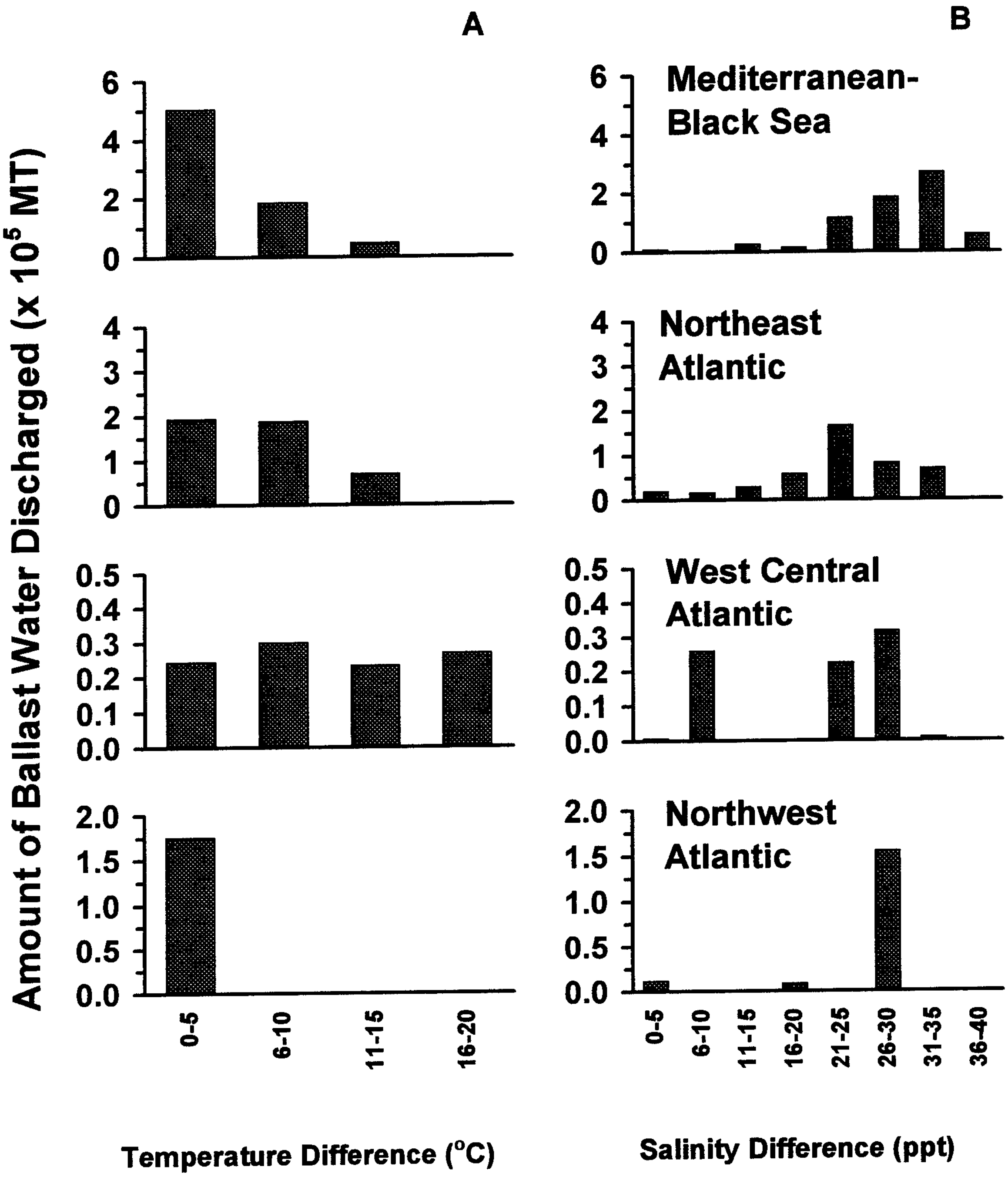

Figure 3-9. Distributions of the amount of ballast water discharged (x 10^5 MT) by bulkers sampled in Baltimore, Maryland as a function of differences in (A) temperature (n = 46 vessels) and (B) salinity (n = 43 vessels) between ballast and port water for the 4 main FAO regions of ballast water origin (August 1993 to August 1994). Y-axes differ among regions.

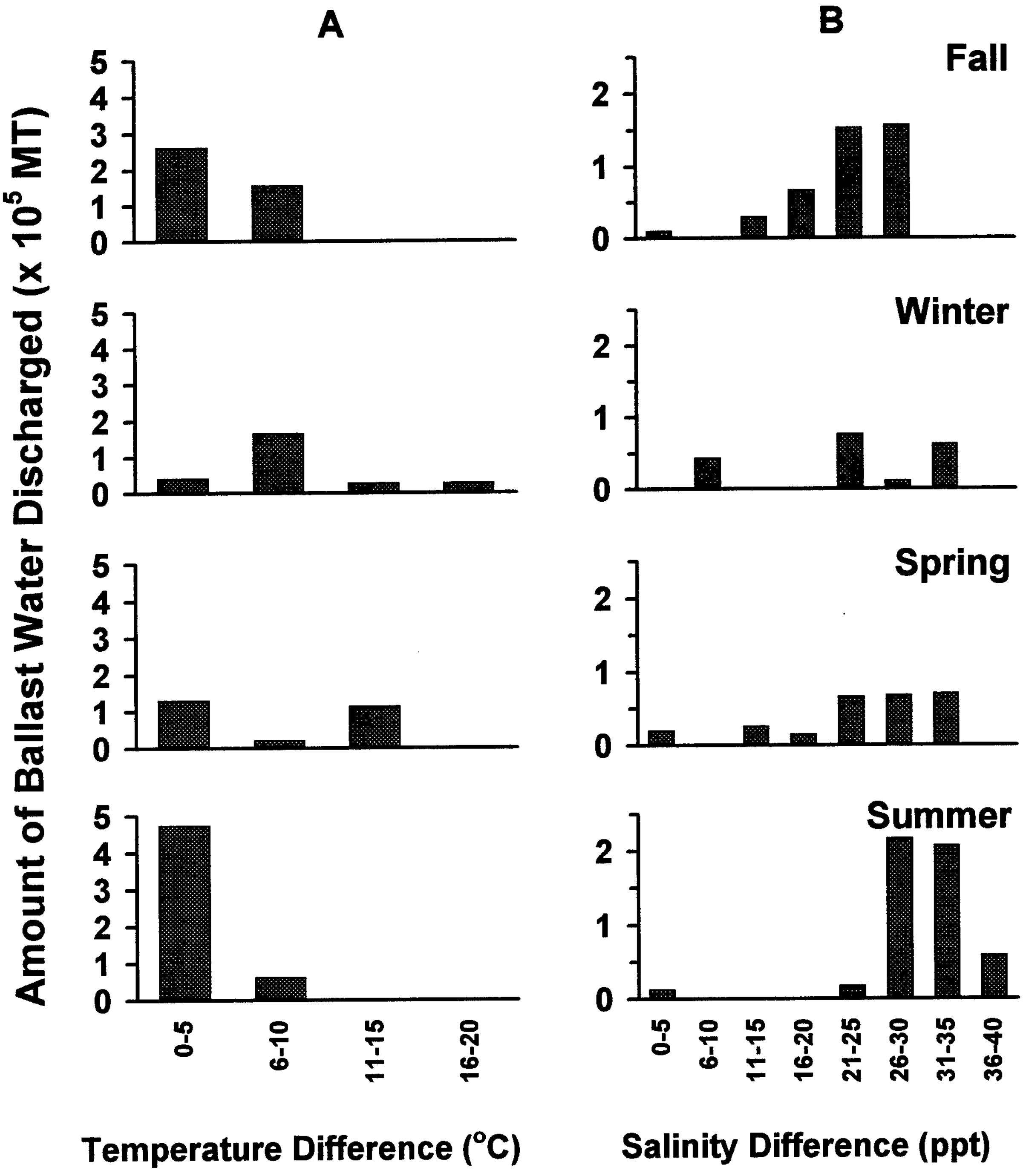

Figure 3-10. Distribution of the amount of ballast water discharged (x 10^5 MT) by bulkers sampled in Baltimore, Maryland as a function of differences in (A) temperature (n = 51 vessels) and (B) salinity (n = 47 vessels) between ballast and port water for each season (August 1993 to August 1994).

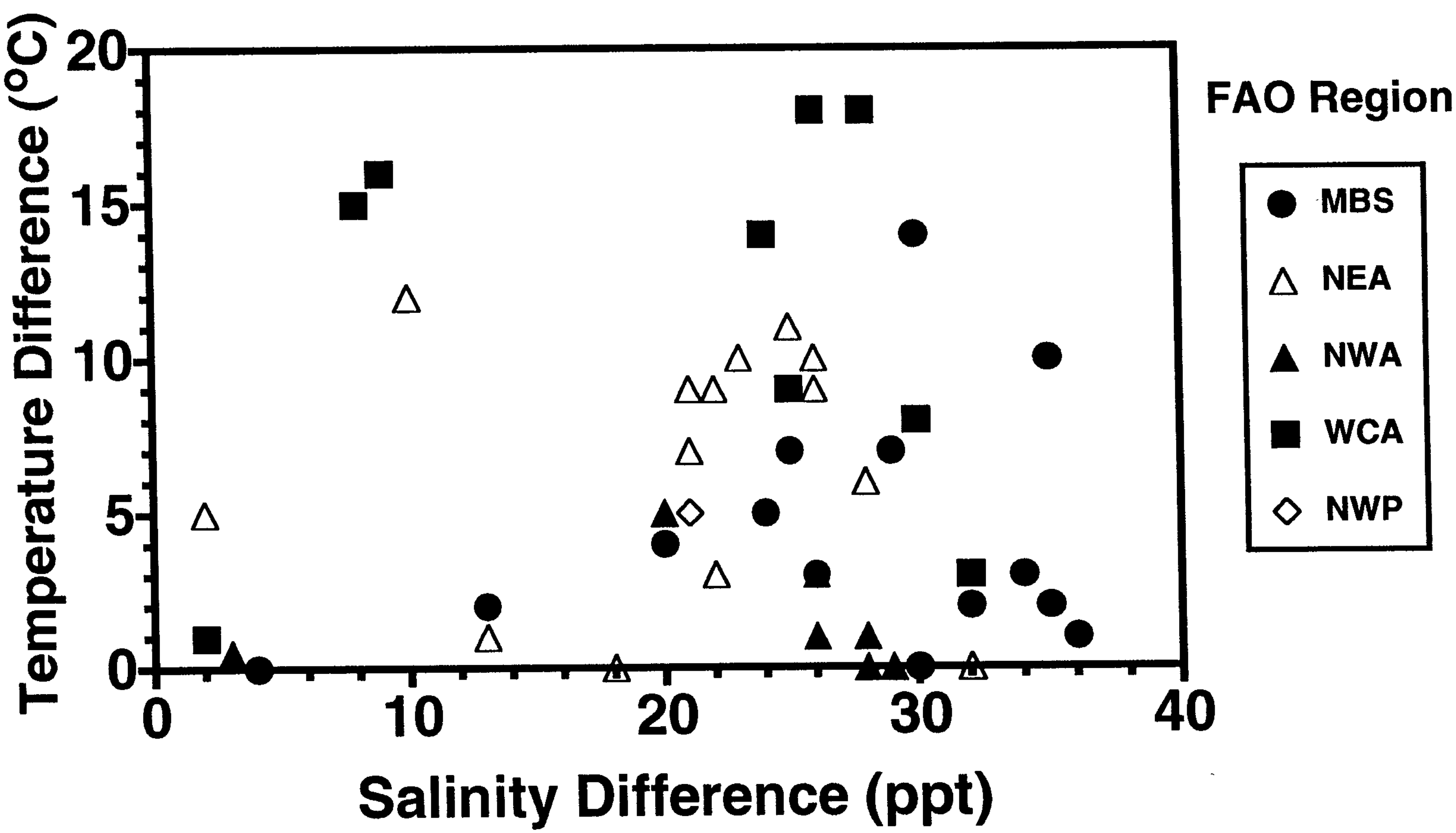

Figure 3-11. Difference in temperature (°C) and salinity (ppt) between port water and deballasted water in Baltimore, Maryland, between August 1993 and August 1994. N = 47 tanks in 43 vessels. FAO regions: MBS, Mediterranean Black Sea; NEA, Northeast Atlantic; NWA, Northwest Atlantic; WCA, West Central Atlantic; NWP, Northwest Pacific.

Table 3-13

Percentage occurrence and abundance of organisms in ballast water of cargo holds (n = 24 vessels) and ballast tanks (n = 47 vessels) on bulkers sampled in Baltimore, Maryland and Norfolk, Virginia between August 1993 and August 1994.

		Cargo Holds					Ballast Tanks				
		Ships (%) in which taxon was					Ships (%) in which taxon was				
		Abundant	Common	Rare	Present		Abundant	Common	Rare	Present	
Taxon	No. Taxa[1]	(> 100/ replicate[2])	(10 to 100/ replicate)	(<10/ replicate)	Quantitative[3]	Qualitative[4]	(> 100/ replicate)	(10 to 100/ replicate)	(<10/ replicate)	Quantitative[3]	Qualitative[4]
Crustacea	***97***	***33.3***	***50.0***	***16.7***	***100.0***	***100.0***	***12.8***	***31.9***	***25.5***	***70.2***	***89.4***
Cirripedia	5	0	20.8	54.2	75.0	75.0	0	14.9	21.3	36.2	48.9
Copepoda	60	58.3	37.5	4.2	100.0	100.0	19.1	31.9	19.1	70.2	89.4
Harpacticoida	17	12.5	33.3	41.7	87.5	87.5	0	8.5	25.5	34.0	53.2
Calanoida	21	25.0	29.2	41.7	95.8	95.8	2.1	14.9	29.8	46.8	57.4
Cyclopoida	11	4.2	12.5	29.2	45.8	45.8	0	10.6	10.6	21.3	29.8
Poecilostomatoida	11	0	37.5	41.7	79.2	79.2	0	6.4	14.9	21.3	36.2
Copepod nauplii & copepodites	-	16.7	50.0	25.0	91.7	95.8	10.6	21.3	14.9	46.8	63.8
Decapoda	9	0	0	29.2	29.2	33.3	0	4.3	8.5	12.8	19.1
Brachyura	4	0	0	8.3	8.3	20.8	0	4.3	6.4	10.6	12.8
Anomura	2	0	0	8.3	8.3	8.3	0	0	2.1	2.1	6.4
Caridea	2	0	0	8.3	8.3	8.3	0	0	2.1	2.1	2.1
Penaeidea	1	0	0	0	0	0	0	0	2.1	2.1	2.1
Decapod zoeae & megalopae	-	0	0	8.3	8.3	8.3	0	0	0	0	0
Euphausiacea	1	0	0	12.5	12.5	12.5	0	0	0	0	0
Stomatopoda	1	0	0	8.3	8.3	8.3	0	0	0	0	0
Cumacea	1	0	0	0	0	0	0	0	2.1	2.1	2.1
Mysidacea	5	0	0	20.8	20.8	20.8	0	0	0	0	0
Isopoda	3	0	0	20.8	20.8	25.0	0	0	4.3	4.3	12.8
Amphipoda	6	0	0	25.0	25.0	25.0	0	0	2.1	2.1	4.3
Gammaridea	2	0	0	16.7	16.7	16.7	0	0	2.1	2.1	2.1
Hyperiidea	3	0	0	8.3	8.3	8.3	0	0	0	0	2.1
Ostracoda	4	0	0	12.5	12.5	12.5	0	0	0	0	0
Branchiopoda	2	0	0	0	0	0	2.1	2.1	0	4.3	10.6
Crustacean nauplii	-	0	0	4.2	4.2	4.2	0	0	0	0	0
Annelida	***27***	***4.2***	***37.5***	***33.3***	***75.0***	***87.5***	***4.3***	***14.9***	***14.9***	***34.0***	***46.8***
Capitellidae	1	0	0	4.2	4.2	4.2	0	0	0	0	0
Chaetopteridae	1	0	0	4.2	4.2	4.2	0	0	2.1	2.1	4.3
Cirratulidae	1	0	0	4.2	4.2	4.2	0	0	0	0	0
Dinophilidae	1	0	0	0	0	0	0	0	0	0	2.1

[1] Minimum number of distinct taxonomic groups identified in quantitative and qualitative samples.

[2] Mean tow volume ± 1 S.D.: cargo hold = 1.32 + 0.32 m^3 (n = 24 holds); ballast tank = 0.22 + 0.16 m^3 (n = 62 ballast tanks).

[3] Percentage occurrence in vessels sampled by replicate quantitative plankton tows.

[4] Percentage occurrence in vessels sampled by replicate, quantitative plankton tows plus occurrence in non-quantitative plankton tows and opportunistic dip net and sediment sampling. Sediment samples from drydocked vessels and whole water samples excluded.

Table 3-13 contd.

Taxon		Cargo Holds: Abundant (> 100/ replicate)	Cargo Holds: Common (10 to 100/ replicate[1])	Cargo Holds: Rare (<10/ replicate)	Cargo Holds: Present Quantitative[2]	Cargo Holds: Present Qualitative[3]	Ballast Tanks: Abundant (> 100/ replicate)	Ballast Tanks: Common (10 to 100/ replicate)	Ballast Tanks: Rare (<10/ replicate)	Ballast Tanks: Present Quantitative[2]	Ballast Tanks: Present Qualitative[3]
Dorveillidae	1	0	0	4.2	4.2	4.2	0	0	0	0	0
Magelonidae	1	0	0	0	0	0	0	0	0	0	2.1
Nereidae	2	0	4.2	8.3	12.5	12.5	0	0	0	0	2.1
Phyllodocidae	3	0	4.2	20.8	25.0	25.0	0	0	4.3	4.3	10.6
Polynoidae	1	0	0	16.7	16.7	16.7	0	0	2.1	2.1	6.4
Sabellaridae	1	0	0	0	0	0	0	0	0	0	2.1
Syllidae	2	0	0	4.2	4.2	8.3	0	0	2.1	2.1	6.4
Spionidae	11	4.2	29.2	33.3	66.7	75.0	4.3	10.6	17.0	31.9	40.4
Terebellidae	1	0	0	8.3	8.3	8.3	0	2.1	4.3	6.4	8.5
Polychaete larvae	-	0	4.2	41.7	45.8	50.0	0	2.1	6.4	8.5	21.3
Platyhelminthes	***3***	***0***	***0***	***45.8***	***45.8***	***50.0***	***0***	***0***	***6.4***	***6.4***	***14.9***
Mollusca	***8***	***8.3***	***8.3***	***41.7***	***58.3***	***58.3***	***4.3***	***6.4***	***21.3***	***31.9***	***44.7***
Bivalvia	2	8.3	8.3	29.2	45.8	45.8	4.3	6.4	21.3	31.9	44.7
Gastropoda	5	0	0	25.0	25.0	29.2	0	4.3	8.5	12.8	21.3
Heteropoda	2	0	0	8.3	8.3	8.3	0	0	0	0	0
Other Prosobranchia	1	0	0	20.8	20.8	25.0	0	4.3	8.5	12.8	19.1
Pteropoda	1	0	0	12.5	12.5	12.5	0	0	0	0	0
Nudibranchia	1	0	0	0	0	0	0	0	0	0	2.1
Polyplacophora	1	0	0	4.2	4.2	4.2	0	0	0	0	0
Cnidaria	***7***	***0***	***8.3***	***33.3***	***41.7***	***45.8***	0	***2.1***	***2.1***	***4.3***	***6.4***
Scyphozoa	1	0	0	8.3	8.3	8.3	0	0	0	0	0
Hydrozoa	6	0	8.3	25.0	33.3	37.5	0	2.1	2.1	4.3	6.4
Chaetognatha	***9***	***0***	***4.2***	***29.2***	***33.3***	***33.3***	***0***	***0***	***4.3***	***4.3***	***8.5***
Ctenophora	***4***	***0***	***0***	***20.8***	***20.8***	***20.8***	***0***	***0***	***2.1***	***2.1***	***2.1***
Echinodermata	***4***	***4.2***	***0***	***12.5***	***16.7***	***16.7***	0	***2.1***	***2.1***	***4.3***	***6.4***
Asteroidea	1	0	0	16.7	16.7	16.7	0	0	0	0	4.3
Echinoidea	2	4.2	0	0	4.2	4.2	0	0	2.1	2.1	2.1
Ophiuroidea	1	0	0	4.2	4.2	4.2	0	2.1	0	2.1	2.1
Echinoderm larvae	-	0	0	4.2	4.2	4.2	0	0	0	0	0
Rotifera	***5***	***0***	***4.2***	***12.5***	***16.7***	***16.7***	***2.1***	***0***	***8.5***	***10.6***	***17.0***
Bryozoa	***1***	***0***	***0***	***12.5***	***12.5***	***12.5***	***0***	***0***	***0***	***0***	***2.1***
Nematoda	***3***	***0***	***0***	***12.5***	***12.5***	***16.7***	***0***	***2.1***	***12.8***	***14.9***	***21.3***

Table 3-13 contd.

Taxon		Cargo Holds: Ships (%) in which taxon was: Abundant (> 100/ replicate[1])	Common (10 to 100/ replicate)	Rare (<10/ replicate)	Present: Quantitative[2]	Present: Qualitative[3]	Ballast Tanks: Ships (%) in which taxon was: Abundant (> 100/ replicate)	Common (10 to 100/ replicate)	Rare (<10/ replicate)	Present: Quantitative[2]	Present: Qualitative[3]
Chordata	***8***	***0***	***4.2***	***4.2***	***8.3***	***25.0***	0	0	***2.1***	***2.1***	***4.3***
Urochordata	2	0	4.2	4.2	8.3	8.3	0	0	2.1	2.1	2.1
Ascidiacea	1	0	4.2	4.2	8.3	8.3	0	0	0	0	0
Larvacea	1	0	0	0	0	0	0	0	2.1	2.1	2.1
Pisces	6	0	0	4.2	4.2	20.8	0	0	0	0	2.1
Carangidae	1	0	0	0	0	4.2	0	0	0	0	0
Clupeidae	1	0	0	0	0	4.2	0	0	0	0	0
Engraulidae	1	0	0	0	0	4.2	0	0	0	0	0
Gasterosteidae	1	0	0	0	0	0	0	0	0	0	2.1
Gobiidae	1	0	0	0	0	4.2	0	0	0	0	0
Soleidae	1	0	0	0	0	4.2	0	0	0	0	0
Pisces (eggs)	-	0	0	4.2	4.2	4.2	0	0	0	0	0
Phoronida	***1***	***0***	***0***	***4.2***	***4.2***	***4.2***	***0***	***0***	***0***	***0***	***0***
Nemertea	***1***	***0***	***4.2***	***0***	***4.2***	***4.2***	***0***	***0***	***6.4***	***6.4***	***6.4***
Sipuncula	***1***	***0***	***0***	***4.2***	***4.2***	***8.3***	***0***	***0***	***0***	***0***	***4.3***
Eggs	***-***	***0***	***12.5***	***16.7***	***29.2***	***29.2***	***0***	***6.4***	***4.3***	***10.6***	***14.9***
Sarcomastigophora	***21***	***16.7***	***37.5***	***25.0***	***79.2***	***83.3***	***2.1***	***8.5***	***10.6***	***21.3***	***21.3***
Dinoflagellida	18*	12.5	41.7	25.0	79.2	83.3	2.1	8.5	8.5	19.1	21.3
Radiolaria & Acantharea	2	4.2	4.2	25.0	33.3	33.3	0	0	2.1	2.1	2.1
Foraminifera	1	0	4.2	16.7	20.8	20.8	0	0	0	0	0
Ciliophora	***6****	***0***	***12.5***	***33.3***	***45.8***	***50.0***	***6.4***	***4.3***	***6.4***	***17.0***	***23.4***
Tintinnida	3	0	0	16.7	16.7	20.8	0	0	0	0	2.1
Other Ciliata	3	0	12.5	20.8	33.3	37.5	6.4	4.3	6.4	17.0	21.3
Diatomacea	***17***	***12.5***	***33.3***	***16.7***	***62.5***	***62.5***	***6.4***	***21.3***	***27.7***	***55.3***	***70.2***
Rhodophyta	***1***	***0***	***0***	***4.2***	***4.2***	***4.2***	***0***	***0***	***0***	***0***	***0***
Cyanobacteria	***1***	***0***	***0***	***0***	***0***	***0***	***2.1***	***0***	***0***	***2.1***	***2.1***

Table 3-14

Summary of taxonomic identifications and regions of ballast water origin. Includes 70 vessels sampled for ballast water and sediments, and 5 vessels sampled for sediment only. Each taxonomic group summarizes the taxa listed below it.

		FAO Region of Ballast Water Origin[3]				
Taxon[1]	Sample[2]	Northeast Atlantic	Northwest Atlantic	Mediterranean - Black Sea	West Central Atlantic	Other
Crustacea	**B,S**	**NEA**	**NWA**	**MBS**	**WCA**	**NWP, LP[4]**
Amphipoda	B	NEA	NWA?			
Dexaminidae	B	NEA				
Gammaridae	B	NEA	NWA?			
Gammarus sp.	B	NEA	NWA?			
Hyperiidae	B	NEA	NWA?			
Lestrigonus bengalensis	B	NEA				
Lestrigonus schizogeneios	B	NEA				
Themisto compressa	B	NEA	NWA?			
Branchiopoda	B	NEA	NWA?		WCA	LP
Daphnia sp.	B	NEA	NWA?		WCA	LP
Cirripedia	B	NEA	NWA	MBS	WCA	
Balanus improvisus	B	NEA	NWA	MBS		
Balanus trigonus	B			MBS		
Balanus venustus	B				WCA	
?*Lepas* sp. or ?*Conchoderma* sp.	B	NEA				

[1] Organisms identified to lowest possible taxonomic level. ? or c.f. denotes uncertain identifications.

[2] Sampling technique: B, ballast water collected by quantitative or qualitative plankton net tow or dip net; W, whole water sample; S, sediment sample or sweep net in deballasted tank.

[3] An unmodified region indicates taxon was collected from one source region only. A question mark indicated a taxon was collected in water of mixed origin (i.e. partially exchanged or topped). In such cases, if the region is enclosed by parentheses, then it is the secondary source (< 50%) of the ballast water in the tank. If question mark is present, but parentheses absent, then region is the primary source (> 50% of the ballast water in tank.

[4] Lake Panama (fresh water; not an FAO region).

* denotes uncertain taxonomic identification in a particular region.

Table 3-14 contd.

Taxon[1]	Sample[2]	FAO Region of Ballast Water Origin[3]				
		Northeast Atlantic	Northwest Atlantic	Mediterranean - Black Sea	West Central Atlantic	Other
Copepoda	B,S	NEA	NWA	MBS	WCA	LP
Calanoida	B	NEA	NWA	MBS	WCA	
Acartiidae	B	NEA	(NWA)?	MBS	WCA	
Acanthacartia tonsa	B			MBS		
Acartia clausi	B	NEA				
Acartia sp.	B	NEA	(NWA)?	MBS		
Acartia tonsa	B	NEA			WCA	
Calanidae	B	NEA	(NWA)?	MBS?	WCA	
Calanus helgolandicus	B	NEA	(NWA)?	MBS?	WCA	
Candaciidae	B	NEA				
Candacia sp.	B	NEA				
Centropagidae	B	NEA				
Centropages hamatus	B	NEA				
Centropages sp.	B	NEA				
Clausocalanidae	B			MBS		
Clausocalanus furcatus	B			MBS		
Sapphirina nigromaculata	B			MBS		
Euchaetidae	B	NEA				
Euchaeta sp.	B	NEA				
Paracalanidae	B	NEA	(NWA)?	MBS?	WCA	
Parvocalanus sp.	B				WCA	
Pontellidae	B	NEA*	(NWA)?	MBS?	WCA	
Labidocera sp.	B				WCA	
Pontellina plumata	B	NEA*	(NWA)?	MBS?		
Scolecitrichidae	B	NEA			WCA	
Phaenna spinifer	B				WCA	
Pseudocalanus elongatus	B	NEA				
Temoridae	B	NEA	(NWA)?	MBS	WCA	
Eurytemora affinis	B				WCA	
Eurytemora hirundoides	B	NEA				
Temora longicornis	B	NEA	(NWA)?		WCA?	
Temora stylifera	B		(NWA)?	MBS		
Cyclopoida	B	NEA	NWA	MBS	WCA	
Cyclopidae	B	NEA		MBS?	WCA	
Acanthocyclops sp.	B	NEA		MBS?		
Acanthoyclops robustus	B				WCA	
Cyclopina ?litoralis	B	NEA				
?*Cyclops* sp.	B	NEA				
Diacyclops bicuspidatus	B				WCA	

Table 3-14 contd.

Taxon[1]	Sample[2]	FAO Region of Ballast Water Origin[3]				
		Northeast Atlantic	Northwest Atlantic	Mediterranean - Black Sea	West Central Atlantic	Other
Oithonidae	B	NEA	(NWA)?	MBS	WCA	
Oithona ?brevicornis	B			MBS		
Oithona nana	B		(NWA)?	MBS		
Oithona ozwaldocruzi	B		(NWA)?		WCA	
Oithona sp.	B	NEA		MBS?		
Sapphirinidae	B	(NEA)?	NWA?			
?*Sappharina* sp.	B	(NEA)?	NWA?			
Harpacticoida	B	NEA	NWA	MBS	WCA	LP*
Clytemnestridae	B		(NWA)?		WCA	
Clytemnestra rostrata	B				WCA	
Clytemnestra sp.	B		(NWA)?		WCA?	
Diosaccidae	B				WCA	
Ectinosomatidae	B		(NWA)?	MBS	WCA	LP*
Microsetella norvegica	B		(NWA)?	MBS		LP*
Microsetella rosea	B		(NWA)?		WCA?	
?*Tetanopsis* ?*mediterranea*	B			MBS		
Laophontidae	B	NEA	(NWA)?	MBS		
Miraciidae	B	NEA	NWA	MBS	WCA	
Macrosetella gracilis	B				WCA	
?*Macrosetella* sp.	B		(NWA)?	MBS?		
?*Miracia* ?*efferata*	B	(NEA)?	NWA?			
Oculosetella gracilis	B	NEA				
Peltidiidae	B	NEA				
Alteutha interrupta	B	NEA				
Tachidiidae	B	NEA	(NWA)?	MBS	WCA	
Euterpina acutifrons	B	NEA	(NWA)?	MBS	WCA	
Tachidius discipes	B	NEA				
Tisbidae	B				WCA	
Tisbe sp.	B				WCA	
Tisbidae scutellidum	B				WCA	
Poecilostomatoida	B	NEA	NWA	MBS	WCA	
Clausidiidae	B	NEA	(NWA)?*	MBS?		
Hemicyclops sp.	B	NEA?	(NWA)?		WCA?	
Leptinogaster sp.	B		(NWA)?	MBS?		
Corycaeidae	B	NEA?	(NWA)?	MBS	WCA	
Conaea sp.	B			MBS		
Corycaeus limbatus	B				WCA	
Corycaeus sp.	B	NEA?	(NWA)?	MBS?	WCA	

Table 3-14 contd.

Taxon[1]	Sample[2]	FAO Region of Ballast Water Origin[3] Northeast Atlantic	Northwest Atlantic	Mediterranean - Black Sea	West Central Atlantic	Other
Farranula carinata	B		(NWA)?	MBS?	WCA	
Oncaeidae	B		(NWA)?	MBS	WCA	
Oncaea media	B			MBS		
Oncaea mediterranea	B		(NWA)?	MBS?	WCA	
Oncaea sp.	B			MBS		
Oncaea venusta	B			MBS		
Cumacea	B				WCA	
Decapoda	B,S	NEA		MBS	WCA	
Anomura	B	NEA			WCA	
?*Pagurus* sp.	B				WCA	
Brachyura	B	NEA		MBS	WCA	
Grapsidae	B			MBS		
Pachygrapsus marmoratus	B			MBS		
Xanthidae	B, S	NEA		MBS	WCA	
Dyspanopeus texanus	B				WCA	
?*Microcassiope* ?*minor*	B			MBS		
Caridea	B	NEA		MBS	WCA	
Crangon crangon	S	NEA				
Penaeidea	B		(NWA)?		WCA?	
?*Trachypenaeus* sp. or ?*Parapenaeus* sp.	B		(NWA)?		WCA?	
Euphausiacea	B	NEA	(NWA)?	MBS		
Isopoda	B	NEA	(NWA)?	MBS	WCA	NWP
Cryptoniscus sp.	B				WCA	
Cymothidae	B				WCA	
Mysidacea	B	NEA	NWA?	MBS		
Anchialina ?*agilis*	B			MBS		
Gastrosaccus spinifer	B	(NEA)?	NWA?			
Mesopodopsis slabberi	B	NEA				
Erythropini	B		(NWA)?	MBS?		

Table 3-14 contd.

Taxon[1]	Sample[2]	FAO Region of Ballast Water Origin[3]				
		Northeast Atlantic	Northwest Atlantic	Mediterranean - Black Sea	West Central Atlantic	Other
Ostracoda	B	NEA	(NWA)?	MBS		LP
Conchoecia oblonga	B	NEA				
Conchoecia spinirostris	B	NEA	(NWA)?	MBS		
Euconchoecia chierchiae	B	NEA				
Stomatopoda	B	NEA	(NWA)?	MBS?		
Annelida	**B,S**	**NEA**	**NWA**	**MBS**	**WCA**	**ECP?**
Capitellidae	B, S	NEA				ECP?
Capitella sp.	B, S	NEA				ECP?
Chaetopteridae	B	NEA			WCA	
?Cirratulidae	B	NEA				
?Dinophilidae	B		(NWA)?	MBS?		
Dorveillidae	B	NEA				
Meiodorvillea sp.	B	NEA				
Magelonidae	B	NEA				
?*Magelona* sp.	B	NEA				
Nereidae	B	NEA				
Nereis sp.	B	NEA				
Phyllodocidae	B	NEA	(NWA)?	MBS?		
?*Nereiphylla* sp.	B		(NWA)?	MBS?		
?*Phyllodoce* sp.	B	NEA				
Polynoidae	B	NEA	(NWA)?			
?Sabellaridae	B				WCA	
Spionidae	B,S	NEA	NWA	MBS	WCA	
Malacoceros sp.	B	NEA				
Minuspio sp.	B			MBS		
Polydora ciliata	B	NEA	NWA?			
Polydora ligni	B	NEA		MBS	WCA	
Polydora sp.	B, S	NEA	NWA?			
Prionospio cirrifera	B			MBS		
Prionospio sp.	B	NEA?	(NWA)?			
Spio filicornis	B				WCA	
Spio sp.	B	NEA?	(NWA)?			
Streblospio benedicti	B	(NEA)?		MBS?		
Syllidae	B	NEA				
Autolytus ?prolifer	B	NEA				
Terebellidae	B	NEA				

Table 3-14 contd.

Taxon[1]	Sample[2]	FAO Region of Ballast Water Origin[3]: Northeast Atlantic	Northwest Atlantic	Mediterranean - Black Sea	West Central Atlantic	Other
Platyhelminthes	**B, S**	**NEA**	**NWA?**	**MBS**	**WCA**	**LP**
Neorhabdocoela	B					LP
Polycladida	B	NEA	(NWA)?	MBS	WCA	
unidentified acotylean	B	NEA	(NWA)?	MBS	WCA	
Mollusca	**B**	**NEA**	**NWA**	**MBS**	**WCA**	
Bivalvia	B	NEA	NWA	MBS	WCA	
Mytilus edulis	B	(NEA)?	NWA?			
Gastropoda	B	NEA	(NWA)?	MBS	WCA	
Heteropoda	B	NEA				
Atlanta sp.	B	NEA				
Other Prosobranchia	B	NEA	(NWA)?	MBS	WCA	
Nudibranchia	B	(NEA)?		MBS?		
Pteropoda	B	NEA				
Polyplacophora	B		(NWA)?	MBS?		
Cnidaria	**B**	**NEA**	**NWA?**	**MBS**	**WCA**	
Hydrozoa	B	NEA	NWA?	MBS	WCA	
Bougainvillia rugosa	B				WCA	
Ectopleura dumortieri	B	NEA		MBS		
Leptomedusa sp.	B	(NEA)?	NWA?			
Obelia sp.	B	NEA?	NWA?		WCA	
Rathkea octopunctata	B	NEA				
Scyphozoa	B	NEA	(NWA)?	MBS?		
Chaetognatha	**B**	**NEA**	**NWA**	**MBS**	**WCA**	
Adhesisagitta hispida	B				WCA	
Flaccisagitta ?enflata	B	NEA?	(NWA)?	MBS		
?Khronitta sp.	B	NEA				
Parasagitta elegans	B	NEA				
Sagitta ?bipunctata	B	NEA				
Sagitta ?tenuis or *?friderici*	B			MBS		
Sagitta setosa	B	NEA?	NWA?			
Spadella ?cephaloptera	B	NEA				

Table 3-14 contd.

Taxon[1]	Sample[2]	FAO Region of Ballast Water Origin[3] Northeast Atlantic	Northwest Atlantic	Mediterranean - Black Sea	West Central Atlantic	Other
Ctenophora	**B**	**NEA**	**(NWA)?**	**MBS**		
?*Haeckelia* sp. or ?*Lampea* sp.	B	NEA?	(NWA)?			
?*Pleurobrachia* ?*pileus*	B	NEA				
?*Pleurobrachia* sp.	B	NEA?	(NWA)?			
Echinodermata	**B**	**NEA**	**NWA?**		**WCA?**	
Asteroidea	B	NEA	NWA?			
Echinoidea		(NEA)?	NWA?		WCA?	
?*Echinocardium* ?*cordatum*	B	(NEA)?	NWA?			
Ophiuroidea	B	NEA				
Rotifera	**B, S**	**NEA**	**NWA**	**MBS**	**WCA**	**LP**
?*Brachionus* sp.	B				WCA	LP
?*Keratella* sp.	B					LP
?*Philodina* sp.	B					LP
?*Synchaeta* sp.	B	NEA	(NWA)?	MBS?		
Bryozoa	**B**	**NEA**	**NWA?**	**MBS**	**WCA**	
Nematoda	**B, S**	**NEA**	**NWA?**	**MBS**	**WCA**	**NWP**
Diplolaimella sp.	B	(NEA)?	NWA?			
Monhystera sp.	S					
Chordata	**B, S**	**NEA**	**NWA**	**MBS**		
Pisces	B	NEA	NWA	MBS		
Carangidae	B		(NWA)?	MBS?		
Alepes djedaba	B		(NWA)?	MBS?		
Clupeidae	B	NEA				
Sprattus sprattus	B	NEA				
Engraulidae	B			MBS		
Engraulis encrasicolus	B			MBS		
Gasterosteidae	B		NWA			
Gasterosteus aculeatus	B		NWA			
Gobiidae	S	NEA				
Pomatoschistus lozanoi	S	NEA				
Soleidae	S	NEA				

Table 3-14 contd.

Taxon[1]	Sample[2]	FAO Region of Ballast Water Origin[3]				
		Northeast Atlantic	Northwest Atlantic	Mediterranean - Black Sea	West Central Atlantic	Other
Urochordata	B	NEA		MBS		
Ascidiacea	B	NEA		MBS		
Larvacea	B	NEA				
Phoronida	**B**	**NEA**				
Nemertea	**B**	**NEA**	**NWA**	**MBS?**		
Sipuncula	**B**	**NEA**		**MBS**		
Sarcomastigophora	**B,S,W**	**NEA**	**NWA**	**MBS**	**WCA**	**NWP**
Dinoflagellida	B, S	NEA	NWA	MBS	WCA	NWP
Ceratocoriidae	B	NEA		MBS?		
Ceratocorys horrida	B	NEA		MBS?		
Dinophysidae	B	NEA				
Ornithocercus magnificus c.f.	B	NEA				
Gymnodiniidae	W	NEA	(NWA)?	MBS?	WCA	
Amphidium sp.	W	NEA	(NWA)?			
Gymnodium sp.	W	NEA?	(NWA)?	MBS?		
Gyrodinium sp.	W	NEA	(NWA)?	MBS?	WCA	
Noctilucidae	B	NEA?	(NWA)?			
Noctiluca sp.	B	NEA?	(NWA)?			
Peridiniidae	B,S,W	NEA	NWA	MBS	WCA	NWP
Ceratium ?horridum	B			MBS		
Ceratium ?tripos	B			MBS		
Ceratium candelabrum	B, W	NEA	(NWA)?	MBS		
Ceratium furca	B			MBS		
Ceratium fusus	B, W	NEA	(NWA?)	MBS		
Ceratium lineatum c.f.	W		(NWA)?	MBS?		
Ceratium longiceps	B	NEA?	(NWA)?			
Ceratium macroceros c.f.	B	NEA	(NWA)?			
Ceratium sp.	B	NEA	NWA	MBS	WCA	NWP
Ceratium sp. 1	B	(NEA)?	NWA?			
Ceratium sp. 2	B		(NWA)?	MBS?		
Diplopsalis sp.	B		(NWA)?	MBS?		
Peridinium sp.	W	(NEA)?		MBS		
Protoperidinium grande c.f.	B	(NEA)?		MBS		
Protoperidinium sp.	B,S,W	NEA	(NWA)?	MBS	WCA	NWP

Table 3-14 contd.

Taxon[1]	Sample[2]	FAO Region of Ballast Water Origin[3]: Northeast Atlantic	Northwest Atlantic	Mediterranean - Black Sea	West Central Atlantic	Other
Scripsiella sp.	W				WCA	
Prorocentridae	W	NEA	(NWA)?			
Prorocentrum minimum	W	NEA?	(NWA)?			
Prorocentrum sp.	W	NEA				
Pyrocystidae	B	NEA				
Pyrocystis noctiluca c.f.	B	NEA				
Foraminifera	B, S	NEA	(NWA)?	MBS?		
Radiolaria & Acantharea	B	NEA	NWA	MBS		
Ciliophora	**B, W**	**NEA**	**NWA**	**MBS**	**WCA**	**NWP**
Litostomatea	W	NEA	(NWA)?	MBS	WCA	
Amphileptus sp.	W	NEA	(NWA)?	MBS	WCA	
Askenasia sp.	W				WCA	
Chaena sp.	W	NEA?	(NWA)?			
Enchelys sp.	W				WCA	
Litinotus sp.	W				WCA	
Nassophorea	W	NEA	(NWA)?	MBS?	WCA	NWP
Aspidisca sp.	W		(NWA)?	MBS?		
Euplotes sp.	W	NEA?	(NWA)?	MBS?	WCA	NWP
Paramecium sp.	W	NEA				
Oligohymenophorea	B, W	NEA	(NWA)?	MBS?	WCA	NWP
Hymenostomatida	W	NEA			WCA	
Scuticociliatida	W	NEA	(NWA)?	MBS?	WCA	NWP
Pleuronema sp.	W					NWP
Porpostoma sp.	W	NEA				
Uronema sp.	W	NEA?	(NWA)?			
unidentified Philasterina	W	NEA				
unidentified species	W	NEA	(NWA)?	MBS?	WCA	NWP
Apostomatida	W	NEA?	(NWA)?			
Sessilida	B, W	NEA	(NWA)?		WCA	
Vorticella sp.	B	NEA				
Zoothamnium pelagicum	B				WCA	
unidentified species	W	NEA	(NWA)?		WCA	
Mobilida	W		(NWA)?	MBS?		

Table 3-14 contd.

Taxon[1]	Sample[2]	FAO Region of Ballast Water Origin[3]				
		Northeast Atlantic	Northwest Atlantic	Mediterranean - Black Sea	West Central Atlantic	Other
Phyllopharyngea	W	NEA	(NWA)?	MBS?	WCA	
Alinostoma sp.	W	NEA				
Chlamydonella sp.	W	NEA				
Orthotrichilia sp.	W	NEA?	(NWA)?			
Pottsiocles sp.	W	NEA?	(NWA)?			
Spiroprorodon sp.	W	NEA	(NWA)?	MBS?	WCA	
Thigmogaster sp.	W	NEA	(NWA)?	MBS?	WCA	
Suctoria	W				WCA	
unidentified genus 1	W				WCA	
unidentified genus 2	W				WCA	
Prostomatea	W	NEA	(NWA)?	MBS?	WCA	NWP
Balanion sp.	W	NEA		MBS?	WCA	NWP
Didinium sp.	W	NEA	(NWA)?			
Mesodinium pulex	W				WCA	
Mesodinium rubrum	W	NEA?	(NWA)?	MBS?	WCA	
Mesodinium sp.	W	NEA	(NWA)?		WCA	
Prorodon sp.	W	NEA				
Urotricha sp.	W	NEA				
Spirotrichea	W	NEA	(NWA)?	MBS?	WCA	
Holosticha sp.	W	NEA?		MBS?		
Strobilidium sp.	W	NEA		MBS?		
Strobilidium sp. 1	W	NEA	(NWA)?	MBS?		
Strobilidium sp. 2	W	NEA	(NWA)?			
Strombidinopsis sp.	W				WCA	
Strombidium sp.	W	NEA	(NWA)?	MBS?		
Strombidium sp. 1	W				WCA	
Strombidium sp. 2	W				WCA	
Strombidium sp. 3	W				WCA	
Strongylidium sp.	W	NEA?	(NWA)?			
Stylonichia sp.	W					NWP
Stichotrichia	W	NEA			WCA	
tintinnids	B, W	NEA	(NWA)?	MBS	WCA	
Tinitinnidium sp.	W	NEA			WCA	
Eutintinnus sp.	W					
Tintinnopsis minuta or *nana*	W	NEA		MBS?		
Tintinnopsis sp.	W	NEA	(NWA)?		WCA	

Table 3-14 contd.

Taxon[1]	Sample[2]	FAO Region of Ballast Water Origin[3]				
		Northeast Atlantic	Northwest Atlantic	Mediterranean - Black Sea	West Central Atlantic	Other
Favella sp.	B	NEA?	(NWA)?			
Diatomacea	**B, S**	**NEA**	**NWA**	**MBS**	**WCA**	**ECP, LP**
Ascillataria sp. or *Pharmidium* sp.	B	NEA?	(NWA)?			
Asterionella ?japonica	B		NWA			
Biddulphia sp.	B	NEA	NWA	MBS?		LP
Chaetoceros sp.	B	NEA	NWA	MBS		
Coscinodiscus ?messanense	B					ECP
Coscinodiscus radiatus	B	NEA?	(NWA)?			
Coscinodiscus robustus	B	NEA?	(NWA)?			
Ditylum brightwellii	B	NEA?	(NWA)?			
Ditylum sp.	B	NEA?	NWA	MBS?	WCA	
Lauderia? sp.	B					LP
Nitzschia sp.	B	NEA?	(NWA)?			
Rhizosolenia sp.	B		(NWA)?	MBS?	WCA	
Skeletonema costatum	B	NEA?	(NWA)?			
Thalassionema sp.	B		(NWA)?	MBS?		
unidentified chain diatom	B	NEA	NWA	MBS	WCA	LP
unidentified discoid	B,S	NEA	NWA	MBS	WCA	
Rhodophyta	**B**	**NEA**				
unidentified filamentous, branched alga	B	NEA				
Cyanobacteria	**B**	**NEA**				
Phaeocystis sp.	B	NEA				
Chlorophyta	**B, S**					**LP**
Pediastrum sp.	B					LP

Diatomacea), we arbitrarily added "1" taxon when we knew we had at least one additional unidentified member of that group; that is, we added 29 species to the total count. To these numbers (192 + 29), we then added 4 additional taxa found solely in benthic sediments in cargo holds (see Table 4-1, footnote), for a total of 225 taxa (Table 3-13). In addition, an additional 8 dinoflagellate and 48 ciliate taxa were found in whole water samples, and 1 additional species of microalga, the chlorophyte *Pediastrum*, was found in water samples of a Ro-Ro vessel from Lake Panama (Table 3-14). Thus, the total number of taxa encountered in the present study was at least 282. This number is an extremely conservative estimate of the diversity reaching Chesapeake Bay. For example, only one bivalve was identified to genus; consequently, we recorded the total number of bivalves as "2" taxa (1 identified + minimum 1 other unidentified species). The actual number of bivalve taxa encountered, considering that veliger larvae occurred in 45% of all cargo holds sampled from many different ports, may well have exceeded 20 or more species.

In the majority of samples, organisms were actively swimming and appeared healthy following their voyage. Evidence of their vigor was demonstrated indirectly by our success in rearing many of these organisms to later stages for identification. The maximum age of ballast water containing a living organism (one copepod nauplius in Rhine River water) was 41 d. Of the 6 vessels in which no life was found, 4 were RoRos whose ballast water was 132 to 730 d old; the other 2 were bulkers (one vessel had undergone mid-ocean exchange; the other was sampled in winter). Both bulkers had water that was 15 d old. *Significantly, the absence of life in one ballast tank did not preclude its existence in other tanks on the same vessel.* On 4 ships in which no living organisms were recorded from one tank, we detected live organisms in other tanks.

Quantitative Plankton Net Samples from Bulkers

Live organisms were collected by quantitative plankton net tows from the ballast water of all bulker cargo holds (Table 3-13, Appendix M). The biota in these holds was diverse taxonomically and included protistans (dinoflagellates, sarcodines, ciliates), plants (diatoms), invertebrates (crustaceans, annelids, molluscs, platyhelminthes, cnidarians, and chaetognaths), and vertebrates (fish). Invertebrate taxa included both meroplanktonic and holoplanktonic representatives. Crustaceans were found in all cargo holds and were abundant (> 100 organisms per net tow) in one-third of the cargo holds sampled (Table 3-13, Fig. 3-12, Appendix M). Additional taxa prevalent in cargo holds included dinoflagellates (79% of holds), annelids (75%), diatoms (62%), molluscs (58%), and platyhelminthes (50%) (Table 3-13, Fig. 3-12). Of the 10 most prevalent taxa, 6 (crustaceans, dinoflagellates, annelids, diatoms, molluscs, sarcodines) were abundant in at least some vessels (Fig. 3-12). We report here the first known occurrence of live ctenophores in ballast water. We also identified larval forms of numerous minor phyla, including sipunculans, phoronids, urochordates, bryozoans, and nemerteans (Tables 3-13, 3-14).

More prevalent taxonomic groups were typically dominated (both in percent occurrence and abundance) by one or two major subclasses. For example, the

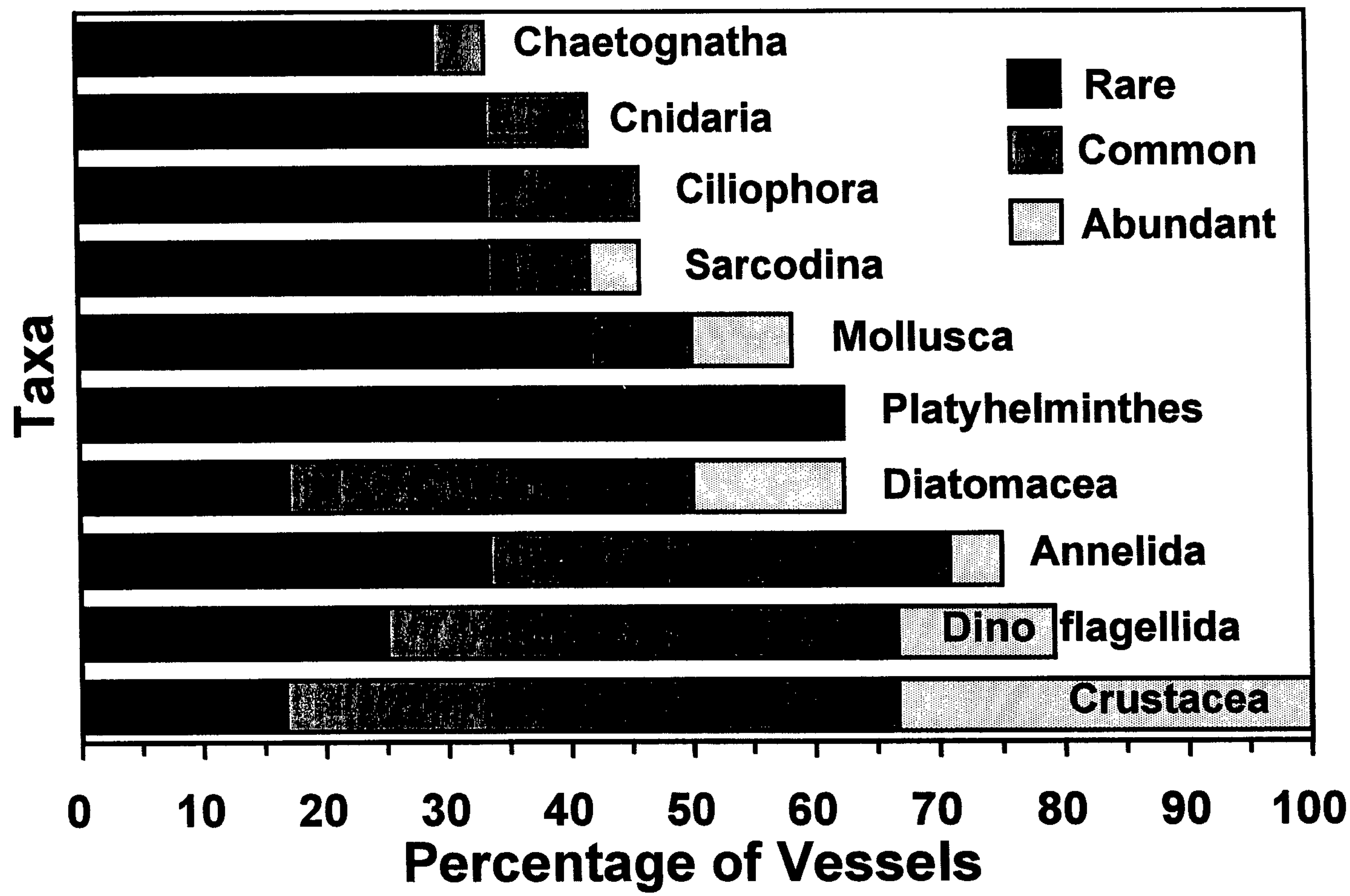

Figure 3-12. Prevalence of 10 most common taxa in ballast water of cargo holds, expressed as percentage occurrence in vessels. Bars are subdivided into the proportion of vessels in which a taxon was rare (< 10 per replicate), common (10 to 100 per replicate), or abundant (> 100 per replicate). Mean tow volume $\pm$ 1 S.D. = 1.32 $\pm$ 0.32 m^3. N = 24 cargo holds sampled in bulkers arriving in Baltimore, Maryland and Norfolk, Virginia between August 1993 and August 1994.

occurrence of crustaceans in all cargo holds was due to the omnipresence of copepods (Table 3-13, Fig. 3-13). We identified copepods from at least four orders (Harpacticoida, Cyclopoida, Calanoida, and Poecilostomatoida), all developmental stages (nauplii, copepodites, and adults), and both sexes in our samples. In terms of abundance, copepods were common or abundant in 96% of the cargo holds (Fig. 3-13). Barnacle nauplii and cyprids were found in 75% of the cargo holds, but were common in only 21% of the cargo holds. Representatives from at least 10 polychaete families were identified from cargo holds (Table 3-13), but larval spionids (Fig. 3-14) were largely responsible for the prevalence of annelids. Similarly, the phylum Mollusca was represented chiefly by bivalve larvae (Fig. 3-15).

Ninety-one percent of bulker ballast tanks sampled by quantitative plankton net tows contained living organisms. As with cargo hold samples, the biota was taxonomically diverse (Table 3-13). Crustaceans (primarily copepods) were most prevalent, occurring in 70% of bulker ballast tanks (Fig. 3-16). Seven of 10 most prevalent taxa in ballast tanks were also found in cargo holds, although the rank order of the taxa differed (Figs. 3-13, 3-16). Some taxa were unique to either cargo holds or ballast tanks. Taxa found in ballast tanks, but not in cargo holds included penaeid shrimp, cumaceans, branchiopods, larvaceans, and cyanobacteria (Table 3-13). Taxa found in cargo holds, but not in ballast tanks included euphausiids, stomatopods, mysids, ostracods, decapod nauplii, nereids, capitellids, heteropods, pteropods, polyplacophorans, scyphozoans, ascidians, phoronids, and foraminiferans (Table 3-13). With few exceptions (e.g., branchiopods) these taxa were rare in abundance; consequently, their apparent tank specificity is almost certainly a matter of chance.

Qualitative, Sediment, and Whole Water Samples

While our quantitative sampling design provided a robust description of the ballast water plankton community, non-quantitative (qualitative) samples yielded valuable information for non-planktonic and benthic taxa. For example, no representative of the 6 fish families we collected in the study were captured in a quantitative plankton net tow. Instead, fish were opportunistically dip netted while they were swimming near the surface of the water, or they were captured after the cargo hold or ballast tank had been emptied. Using the latter technique, we were also able to collect a number of benthic organisms, including shrimp (*Crangon crangon*), juvenile brachyuran crabs, nematodes, and polychaetes (Tables 3-13, 3-14, 3-15).

We encountered living organisms in the still-wet sediments of several ballast tanks that were accessed in dry dock. These included copepods, nematodes, foraminiferans, filamentous green algae, flatworms, and several species of encysted dinoflagellates. In one instance, a barge, stationed for a number of months off of the Pt. Loma sewage treatment plant near Los Angeles, was sampled in drydock in Baltimore, having ballasted in southern California three months earlier. The sediments in the tank were in layers 2.5 - 5 cm deep in places, very fine, slightly anoxic (blackish) with many rust articles and with a clear petroleum-based odor. A thin layer of water covered some of the mud, although the tank had been emptied of water about

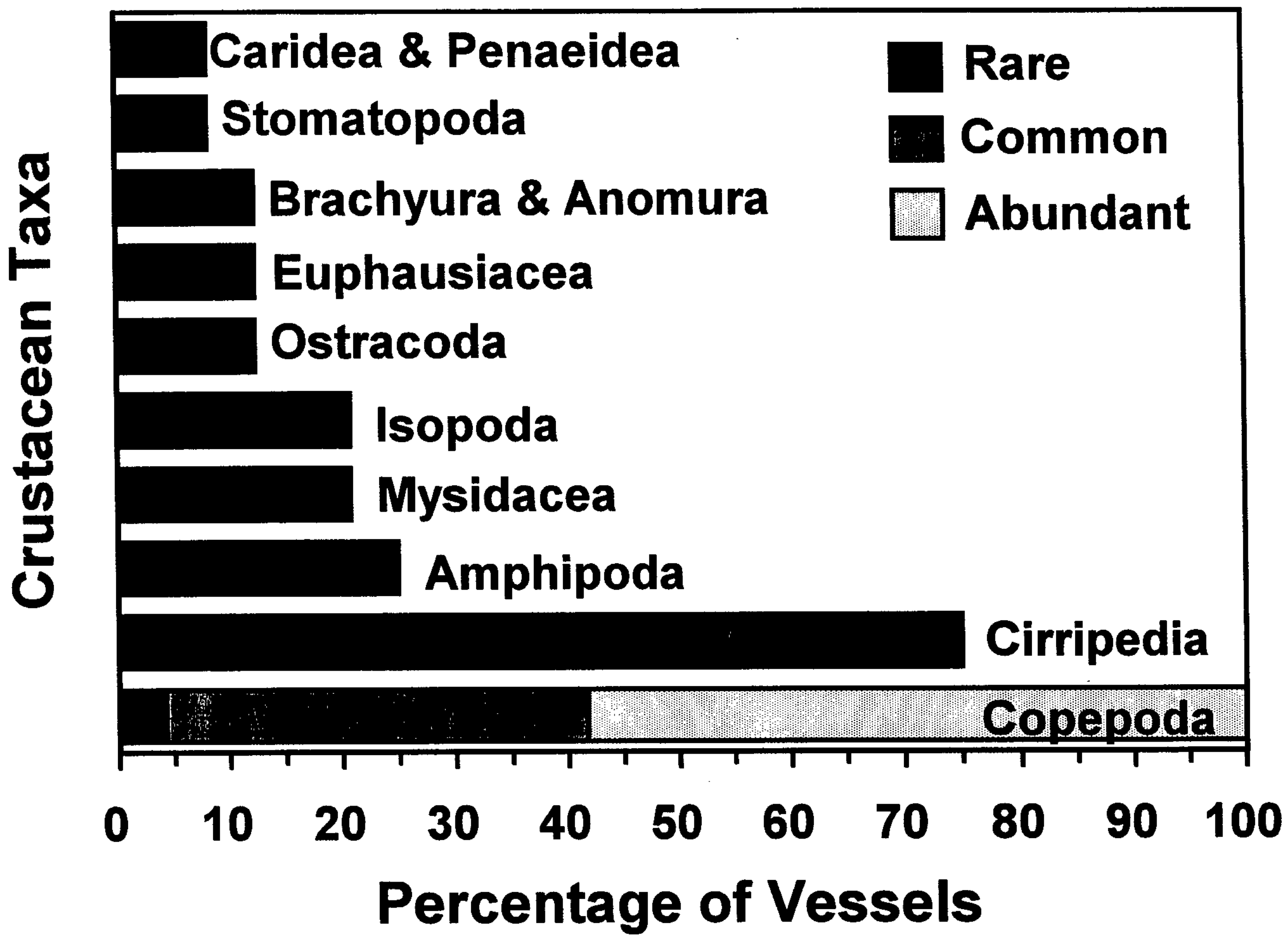

Figure 3-13. Prevalence of crustacean taxa in cargo hold ballast water, expressed as percentage of occurrence. Bars are subdivided into the proportion of vessels in which a taxon was rare (< 10 per replicate), common (10 to 100 per replicate), or abundant (> 100 per replicate). Mean tow volume ± 1 S.D. = 1.32 ± 0.32 m^3. N = 24 cargo holds sampled in bulkers arriving in Baltimore, Maryland and Norfolk, Virginia between August 1993 and August 1994.

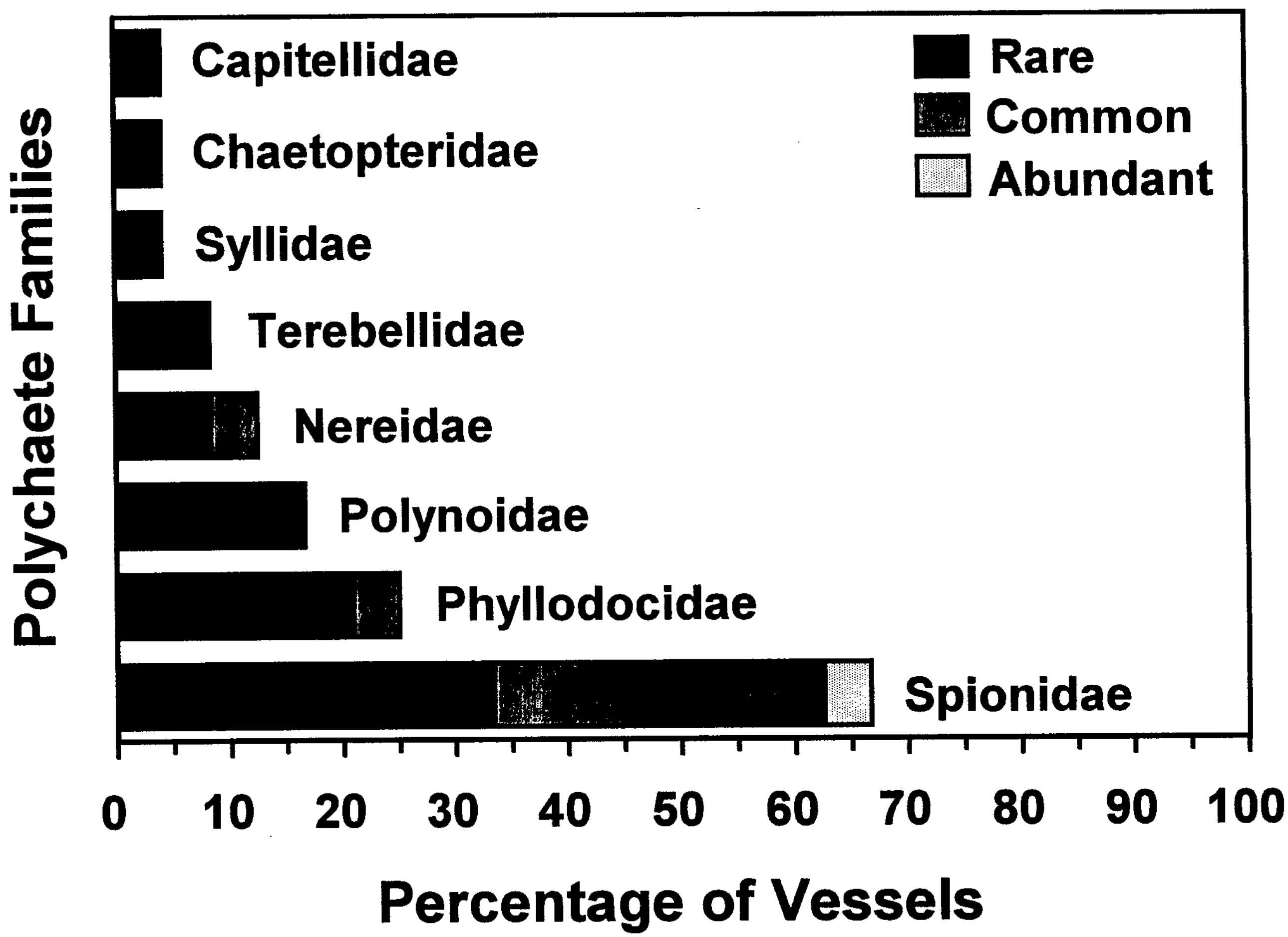

Figure 3-14. Prevalence of polychaete families in cargo hold ballast water, expressed as percentage of occurrence. Bars are subdivided into the proportion of vessels in which a taxon was rare (< 10 per replicate), common (10 to 100 per replicate), or abundant (> 100 per replicate). Mean tow volume $\pm$ 1 S.D. = 1.32 $\pm$ 0.32 m^3. N = 24 cargo holds sampled in bulkers arriving in Baltimore, Maryland and Norfolk, Virginia between August 1993 and August 1994.

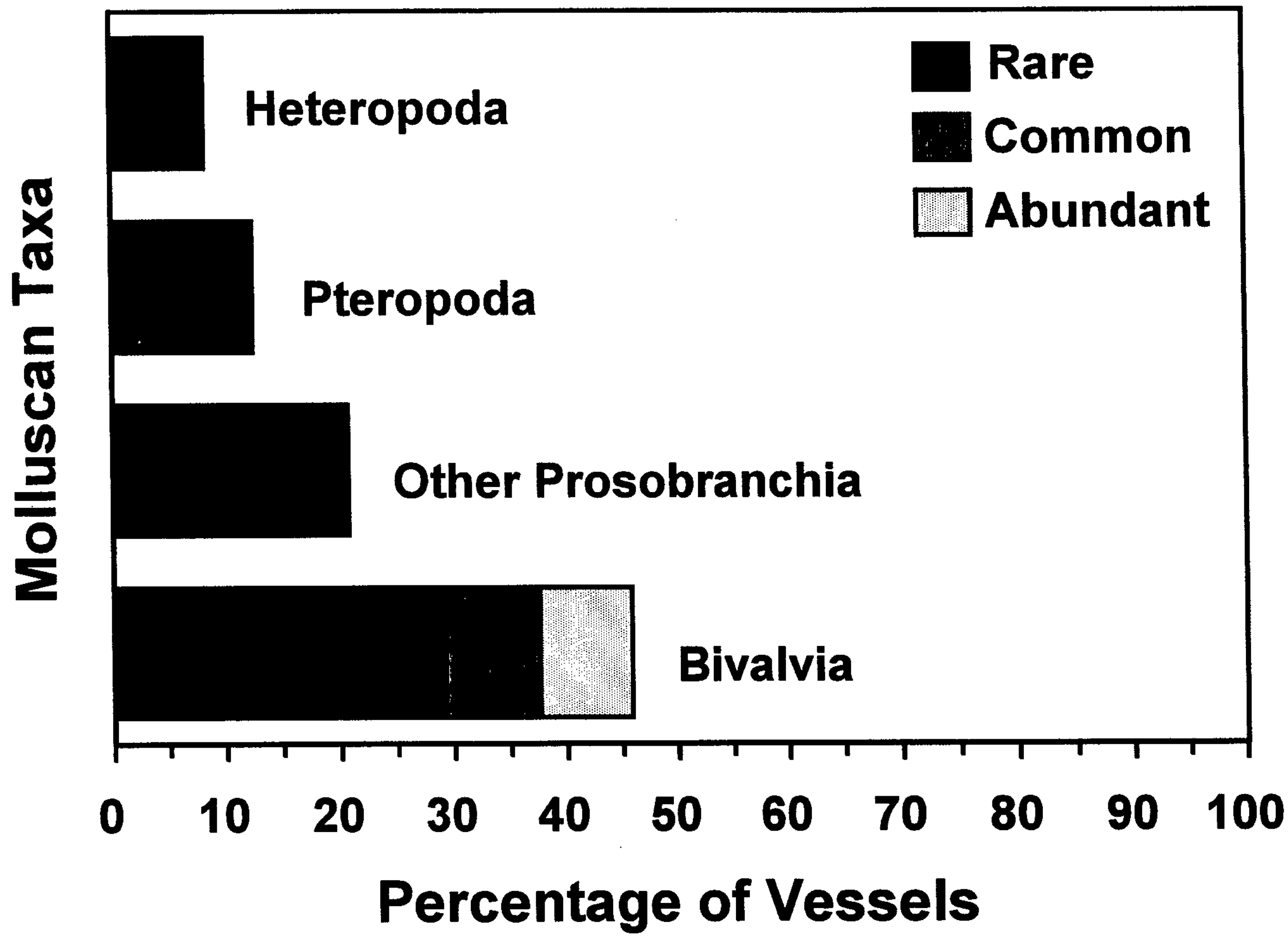

Figure 3-15. Prevalence of molluscan taxa in cargo hold ballast water, expressed as percentage of occurrence. Bars are subdivided into the proportion of vessels in which a taxon was rare (< 10 per replicate), common (10 to 100 per replicate), or abundant (> 100 per replicate). Mean tow volume $\pm$ 1 S.D. = 1.32 $\pm$ 0.32 m^3. N = 24 cargo holds sampled in bulkers arriving in Baltimore, Maryland and Norfolk, Virginia between August 1993 and August 1994.

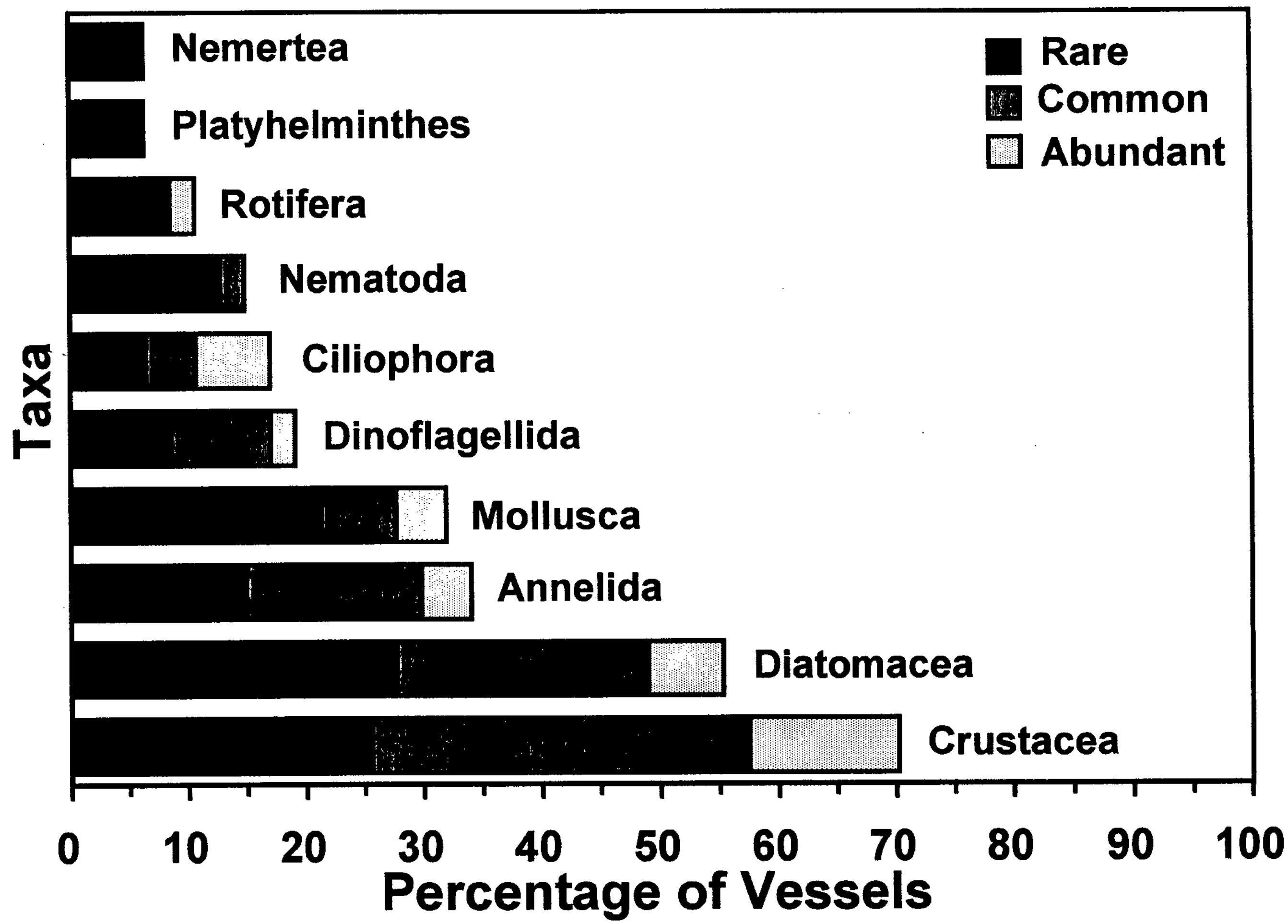

Figure 3-16. Prevalence of 10 most common taxa in ballast water of ballast tanks, expressed as a percentage occurrence in vessels. Bars are subdivided into the proportion of vessels in which a taxon was rare (< 10 per replicate), common (10 to 100 per replicate), or abundant (> 100 per replicate). Mean tow volume $\pm$ 1 S.D. = 0.22 $\pm$ 0.16 m^3. N = 47 ballast tanks sampled in bulkers arriving in Baltimore, Maryland and Norfolk, Virginia between August 1993 and August 1994.

Table 3-15

Frequency of living organisms associated with sediments in deballasted cargo holds and ballast tanks of bulkers sampled in Baltimore, Maryland between August 1993 and August 1994 (n = 10).

Taxon	No. Vessels
Crustacea	4
Copepoda	3
Decapoda	1
Crangon crangon	1
xanthid crab	1
Annelida	2
Capitella sp.	1
Polydora sp.	1
Platyhelminthes	1
Rotifera	1
Nematoda	4
Pisces	1
Pomatoschistus lozanoi	1
soleid flatfish	1
Sarcomastigophora	7
Dinoflagellida	7
Foraminifera	2
Diatomacea	3
Chlorophyta	1

5 days prior to sampling. We found in this sediment one live specimen of a female capitellid polychaete, with eggs along its tube walls. No fouling organisms were seen in the few ballast tanks sampled for sediments in dry dock; however, no concerted effort was made to search for such organisms. The majority of ballast tanks were sampled for planktonic organisms with nets, with no access to the tank bottoms many levels below (where permanent benthic communities or fouling organisms might survive in unpumpable water when the tank was deballasted).

Qualitative diagonal plankton net tows taken in cargo holds yielded little new information given the differences in techniques and volumes of water sampled compared to quantitative tows. In ballast tanks, the threefold increase in water volume sampled by qualitative plankton net tows resulted in higher prevalence for many taxonomic groups (e.g., cirripedia: 36.2% occurrence in quantitative samples vs. 48.9% in qualitative samples; Table 3-13). Few new taxa, however, were added relative to quantitative tows.

Whole water samples contained an additional 48 species of ciliates and 8 species of dinoflagellates not collected in plankton net tows (Table 3-14). We found no identified toxic dinoflagellates in sediment or plankton samples, although the stochastic nature of ballast transport (see Discussion, below) cautions against concluding that Chesapeake Bay is not being inoculated by these organisms.

BIOLOGICAL INFORMATION: QUANTITATIVE COUNTS ON PRESERVED ORGANISMS FROM BULKERS SAMPLED IN BALTIMORE AND NORFOLK:

Effect of Tow Volume on Numbers of Organisms and Taxa

Preserved plankton from 40 tanks from 33 bulkers sampled in Baltimore and Norfolk were classified and enumerated to obtain more detailed quantitative biological information. The volume of water sampled in plankton net tows ranged from 0.07 m^3 to 1.63 m^3 (mean ± 1 S.D. = 0.734 ± 0.59 m^3). There was a tendency for the number of organisms in a sample to increase as tow volume increased (linear regression, df = 1,38; $p = 0.067$), but the correlation was very weak ($R^2 = 0.085$). The number of taxa, however, increased significantly as tow volume increased ($p < 0.001$, $R^2 = 0.27$).

Number of organisms per m^3, per tank, and per ship: Plankton net samples.

The mean number of organisms per cubic meter sampled by quantitative plankton net tows for all regions and all seasons was 159 (range, 0 to 17,979) (Table 3-16). The mean number of organisms per tank (average of the number of organisms/m^3 in tank multiplied by the amount of ballast water in the tank) yielded a mean estimate of 491,765 (range, 0 to 251,780,853). Finally, the mean number of organisms per ship (average of the number of organisms/m^3 in tank multiplied by the amount of ballast water on board the ship) was 3,730,312 (range, 0 to 918,837,899).

Table 3-16

Summary of the mean number of organisms per m^3, per tank, and per ship in bulkers sampled by net tow in Baltimore, Maryland and Norfolk, Virginia between August 1993 and August 1994. Means were back-transformed from $logarithms_{10}$ for presentation. N, number of ballast tanks and cargo holds; Range, upper and lower 95% confidence limits; S. E. M., standard error of the mean.

Density	N	Mean	Range	Log-transformed S. E. M.
No./m^3	40	159	0 - 17,979	0.153
No./tank	38	491,765	0 - 251,780,853	0.281
No./ship	40	3,370,312	0 - 918,837,899	0.282

Whole Water and Sediment Samples: Dinoflagellates and Ciliates

Dinoflagellates were prevalent in whole water samples of ballast water and as cysts in ballast sediments (96% and 70% of the sampled tanks and cargo holds respectively) (Table 3-17). Dinoflagellate densities averaged 0.89 individuals per ml in whole water and 84.6 encysted individuals per gram dry weight in sediments. Ciliates were prevalent in whole water samples (89%). Densities of ciliates averaged 0.51 individuals per ml. Encysted loricate ciliates were absent in sediment samples.

Relative Abundance

Copepods numerically dominated ballast water plankton assemblages (Fig. 3-17, Tables 3-18, 3-19). Cumulatively, copepods comprised 65.3 ± 4.9% (mean ± 1 S.E.M.) of the total number of organisms in samples (Fig.3-17). Bivalves, when present, were the next most abundant taxon (16.4 ± 7.4%), followed by diatoms, polychaetes, dinoflagellates, and cirripeds (Fig. 3-17). Regionally, the relative abundance of copepods ranged from 55% in the Northeast Atlantic to 78% in the West Central Atlantic (Table 3-18). Seasonally, the relative abundance of copepods was lowest in summer (53%) and highest in winter (82%) (Table 3-19). The identity and rank order of abundance of other common taxa varied with region and season (Tables 3-18, 3-19). These differences reflect the stochasticity inherent in ballast transport of plankton; different taxa can be unusually common at certain ports or in certain times of the year (e.g., diatoms made up 100% of the organisms in one of the three winter samples; Table 3-19).

ABUNDANCE AND TAXONOMIC RICHNESS:

Cargo Holds vs. Ballast Tanks

The density of organisms (no./m^3) did not differ significantly between cargo holds and ballast tanks, regardless of whether we compared all available cargo holds and ballast tanks (t-test, $t = 0.48$, $df = 38$, $p = 0.63$) (Table 3-20) or only those paired on the same ships (paired t-test, $t = -0.36$, $df = 5$, $p = 0.74$) (Table 3-21). Significantly more taxa were found in cargo holds than in ballast tanks, whether tanks were unpaired (Wilcoxon test, chi-square = 12.3, $df = 1$, $p < 0.001$) (Table 3-20) or paired (Wilcoxon test, $z = 2.1$, $df = 1$, $p = 0.036$) (Table 3-21). This latter relationship exists because: (1) the number of taxa were not adjusted for tow volume, and (2) longer tows were taken in cargo holds (mean ± 1 S.D. = 18.2 ± 5.1 m) than were possible in ballast tanks (3.3 ± 1.8 m).

Topped vs. Original Ballast Water

The practice of topping (pressing) up original water in cargo holds and ballast tanks during voyages had no noticeable effect on organism densities or number of taxa. The density of organisms did not differ significantly between topped and original (i.e., unmodified) ballast water in cargo holds and ballast tanks (t-test, $t = -0.10$, $df = 16$,

Table 3-17

Occurrence of dinoflagellates and ciliates in ship ballast water and sediments from cargo holds and ballast tanks of vessels sampled in Baltimore, Maryland between August 1993 and August 1994. n, cargo holds and ballast tanks

Taxon	Ballast Water (n = 26)	Ballast Sediment (n = 10)
Dinoflagellates		
% positive samples	96	70
total taxa identified	21	29
no. taxa per ship		
mean	3.2	2.9
range	0 - 10	0 - 9
organism density		
mean	0.89 ml^{-1}	84.6 $(g\ d\ wt)^{-1}$
range	0 - 4.1	0 - 234
Ciliates		
% positive samples	89	0
total taxa identified	47	0
no. taxa per ship		
mean	4.8	0
range	0 - 10	0
organism density		
mean	0.51 ml^{-1}	0
range	0 - 4.3	0

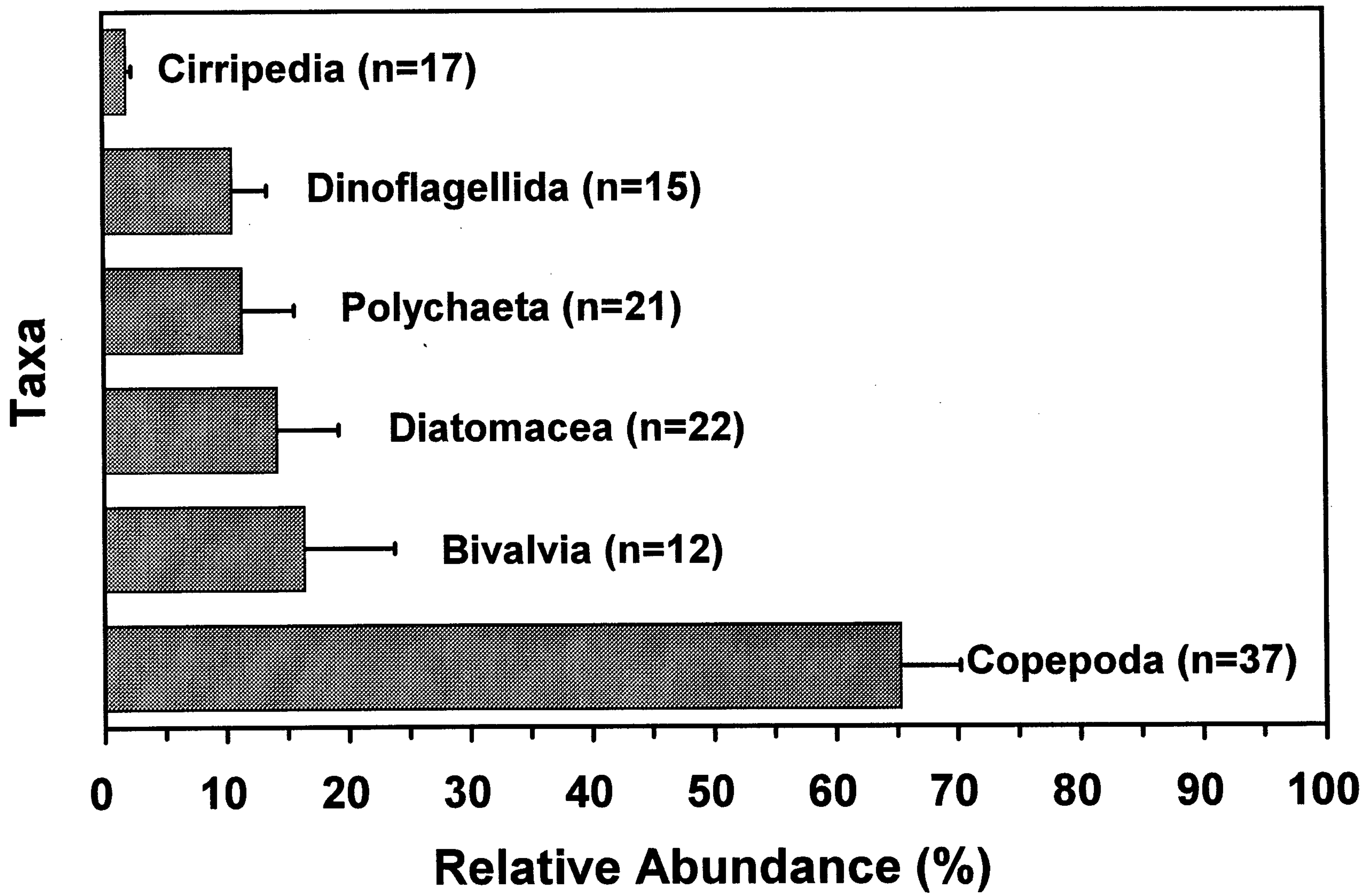

Figure 3-17. Mean relative abundance of common taxa expressed as a percentage of the total density of organisms (no./m^3) in a sample. Common taxa are defined as those present in at least 10 of 40 bulker cargo holds and ballast tanks sampled by net in Baltimore, Maryland and Norfolk, Virginia between August 1993 and August 1994. N = ballast tanks and cargo holds in which a given taxon was present. Error bars represent 1 S.E.M.

Table 3-18

Mean relative abundance ± 1 S.E.M. of the 5 top taxa (present in at least 3 bulkers sampled by net) expressed as a percentage of the total density of organisms (no./m^3) in a sample, by 3 main FAO reigons of ballast water origin. Common taxa are defined as those present in at least 3 bulker cargo holds and ballast tanks in each region. N = number of ballast tanks and cargo holds in which a given taxon was present.

	Region		
Rank	Mediterranean - Black Sea	Northeast Atlantic	West Central Atlantic
1	Copepoda 69.1 ± 6.2 (n = 17)	Copepoda 54.8 ± 11.6 (n = 11)	Copepoda 77.7 ± 8.0 (n = 6)
2	Bivalvia 20.4 ± 7.4 (n = 4)	Diatomacea 33.7 ± 16.0 (n = 6)	Diatomacea 7.1 ± 5.4 (n = 3)
3	Dinoflagellida 14.4 ± 3.7 (n = 10)	Bivalvia 22.9 ± 22.0 (n = 4)	Bivalvia 5.4 ± 2.1 (n = 3)
4	Platyhelminthes 7.4 ± 6.2 (n = 6)	Polychaeta 16.2 ± 9.2 (n = 9)	Gastropoda 1.8 ± 0.7 (n = 3)
5	Polychaeta 5.8 ± 2.6 (n = 8)	Dinoflagellida 3.0 ± 2.1 (n = 3)	Decapoda 1.2 ± 0.6 (n = 3)

Table 3-19

Mean relative abundance ± 1 S.E.M. of the 5 most common taxa expressed as a percentage of the total density of organisms (no./m^3) in a sample, by season. Common taxa are defined as those present in at least 3 bulker cargo holds and ballast tanks in each season. N = number of ballast tanks and cargo holds in which a given taxon was present. In winter, only 4 taxa were present in 3 or more ships.

	Season			
Rank	Fall	Winter	Spring	Summer
1	Copepoda 63.5 ± 10.3 (n = 11)	Copepoda 82.5 ± 5.5 (n = 7)	Copepoda 67.4 ± 8.9 (n = 9)	Copepoda 53.3 ± 10.5 (n = 10)
2	Dinoflagellida 15.8 ± 6.2 (n = 6)	Diatomacea 43.0 ± 28.5 (n = 3)	Diatomacea 9.7 ± 4.0 (n = 6)	Polychaeta 17.6 ± 10.4 (n = 8)
3	Platyhelminthes 13.1 ± 12.7 (n = 3)	Polychaeta 8.1 ± 6.4 (n = 3)	Polychaeta 7.8 ± 3.4 (n = 6)	Bivalvia 17.0 ± 6.6 (n = 5)
4	Isopoda 6.8 ± 5.1 (n = 3)	Dinoflagellida 2.1 ± 0.9 (n = 3)	Hydrozoa 6.6 ± 5.1 (n = 3)	Diatomacea 16.5 ± 11.8 (n = 5)
5	Polychaeta 6.7 ± 5.5 (n = 4)		Bivalvia 5.9 ± 2.6 (n = 3)	Dinoflagellida 6.1 ± 1.2 (n = 4)

Table 3-20

Comparison of the mean[1] number of organisms per cubic meter and mean number of taxa in cargo holds and ballast tanks[2] of bulkers sampled in Baltimore, Maryland and Norfolk, Virginia between August 1993 and August 1994. In general, cargo holds and ballast tanks were not sampled from the same vessel. P-values are given for t-test comparing no./m^3 between tank types and for Wilcoxon 2-sample test comparing no. taxa between tank types.

Ballast Water Organisms	Tank Type: Cargo Hold mean	Cargo Hold n	Tank Type: Ballast Tank mean	Ballast Tank n	p-value
No./m^3	190	19	134	21	0.63
	(78 - 463)		(41 - 437)		
No. taxa	13.5	19	6.8	21	<0.001
	(1.18)		(1.31)		

[1] For no./m^3, means and 95% confidence limits (in parentheses) were back-transformed from $logarithms_{10}$ for presentation. Log-transformed S.E.M. for cargo hold = 0.183; for ballast tanks = 0.243. For no. taxa, untransformed means and S.E.M. (in parentheses) are given.

[2] Ballast tanks include wing, wing bottom, double bottom, fore and aft peak tanks.

Table 3-21

Comparison of the mean[1] number of organisms per cubic meter and mean number of taxa in cargo holds and ballast tanks[2] of bulkers sampled in Baltimore, Maryland and Norfolk, Virginia between August 1993 and August 1994. A single cargo hold and ballast tank were sampled on each of 5 vessels. P-values are given for paired t-test comparing no./m^3 between tank types and for Wilcoxon 2-sample test comparing no. taxa between tank types. N, number of ballast tanks or cargo holds.

Ballast Water Organisms	Tank Type: Cargo Hold mean	Cargo Hold n	Tank Type: Ballast Tank mean	Ballast Tank n	p-value
No./m^3	16 (2 - 89)	5	26 (2 - 232)	5	0.74
No. taxa	11.8 (1.70)	5	4.4 (1.52)	5	0.017

[1] For no./m^3, means and 95% confidence limits (in parentheses) were back-transformed from $logarithms_{10}$ for presentation. Log-transformed S.E.M. for cargo hold = 0.591; for ballast tanks = 0.772. For no. taxa, untransformed means and S.E.M. (in parentheses) are given.

[2] Ballast tanks include wing, wing bottom, double bottom, fore and aft peak tanks.

p = 0.92) (Table 3-22). It should be noted that the two tank conditions were sampled from different vessels (i.e., they were not paired on ships). Samples for each tank condition, however, were equally distributed across fall and winter seasons. The number of taxa also did not differ between topped and original cargo holds and ballast tanks (Wilcoxon test, z = -0.71, df = 1, p = 0.48) (Table 3-22).

Exchanged vs. Original Ballast Water

Several lines of evidence indicate that mid-ocean exchange was effective in reducing the abundance and taxonomic richness of plankton in ballast water. Cargo holds and ballast tanks with original water had significantly higher mean densities of organisms (906 individuals/m^3) (one-tailed t-test, t = -3.1, df = 10, p = 0.006) and greater mean number of taxa (14) (one-tailed t-test, t = -2.0, df = 10, p = 0.037) than did exchanged cargo holds and ballast tanks (43 individuals/m^3 and 7 taxa, respectively) (Fig. 3-18). Some caution must be exercised in interpreting these data, because only one vessel had paired exchanged and unexchanged tanks (see below). The remaining samples were taken from different ships. All vessels, however, originated in the Northeast Atlantic, and only vessels reported to have exchanged water in mid-ocean (i.e., no exchanges over the continental shelf) were used in the comparison. Furthermore, all vessels used had exchanged more than 90% of their water. The proportion of cargo holds and ballast tanks differed between exchanged (2 cargo holds and 4 ballast tanks) and unexchanged (4 cargo holds and 2 ballast tanks) ballast samples and may have biased comparisons of taxonomic richness (i.e., these data were not adjusted for tow volume); however, it should not have affected comparisons of organism density (which were adjusted for tow volume).

Comparison of densities of a signature coastal taxon (spionid polychaetes) in exchanged and original ballast water of a bulker travelling from Belgium to Baltimore provides additional evidence that mid-ocean exchange was effective in removing coastal plankton. In this case, the mean density of spionid polychaetes in a 91% exchanged ballast tank was significantly lower than that in an unexchanged cargo hold (one-tailed t-test, t = -5.6, df = 2, p = 0.015) (Table 3-23). Similarly, the total density of organisms and the total number of taxa were lower in the exchanged than the unexchanged water (one-tailed t-tests, t = -4.6, df = 2, p = 0.022 and t = -5.2, df = 2, p = 0.018 respectively) (Table 3-23). These data, while strongly suggestive, must also be interpreted with caution, because only one tank per exchange condition was sampled and the tank types differed. Samples from replicate exchanged and unexchanged ballast tanks (or cargo holds) on multiple vessels are needed to strengthen statistical arguments concerning the effectiveness of mid-ocean exchange.

Finally, the presence of some coastal taxa (e.g., balanomorph cirripede nauplii and cyprids, bryozoan larvae, most spionid polychaetes) in ships that exchanged 91 to 100% of their ballast water in mid-ocean suggests that the procedure, while effective in reducing abundances, cannot eliminate all traces of the original biota (Table 3-24).

Table 3-22

Comparison of mean[1] number of organisms per cubic meter and mean number of taxa in ballast tanks or cargo holds that had original[2] or topped[3] ballast water during the voyage. Samples were collected by net tow from bulkers arriving in Baltimore, Maryland and Norfolk, Virginia during fall and winter (1993-1994). P-values are given for t-test comparing no./m^3 between tank conditions and for Wilcoxon 2-sample test comparing no. taxa between tank conditions. N, number of ballast tanks and cargo holds.

Ballast Water Organisms	Tank Condition: Original mean	n	Topped mean	n	p-value
No./m^3	126 (45 - 351)	9	295 (24 - 759)	9	0.69
No. taxa	8.6 (1.84)	9	11.1 (2.35)	9	0.48

[1] For no./m3, means and 95% confidence limits (in parentheses) were back-transformed from $logarithms_{10}$ for presentation. Log-transformed S.E.M. for tanks and holds with original water = 0.192; for topped tanks and holds = 0.321. For no. taxa, untransformed means and S.E.M. (in parentheses) are given.

[2] Original: original water in ballast tank or cargo hold unmodified during transit.

[3] Topped: water added to fill ballast tank or cargo hold during voyage ($\leq$15% of ballast amount added).

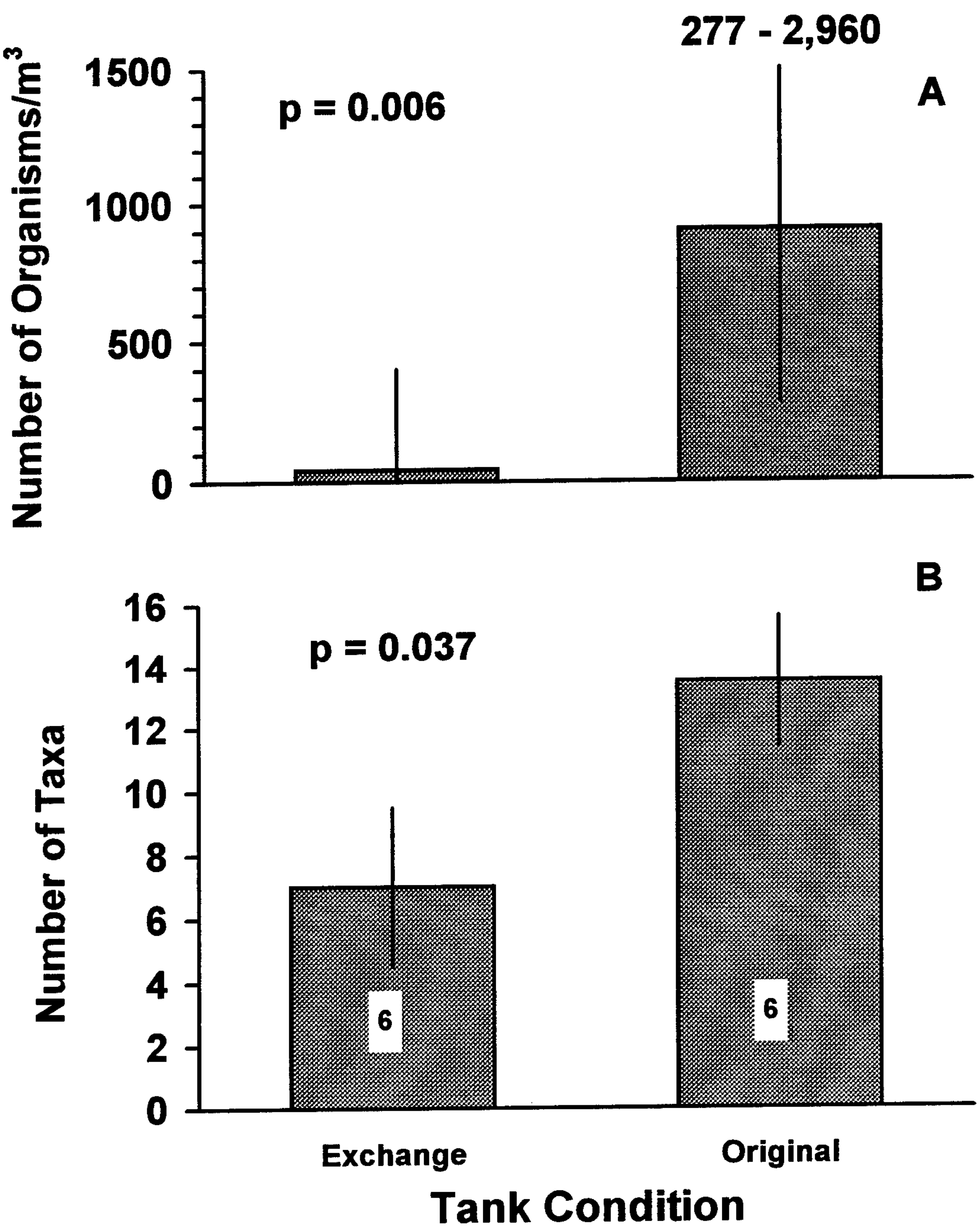

Figure 3-18. Summary of (A) mean number of organisms/m^3 and (B) number of taxa in cargo holds and ballast tanks with original (i.e., unexchanged and unmodified) or exchanged water. For no./m^3, means and 95% confidence limits were back-transformed from $logarithms_{10}$ for presentation. Log-transformed S.E.M for exchanged ballast tanks or cargo holds = 0.374; for tanks or holds that were not exchanged = 0.200. Value for upper confidence limit that is off the scale is given above the bar. For number of taxa, untransformed means and S.E.M. are given. P-values are given for 1-tailed t-tests comparing no./m^3 and no. taxa between tank conditions. The number of vessels for each tank condition are shown inside lower bar.

Table 3-23

Comparison of mean[1] number of spionid polychaetes per cubic meter, total organisms per cubic meter, and number of total taxa in original [2] ballast water from a cargo hold and exchanged[3] ballast water from a ballast tank. Samples were collected by net tow from one bulker in Baltimore, Maryland in summer 1994. P-values are given for one-tailed t-tests comparing densities and no. taxa between tank conditions. **N**, number of replicate samples per tank or hold.

Ballast Water Organisms	Tank Condition: Original mean	n	Exchanged mean	n	p-value
No. spionids/m^3	2,203 (0.242)	2	2.8 (0.454)	2	0.015
Total no./m^3	2,559 (0.242)	2	38.2 (0.315)	2	0.022
Total no. taxa	13.5 (1.5)	2	2.5 (1.5)	2	0.018

[1] For no. spionids/m^3 and no./m^3, back-transformed means and log-transformed S.E.M. (in parentheses) are given. For no. taxa, untransformed means and S.E.M. (in parentheses) are given.

[2] Original: original water in cargo hold unmodified during transit.

[3] Exchanged: original water deballasted and new water added to fill ballast tank during voyage ($\geq$ 90% of ballast amount added).

Table 3-24

Summary information on the sources of original and exchanged[1] ballast water (BW), maximum percentage exchanged[2], and presence of coastal taxa in 6 bulkers that exchanged cargo hold or ballast tank water in mid-ocean.

Source of original BW	Source of exchanged BW	Maximum % exchanged	Coastal taxa
North Sea, Belgium	Northeast Atlantic	91	None identified
New Meuse R., Netherlands	Northeast Atlantic	100	*Polydora* sp., nereid worms, balanomorph cirripede nauplii & cyprids
North Sea, Belgium	Northeast Atlantic	91	*Polydora* sp., balanomorph cirripede nauplii & cyprids
Bay of Biscay, Spain	Northeast Atlantic	97	*Polydora ciliata* , balanomorph cirripede cyprids
North Sea, Germany	Northwest Atlantic	91	Spionid polychaetes, bryozoan larvae, balanomorph cirripede larvae & cyprids
Ghent, Belgium	Northwest Atlantic	100	None identified

1 Only ships that exchanged water $\geq$ 300 km from a coastline are included.

2 Maximum percentage exchanged = (salinity of ballast water / salinity of open ocean, 35 ppt) x 100. Only ships that exchanged $\geq$ 90% of amount in ballast tank included.

Regional and Seasonal Comparisons

We observed regional differences in densities of organisms arriving to Chesapeake Bay in bulkers. Significantly fewer organisms per m^3 were found in ballast water from the Mediterranean/Black Sea than from the Northeast Atlantic or West Central Atlantic (1-way ANOVA, $F = 10.8$, $df = 2, 23$, $p < 0.001$) (Fig. 3-19A) when pooled across seasons. There were no significant differences in plankton density between ballast water samples from the Northeast and West Central Atlantic. The number of taxa did not differ among the 3 primary ballast water source regions (Kruskal-Wallis test, chi-square = 1.88, $df = 2$, $p = 0.39$) (Fig. 3-19B). Neither plankton densities nor number of taxa showed significant seasonal variation when pooled across regions (1-way ANOVA, $F = 0.73$, $df = 3, 25$, $p = 0.55$ and Kruskal-Wallis test, chi-square = 0.71, $df = 3$, $p = 0.87$, respectively) (Fig. 3-20A, B).

Simultaneous comparison of plankton densities by region and season indicated significant regional (2-way ANOVA, $F = 18.5$, $df = 1$, $p < 0.001$) but not seasonal differences ($F = 0.15$, $df = 3$, $p = 0.92$). These analyses compared only the Mediterranean/Black Sea and Northeast Atlantic samples, because samples from the West Central Atlantic were not present in all seasons. As before, plankton densities from the Northeast Atlantic were higher than those from the Mediterranean/Black Sea. Plankton densities for both regions were consistent across seasons (i.e., no region x season interaction, $F = 0.55$, $df = 3$, $p = 0.66$). We also compared the number of taxa by region and season (again, excluding the West Central Atlantic) after controlling for tow volume (covariate). Significantly more taxa were found in ballast water from the Northeast Atlantic (14) than from the Mediterranean/Black Sea (10) (2-way ANCOVA, $F = 5.0$, $df = 1$, $p = 0.044$). The number of taxa did not differ among seasons ($F = 0.58$, $df = 3$, $p = 0.64$) and there was no significant region by season interaction ($F = 1.22$, $df = 3$, $p = 0.34$).

Plankton Density and Environmental Correlates

For combined regions and seasons, log-transformed plankton densities were significantly negatively correlated with the age (Spearman Correlation coefficient, $r = -0.71$, $p < 0.001$; Fig. 3-21), temperature ($r = -0.52$, $p = 0.004$; Fig. 3-22), and salinity ($r = -0.64$, $p < 0.001$; Fig. 3-23) of ballast water. There was no significant relationship between plankton density and the amount of ballast water in tanks ($r = -0.13$, $p = 0.52$) (Fig. 3-24). Potential regional differences in plankton abundance, however, may have confounded several of these relationships. For example, ballast water from the Mediterranean/Black Sea is characterized by low plankton abundances (Fig. 3-19), older water, and higher salinity (Table 3-11). Without comparative information on background plankton densities in each region, it is difficult to determine whether the Mediterranean/Black Sea ballast water samples (Fig. 3-19) are depauperate because of age- (or salinity-) related mortality or because of unrelated regional differences in plankton abundance.

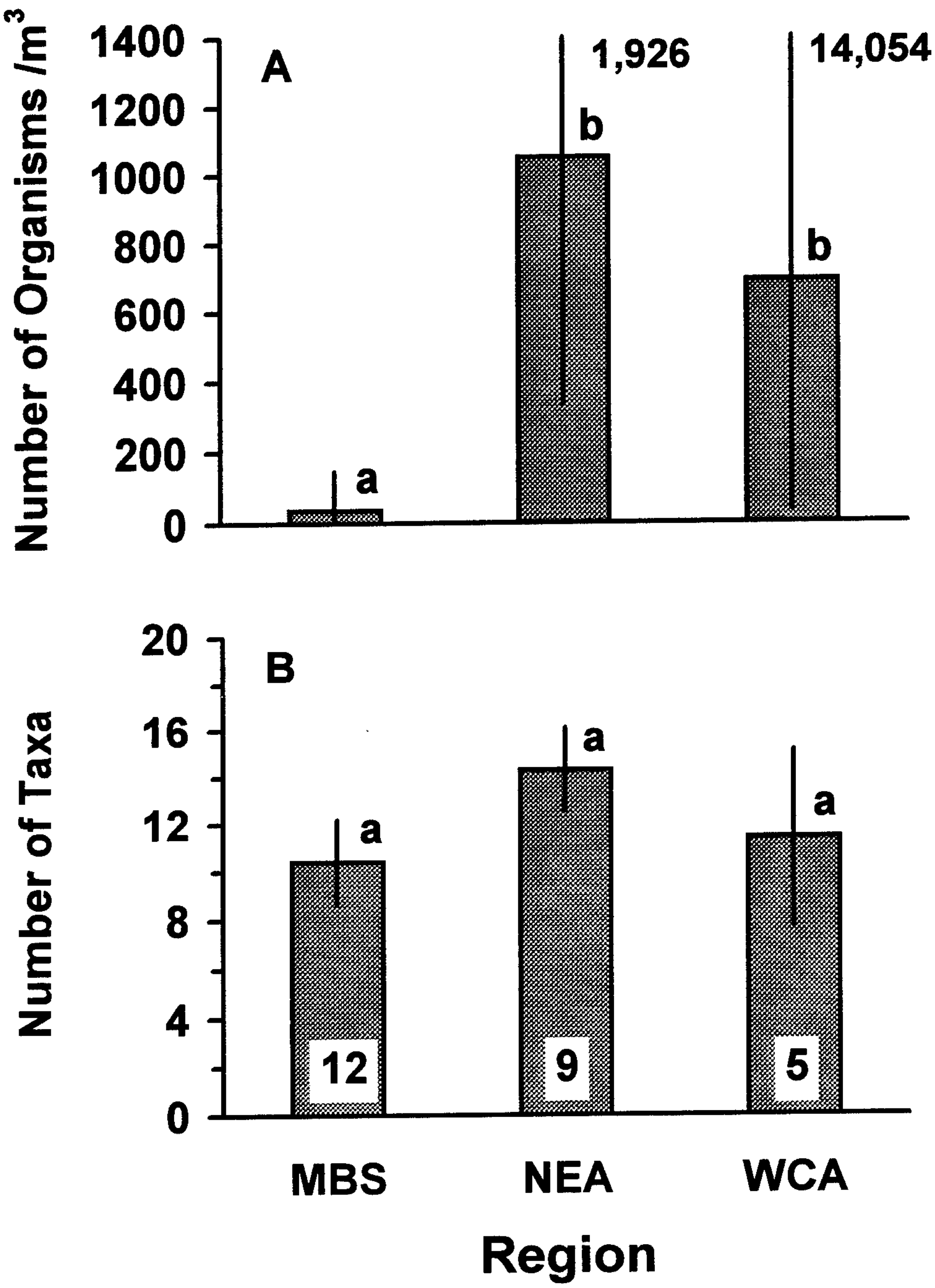

Figure 3-19. Summary of (A) mean number of organisms/m^3 and (B) mean number of taxa in unexchanged ballast water from bulkers sampled by net tow in Baltimore, Maryland and Norfolk, Virginia for the 3 main FAO regions between August 1993 and August 1994. For no./m^3, means and 95% confidence limits were back-transformed from logarithms for presentation. Values for upper confidence limits that are off scale are given above bars. 1-way ANOVA on log-transformed densities: $F = 10.7$, $df = 2, 23$, $p < 0.001$, $MSE = 0.574$. For no. taxa, untransformed means and S.E.M. are given. Kruskal-Wallis test: chi-square = 1.88, $df = 2$, $p = 0.39$. Sample size (n = number of vessels) given inside lower bars. Different letters above bars indicate significant

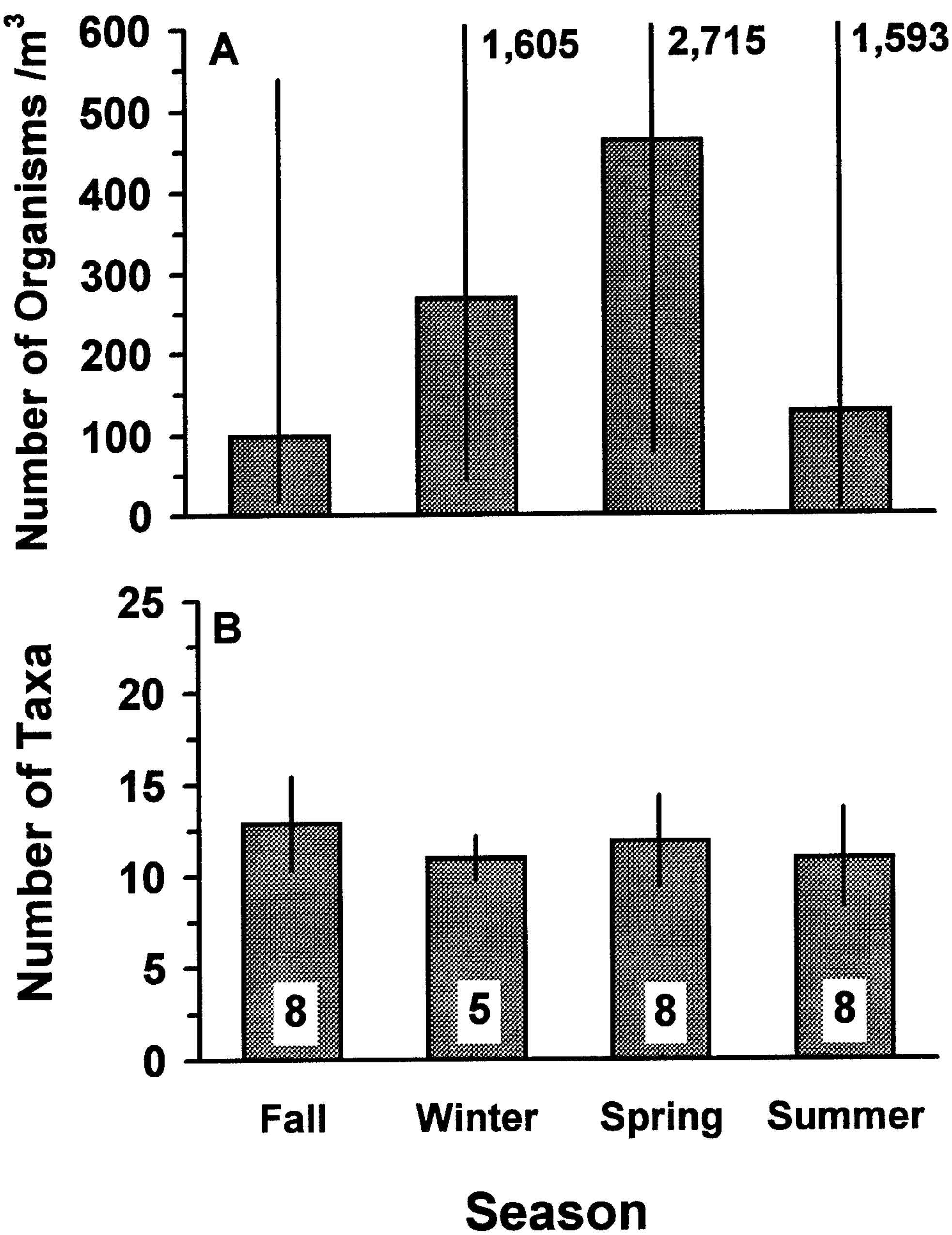

Figure 3-20. Summary of (A) mean number of organisms/m^3 and (B) mean number of taxa in unexchanged ballast water from bulkers sampled by net tow in Baltimore, Maryland and Norfolk, Virginia for each season between August 1993 and August 1994. For no./m^3 means and 95% confidence limits were back-transformed from logarithms for presentation. Values for upper confidence limits that are off scale are given above bars. 1-way ANOVA on log-transformed densities: $F = 0.73$, $df = 3, 25$, $p = 0.55$, $MSE = 0.99$. For no. taxa, untransformed means and S.E.M. are given. Kruskal-Wallis test: chi-square = 0.71, $df = 3$, $p = 0.87$. Sample size (n = number of vessels) given inside lower bars.

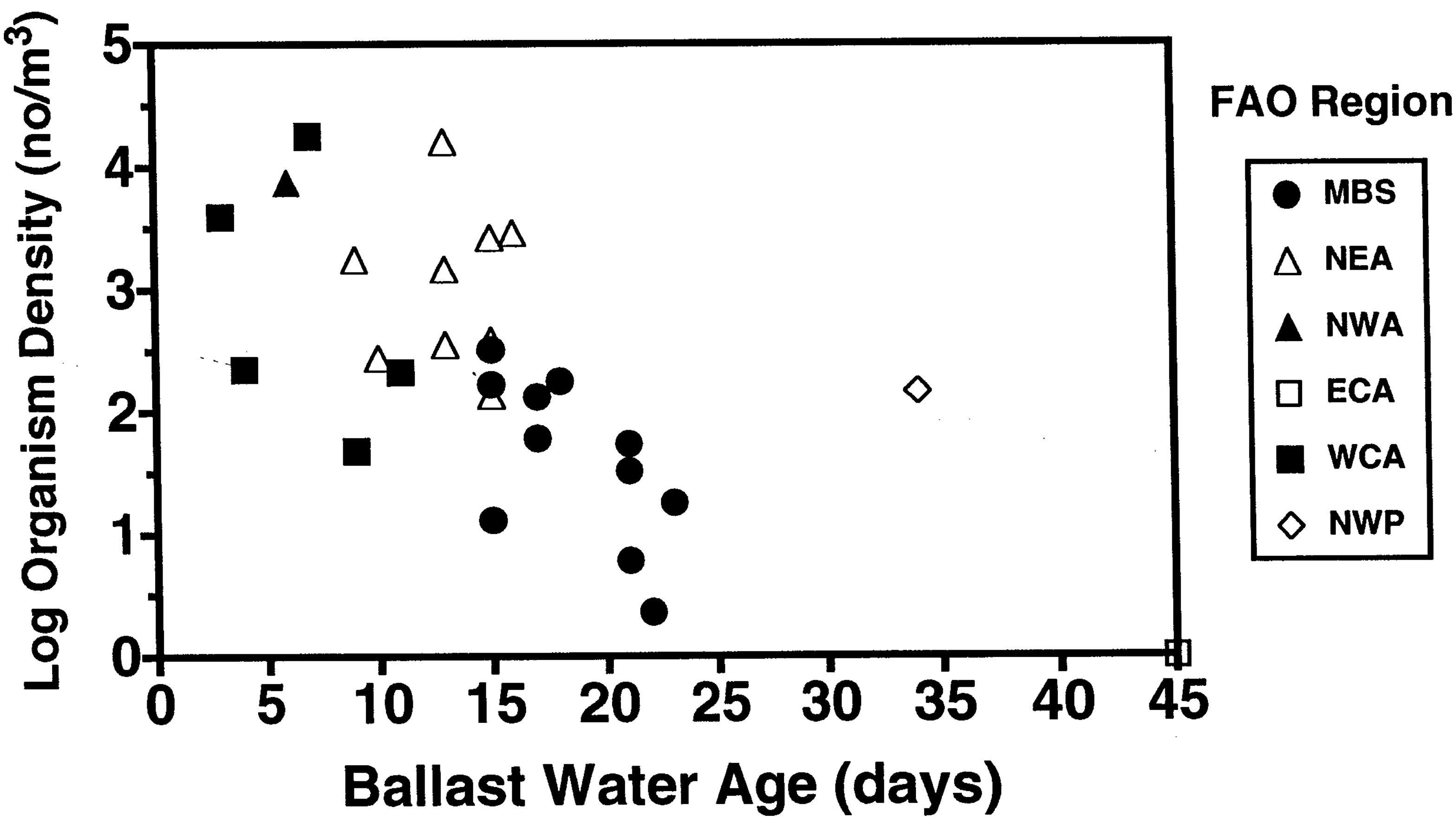

Figure 3-21. Log of organism density (no. organisms/m^3) as a function of ballast water age (in days) in bulkers sampled in Baltimore, Maryland, and Norfolk, Virginia between August 1993 and August 1994. N = 29 vessels. FAO regions: MBS, Mediterranean Black Sea; NEA, Northeast Atlantic; NWA, Northwest Atlantic; ECA, East Central Atlantic; WCA, West Central Atlantic; NWP, Northwest Pacific.

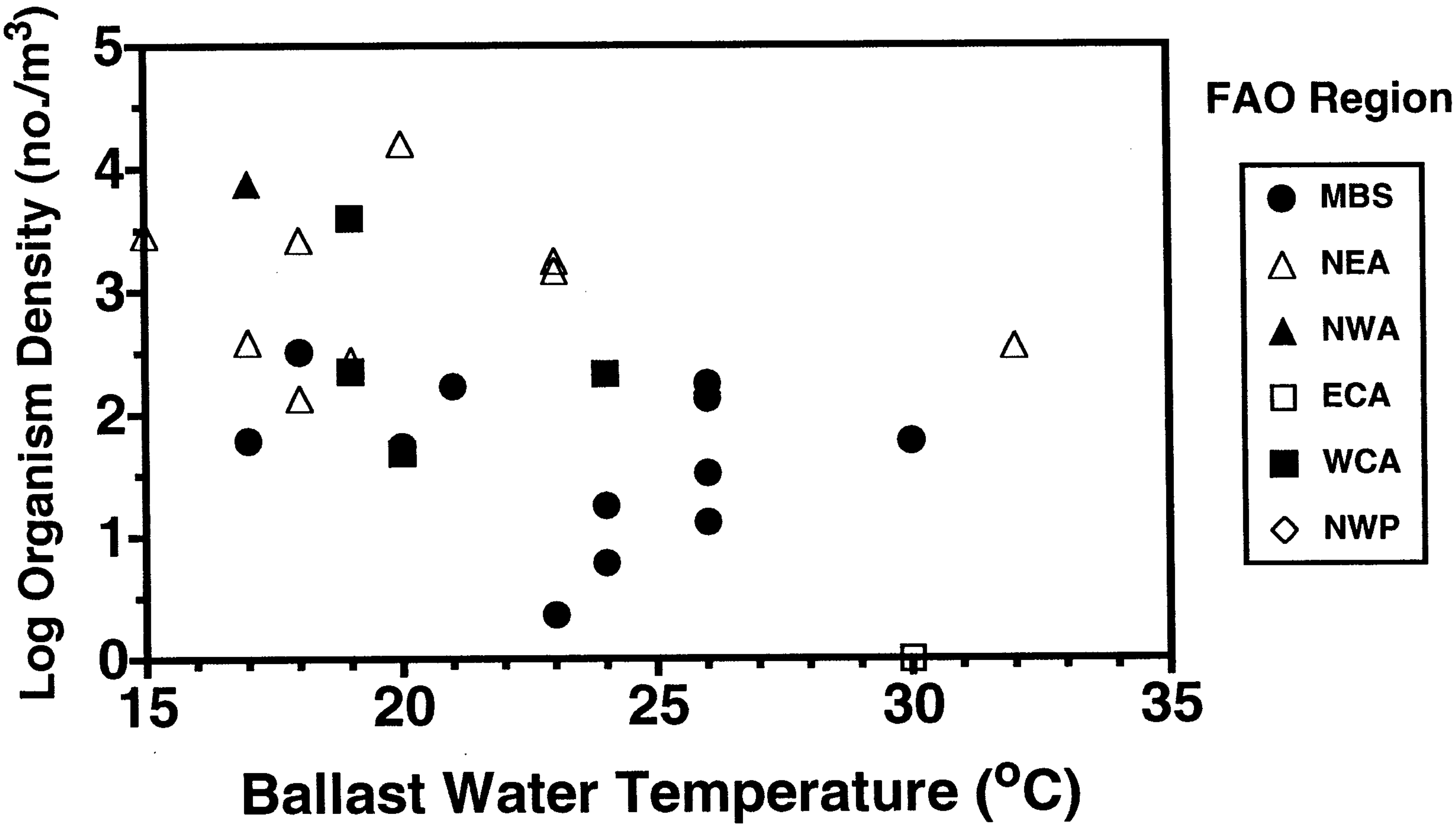

Figure 3-22. Log of organism density (no. organisms/m3) as a function of ballast water temperature (°C) in bulkers sampled in Baltimore, Maryland, and Norfolk, Virginia between August 1993 and August 1994. N = 28 vessels. FAO regions: MBS, Mediterranean/Black Sea; NEA, Northeast Atlantic; NWA, Northwest Atlantic; ECA, East Central Atlantic; WCA, West Central Atlantic; NWP, Northwest Pacific.

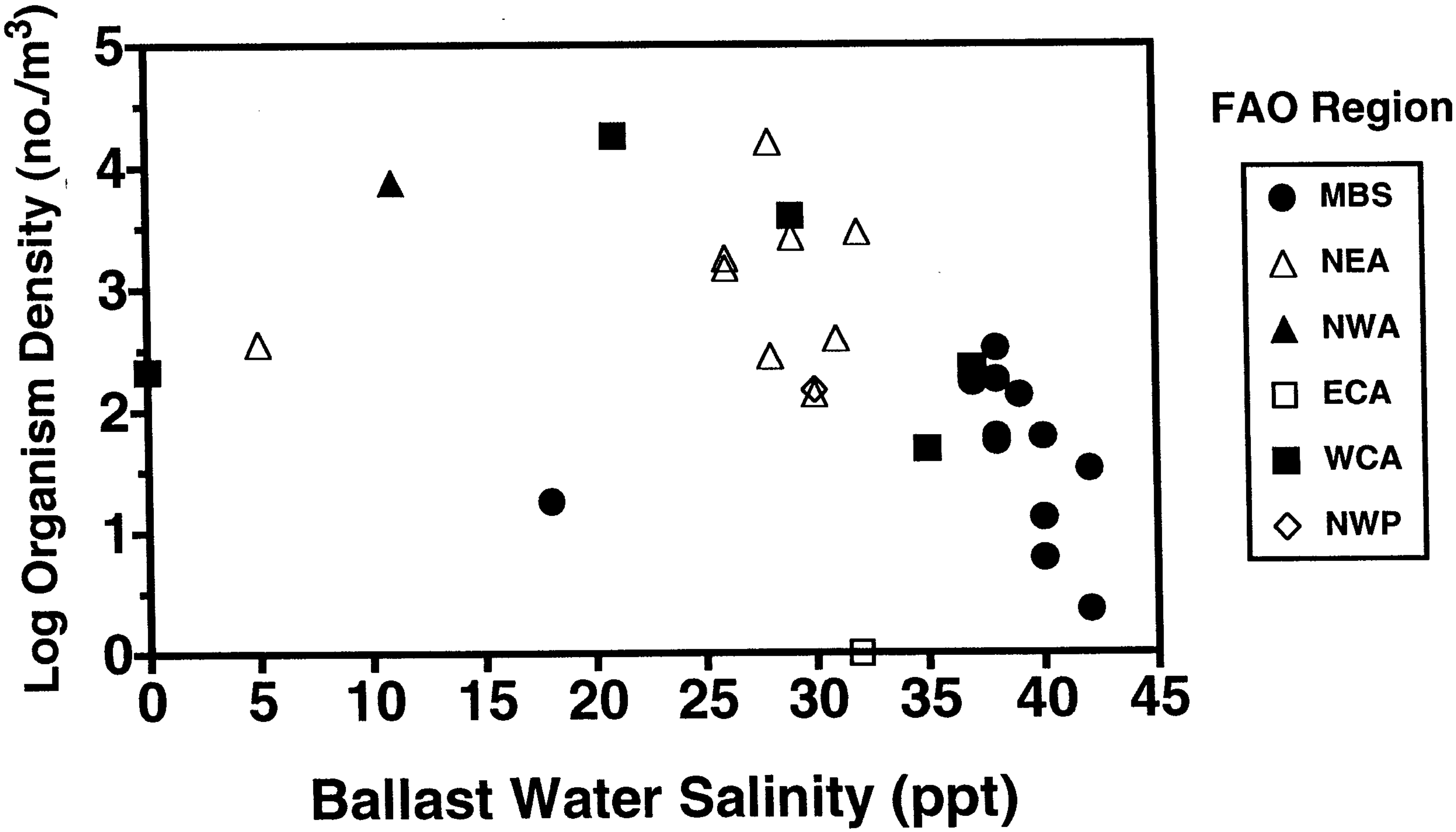

Figure 3-23. Log of organism density (no. organisms/m3) as a function of ballast water salinity (ppt) in bulkers sampled in Baltimore, Maryland, and Norfolk, Virginia between August 1993 and August 1994. N = 29 vessels. FAO regions: MBS, Mediterranean/Black Sea; NEA, Northeast Atlantic; NWA, Northwest Atlantic; ECA, East Central Atlantic; WCA, West Central Atlantic; NWP, Northwest Pacific.

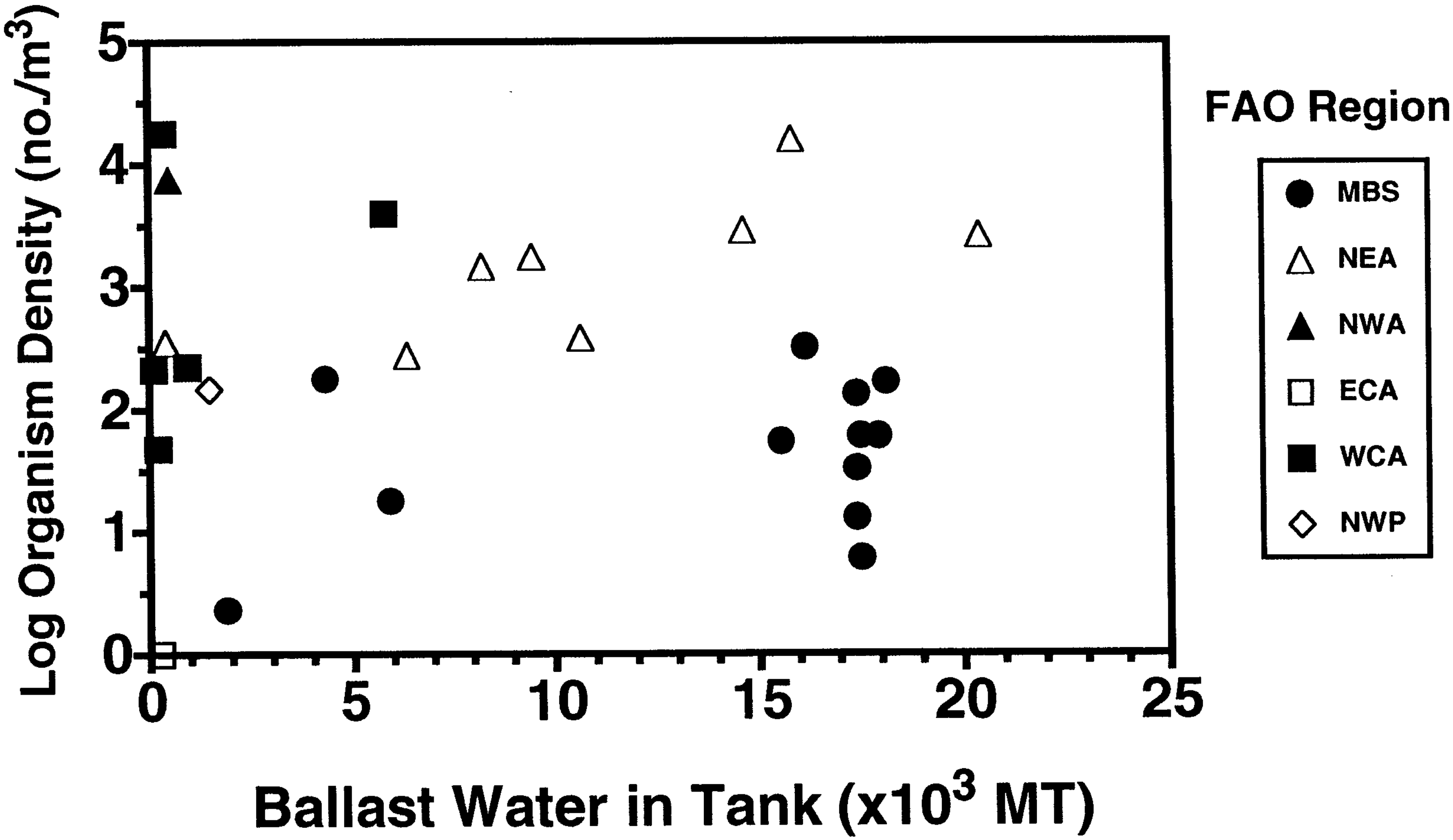

Figure 3-24. Log of organism density (no. organisms/m^3) as a function of amount of ballast water in tank (MT) in bulkers sampled in Baltimore, Maryland, and Norfolk, Virginia between August 1993 and August 1994. N = 28 vessels. FAO regions: MBS, Mediterranean/Black Sea; NEA, Northeast Atlantic; NWA, Northwest Atlantic; ECA, East Central Atlantic; WCA, West Central Atlantic; NWP, Northwest Pacific.

When plankton densities were compared to environmental variables within each of the 3 primary source regions, only one significant correlation was found after sequential Bonferroni correction (Rice 1989). For unknown reasons, plankton densities increased with the amount of ballast water in tanks of bulkers from the Northeast Atlantic ($r = 0.81$, $p = 0.0149$). The absence of significant correlations on a regional basis should not be surprising, because the variation in age, temperature, and salinity of ballast water within a given region is more narrow than between regions.

Undoubtedly, most, if not all, plankton in ballast water will perish if the age of the water increases past a certain time (e.g., 45 d in this study). *More importantly, our data demonstrate that the transit time for most bulkers arriving to Chesapeake Bay from European and Caribbean ports is sufficiently short as to ensure survival of many planktonic organisms entrained in the ballast water.*

Transport of Ballast from Global Hot Spots to Chesapeake Bay

We found one instance of a species transported to Chesapeake Bay that came from a previously invaded region. We observed more than 50 specimens of the fish *Alepes djedaba* Forskaal, known as the Jeddah Jack (family Carangidae) in a vessel from Ashdad, Israel (additional specimens were observed in a vessel from the Eastern Mediterranean after our study was completed). This species is a Lessepsian invader, that is, it moved from the Red Sea through the Suez Canal to the eastern Mediterranean, where it has become an important part of the commercial fishery (Wonham et al., 1996, in preparation). We also encountered in ballast water from Europe certain species that have previously been introduced from North America *to* Europe (e.g., the American barnacle *Balanus improvisus* and the American copepod *Acartia tonsa*) and species that may have been introduced earlier from Europe (e.g., the hydroid *Ectopleura dumortieri*, known on American Atlantic shores since the 1860s). Mussel (*Mytilus*) larvae were encountered in a number of samples from Europe, but whether these represented *Mytilus edulis* or the invasive species *Mytilus galloprovincialis* awaits molecular genetic analyses (J. Geller, personal communication, 1995).

Chapter 4.

DISCUSSION

The transport of ballast water in ships is now recognized as the primary vector for the movement of aquatic organisms within and between oceans (Carlton & Geller 1993). Used to maintain stability during a voyage, ballast water is actively pumped or gravitated into dedicated tanks and cargo holds at one port and released (to varying degrees) at other ports when receiving or delivering cargo (Carlton 1985). The volumes of ballast water being transported and released are immense. In 1991 alone, large commercial vessels released approximately 79 million metric tons of ballast water from foreign ports into U.S. waters (the equivalent of 2.4 million gallons/hour) (Carlton, Reid, and van Leeuwen 1995). Because water is usually ballasted in bays and estuaries rich in plankton and nekton, most ships carry a diverse assemblage of organisms in their cargo holds and ballast tanks (e.g., Medcof 1975, Carlton 1985, Williams et al. 1988, Carlton and Geller 1993). As a consequence, Carlton and Geller (1993) estimated that "on any one day, several thousand species may be in motion in ballast water 'conveyor belts' around the world." At least 57 species are believed to have been introduced to U.S. waters as a result of ballast water and this figure is likely to be a gross underestimate of ballast-mediated invasions (Carlton, Reid, and van Leeuwen 1995). In the Great Lakes alone, the European waterflea *Bythotrephes cederstroemi*, the zebra mussels *Dreissena polymorpha and D. bugensis*, and the fish *Neogobius melanostomus, Proterorhinus marmoratus*, and *Gymnocephalus cernuus* were all introduced by ballast water in the 1980s. In a number of cases, biological invasions have had significant negative impacts on the ecology, economy, and health of aquatic systems (Hallegraeff and Bolch 1991, 1992; Mills et al. 1993; U.S. Congress, O.T.A. 1993). Ballast water continues in the 1990s as the major mechanism for the global transport of aquatic nuisance species.

These considerations mandate comprehensive studies of ballast water as a transport mechanism, in order to facilitate quarantine measures to reduce new exotic species invasions into coastal and aquatic habitats. The data reported here expand considerably our understanding of the ballast-mediated transport of exotic organisms into United States waters. Previously published research has been limited to the arrival of plankton in ballast water in the Pacific Northwest (Coos Bay, Oregon and Port Angeles, Washington) from a single source region (Japan) (Carlton and Geller, 1993; Kelly, 1993), and to two vessels arriving in Boston, Massachusetts and Wilmington, Delaware (Carlton, 1985). In the present study, we provide a picture of the diversity of ballast plankton arriving from multiple source regions into the largest estuary in the United States, the Chesapeake Bay.

Biodiversity of Ballast Water: Taxonomic Diversity

Our data demonstrate conclusively that the Chesapeake Bay is being inoculated by a diverse assemblage of live organisms transported from around the world in ship's ballast water. Given that: (1) the ports of Baltimore, Maryland and

Norfolk, Virginia receive hundreds of bulkers each year; (2) the average bulker we sampled had over 31,000 MT (> 8.1 million gallons) of ballast on board; and (3) 91% of the bulkers sampled contained living organisms, it is evident that these inoculations are occurring on massive and frequent basis. We found at least 278 species of protist, animal, and plant taxa in 70 vessels sampled for ballast water and an additional 4 taxa in 5 vessels sampled for ballast sediments (Table 4-1, footnote). Because we were extremely conservative in our identifications, these numbers substantially underestimate the true diversity of organisms entering Chesapeake Bay. Regardless, all major taxonomic groups, developmental stages, and reproductive modes were represented. Organisms came from freshwater, brackish water, open-ocean, and coastal high-salinity habitats. Although copepods numerically dominated most ballast water samples, other groups, including spionid polychaete worm larvae, bivalve (clam and mussel) larvae, dinoflagellates and diatoms were often present in high numbers. We report here the first occurrence of live ctenophores in ballast water. This finding lends support to the hypothesized role of ballast water in transporting the western Atlantic comb jelly *Mnemiopsis leidyi* to the Black Sea in the early 1980s. Crab zoea and megalopae; barnacle nauplii and cyprids; and 6 families (carangid, clupeid, engraulid, gasterosteid, gobiid, and soleid) of fishes were identified from cargo holds and ballast tanks. Polychaetes were dominated by larvae from the family Spionidae. In particular, a single spionid worm species, *Polydora ligni*, was found in 8 ships from very different regions, including Greece, Italy, Germany, the North Sea, and the Florida Gulf. Whole water samples revealed high protistan diversity and ballast sediment samples yielded encysted dinoflagellates.

The total diversity of organisms being brought to Chesapeake Bay is similar in magnitude to that reported in ballast water studies from 3 other regions. Carlton and Geller (1993) reported 367 species arriving in Coos Bay, Oregon in 159 vessels from Japan. In 31 vessels sampled in Australia, Williams et al. (1988) found 67 taxa of zooplankton and fish. Locke et al. (1991) and Subba Rao et al. (1994) together found a minimum of 213 protist, animal, and plant taxa in 86 vessels arriving in the Great Lakes. The level of taxonomic resolution for specific groups, however, varies significantly among these studies, as shown in Table 4-1. For example, diatoms were emphasized in the studies of Carlton and Geller (1993) and Subba Rao et al. (1994); dinoflagellates were emphasized here and in the studies of Subba Rao et al. (1994). Carlton and Geller (1993) reported 33 flatworm taxa; whereas, Australian studies (Williams et al. 1988) using same-source water from Japan reported only 1 flatworm taxon. We report 54 ciliate protozoans, groups essentially unstudied by other investigators. Subtracting ciliates, flatworms, diatoms, and dinoflagellates, as well as fish and unidentified small protistan or algal taxa, leaves a general but perhaps more manageable category of general "zooplankton", which although including a wide array of holoplanktonic (permanent plankton) and meroplanktonic (larval plankton) taxa, more likely captures uniform biases across all of the studies (e.g., similar underestimates of diversity due to unidentified copepods, and to unidentified bivalve, crustacean, and polychaete larvae). These adjusted numbers and the data from Oregon, the Great Lakes, and our studies, suggest that samples of > 70 ships should yield a minimum biota of > 100 species.

Table 4-1

Examples of levels of taxonomic resolution in ballast studies from different regions (**boldface numbers** indicate special taxonomic emphases). Data summarized for Oregon (Carlton & Geller, 1993), the Great Lakes (Locke et al., 1991; Subba Rao et al., 1993), Australia (Williams et al., 1988), and Chesapeake Bay (present study). In some studies, some taxa were not studied (NS).

	Region			
	Oregon	Great Lakes	Australia	Chesapeake Bay
No. Vessels	159	86	31	70
No. Taxa				
Zooplankton[1]	184	110	64	166
Ciliates[2]	[6]	[3]	NS	**54**
Platyhelminthes	**33**	1	1	3
Fish	2	0	2	6
Diatoms	**128**	**61**	NS	17
Dinoflagellates	4	**30**	NS	**26**
Other[3]	10	8	NS	6
Total	367	213	67	278

[1] Zooplankton captured in plankton net hauls. Excludes benthic taxa, which for Australian studies includes 37 additional taxa, and for studies reported herein includes 4 taxa (unidentified nematode, shrimp *Crangon* , and 2 fish species) found solely in benthic sediments of cargo holds.

[2] Ciliates captured in plankton net hauls or whole water samples. [Numbers] indicate number of taxa estimated in total count, but not extensively studied.

[3] Other includes radiolarians, foraminiferans, green algae, red algae, seagrasses, phytoflagellates, and others.

Factors Affecting Survivorship During Transit

Significantly, our data demonstrate that the transit time for most bulkers arriving to Chesapeake Bay was sufficiently short as to allow survival of planktonic organisms entrained in the ballast water. Furthermore, these organisms appeared to be in good condition after their transoceanic journey; consequently, they were potential invaders upon release. In many cases, invertebrate larvae had sufficient energetic reserves to undergo metamorphosis in the laboratory, even when reared on generic diets (Appendix F). We were thus able to raise many of these newly settled barnacles, worms, crabs, bivalves, and sea urchins to juvenile or adult stages.

Ballasted organisms in cargo holds did well in transit, because bulker cargo holds function as well-mixed, physically constant, ocean-going lakes. They are centrally located in the vessel and contain a large water mass (average > 4 million gallons); consequently, they are usually well buffered from temperature changes caused by surrounding waters. Salinity remains constant unless the holds are exchanged or substantial amounts of water are added (pressed). If the voyage length does not exceed food resources, organisms may not experience environmental conditions dramatically different from those of their native habitat (with the exception of prolonged darkness and perhaps lack of exposure to some predators). Ballast tanks have significantly less capacity than cargo holds (Table 3-2). Whether physical conditions are more variable in ballast tanks than in cargo holds is unknown. Ballast tanks are more exposed to ambient water temperatures, and there is potential for temperature and salinity stratification in wing bottom tanks when pressed, which may effect the health of the biotic assemblage. Biological diversity was lower in ballast tanks than in cargo holds, but we could not rule out sampling bias as a source of the difference. Dissolved oxygen content and densities of organisms were similar between cargo holds and ballast tanks, which suggests that conditions in the latter did not inhibit survival.

Abundances in Ballast Water: Geographic and Seasonal Patterns

Densities of organisms in the ballast water were extraordinarily variable from ship to ship and reflect the stochastic nature of ballast transport. Analysis of a subset of our samples showed densities ranging from 0 to 18,000 organisms per cubic meter of ballast water (excluding bacteria and viruses). If one extrapolates to include the total amount of ballast water on board, a bulker deballasting in Chesapeake Bay could release up to 1 billion organisms. Despite this high variability, we observed significant density differences among source regions. Samples from the Mediterranean/Black Sea (MBS) region had significantly lower numbers of organisms than did samples from the Northeast Atlantic (NEA) or the West Central Atlantic (WCA) (Fig. 3-19). This difference may reflect regional differences in abundance; age-related mortality (voyages from MBS were significantly longer than from NEA or WCA) (Table 3-9); or some combination of both. On-going studies show high mortality for organisms travelling between Israel and Baltimore, but estimates of survivorship for the other two regions are lacking at present (Smith et al., unpubl. data). We detected no seasonal differences in abundances of organisms arriving to Chesapeake Bay.

Comparison of our findings to those of Carlton and Geller (1993) suggests geographic differences in the abundances of organisms received by U.S. ports. For most taxa, Chesapeake Bay had a lower percentage of ballasted cargo holds arriving with abundant organisms (i.e., > 100 per tow) than did Coos Bay, Oregon. This difference may reflect the greater variability in the prevalence and abundance of organisms arriving to Chesapeake Bay from multiple source regions. In contrast, Coos Bay received a more constant supply of organisms from a single source region. Comparisons of initial abundances and survivorship between these and other regions are needed to understand more fully the correlations among inoculation density, inoculation frequency and invasion success.

The Presence of Life History Stages Younger than the Age of the Ballast Water

In a number of cases, larval or juvenile invertebrates were found in ballast samples that were less than the age of the ballast water itself, suggesting *in situ* generation of these individuals. Examples include larval hydromedusan jellyfish, polychaete worm larvae, ascidian (sea squirt) tadpole larvae, barnacle nauplii, and copepod nauplii. Explanations for the presence of these life history stages in the ballast samples include the following:

(1) larvae could be produced from adult organisms in semi-permanent benthic communities or as fouling organisms in ballast tanks,

(2) larvae could be produced from newly-ballasted adult organisms pumped or gravitated into cargo holds (either originally or by pressing up at a later date); this could especially occur if the vessel ballasted tychoplankton (small suspended benthic organisms) in shallow port waters,

(3) larvae could be produced by adult fouling organisms in the ships' sea chests,

(4) larvae could be produced by species with a very short generational time [e.g., the hydroid *Tubularia crocea* is reported to have settled on a ship's hull in Hawaii, and grown to reproductive maturity by the time the ship reached Panama 10 days later (WHOI 1952)].

In support of hypothesis 1, we found an ovigerous female capitellid worm in ballast tank sediments of one vessel, and settled adult barnacles have been observed in sea chests (hypothesis 3) of drydocked vessels (W.Walton, personal communication). Adult organisms capable of producing planktonic offspring (hypothesis 2) were collected from ballasted cargo holds: these included fish, worms, and copepods (NABISS 2 data) as well as ovigerous crabs (G. Ruiz, personal communication). Relative to hypothesis 4, we found medusae of the tubularian hydroid *Ectopleura dumortieri* that were one to two days old (C. Mills, personal communication) in cargo hold ballast water that was 17 days old, suggesting that this species has a fairly short life cycle.

Life in Ballast Sediments

The benthic sediments of both ballasted cargo holds and ballast tanks contained living organisms. In the case of ballasted cargo holds, these sediments act as a temporary sink or habitat for a number of benthic organisms and/or their life history stages, such as dinoflagellate cysts, crabs, shrimp, and bottom-dwelling fish. These sediment "communities", however, are only as old as the ballast leg, because the cargo hold is cleaned out whenever cargo is to be loaded. In the case of deballasted ballast tanks, access to (presumably) older sediments that were still wet yielded specimens of copepods, nematodes, foraminiferans, filamentous green algae, flatworms, several species of encysted dinoflagellates, and, in one vessel, an ovigerous capitellid polychaete worm. Because ballast tanks often are not cleaned out for extended periods (e.g., months to years); the potential to build a stable benthic community is much greater than in a cargo hold. We generally, however, had little access to these longer-term or semi-permanent ballast tank sediments. The potential importance of these communities is that they may act as a source of ciliates, dinoflagellates, and invertebrate larvae.

Transport of Ballast from Global Hot Spots to Chesapeake Bay

Our efforts to identify known invasive (or otherwise nuisance) species in ballast water and ballast sediments were hampered by the logistical difficulty of identifying most ballast organisms (especially larvae) to species (see Recommendations). For example, we reared bivalve larvae found in samples of predominately European ballast water (tanks were pressed outside of Chesapeake Bay) to settled juveniles over the course of several months. Without genetic analysis, however, we could not determine whether these were the native mussel *Mytilus edulis* or the invasive Mediterranean mussel *M. galloprovincialis.* The latter species has proved to be a highly invasive species in western Europe, South Africa, southern California, Japan, and elsewhere, and warm water shores of the Atlantic American coast remain the only continental margin of the Northern Hemisphere now uninvaded.

We documented one instance of a known invader. We found the Red Sea fish *Alepes djedaba* (Jedda jack), which invaded the Mediterranean via the Suez Canal, being ballasted in Israel and transported to Chesapeake Bay. This fish was unlikely to have survived the low salinities in Baltimore harbor or the colder winter waters of Chesapeake Bay. However, its path (Red Sea to Eastern Mediterranean to North America) is of interest in light of the recent invasion of the portunid crab *Charybdis helleri* into the greater Caribbean region and the Atlantic coast of Florida (Lemaitre 1995). This carnivorous crab is also believed to have come from the Red Sea via the Suez Canal into the Mediterranean and then to the Americas. *Charybdis* could have significant impact on mollusc and decapod communities in newly invaded areas.

The transport of specific nuisance taxa from a recognized global hot spot to another port remains poorly documented, not only because of difficulties in taxonomic resolution, but also because of the stochastic nature of vessel traffic and sampling. Nevertheless, the potential for nuisance species to 'leap-frog' from one region to another is great as evidenced by the apparent spread of *Vibrio cholera* introduced to South America to Mobile Bay, Alabama (Centers for Disease Control, 1991) and the dispersal of toxic dinoflagellates from Japanese to Australian and New Zealand waters (Hallegraeff and Bolch 1991, Baldwin 1992). Dedicated route studies and experimental programs that focus on this phenomenon would be of a significant value.

The Role of Long-Distance and/or Longer-Term Voyages in Ballast Biota Survival

Our data suggest that densities of organisms in ballast water decrease as the age of the water (i.e., voyage duration) increases. In particular, ballast water less than 14 days old had higher densities of plankton than did 14 to 24 day old ballast water (Figure 3-21). Similarly, Williams et al. (1988) reported that "few, if any animals are likely to be present after a transit time of about 24 days." Whether these apparent thresholds reflect a decay in food resources or other factors remains unclear. Caution, however, must be exercised before concluding that older water is necessarily of 'lower risk'. First, given existing trade patterns, we could not distinguish whether low abundances in 14-24 day old water were due to regional differences (most of these vessels were from a single, lower diversity region in the eastern Mediterranean) or whether they, in fact, represented age-dependent mortality. In order to tease apart these alternative hypotheses, we would need either to (1) monitor one or more additional trade routes from other regions that experience voyages of similar length, or (2) measure plankton survivorship on existing transoceanic routes directly. We are presently testing the latter alternative by sampling ballast biotic assemblages in the same ships at the start and finish of voyages (Smith et al., unpubl. data). Second, living organisms were found in our study in vessels with water 33 days old (from Ulsan, Korea), and live copepods were found in ballast water up to 95 days old (Carlton, 1985). In the latter case, however, these organisms may have arisen via excystment from resting eggs (as suggested by Williams et al., 1988), or alternatively through *in situ* reproduction. Finally, evidence that benthic and fouling communities exist in permanent ballast tanks (Williams et al., 1988; J. T. Carlton and J. Weis, personal communication) suggests that adult populations could generate larvae into the ballast water column for many weeks or months. The available data do not permit, at this time, setting a minimum or maximum "safe" time threshold for water age.

Invasion Risk: Port Trade Profiles and Volumes and Sources of Water Received

Ports in the United States differed dramatically in the amounts of foreign ballast water they received in 1991 (NABISS 1; Carlton, Reid, and van Leeuwen 1995); these differences reflected port-specific trade profiles. Undoubtedly, the type, volume, and source of commercial shipping traffic in each port influences the frequency and quantity of the biological inoculum received. As indicated in NABISS 1, the total number of ships arriving in ballast to a port is a poor indicator of the amount of ballast water received. Instead, a more reliable measure of ballast water amounts received in a port is the number of bulk cargo ships coming to load products. For example, the ports of New York and Miami received greater numbers of ships in ballast in 1991 (4058 and 5984, respectively) than did the ports of Norfolk, Virginia (2347) and Baltimore, Maryland (2043) (Carlton, Reid, and van Leeuwen, 1995). Norfolk and Baltimore, however, ranked second and fifth nationally in terms of the amount of ballast water received (vs. 10th and 13th for New York and Miami respectively) (Carlton, Reid, and van Leeuwen, 1995). Their high rankings reflected the fact that Baltimore and Norfolk are the principal exporters of bulk cargo (primarily coal) on the U.S. Atlantic coast. In contrast, New York and Miami received primarily cruise ships which carry and release relatively little ballast water. In our survey, Baltimore received considerable traffic from RoRos and both Baltimore and Norfolk received substantial numbers of container ships (Fig. 3-1). As with cruise ships, these vessels carried and released relatively little ballast water in comparison to bulkers. We caution, however, that this does not mean that such vessels are low risk relative to their ability to transport and release exotic species.

From 1993 to 1994, the port of Norfolk received over 2.5 times more ballast water than did Baltimore, despite receiving a similar number of bulkers. The difference reflects the fact that a higher percentage of bulkers was arriving in Norfolk to load cargo than in Baltimore (Fig. 3-2). Thus, even for ports within a single estuary, the type of commodities being imported and exported greatly influences the amount of ballast water each received.

The relative amounts of ballast water arriving in Norfolk and Baltimore in our study match those reported in NABISS 1 by Carlton, Reid, and van Leeuwen (1995) for 1991. The absolute amounts, however, differ between the two studies. Over the 12 month span of our study (NABISS 2), we estimated that the ports of Baltimore and Norfolk received 5,882,459 MT and 15,225,188 MT of ballast water, respectively, from bulkers alone. These amounts are significantly greater than estimates by Carlton, Reid, and van Leeuwen (1995) (2,833,729 MT in Baltimore and 9,325,145 MT in Norfolk), even though the total number of vessels used in the calculations was similar between the studies. The difference is explained by the fact that NABISS 1 used data drawn from a more variable, nationwide sample of vessels. The average amount of ballast water on board in our calculations was larger than that used in NABISS 1, because vessels arriving in Baltimore and Norfolk were larger than the national average. Thus, while the relative port rankings generated in NABISS 1 remain unchanged, the amount of ballast water Carlton, Reid, and van Leeuwen (1995) estimated entering U.S. ports (or at the very least, Chesapeake Bay ports) is even more conservative than originally proposed.

The ports of Norfolk, Virginia and Baltimore, Maryland received water from multiple source regions; vessels sampled in our survey originated from 6 global ocean regions. Bulk cargo carriers arrived in ballast from 25 countries and 39 last ports of call. The vast majority of the water released into Chesapeake Bay came from three regions: the Northeast Atlantic Ocean, the Mediterranean-Black Sea Region, and the Western Central Atlantic Ocean. This is in contrast to the previous data set available in the United States, which reported plankton arriving on the Pacific coast of America from 1 ocean region (Northwest Pacific), 1 country (Japan), and 25 last ports of call within that country (Carlton and Geller, 1993). This large number of source regions contributing nonindigenous taxa into one estuary reflects one of the greatest difficulties in assessing invasion risk due to ballast water transport. Local and regional variations (e.g., tidal, hydrographic, physical-chemical), spatial variations (e.g., different regions within a harbor, proximity to a sewage outfall), and temporal variations (diurnal, lunar, seasonal, annual, decadal) could and do generate extensive variation in the composition and abundance of plankton carried out of a port by a departing ship. A second layer of temporal variation is then added upon these earlier parameters, because different vessels retain ballast water for different lengths of time, depending upon many factors, including length of voyage, cargo requirements, and weather conditions. Thus RoRos and container vessels frequently contain older water, in contrast to bulker cargo hold water, which is often no older than the length of the voyage from the last port of call. When these complexities are considered against the larger backdrop of many different global source regions the scale of complexity becomes enormous.

Invasion Risk: Discharge Port Compatibility

A critical factor influencing the survival of the biological inoculum after deballasting is the compatibility of physical conditions between donor and recipient ports. Certainly, a port will be at greater risk of a ballast-mediated invasion if the temperature and salinity of its water are similar those of the donor ports.

Our data show a striking difference between the salinity of Baltimore harbor and that of the majority of ballast water it receives (Figs.3-8, 3-11). Much of the ballast water arriving from the Mediterranean, Northeast Atlantic and West Central Atlantic regions was greater than 21 ppt (Fig. 3-9). With the exception of very euryhaline species or those with resistant stages, most ballast water organisms should perish following their release into Baltimore's low salinity (3 to 8 ppt) water. In contrast, organisms deballasted in the higher salinity waters (20 to 28 ppt) in Norfolk should have a greater probability of survival. The latter conclusion assumes that the salinity profile of the ballast water reaching Norfolk, where we had few samples, is similar to that arriving in Baltimore.

Differences in temperature between Baltimore harbor and deballasted water were less extreme than those for salinity. Not surprisingly, differences were least in summer, when temperatures in Chesapeake Bay most closely matched the warm water ports of the eastern Mediterranean and West Central Atlantic. Survivorship of deballasted organisms should be higher in summer. In contrast, organisms arriving from these ports to Chesapeake Bay between late fall and early spring, however, would likely experience significant temperature-related physiological stress.

Frequency and Effectiveness of Ballast Exchange

Despite calls from the International Maritime Organization for voluntary open-ocean exchange of ballast water, exchanges were reported in only 17% of the bulkers sampled in our study (Table 3-4). Furthermore, in these instances, ship's officers often overestimated the effectiveness of the exchange (Table 3-5). Reasons for conducting an exchange included a desire to flush tanks of sediment-laden water taken on in port and the assumption that exchange was 'required' before entering U.S. coastal ports. In our interviews with ship's officers, they indicated that exchange of cargo holds was more difficult than that of ballast tanks. In our survey, exchanges were reported more frequently for the latter.

We found, as did Locke et al. (1991, 1993) that original port and coastal organisms (e.g., coastal spionid polychaetes) remained in exchanged ballast tanks and cargo holds, although in far fewer numbers (Fig. 3-18, Tables 3-23, 3-24). While strongly suggestive that exchange is effective in reducing (but not eliminating) coastal taxa, these data must be interpreted with caution because of the small sample sizes and nature of the samples (i.e., they were generally not taken from paired tanks on the same vessels; see Results).

Ballast exchange remains the primary means of ballast management in the mid-1990s. While undoubtedly acting to reduce significantly the numbers of original organisms, and while saltwater exchange for freshwater ballast has both a flushing and a biocidal effect (but see Carlton, Reid, and van Leeuwen, 1995 for exceptions), the fact that aboriginal taxa remain (due to inadequate exchange) argues that ballast exchange is not a complete solution. In addition, many vessels, for safety reasons, may not be able to undertake ballast exchange. These considerations are some of the primary motivations for seeking ballast management options other than, or in addition to, ballast exchange (Carlton, Reid, and van Leeuwen 1995).

Conclusions

Our study suggests that the risk of ballast-mediated invasion in United States coastal waters remains extraordinarily high. In the estuary studied here, Norfolk and the lower Chesapeake Bay appear to be at greater risk to ballast-mediated invasion than Baltimore and the upper Bay, because: (1) Norfolk receives greater amounts of foreign ballast water than does Baltimore, and (2) there is greater similarity in salinity between deballasted water and Norfolk harbor water. That said, it is ill-advised to conclude that ports such as Baltimore are 'safe' from ballast-mediated invasions. At present, little is known of the processes that mediate successful ballast invasions. It is not clear whether repeated inoculations are needed over time or whether a single vessel, densely packed with organisms is sufficient to establish a population. If the latter is the case, then no port receiving water from an exogenous source is immune. There is circumstantial evidence that both mechanisms may be in operation. Many marine invasive species, apparently distributed by ballast water (or by hull fouling), are found in ports and harbors in much of the world, suggesting their constant (multiple inoculation) and extensive (massive inoculation) transport. Other invasions appear in only one site, suggesting rare and inconsistent transport. Thus the common Japanese shore crab *Hemigrapsus sanguineus*, otherwise known only from western Pacific shores, has invaded the mid-Atlantic coast of North America (from an initial colonization site in New Jersey, near the mouth of Delaware Bay), rather than any site in the Pacific Ocean (McDermott 1991). That few vessels from the western Pacific discharge ballast at the entrance to Delaware Bay suggests the possibility that the inoculation may have been due to a single vessel release. Thus, while the water that Baltimore receives is generally mismatched with the environmental conditions of the port, reasonable chances remain that an invasion could occur from a vessel releasing fresh- or brackish-water organisms. Five ships in our study, in fact, deballasted water that closely matched both the temperature and salinity of Baltimore harbor water. These vessels came from 4 different ocean regions (Fig. 3-11; one point is covered by another), which argues against classifying certain donor regions as hazard-free. Temperatures and salinities within any region can vary substantially depending on the donor port's location. It also is important to recognize that ballast water inoculations in Chesapeake Bay are not restricted to the ports of Baltimore and Norfolk. Many bulkers travelling to Baltimore released ballast water continuously as they moved up the shipping channel; hence, deballasted organisms could be distributed throughout the Bay. Given the constant threat of a ballast-mediated introduction, control measures are critically needed.

The enormous variability in the composition and abundance of organisms encountered in ballast water of ships coming to Chesapeake Bay coupled with the extreme difficulty (if not impossibility) in identifying most organisms to species argue against the establishment of biomonitoring programs that assess whether incoming ships are safe to deballast in port. First, morphological identification of the myriad larvae from different parts of the world could not be accomplished in timely fashion. Although relatively rapid assays to detect the presence of some harmful organisms, such as *Vibrio cholera* may exist, these specific tests cannot guarantee that other (current or future) nuisance species are not present. Third, it is difficult, if not impossible, to sample all tanks for organisms. Ballast tanks and cargo holds on a single ship can have water from different sources. Furthermore, access to the lower levels of ballast tanks is often not possible, which means many organisms may be missed.

In the short term, the ballast micromanagement practices recommended in NABISS 1 (Carlton, Reid and van Leeuwen 1995) combined with ballast exchange may be the best method for reducing the risk of ballast-mediated invasions. While our data suggest that mid-ocean exchange was effective in removing most (but not all) coastal plankton, more rigorous experiments are needed that compare matched exchanged and unexchanged tanks on the same ship. At the least, vessels coming from known hot spots or with water similar in salinity to that of the recipient port should be requested to attempt open-ocean exchange. In the long-term, technological innovations are needed either to prevent intake of aquatic organisms into ballast tanks, or eliminate them once they are on board.

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Appendix A

Acronyms and Abbreviations

α	Type I error rate
ANOVA	Analysis of variance
ANCOVA	Analysis of covariance
BME	Baltimore Maritime Exchange
BW	Ballast water
BWCAP	Ballast water capacity of vessel, in metric tons (MT); % BWCAP, percent of vessel ballast capacity filled
BWTCAP	Ballast water tank capacity, in metric tons (MT); % BWTCAP, percent of tank filled with ballast water
BWOB	Ballast water on board vessel, in metric tons (MT)
BWIT	Ballast water in tank, in metric tons (MT)
C	Celsius degrees
df	Degrees of freedom
D. O.	Dissolved oxygen, in milligrams per liter (mg/l)
ETA	Estimated time of arrival
FAO	United Nations' Food and Agriculture Organization (standardized ocean regions of the world)
g d wgt-1	Per grams dry weight
GRT	Gross register tonnage, a measure of vessel volume in register tons (1 RT = 1000 cubic feet), including the hull and all enclosed above deck space
HRMA	Hampton Roads Maritime Association
IO	Indian Ocean (FAO region)
LFPOC	Last foreign port of call
LP	Lake Panama (not an FAO region, but listed separately because none of the regions was appropriate for this water source)
LPOC	Last port of call
LT	Long tons, a unit of ballast water measurement (1 LT = 1.0162 MT)
m	meters
m^3	Cubic meters, a measure of ballast water amount (1 m^3 of fresh water = 1 MT; for conversion factors for brackish and saline water see Methods)
MD	Maryland
mg/l	milligrams per liter

ml	milliliter
MBS	Mediterranean - Black Sea (FAO region)
MSO	Marine Safety Office
MT	Metric ton, a unit of ballast water mass (also measured in LT, m^3)
n	sample size
NAA	North American Atlantic
NABISS	National Biological Invasions Shipping Study
NEA	Northeast Atlantic (FAO region)
No.	Number
NWA	Northwest Atlantic (FAO region)
NWP	Northwest Pacific (FAO region)
p	probability
ppt	Parts per thousand, a measure of salinity
Q?	Qualitative sample
RoRo	Roll-on, Roll-off vessel
S. D.	Standard deviation
S. E. M.	Standard error of the mean
SERC	Smithsonian Environmental Research Center
STP	Simultaneous Test Procedure
U.S.	United States
USCG	United States Coast Guard
VA	Virginia
WCA	West Central Atlantic (FAO region)
WCMS	Williams College-Mystic Seaport

Appendix B

Letter of Introduction

SMITHSONIAN ENVIRONMENTAL
RESEARCH CENTER
P.O. Box 28, Edgewater, MD 21037-0028

MARITIME STUDIES PROGRAM
WILLIAMS COLLEGE - MYSTIC SEAPORT
P.O. Box 6000, Mystic, CT 06355-0990

SCIENTIFIC STUDY OF BALLAST WATER

** We are studying the aquatic salt and freshwater life in ballast water and sediments. This U.S. Coast Guard sponsored study was requested by the United States Congress. Your participation in this program is voluntary. The purpose of our study is to determine what kinds of organisms are taken up in ballast and what kinds of organisms survive voyages from port to port.

** This study is not an inspection or examination. We are not studying water pollution. This is a scientific study on the transport of living organisms into U.S. waters by ballast water.

** We sample the living organisms in ballast water. We normally collect these organisms from the ballast water **by lowering a net through a deck hatch or manhole cover**. Your cooperation in gaining access to the water is appreciated.

** This is a cooperative research program between scientists from Williams College-Mystic Seaport (WCMS) and the Smithsonian Environmental Research Center (SERC); the program is based locally at SERC.

** If you have any questions, please do not hesitate to contact us at the SERC numbers listed below. Again, we thank you for your time and cooperation in this important study.

Sincerely,

Dr. David Smith (WCMS)
Ms. Linda McCann (WCMS)
Ms. Marjorie Wonham (WCMS)
Dr. Gregory Ruiz (SERC)
Dr. Anson Hines (SERC)

Baltimore Area - Phone: 410-269-1412 Washington Area - Phone: 301-261-4190 Annapolis Area - Phone: 410-798-4424
FAX: 301-261-7954

Appendix C

Ship-Boarding Questionnaire

BALLAST DATA SHEET

NAA-

Date/Time:
Field Party:

VESSEL INFORMATION: Vessel name:

Registry: Type: GRT:
Agency: Officers & crew:
Arrival port: Berth: LPOC:
Arrival date: Time: LFPOC:
Departure date: Next Ports:

BALLAST WATER INFORMATION: Specify units: m3, MT, LT, ST

Total BW on board:
Total BW capacity:

DEBALLASTING	Here	Y / N
(when, where,	Later	Y / N
BW sources?)	Earlier	Y / N

BW SOURCES:

Dates	Locations	Tanks	Ballasted / Pressed up

BW EXCHANGED: Y / N (specify quantities, locations, dates, reasons)

SAMPLING:

	Tank 1 (blue)						Tank 2 (orange)					
Tank/hold (number, name)												
BW capacity of tank												
BW quantity in tank												
BW source(s)												
Date(s) ballasted												
Sample Net	Diam:			Mesh:			Diam:			Mesh:		
method	Water depth in tank:						Water depth in tank:					
	jar	tow ht	Q?	jar	tow ht	Q?	jar	tow ht	Q?	jar	tow ht	Q?
	1-1			1-4			2-1			2-4		
	1-2			1-5			2-2			2-5		
	1-3			1-6			2-3			2-6		
Pump (volume / rate)												
Dip net? (write details on back)												
Wayne's samples (check)	dead			live			dead			live		
Salinity (o/oo)												
Temp (oC)												
Water colour, other notes												
Deballasting during sampling?	Y / N	depth water dropped:					Y / N	depth water dropped:				

SHIPSIDE: Salinity (o/oo) Temp (oC)

PHOTOS: Vessel Sampling

MJW 6/94

VERTICAL PROFILES

Tank 1 (blue)

Salinity Meter			DO Meter		
Depth (m)	Temp (oC)	Salinity (o/oo)	Depth (m)	Temp (oC)	DO (mg/l)

Tank 2 (orange)

Salinity Meter			DO Meter		
Depth (m)	Temp (oC)	Salinity (o/oo)	Depth (m)	Temp (oC)	DO (mg/l)

SHIPSIDE

Salinity Meter			DO Meter		
Depth (m)	Temp (oC)	Salinity (o/oo)	Depth (m)	Temp (oC)	DO (mg/l)

NOTES:

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Appendix D

Containment Protocol for Research on Nonindigenous Aquatic Species

CONTAINMENT PROTOCOL

HANDLING, QUARANTINE, AND DISPOSAL PROTOCOLS FOR NONINDIGENOUS SPECIES AT THE SMITHSONIAN ENVIRONMENTAL RESEARCH CENTER
October 1993

Project Specific Research Containment Protocol for: The National Biological Invasions Shipping Study (NABISS): Biological Invasions by Nonindigenous Species in United States Waters: Quantifying the Role of Ballast Water and Sediments

The NABISS Project at SERC involves the collection of living plankton and benthos from the ballast water and sediments of foreign vessels arriving in Chesapeake Bay, and the transportation of these organisms back to specific and authorized laboratories at SERC. The following handling, quarantine, disposal, and termination protocols will be observed for this project. Some of these procedures are adopted in part from the "Zebra Mussel Containment Protocols", by D. P. Reid et al. (1993).

Definition of "Specific and Authorized Laboratory Sites"

Specific and authorized laboratory sites means those locations within the SERC complex that Center authorities have identified and designated as sites within which research may be conducted on living and preserved organisms. A site map will indicate these authorized locations. In the following document, "the laboratory" or "laboratory" means these specifically designated locations within SERC.

HANDLING AND QUARANTINE

Facility Containment Protocol

1. Samples with living ballast organisms brought to SERC will be held in specifically designated rooms with lockable doors. These rooms will be designated as "Restricted Areas" and be so posted. Doors are to be locked when responsible personnel are absent for extended periods of time (such as evenings, weekends, and holidays). These laboratory rooms will be used for (a) the examination of living and preserved samples of ballast organisms and (b) the culture of organisms derived from the ballast.

2. In no case will nonindigenous organisms be held in flowing water systems which do or could drain to the outside environment.

3. All research and staff personnel with involvement in the NABISS project and have key access to the designated laboratory sites will be briefed about the project and will indicate that they understand that living nonindigenous organisms are in the laboratory and may not be removed from the laboratory. All such personnel will sign a documentation sheet indicating that they have been so briefed and that they understand these protocols.

Field and Laboratory Equipment Use Protocol

4. Nets, plankton bottles, and any other equipment used in the collection of plankton from ballast tanks will not be used in any other field work.

5. Culture dishes, buckets, forceps, and any other equipment used in the handling, culturing, or storing of nonindigenous organisms in the laboratory will not be used in any other laboratory work.

6. All such field and laboratory equipment will be clearly labeled as 'EX' (live exotic), 'FX' (Formalin or fixed exotic); or 'BW' (Ballast Water).

Equipment Cleaning and Disinfectant Protocol

7. All equipment, or other devices used in the collecting, handling, and processing of ballast water plankton (including but not limited to nets, field containers, buckets, glassware, plastic ware and examination tools) will be rinsed and washed in sinks or other washing areas whose drainage leads solely to septic systems. Chlorine baths will be available for the dipping and rinsing of laboratory and collecting equipment.

ACCIDENTAL SPILLS PROTOCOL

1. A spill of specimens of nonindigenous organisms will be considered an emergency situation.

2. A procedure to handle such spills shall be posted conspicuously in all nonindigenous organisms work areas.

NORMAL TERMINATION PROTOCOL

1. All living ballast plankton brought to SERC will be preserved after examination, after culturing, or after experimental work.

2. The sole exception to (1) will be the hand carrying of, or express mail shipment of living specimens to consulting systematists (taxonomists) for identification, with the understanding that these specimens will subsequently be preserved. A log will be maintained of the organisms (species) and numbers of all living specimens provided to consulting systematists, the methods of conveyance, and to whom and when they are carried or sent.

3. The sole exception to (2) will be when the consulting systematists finds it necessary to maintain the organisms in question alive for observation, culturing, or other purposes. In this case it is the responsibility of NABISS personnel to inform the consulting laboratory of the minimum handling, quarantine, and disposal protocols recommended when nonindigenous organisms are involved in research programs. A copy of these protocols must be provided to the consulting laboratory.

PROJECT TERMINATION PROTOCOL

1. Upon final completion of the NABISS project (or related ballast research projects) or removal from the project, all equipment, supplies, and other materials which may or could be reused in future research projects must be sterilized by chlorinating, autoclaving, freezing, acid bath, or other suitable means.

2. All other equipment, supplies, and other materials for which there are no plans for future use must be treated as in (1) or destroyed.

EMERGENCY TERMINATION PROTOCOL

1. If the integrity of the research facility at SERC is threatened by imminent and confirmed destruction by hurricanes, flood, or other event, and if time allows (without threat to personnel safety), all living nonindigenous organisms (species) cultures and experiments shall be either (a) transported off-site in sealed and labeled containers to a suitable facility, or (b) terminated by adding chlorine bleach to all pertinent systems at a volume:volume ration of 1:50 (1 part bleach for every 50 parts water).

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Appendix E

Data Sheets for Live Analysis of Ballast Samples

NAA - ____________

BALLAST LIVE ANALYSIS DATA SHEET

Analysis Date: ____________ Ship Name: ____________

Analysis Time: ____________ Your Initials: ____________

General Notes: ____________

TAXON	Present?	Abundant (>100)	Common (10-100)	Rare (<10)	Voucher? (how many?)	Photos? (roll/slide#)	Cultured? (C-#)	NOTES
DINOFLAGELLATA								
Ceratium								
DIATOMACEA								
Discoid								
"PROTOZOA"								
Radiolaria								
Foraminifera								
Tintinnida								
Ciliates								
CNIDARIA								
Hydrozoa								
Anthozoa								
Scyphozoa								
CTENOPHORA								
PLATYHELMINTHES								
Turbellaria								
Müller's or Götte's larvae								
NEMATODA								
ROTIFERA								
GASTROTRICHA								
SIPUNCULA								

TAXON	Present?	Abundant (>100)	Common (10-100)	Rare (<10)	Voucher? (how many?)	Photos? (roll/slide#)	Cultured? (C-#)	NOTES
NEMERTEA								
ANNELIDA								
Polynoidae								
Phyllodocidae								
Spionidae								
MOLLUSCA								
Gastropoda								
Bivalvia								
Pteropoda								
CRUSTACEA								
Cirripedia								
nauplii								
cyprids								
Copepoda								
nauplii								
copepodites								
Harpacticoida								
Calanoida								
Cyclopoida								
Poecilostome								
other								
Amphipoda								
Gammaridea								
Hyperiidea								
Caprellidea								
Isopoda								
Decapoda								
Brachyura								
Caridea								

TAXON	Present?	Abundant (>100)	Common (10-100)	Rare (<10)	Voucher? (how many?)	Photos? (roll/slide#)	Cultured? (C-#)	NOTES
Anomura								
Tanaidacea								
Mysidacea								
Cumacea								
Stomatopoda								
Euphausiacea								
Ostracoda								
other Crustacean nauplii								
CHELICERATA								
PHORONIDA								
BRACHIOPODA								
CHAETOGNATHA								
ECHINODERMATA								
Asteroidea								
Ophiuroidea								
Echinoidea								
Holothuroidea								
Unknown								
HEMICHORDATA								
CHORDATA								
Ascidiacea								
Thaliacea								
Larvacea								
Fish								
OTHER								
Eggs								
Planuloids								
"PLANTAE"								
Rhodophyta								
Chlorophyta								
Phaeophyta								
Zosteraceae								

Appendix F

LARVAL CULTURING

Protocols for rearing barnacles, spionid polychaetes, and bivalves are sketched briefly below; they draw largely on the expertise of scientists involved in the various ballast water projects at SERC. We used 10 µm filtered river water for culturing and adjusted salinity by dilution or addition of Instant Ocean™. Larvae were cultured in pyrex dishes (80 mm width x 40 mm depth) in 100 ml of water in temperature- and light-controlled chambers.

Barnacles. We used Dineen's & Hines' (1992) protocol to rear larval barnacles to adulthood. Nauplii were placed in microfiltered river water and fed a combination of *Dunaliella tertiolecta* and *Isochrysis galbana* (Caribbean strain). Antibiotics were added daily. Barnacle nauplii metamorphosed to cyprids in as little as 6 d, and cyprids settled on slate plates or the sides of the dish after 4-5 d.

Spionid polychaetes. We reared larvae of spionid polychaetes using the protocol described in McCann & Levin (1989). Larvae were kept in culture dishes (25 individuals/dish) containing microfiltered sea water, but lacking sediment. Water was changed and food (1:1 mixture of *Dunaliela* sp. and *Isochrysis galbana*) added every other day. Planktotrophic polychaetes (e.g., *Streblospio benedicti*) could remain in the water column at least 1-2 wks before settling. When larvae were ≥ 10 segments in length, a thin layer of sediment was added to promote settlement. Sediment for tubiculous polychaetes was collected locally; sieved through a 500 µm mesh screen and frozen for 1 wk to kill all resident organisms. Prior to use, we covered the thawed sediment with a thin layer of seawater to encourage bacterial growth. Settled polychaetes were kept in culture dishes with a thin layer of mud and were fed a tablespoon of mud 1-2 times per wk.

Bivalves. We use protocols provided by D. Carlton (pers. comm.) and Strathmann (1987) to culture bivalves. Veligers were reared in culture dishes containing microfiltered sea water at densities of 25 individuals/dish. Water was changed and food (1:1 mixture of *Dunaliela* and *Isochrysis*) added each day. Antibiotics were added daily. When veligers showed signs of 'feeling' the bottom a small amount of sediment (1 ml) was added to stimulate settling and metamorphosis.

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Appendix G

Vessel Types by Season

Baltimore, MD
Norfolk, VA

BALTIMORE, MD

Number of vessels arriving in Baltimore, Maryland between January and December 1994 by season[1] and vessel type. Data are summarized from Baltimore Maritime Exchange weekly vessel traffic reports. Percentages (in parentheses) denote distribution of vessel types within each season and for entire year.

Vessel Type	Season				Total
	Fall	Winter	Spring	Summer	
Bulker	144 (26.2)	118 (30.3)	184 (30.9)	177 (31.2)	623 (29.7)
RoRo	138 (25.1)	98 (25.2)	147 (24.7)	134 (23.6)	517 (24.6)
Container	229 (41.7)	140 (36.0)	221 (37.1)	223 (39.3)	813 (38.7)
Other[2]	38 (6.9)	33 (8.5)	44 (7.4)	33 (5.8)	148 (7.0)
Total	549	389	596	567	2101

[1] Fall, Sept. - Nov.; Winter, Dec. - Feb.; Spring, Mar. - May; Summer, June - Aug.

[2] Other includes tankers, tugs, barges, ice breakers, passenger ships, and cable ships.

NORFOLK, VA

Number of vessels arriving in Norfolk, Virginia between January and December 1994. Data are summarized from Hampton Roads Maritime Association weekly vessel traffic reports. Percentages (in parentheses) denote distribution of vessel types within each season and for entire year.

	Season				
Vessel Type	Fall	Winter	Spring	Summer	Total
Bulker	211 (32.5)	187 (33.0)	173 (30.9)	150 (28.5)	721 (31.3)
RoRo	15 (2.3)	6 (1.0)	3 (0.5)	10 (1.9)	34 (1.5)
Container	309 (47.5)	293 (51.7)	296 (52.9)	290 (55.0)	1188 (51.6)
Other[2]	115 (17.7)	81 (14.3)	88 (15.7)	77 (14.6)	361 (15.7)
Total	650	567	560	527	2304

[1] Fall, Sept. - Nov.; Winter, Dec. - Feb.; Spring, Mar. - May; Summer, June - Aug.

[2] Other includes tankers, tugs, barges, and combinations.

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Appendix H

Vessel Load and Discharge Statistics for Baltimore and Norfolk by Season

Number of bulkers loading and discharging cargo by month in Baltimore, Maryland (January to December 1994, and in Norfolk, Virginia (September 1993 to August 1994). Data are summarized from Baltimore Maritime Exchange and Hampton Roads Maritime Association weekly vessel traffic reports.

	Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec	Total
Baltimore:													
Load	22	15	24	20	22	10	20	14	7	15	11	7	187
Discharge	34	27	31	38	36	39	42	43	36	28	39	7	400
Load & Discharge	2	2	2	2	3	2	2	2	2	2	2	1	24
Unknown	1	1	1	3	2	0	1	2	2	0	0	0	13
Total	59	45	58	63	63	51	65	61	47	45	52	15	624
Norfolk:													
Load	48	42	45	36	34	42	40	37	50	12	42	56	484
Discharge	10	10	18	18	8	8	9	12	10	6	11	15	135
Load & Discharge	0	1	3	3	4	1	0	1	1	14	3	5	36
Unknown	0	0	0	0	4	0	0	0	1	0	0	0	5
Total	58	53	66	57	50	51	49	50	62	32	56	76	660

Appendix I

Last Region, Country, and Port of Call of Bulkers

Frequency and percentage of bulkers sampled in Baltimore, Maryland and Norfolk, Virginia between August 1993 and August 1994 (n = 60) listed by last FAO region[1], country, and port of call.

FAO Region	Last Country of Call	Last Port of Call	Vessels No.	Vessels %
Mediterranean-Black Sea	Egypt	Alexandria	1	2
	Greece	Pylos	1	2
	Israel	Ashdod	7	12
		Hadera	2	3
	Italy	Trieste	1	2
	Spain	Tarragona	2	3
	Turkey	Istanbul	2	3
		Tuzla	1	2
Northeast Atlantic	Belgium	Antwerp	2	3
		Gent	2	3
		Zeebrugge	2	3
	Britain	Gibraltar	1	2
		Immingham	1	2
		Liverpool	1	2
		Port Talbot	1	2
	France	Dunkirk	4	7
	Germany	Bremen	1	2
		Wilhelmshaven	1	2
	Morocco	Mohammedia	1	2
	Netherlands	Rotterdam	4	7
	Portugal	Sines	1	2
	Russia	St. Petersburg	1	2
	Spain	Bilbao	1	2
		Gijon	1	2
Northwest Atlantic	Canada	Port Alfred	1	2
	USA	New York, NY	1	2
West Central Atlantic	Dominican Republic	Rio Haina	1	2
	Guatemala	Puerto Barrios	1	2
	Mexico	Veracruz	2	3
	Trinidad	Point Lisas	1	2
		Unknown	1	2
	USA	Port Manatee, FL	1	2
		New Orleans, LA	2	3
		Houston, TX	2	3
	Venezuela	La Guaira	1	2
Northwest Pacific	Japan	Hibikinada	1	2
	South Korea	Ulsan	1	2
Indian Ocean	Australia	Fremantle	1	2
		Unknown	1	2
Total	25	39	60	

[1] United Nations' Food and Agriculture Organization (FAO) standardized ocean regions of the world.

Appendix J

Bulker Statistics by Season and Source Region

Gross Register Tonnage
Ballast Water Capacity
Ballast Water on Board

Gross Register Tonnage

Summary of mean gross register tonnage[1] of bulkers sampled in Baltimore, Maryland and Norfolk, Virginia between August 1993 and August 1994 by season[2] and by FAO region[3] of ballast water origin. N, number of vessels; S. D., 1 standard deviation.

FAO Region		Season				Total
		Fall	Winter	Spring	Summer	
Mediterranean-Black Sea	Mean	53,661	67,258	51,462	69,551	59,546
	N	7	2	3	5	17
	S. D.	20,556	6,413	9,752	9,766	16,213
Northeast Atlantic	Mean	49,128	46,998	69,773	63,394	59,177
	N	3	5	7	4	19
	S. D.	23,663	35,051	17,264	19,650	24,588
Northwest Atlantic	Mean	19,684	-	38,299	50,216	44,152
	N	1		1	5	7
	S. D.	-	-	-	22,448	21,727
East Central Atlantic	Mean	-	-	-	7,189	7,189
	N				1	1
	S. D.	-	-	-	-	-
West Central Atlantic	Mean	17,673	45,041	27,714	23,148	30,281
	N	1	3	4	3	11
	S. D.	-	27,478	16,646	21,411	20,615
Northwest Pacific	Mean	19,702				19,702
	N	1				1
	S. D.	-	-	-	-	-
East Central Pacific	Mean	-	-	-	37,519	37,519
	N				1	1
	S. D.	-	-	-	-	-
Indian Ocean	Mean	22,629	-	-	36,983	29,806
	N	1			1	2
	S. D.	-	-	-	-	10,150
Total	Mean	43,050	50,463	52,797	50,177	49,200
	N	14	10	15	20	59
	S. D.	22,713	28,241	23,237	24,518	24,077

[1] 1 register ton = 1000 ft^3.

[2] Fall, Sept. - Nov.; Winter, Dec. - Feb.; Spring, Mar. - May; Summer, June - Aug.

[3] United Nations' Food and Agriculture Organization (FAO) standardized ocean regions of the world.

Ballast Water Capacity

Summary of mean ballast water capacity (MT) of bulkers sampled in Baltimore, Maryland and Norfolk, Virginia between August 1993 and August 1994 by season[1] and by FAO region[2] of ballast water origin. N, number of vessels; S. D., 1 standard deviation.

FAO Region		Season				Total
		Fall	Winter	Spring	Summer	
Mediterranean-Black Sea	Mean	53,018	75,883	45,211	72,793	60,146
	N	7	2	3	5	17
	S. D.	27,537	793	26,077	7,245	22,968
Northeast Atlantic	Mean	44,154	36,972	64,160	56,272	52,376
	N	3	4	6	4	17
	S. D.	21,443	34,156	19,190	26,389	25,490
Northwest Atlantic	Mean	14,675	-	24,300	39,996	34,136
	N	1		1	5	7
	S. D.	-	-	-	20,132	19,444
East Central Atlantic	Mean	-	-	-	1,368	1,368
	N				1	1
	S. D.	-	-	-	-	-
West Central Atlantic	Mean	11,000	28,355	16,116	16,448	19,079
	N	1	3	4	3	11
	S. D.	-	7,557	17,928	13,761	13,552
Northwest Pacific	Mean	15,194	-	-	-	15,194
	N	1				1
	S. D.	-	-	-	-	-
East Central Pacific	Mean	-	-	-	22,000	22,000
	N				1	1
	S. D.	-	-	-	-	-
Indian Ocean	Mean	21,249	-	-	20,000	20,625
	N	1			1	2
	S. D.	-	-	-	-	883
Total	Mean	40,407	42,746	43,525	44,087	42,834
	N	14	9	14	20	57
	S. D.	26,536	28,648	27,878	27,416	26,801

[1] Fall, Sept. - Nov.; Winter, Dec. - Feb.; Spring, Mar. - May; Summer, June - Aug.
[2] United Nations' Food and Agriculture Organization (FAO) standardized ocean regions of the world.

Ballast Water on Board

Summary of mean ballast water on board (MT) of bulkers sampled in Baltimore, Maryland and Norfolk, Virginia between August 1993 and August 1994 by season[1] and by FAO region[2] of ballast water origin. N, number of vessels; S. D., 1 standard deviation.

FAO Region		Season				
		Fall	Winter	Spring	Summer	Total
Mediterranean-Black Sea	Mean	44,045	59,838	29,667	62,531	48,803
	N	7	2	3	5	17
	S. D.	24,459	4,191	16,803	13,903	21,488
Northeast Atlantic	Mean	35,610	26,169	53,176	43,414	40,690
	N	3	4	5	4	16
	S. D.	19,667	34,573	19,795	18,401	24,045
Northwest Atlantic	Mean	8,400	-	23,850	23,270	21,229
	N	1		1	5	7
	S. D.	-	-	-	14,859	13,388
East Central Atlantic	Mean	-	-	-	1,128	1,128
	N				1	1
	S. D.	-	-	-	-	-
West Central Atlantic	Mean	10,000	12,923	8,573	12,658	11,003
	N	1	3	4	3	11
	S. D.	-	8,798	9,379	11,246	8,459
Northwest Pacific	Mean	9,400	-	-	-	9,400
	N	1				1
	S. D.	-	-	-	-	-
East Central Pacific	Mean	-	-	-	400	400
	N				1	1
	S. D.	-	-	-	-	-
Indian Ocean	Mean	320	-	-	22	171
	N	1			1	2
	S. D.	-	-	-	-	210
Total	Mean	31,662	29,236	31,771	32,109	31,457
	N	14	9	13	20	56
	S. D.	24,763	28,432	24,006	25,736	24,861

[1] Fall, Sept. - Nov.; Winter, Dec. - Feb.; Spring, Mar. - May; Summer, June - Aug.

[2] United Nations' Food and Agriculture Organization (FAO) standardized ocean regions of the world.

Appendix K

Ballast Water Characteristics
By Season and Source Region

Temperature
Salinity
Age

Temperature

Summary of mean temperature (°C) of ballast water in ballast tanks and cargo holds of bulkers sampled in Baltimore, Maryland and Norfolk, Virginia between August 1993 and August 1994 by season[1] and by FAO region[2] of ballast water origin. N, number of ballast tanks and cargo holds; S. D., 1 standard deviation.

FAO Region		Season				Total
		Fall	Winter	Spring	Summer	
Mediterranean-Black Sea	Mean	21.9	16.3	18.8	26.0	21.6
	N	7	3	3	5	18
	S. D.	3.3	3.9	3.6	2.5	4.5
Northeast Atlantic	Mean	20.7	15.3	18.6	24.9	19.6
	N	6	5	7	4	22
	S. D.	2.3	3.1	3.0	4.8	4.4
Northwest Atlantic	Mean	22.0	-	16.5	24.6	23.5
	N	1	0	1	8	10
	S. D.	-	-	-	3.7	4.2
East Central Atlantic	Mean	-	-	-	30.0	30.0
	N	0	0	0	1	1
	S. D.	-	-	-	-	-
West Central Atlantic	Mean	20.0	18.5	22.8	30.0	22.9
	N	1	4	4	3	12
	S. D.	-	1.9	4.1	1.0	5.2
Northwest Pacific	Mean	26.3	-	-	-	26.3
	N	1	0	0	0	1
	S. D.	-	-	-	-	-
East Central Pacific	Mean	-	-	-	26.2	26.2
	N	0	0	0	1	1
	S. D.	-	-	-	-	-
Indian Ocean	Mean	16.0	-	-	27.0	21.5
	N	1	0	0	1	2
	S. D.	-	-	-	-	8
Total	Mean	21.3	16.6	19.6	26.1	21.7
	N	17	12	15	23	67
	S. D.	3.1	3.0	3.7	3.6	4.8

[1] Fall, Sept.-Nov.; Winter, Dec. - Feb.; Spring, Mar. - May; Summer, June - Aug.
[2] United Nations' Food and Agriculture Organization (FAO) standardized ocean regions of the world.

Salinity

Summary of mean salinity (ppt) of ballast water in ballast tanks and cargo holds of bulkers sampled in Baltimore, Maryland and Norfolk, Virginia between August 1993 and August 1994 by season[1] and by FAO region[2] of ballast water origin. N, number of ballast tanks and cargo holds; S.D., 1 standard deviation.

FAO Region		Season Fall	Winter	Spring	Summer	Total
Mediterranean-Black Sea	Mean	33.4	37.3	25.8	39.5	34.5
	N	7	3	3	5	18
	S. D.	9.0	1.2	10.8	1.5	8.1
Northeast Atlantic	Mean	31.5	19.3	27.1	23.9	26.3
	N	6	4	7	4	21
	S. D.	3.6	13.9	12.3	12.9	11.1
Northwest Atlantic	Mean	30.0	-	16.0	26.1	25.5
	N	1	0	1	8	10
	S. D.	-	-	-	13.0	12.0
East Central Atlantic	Mean	-	-	-	32.0	32.0
	N	0	0	0	1	1
	S. D.	-	-	-	-	-
West Central Atlantic	Mean	35.0	21.8	24.3	16.0	22.2
	N	1	4	4	3	12
	S. D.	-	16.2	16.3	12.3	14.1
Northwest Pacific	Mean	30.0	-	-	-	30.0
	N	1	0	0	0	1
	S. D.	-	-	-	-	-
East Central Pacific	Mean	-	-	-	30.0	30.0
	N	0	0	0	1	1
	S. D.	-	-	-	-	-
Indian Ocean	Mean	34.0	-	-	34.0	34.0
	N	1	0	0	1	2
	S. D.	-	-	-	-	0.0
Total	Mean	32.5	25.1	25.4	28.1	28.1
	N	17	11	15	23	66
	S. D.	6.0	14.1	12.1	12.1	11.4

[1] Fall, Sept.-Nov.; Winter, Dec. - Feb.; Spring, Mar. - May; Summer, June - Aug.

[2] United Nations' Food and Agriculture Organization (FAO) standardized ocean regions of the world.

Age

Summary of mean age (days) of ballast water in ballast tanks and cargo holds of bulkers sampled in Baltimore, Maryland and Norfolk, Virginia between August 1993 and August 1994 by season[1] and by FAO region[2] of ballast water origin. N, number of ballast tanks and cargo holds; S. D., 1 standard deviation.

FAO Region		Season				Total
		Fall	Winter	Spring	Summer	
Mediterranean-Black Sea	Mean	18.9	17.0	20.3	18.8	18.8
	N	7	3	3	5	18
	S. D.	4.3	3.5	3.1	2.5	3.4
Northeast Atlantic	Mean	9.9	19.6	14.1	11.0	13.8
	N	6	5	7	3	21
	S. D.	5.1	12.0	2.9	3.5	7.3
Northwest Atlantic	Mean	2.0	-	2.0	6.0	5.2
	N	1	0	1	8	10
	S. D.	-	-	-	4.7	4.5
East Central Atlantic	Mean	-	-	-	75.0	75.0
	N	0	0	0	1	1
	S. D.	-	-	-	-	-
West Central Atlantic	Mean	8.0	5.5	6.3	7.7	6.5
	N	1	4	4	3	12
	S. D.	-	1.3	4.2	3.1	2.6
Northwest Pacific	Mean	34.0	-	-	-	34.0
	N	1	0	0	0	1
	S. D.	-	-	-	-	-
East Central Pacific	Mean	-	-	-	6.0	6.0
	N	0	0	0	1	1
	S. D.	-	-	-	-	-
Indian Ocean	Mean	37.0	-	-	20.0	28.5
	N	1	0	0	1	2
	S. D.	-	-	-	-	12.0
Total	Mean	16.0	14.3	12.9	13.6	14.2
	N	17	12	15	22	66
	S. D.	9.8	9.9	6.4	15.1	11.2

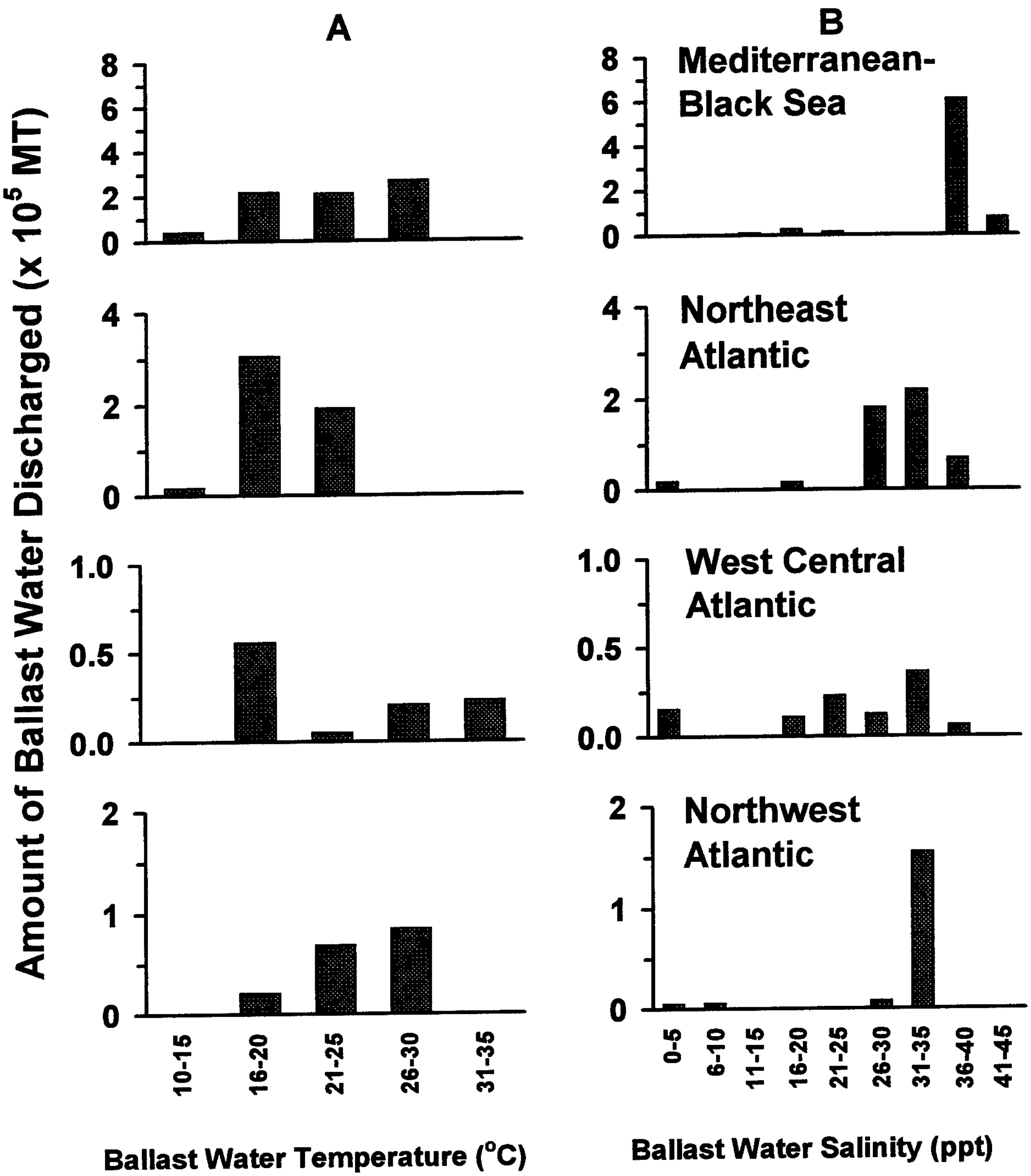
[1] Fall, Sept.-Nov.; Winter, Dec. - Feb.; Spring, Mar. - May; Summer, June - Aug.

[2] United Nations' Food and Agriculture Organization (FAO) standardized ocean regions of the world.

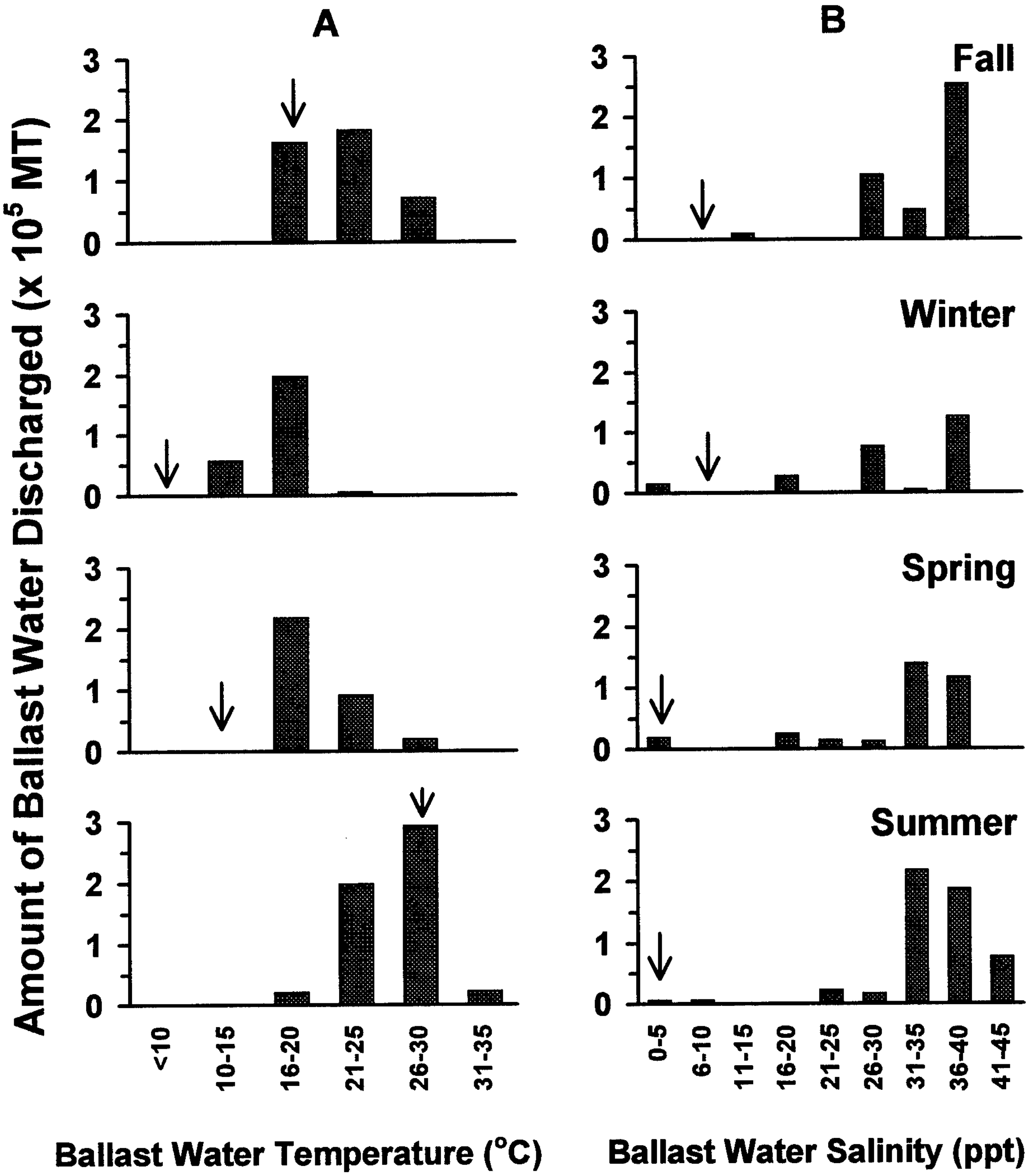
Appendix L

Temperature and Salinity Distributions of Bulker Ballast Water Discharged in Baltimore

by Source Region
by Season



Distribution of the amount of ballast water discharged (x 10^5 MT) by bulkers sampled in Baltimore, Maryland as a function of ballast water (A) temperature (n = 48 vessels) and (B) salinity (n = 47 vessels) for the 4 main FAO regions of ballast water origin (August 1993 to August 1994). Y-axis scales differ among regions.



Distributions of the amount of ballast water discharged (x 10^5 MT) by bulkers sampled in Baltimore, Maryland as a function of ballast water (A) temperature (n = 54 vessels) and (B) salinity (n = 53) for each season from August 1993 to August 1994. Arrows denote mean temperature or salinity of port water for each season. Y-axis scales differ among seasons.

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Appendix M

Summary of Frequencies and Abundances of Organisms in Bulker Cargo Holds

Percentage occurrence and abundance of organisms in ballast water of cargo holds from bulkers (n = 24) sampled in Baltimore, Maryland and Norfolk, Virginia between August 1993 and August 1994.

Taxon	Ships (%) in which taxon was Abundant (>100/ replicate[1])	Common (10 to 100/ replicate)	Rare (< 10/ replicate)	Present[2]
Crustacea	***33.3***	***50.0***	***16.7***	***100.0***
Cirripedia	0	20.8	54.2	75.0
Harpacticoida	12.5	33.3	41.7	87.5
Calanoida & Cyclopoida	29.2	25.0	45.8	100.0
Poecilostomatoida	0	37.5	41.7	79.2
Copepoda nauplii & copepodites	16.7	50.0	25.0	91.7
Decapoda	0	0	29.2	29.2
Euphausiacea	0	0	12.5	12.5
Stomatopoda	0	0	8.3	8.3
Mysidacea	0	0	20.8	20.8
Isopoda	0	0	20.8	20.8
Gammaridea	0	0	16.7	16.7
Hyperiidea	0	0	8.3	8.3
Ostracoda	0	0	12.5	12.5
Annelida	***4.2***	***37.5***	***33.3***	***75.0***
Spionidae	4.2	29.2	33.3	66.7
Polynoidae	0	0	16.7	16.7
Other Polychaeta	0	16.7	33.3	50.0
Platyhelminthes	***0***	***0***	***45.8***	***50.0***
Mollusca	***8.3***	***8.3***	***41.7***	***58.3***
Bivalvia	8.3	8.3	29.2	45.8
Gastropoda	0	0	25.0	25.0
Cnidaria	***0***	***8.3***	***33.3***	***41.7***
Scyphozoa	0	0	8.3	8.3
Hydrozoa	0	8.3	25.0	33.3
Chaetognatha	***0***	***4.2***	***29.2***	***33.3***
Ctenophora	***0***	***0***	***20.8***	***20.8***
Echinodermata	***4.2***	***0***	***12.5***	***16.7***
Asteroidea	0	0	16.7	16.7
Echinoidea	4.2	0	0	4.2
Ophiuroidea	0	0	4.2	4.2
Rotifera	***0***	***4.2***	***12.5***	***16.7***
Bryozoa	***0***	***0***	***12.5***	***12.5***
Nematoda	***0***	***0***	***12.5***	***12.5***
Chordata	***0***	***4.2***	***4.2***	***8.3***
Urochordata	0	4.2	4.2	8.3
Pisces	0	0	4.2	4.2
Phoronida	***0***	***0***	***4.2***	***4.2***
Nemertea	***0***	***4.2***	***0***	***4.2***
Sipuncula	***0***	***0***	***4.2***	***4.2***
Sarcomastigophora	***16.7***	***37.5***	***25.0***	***79.2***
Dinoflagellida	12.5	41.7	25.0	79.2
Radiolaria	4.2	4.2	25.0	33.3
Foraminifera	0	4.2	16.7	20.8
Ciliophora	***0***	***12.5***	***33.3***	***45.8***
Tintinnida	0	0	16.7	16.7
Other Ciliata	0	12.5	20.8	33.3
Diatomacea	***12.5***	***33.3***	***16.7***	***62.5***
Rhodophyta	***0***	***0***	***4.2***	***4.2***

[1] Mean tow volume $\pm$ 1 S.D. = 1.32 $\pm$ 0.32 m^3 (n = 24 cargo holds).

[2] Total percentage occurrence in vessels sampled by replicate, quantitative plankton tows.