

## Invasion pressure to a ballast-flooded estuary and an assessment of inoculant survival

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### Abstract

The relationships between invasion pressure, post-transport inoculant survival, and regional susceptibility to invasion are poorly understood. In marine ecosystems, the movement and release of ballast water from ocean-going ships provides a model system by which to examine the interplay among these factors. One of the largest estuaries in North America, the Chesapeake Bay, receives tremendous amounts of foreign ballast water annually and thus should be at high invasion risk. To date, however, few introductions in Chesapeake Bay have been attributed to ballast release. To understand better the dynamics of this invasion process, we (1) characterized and quantified the biota arriving to Chesapeake Bay in foreign ballast water, (2) compared temperatures and salinities of ballast water and harbor water in upper Chesapeake Bay, and (3) tested experimentally survival of organisms collected from ballast water in temperatures and salinities characteristic of the region. From 1993 to 1994, we sampled planktonic and benthic organisms from 60 foreign vessels arriving to Chesapeake Bay. Our data show that the estuary is being inoculated by a diverse assemblage of aquatic organisms from around the world. Furthermore, the short transit time ( $\leq 15$  d) for most vessels ensured that substantial numbers of larval and post-larval organisms were being deballasted alive. Most of the ballast water discharged into the upper Chesapeake Bay, however, was significantly higher in salinity ( $>20\text{‰}$ ) than that of the receiving harbor. In laboratory tolerance experiments, ballast water organisms perished under such conditions. Thus, a mismatch in physical conditions between donor and receiver regions may explain the dearth of invasions in the upper Bay. It is likely that the lower Chesapeake Bay, which is more saline, remains at higher risk to ballast water invasion. Recognition of such intraregional differences should allow more focused predictions for monitoring and management.

### Introduction

Global transfers and introductions of nonindigenous species by human activities are fundamentally altering the earth's biota (Elton 1958; Carlton 1989; Lodge 1993; Norse 1993). To understand better the processes that mediate biological invasions, it is critical to define the interplay between several important factors,

including (1) invasion pressure (i.e., the density, diversity, and frequency of organisms being inoculated into a new environment), (2) post-transport inoculant survival (an approximation of colonization potential) and (3) intra- and interregional variation in susceptibility to invasion. In marine ecosystems, numerous vectors continually move animals, plants, and other organisms across oceans and between continents (Carlton 1985,

1992, 1994). These vectors and the target regions that receive nonindigenous organisms may serve as useful templates through which to examine the dynamics of the invasion process.

In the last half of the 20th century, a primary mode of inoculation in marine systems has been the movement of organisms in the ballast water of ships (Medcof 1975; Carlton 1985; Williams et al. 1988; Carlton and Geller 1993). Ballast water is used to maintain vessel stability. The water is pumped or gravitated into cargo holds or ballast tanks at one port and then released, to varying degrees, at other ports when receiving (or at times even delivering) cargo (Carlton 1985; Carlton et al. 1995). As ballast water is typically drawn from biologically rich coastal areas, ships often carry a diverse assemblage of organisms in their ballast tanks (Carlton 1985; Williams et al. 1988; Baldwin 1992; Carlton and Geller 1993; Smith et al. 1996). Upon release, surviving organisms have the potential to colonize new habitats, and, in a number of instances, ballast-mediated introductions have had significant ecological and economic impacts (Nichols et al. 1990; Alpine and Cloern 1992; Office of Technology Assessment 1993; Mackie and Schloesser 1996; Wilcove et al. 1998). One of North America's largest estuaries, the Chesapeake Bay, receives tremendous amounts of ballast water annually (>21 million metric tons discharged in 1993–1994; Smith et al. 1996). Located in the middle Atlantic American bight, Chesapeake Bay provides a model system to examine invasion potential by ballast water, and it is the focus of the present study.

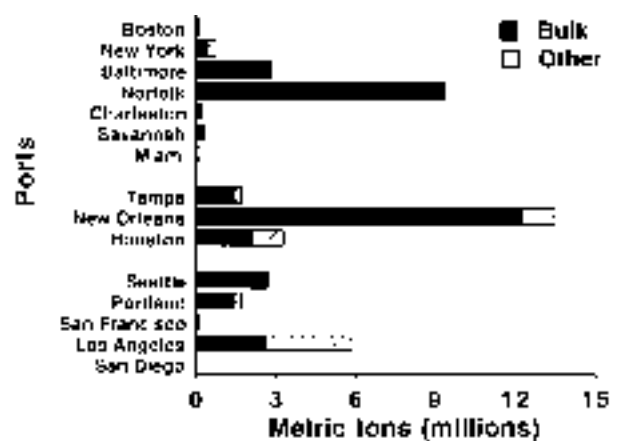
#### *The ballast water vector and receiver area vulnerability*

A successful ballast-mediated invasion is a multi-stage process. It is one that requires intake of potential invaders along with ballast water into the ship, survival of species during the voyage, colonization of the new environment, and continued reproduction of the introduced species (Carlton 1985). At present we have few quantitative data to evaluate which factors are critical to success across each stage. For example, the likelihood of invasion may be influenced by the amount of ballast water released, the diversity and abundance of organisms in the ballast water, and the degree of similarity in abiotic and biotic conditions in the donor and receiving regions (Carlton 1996a). A more complete understanding of ballast-mediated invasions requires, at a minimum, comparative data among regions that measure the (1) amount and frequency of ballast water

release, (2) taxonomic composition and abundance of organisms in the ballast water, (3) degree of similarity in environmental conditions in the donor and recipient regions, (4) likelihood of survival and establishment of invading taxa in the receiving region, and (5) patterns and rates of invasions.

Ports in the United States differ substantially in the amounts of ballast water they receive from foreign sources and, therefore, may be at different risk of ballast-mediated invasion (Carlton et al. 1995; Ruiz et al. 1997). For example, in a survey of 22 US ports, the Ports of Baltimore, Maryland and Norfolk, Virginia in Chesapeake Bay ranked fifth and second respectively in the amounts of foreign ballast water received (Figure 1) (Carlton et al. 1995). If the amount of ballast water received is an important determinant of invasion success, then high traffic regions such as the Chesapeake Bay should be at great risk to invasion. To date, however, relatively few invasions in Chesapeake Bay have been attributed to ballast water release (G. Ruiz, unpublished data). In comparison, approximately one-quarter of the aquatic invasions in San Francisco Bay (48 species) have been linked to ballast water transport (Cohen and Carlton 1995), even though the estuary receives comparatively little foreign ballast water (Figure 1) (Carlton et al. 1995).

A number of scenarios, singly or in concert, may explain regional differences in the frequency of ballast-mediated invasions. First, in many systems, it



*Figure 1.* Amounts of acknowledged ballast water (in millions of metric tons) released by foreign commercial vessels in 1991 in United States ports. Black portion of bars indicates amount of ballast water released by bulk cargo carriers; hatched portion indicates amount released by all other vessel types (e.g., containers, tankers, roll on/roll off ships, cruise ships). Data are summarized from Carlton et al. (1995).

is possible that ballast-mediated invasions have in fact occurred, but have gone unnoticed (Carlton 1996b). Second, it may be that in estuaries receiving large amounts of ballast water, such as Chesapeake Bay, the organisms that arrive are too (a) few numerically, (b) depauperate taxonomically, or (c) weakened physiologically to establish. Third, if a substantial percentage of incoming vessels have attempted ballast control measures (e.g., exchange of ballast water with ocean water prior to arrival; Locke et al. 1991, 1993), few coastal species would be expected to persist. Finally, conditions in the receiving port may not favor survival of organisms following their release. For example, if temperatures and salinities differ substantially from those of the annual variability of the source water, then survival of most immigrants following release may be low.

To determine key factors influencing the vulnerability of a region to ballast-mediated invasion, we examined foreign commercial ships arriving to the Ports of Baltimore and Norfolk for the presence, taxonomic composition and abundance of live organisms in their ballast water. Previous ballast water surveys (e.g., Williams et al. 1988; Hallegraeff and Bolch 1991, 1992; Carlton and Geller 1993; Galil and Hülsmann 1997; Pierce et al. 1997) have documented the taxonomic diversity of organisms in ships' ballast water without extensively quantifying abundances. In this study, we (1) enumerated plankton abundance, (2) tested for regional or seasonal differences in the ballast biota, and (3) compared abundances against other variables such as voyage duration and ballast water temperature and salinity. We also wished to determine the likelihood of deballasted organisms establishing in Chesapeake Bay based on the immediate physicochemical regime. To this end, we compared the temperature and salinity of discharged ballast water with that of Baltimore harbor and tested experimentally the survivorship of organisms collected from ballast water in temperature and salinity combinations characteristic of Chesapeake Bay.

## Materials and methods

### *Study sites*

Between August 1993 and August 1994, we surveyed and sampled foreign ballast water from commercial vessels arriving in the Port of Baltimore, Maryland (39°14' N, 76°33' W) in upper Chesapeake Bay and in the Port of Norfolk, Virginia (36°51' N, 76°19' W)

in lower Chesapeake Bay. We focused our sampling efforts on bulk cargo carriers, because this class of vessels is responsible for most of the foreign ballast water released in Chesapeake Bay and in the United States more generally (Figure 1) (Carlton et al. 1995).

### *Sampling protocol*

We identified and tracked vessels with the assistance of the United States Coast Guard, local shipping agents, and regional maritime exchanges. On average, we sampled 1 to 2 vessels per week. Vessels were chosen at random with respect to last port of call (i.e., probable source of ballast water). Ballast water was sampled from one or two available cargo holds or ballast tanks per ship. In each of these, we measured water temperature and salinity using a salinity-conductivity-temperature meter (YSI Model 133) and dissolved oxygen content using an oxygen meter (YSI Model 57). Measurements were taken at the surface and at 5 m (cargo holds) or 1 m (ballast tanks) depth intervals. Temperature, salinity and dissolved oxygen content of the harbor water adjacent to the ship were recorded at the surface and at 5 m intervals to 15 m depth.

We sampled ballast water for planktonic organisms using replicate vertical tows of a plankton net (net length, 0.9 m; diameter of opening, 0.3 m; mesh size, 80  $\mu$ m). Each tow was pulled through a known depth of the water column at approximately 0.5 m/s. In cargo holds, three samples were taken (typically to 18–20 m depth) at evenly spaced intervals along the length of the hold. To sample rare organisms, an additional single qualitative tow was taken diagonally from the bottom to the surface along the entire side of the cargo hold. Ballast tanks are smaller and located fore, aft and along the top sides of the vessel. These tanks, which are accessible through a deck hatch opening, are typically divided into compartments by vertical supports and horizontal platforms. Thus, in most cases, the net could not be lowered below the uppermost level ( $\approx$  3 m depth). In ballast tanks, two samples were taken through the deck hatch opening, each sample consisting of a single vertical tow from near the bottom of the compartment to the water's surface. These were followed by an additional qualitative tow to sample rare organisms, where the plankton net was drawn through the water column repeatedly for a total tow height of 10 m.

Whenever isopods, amphipods, or fish were observed at the water surface, we collected them with small aquarium nets. On several occasions, we returned

to a vessel after sampling the ballast water to examine the deballasted cargo hold. Larger organisms were captured in the deballasted cargo holds by hand or aquarium net, and small quantities of sediment were scooped in plastic containers. As search time in the deballasted cargo hold was limited by the vessel's operational constraints, these collections were not conducted in a quantitative fashion.

All biological samples were placed in insulated coolers and transported by vehicle from Baltimore (1 h) or Norfolk (4 h) to the Smithsonian Environmental Research Center in Edgewater, Maryland for analysis. Ice packs were used to reduce metabolic activity of the plankton. Collections of plankton from Norfolk were diluted by half to ensure the survival of organisms during the journey to the laboratory. Samples were aerated using a battery powered air supply during transport.

#### *Biological analyses*

All net tow samples were aerated upon return to the laboratory. Within 1 to 24 h after collection (usually <4 h), samples were analyzed under a stereomicroscope (Leica MZ8) for the presence of live plankton. All organisms were categorized to the lowest identifiable taxonomic level possible and then by morphotype (e.g. calanoid copepod 'A'). For quantitative samples, the abundance of each morphotype was estimated on a logarithmic scale (rare, <10 individuals per sample; common, 10–100 per sample; or abundant, >100 per sample). Qualitative tows were scanned for new organisms, or used to collect specimens for culturing and identification or for survival experiments (described below). Sediments were examined under a stereomicroscope for living macro- and meiofaunal organisms.

After completion of the live analysis, most organisms in a sample were preserved collectively in 75% ethanol. Unidentified, unusual, or fragile specimens, however, were labelled, preserved, and stored separately. Whenever possible, these specimens were sent to appropriate taxonomists for identification. When greater numbers than 10 live individuals of an unidentified larval morphotype were present in samples, they were removed and cultured to juvenile or adult stages for the purpose of identification. Individuals were raised in 100 ml glass dishes at a density of approximately 10 organisms per dish. Organisms were kept in 80 µm-filtered ballast water for the first week; 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 *Isochrysis galbana* and *Dunaliella tertiolecta*. Carnivorous larvae were provided *Artemia* nauplii or the rotifer *Brachionus plicatilis*. The culture water and food were changed every other day for certain taxa (e.g., decapod zoea) and every third day for other groups or later developmental stages.

For cargo holds and ballast tanks with ballast water from single sources (i.e., no additional water was added en route), we enumerated organisms in plankton tow samples after preservation to generate estimates of density for statistical analyses. Samples were transferred from the preservative to water and stained with Rose Bengal. For taxa whose abundances were low (<500 organisms), all stained individuals were counted. For denser samples, taxon abundances were estimated by counting and averaging six replicate 1 ml subsamples that had been pipetted from a well-mixed known volume (80 ml).

#### *Tolerance experiments*

When sufficient numbers (>90 live individuals) of a taxon or morphotype were present, we tested their survival in salinity and temperature combinations characteristic of Chesapeake Bay. Ten individuals of a given taxon were placed in each of three (or, if numbers permitted, four) replicate 100 ml glass dishes containing artificial seawater. These organisms were then kept for 14 d in incubators at 9 combinations of three temperatures (5 °C, 15 °C, 25 °C) and three salinities (5‰, 15‰, 25‰). Organisms were fed and maintained under conditions described above for culturing larvae. Survivorship of organisms in all dishes was assessed on days 1, 3, 5, 7, and 14 after onset of each experiment.

#### *Data analyses*

##### *Vessel and ballast water analyses*

Data from Baltimore and Norfolk were combined for analyses unless specified otherwise. We converted ballast water capacities of all ships to metric tons (MT). Geographic sources of the ballast water and last ports of call were classified by region (Figure 2). We defined 'topped' cargo holds and ballast tanks as those in which at least half of the original amount of water remained prior to new water being added. 'Exchanged' cargo holds and ballast tanks were defined as those in which >50% of the original water was flushed before being replaced with ocean water.

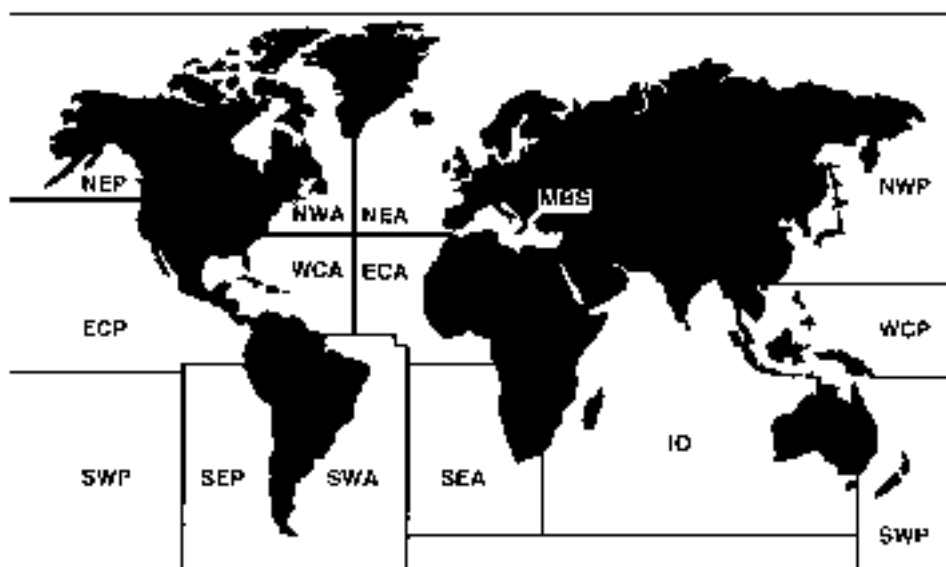


Figure 2. Ocean regions as classified by United Nations' Food and Agriculture Organization (FAO). ECP, Eastern Central Pacific; IO, Indian Ocean; MBS, Mediterranean-Black Sea; NEA, Northeast Atlantic; NEP, Northeast Pacific; NWA, Northwest Atlantic; NWP, Northwest Pacific; SEA, Southeast Atlantic; SEP,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

### Biological analyses

The use of log-scale categories of abundance in our live analyses prevented averaging replicate tow 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. Because tow volumes differed significantly between cargo holds and ballast tanks ( $t$ -test,  $P < 0.001$ ), live analysis data from these tank types were analyzed separately. If larvae could not be subclassified, they were instead pooled into the next lowest known taxonomic category (e.g., polychaete larvae rather than capitellid larvae).

We were conservative in estimating the number of species within a taxonomic group. By definition, a minimum of one species was present for any taxonomic level (be it phylum, class, order, family, or genus) identified. To this, we added the number of distinct lower taxonomic levels identified. For example, if no taxonomic level lower than *Bivalvia* was identified, the total number of bivalve species was considered as 1. If we were able to identify 3 species of bivalves positively and additional unidentified bivalves were also present, then the total number of bivalve taxa was listed as 4 (the base number of 1 + 3 identified species). This convention was retained, even if multiple species were probable (e.g., bivalve specimens came from several ocean regions). Our data, then, provide a conservative

estimate of overall taxonomic diversity in the ballast water assemblages arriving in Chesapeake Bay.

For vessels in which preserved samples were enumerated, 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., tow depth multiplied by the area of the net opening). Plankton densities were  $\log_{10}$  transformed prior to statistical analysis, then back-transformed for presentation. Standard errors are reported around the back-transformed means (Sokal and Rohlf 1981). Statistical comparisons of the number of taxa in ballast tanks were calculated using untransformed data.

### Statistical analyses

We used Statistical Analysis Systems software (SAS Institute 1985) for parametric and nonparametric 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 analysed using analysis of variance (ANOVA), and, for frequency data, G-tests of independence (Sokal and Rohlf 1981). If ANOVA models were significant, unplanned multiple comparisons were used to distinguish group differences (Ryan's Q-test; Day and Quinn 1989). For frequency data, among-treatment differences were

compared using a simultaneous test procedure (Sokal and Rohlf 1981). If transformations failed to correct for non-normal or heteroscedastic data, appropriate non-parametric tests 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 ( $\alpha$ ) was 0.05. When separate tests of association were performed for a given variable (e.g., plankton density vs. temperature, salinity, or age of ballast water), significance levels of individual comparisons were interpreted after sequential Bonferroni correction (Rice 1989).

## Results

We obtained ballast water samples from cargo holds and ballast tanks of 60 foreign vessels, all arriving in ballast to load cargo (primarily coal). Fifty-four vessels were sampled in Baltimore and 6 vessels in Norfolk. In all, 26 cargo holds and 62 ballast tanks were sampled from these vessels. In most cases, when multiple tanks were sampled on a ship, the water in each tank was from the same source region and had identical temperature and salinity characteristics. In a few cases, the water sources in holds and tanks on a single vessel differed (e.g., a tank had been exchanged, newly filled, or topped up en route) resulting in between-tank differences in physical characteristics (defined as  $\geq 3\%$  or  $3^\circ\text{C}$ ). As a consequence, 67 of the 88 cargo holds and ballast tanks sampled on vessels were considered ‘distinctly different’.

### *Vessel and ballast water origin*

Vessels arrived at Baltimore and Norfolk from 39 foreign ports, principally representing the Atlantic Ocean and its Seas, yet representing all major regions of the world (see Smith et al. 1996 for details). We sampled most vessels in the summer ( $n = 20$ ) and fewest in winter ( $n = 10$ ) (Table 1). For the majority of vessels (42%), the last port of call was in the Northeast Atlantic (NEA) (Figure 3A). Most of the remaining vessels had a last port of call in either the Mediterranean/Black Sea (MBS) (28%) or West Central Atlantic (WCA) (20%) regions. For 56 of 60 vessels, the ballast water source was the same as the region for last port of call. For the remaining 7%, the source of the ballast water and the last region of call differed because cargo holds or ballast tanks were either filled or exchanged later in the voyage. Vessels arriving from the NEA and MBS regions were significantly larger, had greater ballast water capacity, and carried more ballast water on board than did vessels carrying water from the West Central Atlantic (WCA) (Smith et al. 1996). As a consequence, most of the ballast water on board vessels that discharged in Baltimore and Norfolk was either of MBS (47.1%) or NEA (37.0%) origin (Figure 3B).

### *Ballasting operations*

Vessels varied substantially in the amount of ballast water carried on board (mean  $\pm$  1 SD;  $31,457 \pm 24,861$  MT;  $n = 56$ ). This variation reflected (1) the

Table 1. Frequency of bulk cargo carriers sampled in Baltimore, Maryland and Norfolk, Virginia between August 1993 and August 1994 by season<sup>a</sup> and by region<sup>b</sup> of ballast water origin.

Region	Season				Total
	Fall	Winter	Spring	Summer	
Mediterranean-Black Sea	7	2	3	5	17
Northeast Atlantic	4	5	7	4	20
West Central Atlantic	1	3	4	3	11
Northwest Atlantic	1	0	1	5	7
Other <sup>c</sup>	2	0	0	3	5
Total	15	10	15	20	60

<sup>a</sup>Fall, Sept.–Nov.; Winter, Dec.–Feb.; Spring, Mar.–May; Summer, June–Aug.

<sup>b</sup>United Nations’ Food and Agriculture Organization (FAO) standardized ocean regions of the world.

<sup>c</sup>Other includes ballast from East Central Atlantic ( $n = 1$ ), Northwest Pacific ( $n = 1$ ), East Central Pacific ( $n = 1$ ), and Indian Ocean ( $n = 2$ ) regions.

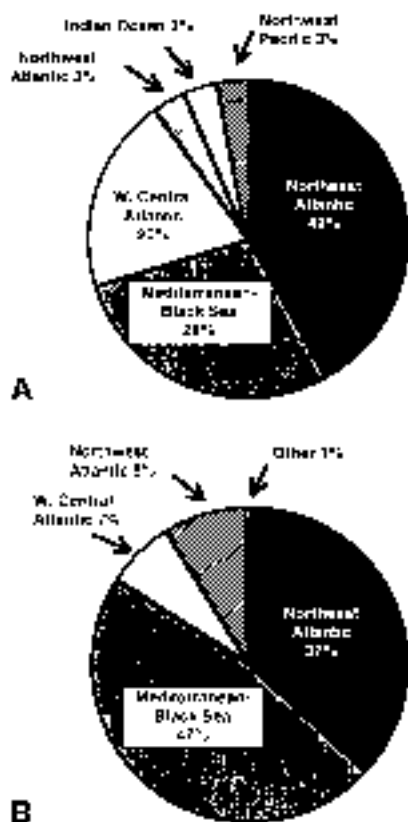


Figure 3. (A) Frequency of bulk cargo carriers sampled in Baltimore and Norfolk by last region of call ( $n = 60$  vessels) and (B) relative amounts of ballast water discharged from bulk cargo carriers by region of ballast water origin (total amount of ballast water discharged = 1,761,593 metric tons).

ship's total ballast water capacity, which was a function of vessel size, (2) port- and ship-specific ballasting procedures, and (3) amount of cargo. On average ( $\pm 1$  SD), vessels arrived in port carrying  $71 \pm 26\%$  of their total ballast water capacity. The average amount of ballast water in a single cargo hold typically comprised 50% of the total ballast water carried on vessels.

Eighty-eight percent of the 60 vessels reported deballasting some or all of their ballast water in port. Nearly half (48%) of the vessels we sampled arrived with their original ballast water unmodified (i.e., not topped up or exchanged during the voyage). Thirty-five percent of the vessels topped up their cargo holds or ballast tanks to replace water lost in transit to overflow. Approximately 1 in 6 vessels (17%) attempted full exchange of one or more of their cargo holds or ballast tanks. While ships' officers generally reported '100%' exchange of ballast water, the salinity of water in exchanged cargo holds and ballast tanks was often

less than that of open-ocean water, suggesting that exchange was not complete (Smith et al. 1996).

#### Physical characteristics of the ballast water

##### Cargo holds and ballast tanks

There was no evidence of vertical stratification of temperature, salinity, or dissolved oxygen (0 to 15 m depth) in the ballast water in cargo holds (Smith et al., unpublished data). 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 depth). On one occasion, we were able to measure water in a ballast tank to a depth of 14 m. This vessel, which ballasted in St Petersburg, Russia, attempted an exchange in the mid-Atlantic. Water salinity in the ballast tank ranged from 14‰ at 7 m depth to 30‰ at 13.5 m depth, indicating the potential for stratification in these subdivided tanks. Dissolved oxygen (mean  $\pm 1$  SD) was not limiting to life in either cargo holds ( $7.2 \pm 1.4$  mg l<sup>-1</sup>;  $n = 11$  holds) or uppermost compartments of ballast tanks ( $6.9 \pm 2.2$  mg l<sup>-1</sup>;  $n = 22$  tanks).

##### Geographic and seasonal patterns

Mean temperature of ballast water in vessels differed by season (2-way ANOVA,  $F = 20.7$ ;  $df = 3, 40$ ;  $P < 0.001$ ), but not by region ( $F = 2.51$ ;  $df = 2, 40$ ;  $P = 0.094$ ) for the 3 primary regions (NEA, MBS, WCA). Mean temperatures ( $\pm 1$  SD) were highest in summer ( $26.8 \pm 3.5$  °C;  $n = 23$  cargo holds and ballast tanks), intermediate in fall ( $21.3 \pm 2.8$  °C;  $n = 17$ ) and spring ( $19.8 \pm 3.7$  °C;  $n = 15$ ), and lowest in winter ( $16.8 \pm 3.1$  °C;  $n = 11$ ). Seasonal temperature differences were consistent among regions (region  $\times$  season interaction,  $F = 0.42$ ;  $df = 6, 40$ ;  $P = 0.86$ ).

Mean salinity of ballast water in vessels differed by region (2-way ANOVA,  $F = 3.50$ ;  $df = 2, 39$ ;  $P = 0.04$ ), but not by season ( $F = 0.84$ ;  $df = 2, 39$ ;  $P = 0.48$ ) for the 3 primary regions. Mean salinities were higher from the MBS ( $34.6 \pm 8.0$ ‰;  $n = 18$  cargo holds and ballast tanks) than from the NEA ( $26.3 \pm 11.1$ ‰;  $n = 21$ ) or WCA ( $22.3 \pm 14.1$ ‰;  $n = 12$ ). These regional differences in salinity were consistent across seasons (region  $\times$  season interaction,  $F = 1.25$ ;  $df = 6, 39$ ;  $P = 0.30$ ).

##### Age of ballast water

The age of the ballast water in vessels differed significantly among the regions of ballast water origin

(Kruskal–Wallis test, chi-square = 39.9,  $df = 3$ ,  $P < 0.001$ ). Mean age ( $\pm 1$  SD) was greatest for ballast water arriving from the MBS region ( $19 \pm 3$  d;  $n = 18$  cargo holds and ballast tanks). Ballast water arriving from the NEA region was of intermediate age ( $14 \pm 7$  d;  $n = 21$ ). The shortest residence times for ballast water occurred in vessels carrying water from the WCA ( $7 \pm 3$  d;  $n = 12$ ) and the Northwest Atlantic (NWA;  $5 \pm 4$  d;  $n = 10$ ).

#### Comparisons between ballast water and Baltimore Harbor water

In general, the temperature and salinity of most of the ballast water discharged into Baltimore Harbor showed little similarity with that of the harbor water (too few ships were sampled in Norfolk to conduct a similar analysis). Approximately 1,468,285 MT of ballast water from the MBS, NEA, WCA, and NWA were released by sampled vessels into Baltimore Harbor (Smith et al. 1996), and this ballast water ranged widely in temperature and salinity. Between August 1993 and August 1994, temperatures of discharged ballast water were between  $12^\circ\text{C}$  and  $32^\circ\text{C}$  (Figure 4A) and salinities were between  $0\text{‰}$  and  $42\text{‰}$  (Figure 4B). During this period, mean water temperatures in Baltimore Harbor showed considerable seasonal variation (see arrows, Figure 4A), while mean salinities were consistently low ( $<10\text{‰}$ ) (see arrows, Figure 4B). Ballast and harbor water temperatures were most similar during summer (Figure 4A) and for water from the NWA (Figure 5A). In contrast, salinities differed substantially regardless of the season (Figure 4B) or region of ballast water origin (Figure 5B). Almost 95% of the ballast water discharged into Baltimore Harbor had a salinity that was higher by at least  $11\text{‰}$  (Figure 5B). 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, only 5 had ballast water temperatures and salinities differing by  $\leq 5^\circ\text{C}$  and  $\leq 5\text{‰}$ , respectively, from that of the harbor water. These ‘matches’, all from different ships, came from 4 different ocean regions (MBS, NEA, NWA, WCA).

#### Biological information

##### Analyses of live organisms

Ninety-seven percent of the 60 vessels sampled in our survey contained live aquatic organisms. Living organisms were collected by quantitative plankton net tows

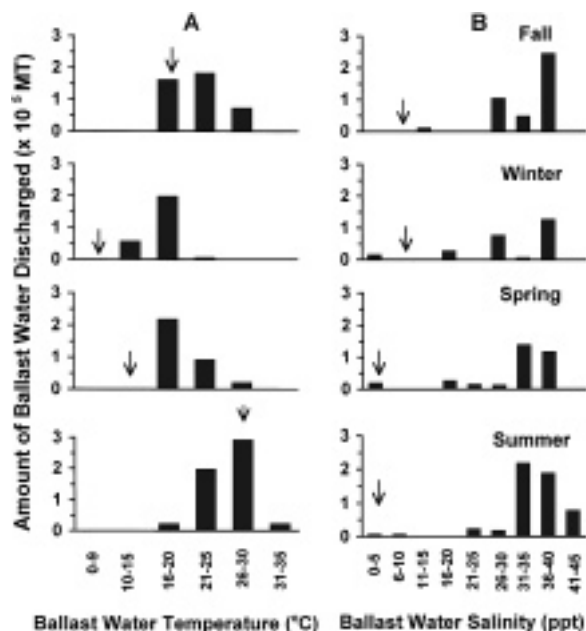


Figure 4. Seasonal distribution of the amount of ballast water discharged ( $\times 10^5$  metric tons, MT) by bulk cargo carriers sampled in Baltimore as a function of ballast water (A) temperature ( $n = 54$  vessels) and (B) salinity ( $n = 53$ ). Arrows denote mean temperature or salinity of port water for each season.

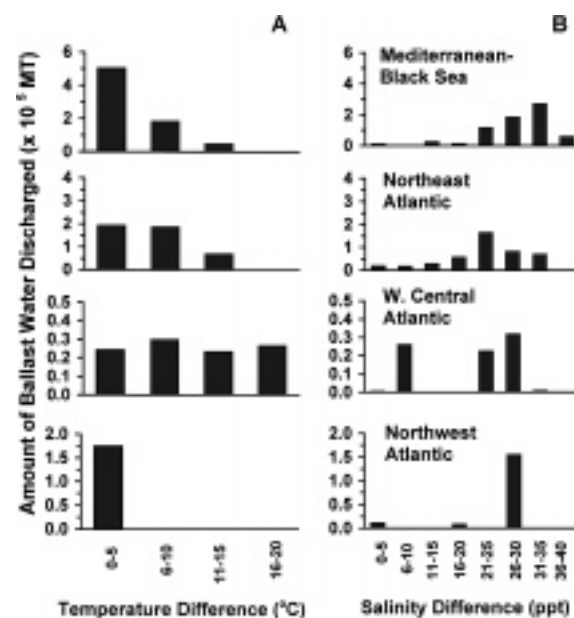


Figure 5. Distribution of the amount of ballast water discharged ( $\times 10^5$  metric tons, MT) by bulk cargo carriers sampled in Baltimore as a function of differences in (A) temperature ( $n = 46$  vessels) and (B) salinity ( $n = 43$  vessels) between ballast and port water for the 4 main regions of ballast water origin. y-axes differ among regions.



from the ballast water of all cargo holds ( $n = 24$ ) and 91% (43 of 47) of the ballast tanks (Table 2). Living organisms were collected in all seasons and from 6 of 8 ocean regions (MBS, NEA, NWA, WCA, NWP, ECP, but not ECA or IO; see Figure 2 legend).

The biota in both the cargo holds and ballast tanks was diverse taxonomically and included representatives from 15 animal and 3 protist phyla, 2 plant divisions and cyanobacteria (Table 2). We report here the first known occurrence of live ctenophores in ballast water (Table 2). A minimum of 221 distinctly different taxa were identified; 188 taxa were from plankton samples. For 29 groups from plankton samples, we added '1' taxon when we knew we had at least one additional unidentified member of that group. To these numbers (188 + 29), we then added 4 additional taxa found solely in benthic sediments in cargo holds for a total of 221 taxa.

Invertebrate taxa included both meroplanktonic and holoplanktonic representatives. Crustaceans were found in all cargo holds and most (70%) ballast tanks (see Table 2; Present, Quantitative). Crustaceans were abundant (i.e., >100 organisms per net tow) in 33% of the cargo holds and in 13% of the ballast tanks (Table 2). Other prevalent taxa (see Table 2; Present, Quantitative) in cargo holds and ballast tanks respectively, included annelids (75% and 34%), platyhelminthes (46% and 6%), molluscs (58% and 32%), dinoflagellates (79% and 19%), and diatoms (63% and 55%). We also identified larval forms of numerous other phyla, including bryozoans, urochordates, phoronids, nemertean, and sipunculans (Table 2). Some taxa were unique to either cargo holds or ballast tanks. With few exceptions (e.g., Crustacea: Branchiopoda; Table 2), these taxa were always rare in abundance; consequently, their apparent tank specificity is a matter of chance.

More prevalent taxonomic groups were typically dominated (both in percent occurrence and in abundance) by one or two major subclasses. For example, the occurrence of crustaceans in all cargo holds was due to the omnipresence of copepods (Table 2). We identified copepods from four orders (Harpacticoida, Cyclopoida, Calanoida, and Poecilostomatoida), all developmental stages (nauplii, copepodites, and adults), and both sexes in our samples. Copepods were common or abundant in 96% of the cargo holds. Cirripede 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 2), but

larval spionids predominated. Similarly, the phylum Mollusca was represented chiefly by bivalve larvae.

While our quantitative sampling design provided a robust description of the ballast water plankton community, non-quantitative samples yielded valuable information for non-planktonic and benthic taxa (little new information was gained for plankton diversity from the qualitative plankton tows). For example, no representatives of the six 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 had been emptied (see Table 2; Present, Qualitative). Using the latter technique, we were also able to collect a number of benthic organisms, including shrimp (e.g., *Crangon crangon*), brachyuran crabs, nematodes, and polychaetes.

#### *Analyses of preserved organisms*

##### *Relative abundance*

Detailed quantitative counts of plankton samples indicated that copepods numerically dominated ballast water plankton assemblages (Table 3). Regionally, the mean relative abundance of copepods ranged from 55% in the NEA to 78% in the WCA (Table 3). Seasonally, the mean relative abundance of copepods was lowest in summer (53%) and highest in winter (82%) (Table 3). The identity and rank order of abundance of other common taxa (e.g., Bivalvia, Diatomacea, Dinoflagellida, Polychaeta) varied with region and season (Table 3). These differences reflect the variability inherent in ballast transport of plankton; different taxa can be common at certain locations or in certain times of the year.

##### *Regional and seasonal comparisons*

Regional differences existed in densities of organisms arriving to Chesapeake Bay. Significantly fewer organisms per cubic meter were found in ballast water from the MBS than from the NEA or WCA (1-way ANOVA,  $F = 10.8$ ;  $df = 2, 23$ ;  $P < 0.001$ ) (Figure 6A) when pooled across seasons (note that simultaneous comparison of 3 regions and 4 seasons was not possible, because samples for the WCA were not present in all seasons). There were no significant differences in plankton density between ballast water samples from the NEA and WCA. The number of taxa did not differ among the 3 primary ballast water source regions (Kruskal-Wallis test, chi-square = 1.88;  $df = 2$ ;  $P = 0.39$ ) (Figure 6B). Neither plankton densities (1-way



Dorvilleidae	1	0	0	4.2	4.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mageloniidae	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2.1
Nereidae	2	0	4.2	8.3	12.5	4.2	0	0	0	0	0	0	0	0	0	0	0	0	0	2.1
Phyllocoelidae	3	0	4.2	20.8	25.0	4.2	0	0	0	0	0	0	0	0	4.3	4.3	0	0	0	10.6
Polynoidae	1	0	0	16.7	16.7	0	0	0	0	0	0	0	0	0	2.1	2.1	0	0	0	6.4
Sabelliariidae	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2.1
Syllidae	2	0	0	4.2	8.3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6.4
Spionidae	11	4.2	29.2	33.3	66.7	75.0	4.3	4.3	10.6	17.0	31.9	0	0	0	2.1	2.1	0	0	0	40.4
Terebellidae	1	0	0	8.3	8.3	0	0	0	2.1	4.3	6.4	0	0	0	6.4	6.4	0	0	0	8.5
Polychaete larvae	-	0	4.2	41.7	45.8	0	0	0	2.1	6.4	8.5	0	0	0	8.5	8.5	0	0	0	21.3
<b>Platyhelminthes</b>	<b>2</b>	<b>0</b>	<b>0</b>	<b>45.8</b>	<b>50.0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>6.4</b>	<b>6.4</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>6.4</b>	<b>6.4</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>14.9</b>
<b>Mollusca</b>	<b>8</b>	<b>8.3</b>	<b>8.3</b>	<b>41.7</b>	<b>58.3</b>	<b>58.3</b>	<b>4.3</b>	<b>4.3</b>	<b>6.4</b>	<b>21.3</b>	<b>31.9</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>21.3</b>	<b>31.9</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>44.7</b>
Bivalvia	2	8.3	8.3	29.2	45.8	45.8	4.3	4.3	6.4	21.3	31.9	0	0	0	31.9	31.9	0	0	0	44.7
Gastropoda	5	0	0	25.0	29.2	25.0	0	0	4.3	8.5	12.8	0	0	0	12.8	12.8	0	0	0	21.3
Heteropoda	2	0	0	8.3	8.3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Other Prosobranchia	1	0	0	20.8	25.0	20.8	0	0	4.3	8.5	12.8	0	0	0	12.8	12.8	0	0	0	19.1
Pteropoda	1	0	0	12.5	12.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nudibranchia	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2.1
Polyplocophora	1	0	0	4.2	4.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<b>Cnidaria</b>	<b>7</b>	<b>0</b>	<b>8.3</b>	<b>33.3</b>	<b>41.7</b>	<b>45.8</b>	<b>0</b>	<b>0</b>	<b>2.1</b>	<b>2.1</b>	<b>4.3</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>4.3</b>	<b>4.3</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>6.4</b>
Scyphozoa	1	0	0	8.3	8.3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrozoa	6	0	8.3	25.0	33.3	37.5	0	0	2.1	2.1	4.3	0	0	0	4.3	4.3	0	0	0	6.4
<b>Chaetognatha</b>	<b>9</b>	<b>0</b>	<b>4.2</b>	<b>29.2</b>	<b>33.3</b>	<b>33.3</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>4.3</b>	<b>4.3</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>4.3</b>	<b>4.3</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>8.5</b>
<b>Ctenophora</b>	<b>4</b>	<b>0</b>	<b>0</b>	<b>20.8</b>	<b>20.8</b>	<b>20.8</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>2.1</b>	<b>2.1</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>2.1</b>	<b>2.1</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>2.1</b>
<b>Echinodermata</b>	<b>4</b>	<b>4.2</b>	<b>0</b>	<b>12.5</b>	<b>16.7</b>	<b>12.5</b>	<b>0</b>	<b>0</b>	<b>2.1</b>	<b>2.1</b>	<b>4.3</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>4.3</b>	<b>4.3</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>6.4</b>
Asteroidea	1	0	0	16.7	16.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4.3
Echinoidea	2	4.2	0	4.2	4.2	0	0	0	0	2.1	2.1	0	0	0	2.1	2.1	0	0	0	2.1
Ophiuroidea	1	0	0	4.2	4.2	0	0	0	2.1	2.1	2.1	0	0	0	2.1	2.1	0	0	0	2.1
Echinoderm larvae	-	0	0	4.2	4.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<b>Rotifera</b>	<b>3</b>	<b>0</b>	<b>4.2</b>	<b>12.5</b>	<b>16.7</b>	<b>16.7</b>	<b>2.1</b>	<b>2.1</b>	<b>0</b>	<b>8.5</b>	<b>10.6</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>8.5</b>	<b>10.6</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>17.0</b>
<b>Bryozoa</b>	<b>1</b>	<b>0</b>	<b>0</b>	<b>12.5</b>	<b>12.5</b>	<b>12.5</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>2.1</b>
<b>Nematoda</b>	<b>3</b>	<b>0</b>	<b>0</b>	<b>12.5</b>	<b>12.5</b>	<b>12.5</b>	<b>0</b>	<b>0</b>	<b>2.1</b>	<b>12.8</b>	<b>14.9</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>12.8</b>	<b>14.9</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>21.3</b>

Table 2. Continued.

Taxon	No. taxa <sup>a</sup>	Cargo holds					Ballast tanks				
		Ships (%) in which taxon was					Ships (%) in which taxon was				
		Abundant (>100/ replicate <sup>b</sup> )	Common (10 to 100/ replicate)	Rare (<10/ replicate)	Present		Abundant (>100/ replicate)	Common (10 to 100/ replicate)	Rare (<10/ replicate)	Present	
			Quantitative <sup>c</sup>	Qualitative <sup>d</sup>				Quantitative <sup>c</sup>	Qualitative <sup>d</sup>		
<b>Chordata</b>	<b>8</b>	<b>0</b>	<b>4.2</b>	<b>4.2</b>	<b>8.3</b>	<b>25.0</b>	0	0	<b>2.1</b>	<b>2.1</b>	<b>4.3</b>
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
<b>Phoronida</b>	<b>1</b>	<b>0</b>	<b>0</b>	<b>4.2</b>	<b>4.2</b>	<b>4.2</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>
<b>Nemertea</b>	<b>1</b>	<b>0</b>	<b>4.2</b>	<b>0</b>	<b>4.2</b>	<b>4.2</b>	<b>0</b>	<b>0</b>	<b>6.4</b>	<b>6.4</b>	<b>6.4</b>
<b>Sipuncula</b>	<b>1</b>	<b>0</b>	<b>0</b>	<b>4.2</b>	<b>4.2</b>	<b>8.3</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>4.3</b>
<b>Eggs</b>	<b>–</b>	<b>0</b>	<b>12.5</b>	<b>16.7</b>	<b>29.2</b>	<b>29.2</b>	<b>0</b>	<b>6.4</b>	<b>4.3</b>	<b>10.6</b>	<b>14.9</b>
<b>Sarcomastigophora</b>	<b>21</b>	<b>16.7</b>	<b>37.5</b>	<b>25.0</b>	<b>79.2</b>	<b>83.3</b>	<b>2.1</b>	<b>8.5</b>	<b>10.6</b>	<b>21.3</b>	<b>21.3</b>
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
<b>Ciliophora</b>	<b>6</b>	<b>0</b>	<b>12.5</b>	<b>33.3</b>	<b>45.8</b>	<b>50.0</b>	<b>6.4</b>	<b>4.3</b>	<b>6.4</b>	<b>17.0</b>	<b>23.4</b>
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
<b>Diatomacea</b>	<b>16</b>	<b>12.5</b>	<b>33.3</b>	<b>16.7</b>	<b>62.5</b>	<b>62.5</b>	<b>6.4</b>	<b>21.3</b>	<b>27.7</b>	<b>55.3</b>	<b>70.2</b>
<b>Rhodophyta</b>	<b>1</b>	<b>0</b>	<b>0</b>	<b>4.2</b>	<b>4.2</b>	<b>4.2</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>
<b>Cyanobacteria</b>	<b>1</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>2.1</b>	<b>0</b>	<b>0</b>	<b>2.1</b>	<b>2.1</b>

<sup>a</sup>Minimum number of distinct taxonomic groups identified in quantitative and qualitative samples.

<sup>b</sup>Mean tow volume  $\pm$  1 SD: cargo hold =  $1.32 \pm 0.32 \text{ m}^3$  ( $n = 24$  holds); ballast tank =  $0.22 \pm 16 \text{ m}^3$  ( $n = 62$  ballast tanks).

<sup>c</sup>% occurrence in vessels sampled by replicate quantitative plankton tows.

<sup>d</sup>% occurrence in vessels sampled as above plus occurrences in non-quantitative plankton tows and opportunistic dip net and sediment sampling.

Table 3. Mean relative abundance  $\pm$  SEM of the 5 top taxa (present in at least 3 bulk cargo carriers sampled by net) expressed as a percentage of the total density of organisms ( $\text{no. m}^{-3}$ ) in a sample, by 3 main regions and season of ballast water origin. Common taxa are defined as those present in at least 3 cargo vessel holds and ballast tanks in each region or season. In winter, only 4 taxa were present in 3 or more ships.  $n$  = number of ballast tanks and cargo holds in which a given taxon was present.

Rank	Region			Season			
	Mediterranean Black Sea	Northeast Atlantic	West Central Atlantic	Fall	Winter	Spring	Summer
1	Copepoda $69.1 \pm 6.2$ ( $n = 17$ )	Copepoda $54.8 \pm 11.6$ ( $n = 11$ )	Copepoda $77.7 \pm 8.0$ ( $n = 6$ )	Copepoda $63.5 \pm 10.3$ ( $n = 11$ )	Copepoda $82.5 \pm 5.5$ ( $n = 7$ )	Copepoda $67.4 \pm 8.9$ ( $n = 9$ )	Copepoda $53.3 \pm 10.5$ ( $n = 10$ )
2	Bivalvia $20.4 \pm 7.4$ ( $n = 4$ )	Diatomacea $33.7 \pm 16.0$ ( $n = 6$ )	Diatomacea $7.1 \pm 5.4$ ( $n = 3$ )	Dinoflagellida $15.8 \pm 6.2$ ( $n = 6$ )	Diatomacea $43.0 \pm 28.5$ ( $n = 3$ )	Diatomacea $9.8 \pm 4.0$ ( $n = 6$ )	Polychaeta $17.6 \pm 10.4$ ( $n = 8$ )
3	Dinoflagellida $14.4 \pm 3.7$ ( $n = 10$ )	Bivalvia $22.9 \pm 22.0$ ( $n = 4$ )	Bivalvia $5.4 \pm 2.1$ ( $n = 3$ )	Platyhelminthes $13.1 \pm 12.7$ ( $n = 3$ )	Polychaeta $8.1 \pm 6.4$ ( $n = 3$ )	Polychaeta $7.8 \pm 3.4$ ( $n = 6$ )	Bivalvia $17.0 \pm 6.6$ ( $n = 5$ )
4	Platyhelminthes $7.4 \pm 6.2$ ( $n = 6$ )	Polychaeta $16.2 \pm 9.2$ ( $n = 9$ )	Gastropoda $1.8 \pm 0.7$ ( $n = 3$ )	Isopoda $6.8 \pm 5.1$ ( $n = 3$ )	Dinoflagellida $2.1 \pm 0.9$ ( $n = 3$ )	Hydrozoa $6.6 \pm 5.1$ ( $n = 3$ )	Diatomacea $16.5 \pm 11.8$ ( $n = 5$ )
5	Polychaeta $5.8 \pm 2.6$ ( $n = 8$ )	Dinoflagellida $3.0 \pm 2.1$ ( $n = 3$ )	Decapoda $1.2 \pm 0.6$ ( $n = 3$ )	Polychaeta $6.7 \pm 5.5$ ( $n = 4$ )		Bivalvia $5.9 \pm 2.6$ ( $n = 3$ )	Dinoflagellida $6.1 \pm 1.2$ ( $n = 4$ )

ANOVA,  $F = 0.73$ ;  $df = 3, 25$ ;  $P = 0.55$ ) nor number of taxa (Kruskal–Wallis test, chi-square =  $0.71$ ;  $df = 3$ ;  $P = 0.87$ ) showed significant seasonal variation when pooled across the 3 regions.

#### Plankton density and environmental correlates

For combined regions and seasons, log-transformed plankton densities were significantly negatively correlated with the age (Spearman Correlation coefficient,  $r = -0.71$ ,  $P < 0.001$ ; Figure 7A), temperature ( $r = -0.52$ ,  $P = 0.004$ ; Figure 7B), and salinity ( $r = -0.64$ ,  $P < 0.001$ ; Figure 7C) of ballast water. There was no significant relationship between plankton density and the amount of ballast water in cargo holds and ballast tanks ( $r = -0.13$ ,  $P = 0.52$ ) (Figure 7D). Potential regional differences in plankton abundance, however, may have confounded several of these relationships. For example, ballast water from the eastern MBS (where many of this region's samples originated) was characterized by low plankton abundances (Figure 6A), older water, and higher salinity. Whether the MBS ballast water samples (Figure 7, closed circles) were depauperate because of age-, temperature-, or salinity-related mortality during transit or because of unrelated regional differences in initial plankton abundance remains to be determined.

#### Tolerance experiments

We assessed survivorship of polychaetes, bivalves, and crustaceans collected from ballast water and raised at temperatures and salinities characteristic of Chesapeake Bay (Figures 8 and 9). Polychaete survival was not consistent among temperature and salinity treatments after 2 wk (i.e., there were significant temperature  $\times$  salinity interactions in 5 of 6 experiments) (Figures 8A–F). In all but one case ( $25^\circ\text{C}$  at 5‰; Figure 8F), high mortality was associated with the lowest salinity treatment. In 4 of 6 experiments, the lowest temperature treatment ( $5^\circ\text{C}$ ) was also fatal to polychaetes regardless of salinity (Figures 8A, C, E, F). Given that the original ballast water samples containing these polychaetes were at least 25‰ and  $14^\circ\text{C}$ , mortality under these low salinity and temperature experimental treatment levels is not surprising and is likely to be independent of the species involved. Higher survivorship was observed in treatments with medium to high salinities and temperatures (Figure 8). Low salinities also negatively affected the bivalve *Mytilus* sp. (Figures 9A, B) and the copepod *Eurytemora velox* (Figure 9E). An unidentified species of bivalve from Sweden showed uniformly low survivorship across all temperature and salinity combinations (Figure 9C). Mysids (*Neomysis* sp.)

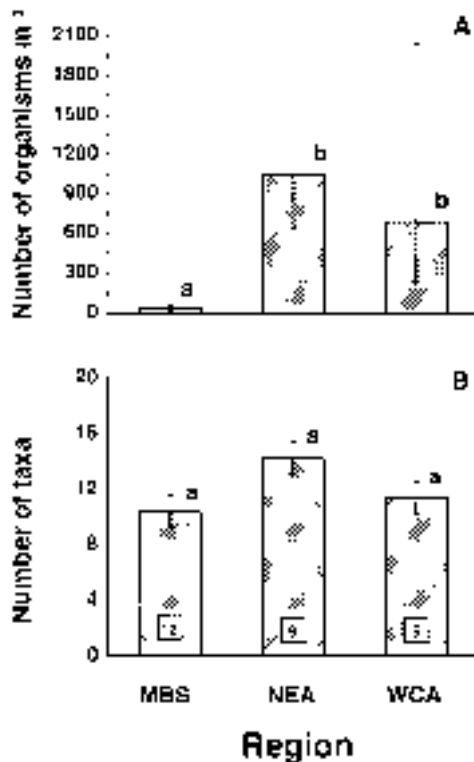


Figure 6. Summary of mean (A) number of organisms  $m^{-3}$  and (B) number of taxa in unexchanged ballast water from bulk cargo carriers sampled by net tow in Baltimore and Norfolk for the 3 main regions. For number of organisms  $m^{-3}$ , means and standard error of means (SEM) were back-transformed from logarithms for presentation. 1-way ANOVA on log-transformed densities:  $F = 10.8$ ,  $df = 2, 23$ ,  $P < 0.001$ ,  $MSE = 0.574$ . For number of taxa, untransformed means and SEM are given. Kruskal-Wallis test comparing number of taxa: chi-square = 1.88,  $df = 2$ ,  $P = 0.39$ . Different letters above bars indicate significant differences among means ( $P < 0.05$ ). The number of vessels is given inside lower bars. MBS, Mediterranean-Black Sea; NEA, Northeast Atlantic; WCA, West Central Atlantic.

from Belgium survived only at temperatures of 15 °C (Figure 9D).

## Discussion

### *Biological diversity of the inoculant pool*

Our data demonstrate conclusively that a ballast flooded estuary, the Chesapeake Bay, is being inoculated by a diverse assemblage of live organisms transported from around the world. Furthermore, it is evident that these inoculations are occurring on a massive and

frequent basis. Hundreds of bulk cargo vessels arrive in ballast in the Ports of Baltimore and Norfolk each year (Carlton et al. 1995; Smith et al. 1996). Each of these is capable of carrying tens of thousands of metric tons of foreign ballast water. Most vessels sampled contained live organisms, and ballast water was discharged in all seasons. Taken together, these data suggest that the opportunity for ballast-mediated introduction is great.

We found at least 221 species of protists, animals, and plants in 60 vessels sampled for ballast water and ballast sediments (Table 2). 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 and their developmental and reproductive stages were represented. Organisms originated from freshwater, brackish water, open ocean, and coastal high-salinity habitats. Although copepods dominated most ballast water samples, both in percent occurrence and abundance (Tables 2 and 3), other groups, including spionid polychaete larvae, bivalve larvae, dinoflagellates and diatoms were often present in high numbers.

The total diversity of organisms being brought to Chesapeake Bay is similar in magnitude to that reported in ballast water studies from three other regions. Carlton and Geller (1993) reported 367 species arriving in Coos Bay, Oregon in 159 vessels from Japan (Table 4). 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, varied significantly among these studies (Table 4). For example, diatoms were emphasized in the studies of Carlton and Geller (1993) and Subba Rao et al. (1994); dinoflagellates were further emphasized in the studies of Subba Rao et al. (1994). Carlton and Geller (1993) reported 33 flatworm taxa; in contrast, Australian studies (Williams et al. 1988) using same-source water from Japan reported only 1 flatworm taxon. These differences are almost certainly the result of analyzing live versus preserved specimens respectively. Subtracting ciliates, flatworms, diatoms, and dinoflagellates, as well as fish and unidentified small protistan or algal taxa, leaves perhaps a more comparable category of general 'zooplankton', which more likely captures uniform biases across all of the studies. These adjusted numbers and the data from Oregon, the Great Lakes, and our study, suggest that samples of greater

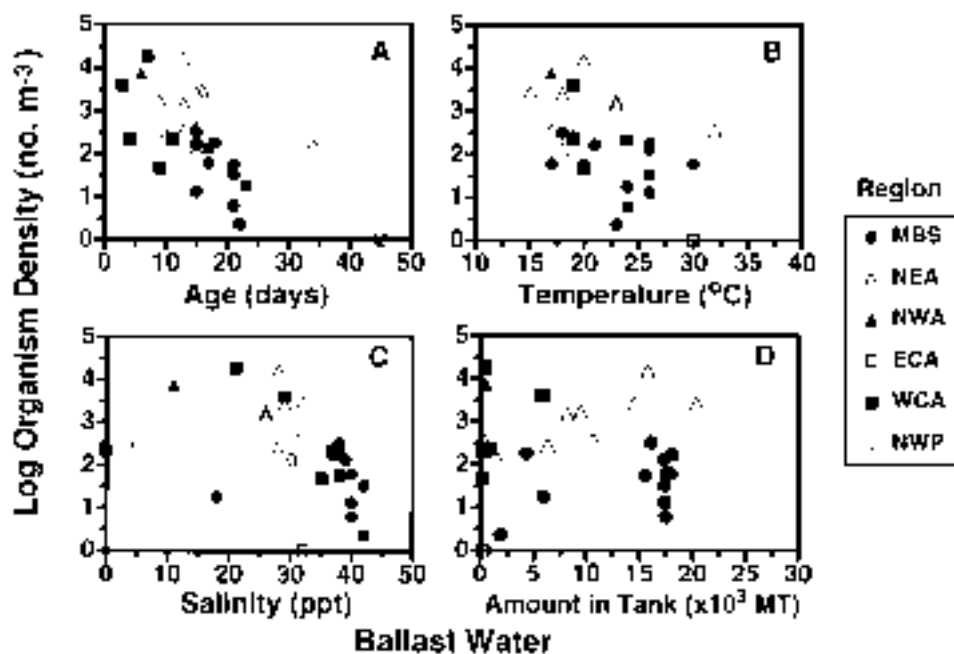


Figure 7.  $\text{Log}_{10}$  of organism density ( $\text{no. m}^{-3}$ ) as a function of ballast water: (A) age (days), (B) temperature ( $^{\circ}\text{C}$ ), (C) salinity (ppt), and (D) amount in cargo hold or ballast tank ( $\times 10^3$  MT) in bulk cargo carriers sampled in Baltimore and Norfolk. See Figure 1 legend for FAO regions.

than 60 ships should yield a minimum biota of 100 species.

The use of several sampling methodologies in the present study allowed us to document previously uncollected or undersampled organisms from ballast water. For example, plankton net sampling followed by live analysis provided the first documented occurrence of live ctenophores in ballast water. The discovery of ctenophores in ballast water 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 (Harbison and Volovik 1994). Opportunistic dip-netting yielded six families of fishes from cargo holds and ballast tanks (Table 2). Significantly, our sampling of recently deballasted cargo holds revealed the existence of post-settlement, and in some cases gravid, benthic organisms. Their presence should increase the chance of a successful invasion, because more vulnerable life-history stages (e.g., zygote, larva) are bypassed during transit.

#### Patterns of abundance in ballast water

Densities of organisms in the ballast water were highly variable from ship to ship (0 to 18,000 organisms  $\text{m}^{-3}$ )

and reflect the stochastic nature of ballast water transport. The high variability in inoculant abundances may be attributed, in large part, to Chesapeake Bay receiving foreign water from multiple source regions and distances (Figure 3). We observed significant density differences among source regions (Figure 6A), but it is unclear whether this pattern reflects regional differences in (1) initial plankton abundance, (2) age-related mortality (voyages from the MBS were significantly longer than from the NEA or WCA; Figure 7A), or (3) some combination of both. Studies show high mortality ( $>90\%$ ) for organisms travelling between Israel and Baltimore (Smith et al. in preparation; Wonham et al. in preparation), but estimates of initial abundances and survival for the other two regions are lacking. Comparable surveys of plankton survivorship along these and other major shipping routes are needed to determine the major factors influencing inoculant abundance.

Significantly, the transit time for most vessels arriving to Chesapeake Bay was sufficiently short ( $\leq 15$  d) as to allow survival of many organisms entrained in the ballast water (Figure 7A). In most cases, surviving organisms appeared viable and many were reared or cultured successfully in the laboratory. Thus, if one extrapolates our estimates of organism abundance to

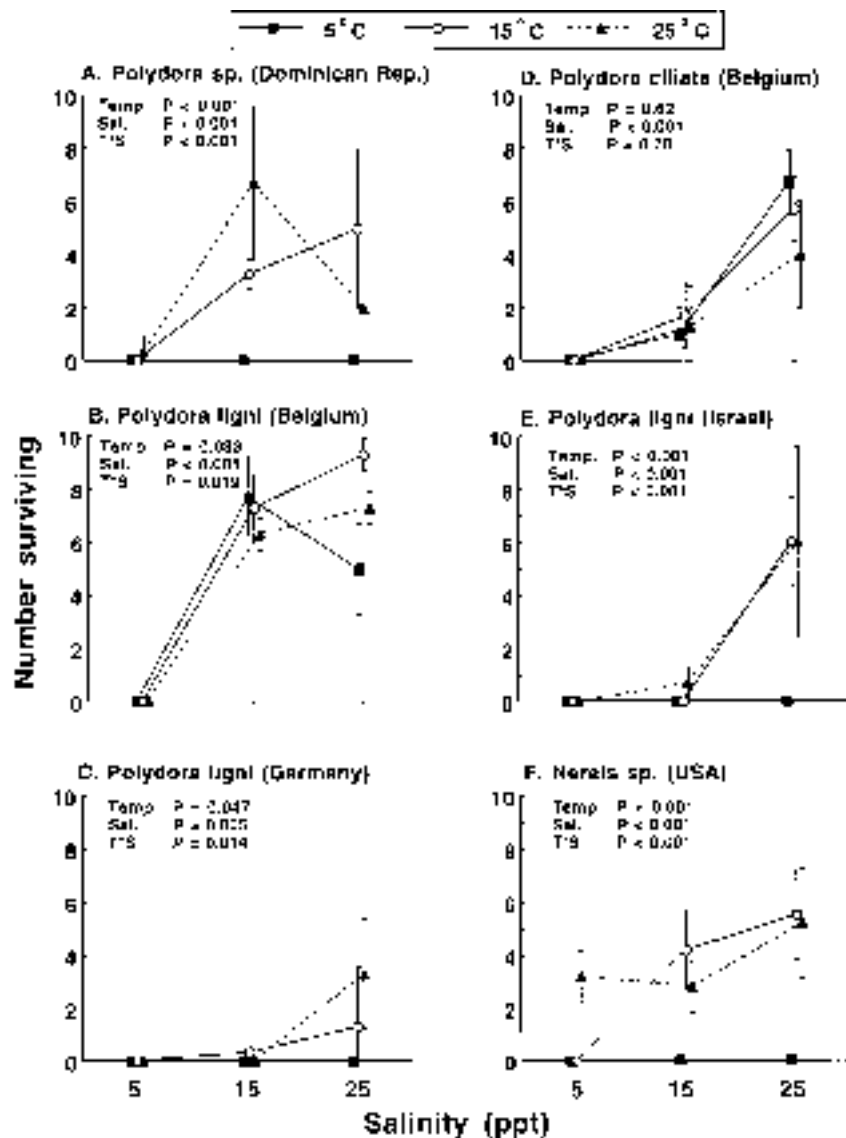


Figure 8. Mean numbers ( $\pm 1$  standard deviation) of polychaete individuals surviving in 9 combinations of temperature and salinity in the laboratory after 2 weeks. Species and source country of water (in parenthesis) are provided. Organisms were collected from ballast water with an original salinity and temperature, respectively, of (A) 34‰, 20°C; (B) 26‰, 23°C; (C) 31‰, 26°C; (D) 27‰, 14°C; (E) 40‰, 20°C; (F) 25‰, 29°C. Temperature treatment levels were 5°C, 15°C, and 25°C and salinity treatment levels, 5‰, 15‰, 25‰. Initially 10 individuals were placed in each of 3 replicate dishes (4 replicate dishes for *Nereis* sp.). *P*-values for temperature (Temp.) and salinity (Sal.) and temperature  $\times$  salinity interaction (T\*S) from 2-way analysis of variance models are provided for each experiment.

the total amount of ballast water on board an average bulk cargo carrier, a single deballasting vessel could release up to 1 billion organisms in a port. Multiplying these densities by the number of bulk cargo carriers that deballast annually in Baltimore (e.g., >200 in 1994) and Norfolk (>500 in 1994) (Smith et al. 1996), one would predict that ballast-mediated invasions would be occurring often. Why then, have so few been noted?

#### *Factors limiting ballast water invasions in Chesapeake Bay*

In developing models to predict invasions, Lodge (1993, p. 133) stressed the need to understand “the critical interaction of invader and the target community”. In the case of ballast-mediated invasions, neither inter- nor intraregional vulnerability can be predicted solely from



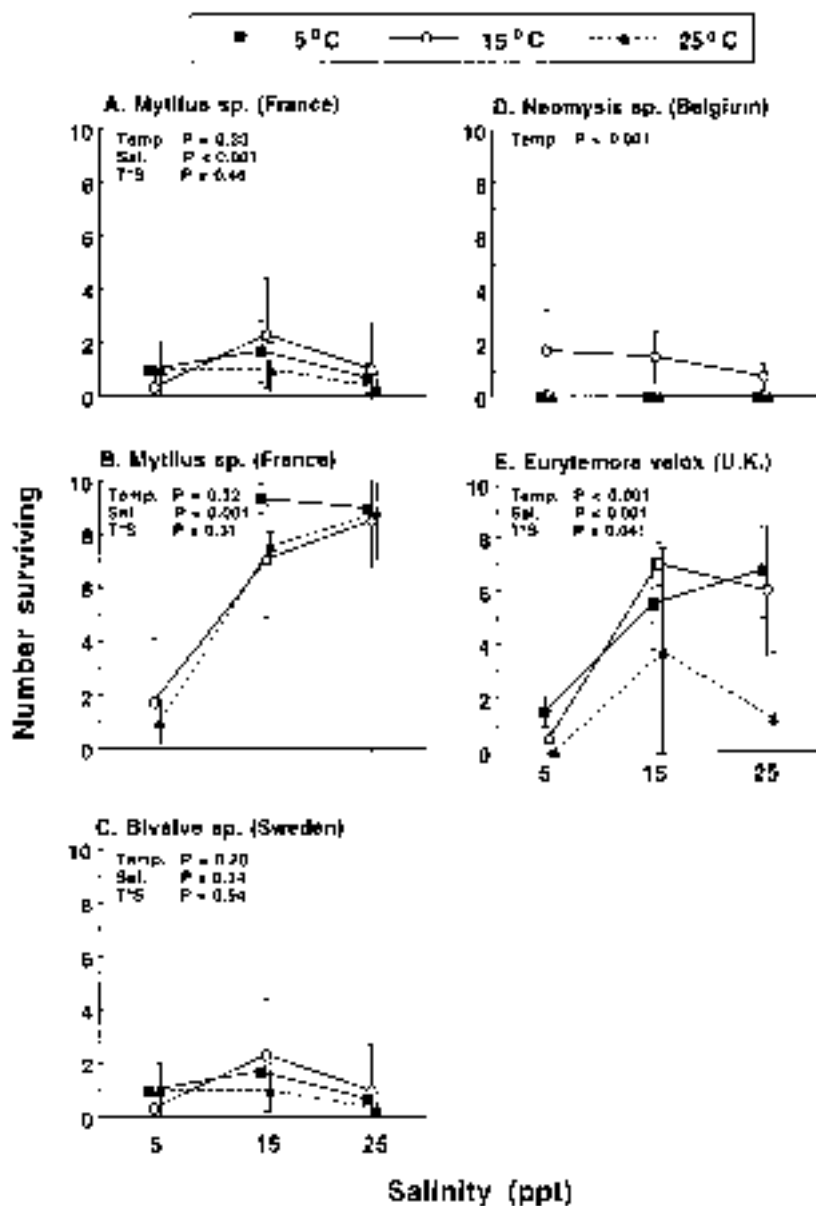


Figure 9. Mean numbers ( $\pm 1$  standard deviation) of bivalves (A, B, C), mysids (D), and copepods (E) surviving in 9 combinations of temperature and salinity in the laboratory after 2 weeks. Organisms were collected from ballast water with an original salinity and temperature, respectively, of (A) 32‰, 19 °C; (B) 22‰, 15 °C; (C) 9‰, 15 °C; (D) 5‰, 26 °C; (E) 22‰, 7 °C. See Figure 8 legend for other details. Three replicate dishes were used in experiments shown in A and C; 4 replicate dishes were used in experiments shown in B, D, and E.

knowledge of the amount of ballast water received or the density and diversity of organisms entrained (i.e., the ‘invasion or propagule pressure’, sensu Williamson 1996). One critical factor influencing the survival of any biological invader is its compatibility with abiotic conditions in the invaded habitat (e.g., van den Brink et al. 1993; Baltz and Moyle 1993; Lodge 1993;

Moyle and Light 1996). Of the factors that could limit ballast water invasions, the mismatch between donor and receiving water salinity (and, to some extent, temperature) may be paramount in the *upper* Chesapeake Bay. Most of the foreign water being deballasted in Baltimore Harbor was substantially higher in salinity (>20‰) than that of the receiving water regardless

Table 4. Examples of levels of taxonomic resolution in ballast studies from different regions (boldface numbers indicate special taxonomic emphases). Data summarized for Oregon (Carlton and 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	60
No. taxa				
Zooplankton <sup>a</sup>	184	110	64	168
Ciliates <sup>b</sup>	[6]	[3]	NS	6
Platyhelminthes	<b>33</b>	1	1	2
Fish	2	0	2	6
Diatoms	<b>128</b>	<b>61</b>	NS	16
Dinoflagellates	4	<b>30</b>	NS	18
Other <sup>c</sup>	10	8	NS	5
Total	367	213	67	221

<sup>a</sup>Zooplankton captured in plankton net hauls. Excludes benthic taxa, which for Australian studies includes 37 additional taxa, and in the present study includes 4 taxa (unidentified nematode, shrimp *Crangon*, and 2 fish species) found solely in benthic sediments of cargo holds.

<sup>b</sup>[Numbers] indicate number of taxa estimated in total count, but not extensively studied.

<sup>c</sup>Other includes radiolarians, foraminiferans, green algae, red algae, seagrasses and phytoflagellates.

of season (Figure 4) or source region (Figure 5). Our laboratory tolerance experiments suggest such salinity differences would be fatal for many organisms; polychaetes, bivalves, and a copepod species showed high rates of mortality at 5‰ (Figures 8 and 9). Differences in temperature between Baltimore Harbor and deballasted water, although less extreme than those for salinity, were still substantial (Figures 4A and 5A). Temperature differences were lowest in summer (Figure 4A), but organisms arriving between late fall and early spring would likely experience significant temperature-related physiological stress (e.g., 5 °C ‘wintertime’ conditions in our laboratory experiments Figure 8A).

Our data suggest the potential for within-region differences in susceptibility to ballast water invasion. Although Norfolk and Baltimore both export coal and grain to (and thus import ballast water from) the same geographic regions, we predict that Norfolk and the lower Chesapeake Bay are at higher risk of invasion than Baltimore and the upper Chesapeake Bay. First, the invasion pressure is greater in the lower Chesapeake Bay, because it receives significantly more foreign ballast water (e.g.,  $>15.2 \times 10^6$  MT in 1994) than the upper Chesapeake Bay ( $>5.9 \times 10^6$  MT) (Smith et al.

1996). Second, the Port of Norfolk has higher salinity water (20‰ to 28‰) than does Baltimore (3‰ to 8‰), thus, the physical characteristics of the receiving water should be more amenable in the former. In our laboratory experiments, we typically observed highest survivorship of ballast water organisms at 25‰ and 15 °C to 25 °C (Figures 8 and 9). Based on these two fundamental physical criteria, deballasted organisms are more likely to survive in the lower Chesapeake Bay.

#### Comparison with other invaded systems

Given the substantial invasion pathway to and the amenable physical conditions that exist in at least part of Chesapeake Bay, why is there no greater evidence of ballast-mediated invasions compared to the Great Lakes (29% of 139 introduced species are thought to have arrived in ballast water; Mills et al. 1993) or San Francisco Bay (23% of 212 introduced species; Cohen and Carlton 1995)? Recent evidence indicates that Chesapeake Bay is not immune to invasion by other mechanisms (G.M. Ruiz et al., unpublished data). Consequently, other factors besides salinity and temperature must be influencing invasion success. In aquatic systems, factors such as substratum type (e.g., for

benthic organisms), water quality (Moyle and Light 1996), disturbance frequency and magnitude (Nichols et al. 1990; van den Brink et al. 1993), previous invasion history (Robinson and Edgemon 1988), and community composition and resistance (Case 1990, 1991; Baltz and Moyle 1993) can determine the success or failure of an invasion. Whether any of these factors differ systematically among regions to explain existing variation in the numbers of ballast-mediated invasions will require detailed studies over broad temporal and spatial scales.

*Are some coastal areas 'safe' from ballast-mediated invasion?*

Despite the poor match between invaders and abiotic conditions in the upper Chesapeake Bay, it would be 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 (Carlton 1996a). For example, 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. Thus, while the water that Baltimore receives is generally mismatched with environmental conditions in the port, reasonable chances remain that an invasion could occur from a vessel releasing freshwater or brackish-water organisms or resting stages.

Similarly, it would be a mistake to interpret the absence of a high-impact ballast-mediated invasion in Chesapeake Bay (or in any other coastal region receiving ballast water) to mean that no future invasion will occur. Numerous authors have pointed to the difficulty in predicting which species will invade and when (Ehrlich 1986; Roughgarden 1986; Simberloff 1989; Crawley 1989; Lodge 1993; Carlton 1996a). For example, conditions for the ballast water dispersal of the zebra mussel *Dreissena polymorpha* to the Great Lakes existed for decades before the invasion actually occurred (Carlton 1996a). Changes in shipping patterns could result in increased trade with ports harboring more compatible water, a novel biota, or other nonindigenous species (Carlton 1996a), thereby increasing the potential for successful establishment.

If a large number of source regions contribute non-indigenous taxa into one estuary, as is the case in Chesapeake Bay, then it will be extremely difficult to assess invasion risk from ballast water transport. Local and

regional variations (e.g., tidal, hydrographic, physico-chemical), spatial variations (e.g., harbors within regions), and temporal variations (diurnal, lunar, seasonal) 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, because different vessels (and tank types within vessels) retain ballast water for different lengths of time, depending upon many factors, including length of voyage, cargo requirements, and sea-state conditions while vessels are in transit. Finally, tank inaccessibility, sampling biases, and clustering of organisms in ballast water may distort estimates of diversity and abundance. When these factors are considered against the larger backdrop of many different global source regions, the scale of complexity becomes enormous. At the least, however, it is clear that within-system invasion pressure can vary considerably, and this observation may allow for more focused intraregional prediction, monitoring, and management.

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