INTRODUCTION

Ballast-water transport

One of the largest pathways of marine introductions today is ballast-water transport along commercial shipping routes (Carlton & Geller 1993, NRC 1996, Carlton 1999). Ballast water is ambient water loaded into ballast tanks of commercial vessels for trim and stability. A typical bulk cargo vessel may load ballast water while unloading cargo, and discharge that ballast water en route or while loading cargo in the next port. In this manner, dense and diverse assemblages of zooplankton (Carlton & Geller 1993, Smith et al. 1999, Gollasch et al. 2000), phytoplankton (Hallegraeff & Bolch 1991, Subba Rao et al. 1994), fish (Wonham et al. 2000) and protists (Galil & Hülsmann 1997), are continuously being transferred within and among ocean basins.

For organisms to be introduced successfully via ballast-water transport, they must pass through a series of stages (Carlton 1985, 1996). Briefly, potential invaders must (1) survive ballasting into a ship’s tank; (2) survive the voyage; and (3) survive discharge into the recipient port. At each stage, species are filtered out of the ballasted assemblage by abiotic and biotic sources of mortality. The selectivity of these mortality sources determines the final abundance and diversity of potential invaders delivered to a recipient port (Carlton 1985, 1996, Smith et al. 1999, Lavoie et al. 1999).

Sources of mortality at each stage are poorly understood. The selectivity of stages 1 and 3, plankton uptake and release, is for the most part undescribed (Carlton et al. 1982). Somewhat more is known about stage 2, the ballast voyage, where heavy mortality may occur (Carlton et al. 1982, Rigby & Hallegraeff 1994, Lavoie et al. 1999, Gollasch et al. 2000). Some taxa clearly survive better than others (Lavoie et al. 1999, Gollasch et al. 2000), but taxon and ballast tank-specific trends in plankton density during voyages remain largely unquantified. Potential sources of mortality during transport include biological factors such as (1) starvation and (2) predation; physical factors such as (3) light limitation, (4) temperature change, (5) injury during ballasting, (6) injury from turbulence during the voyage and (7) lack of settlement substrate; and chemical factors such as (8) oxygen limitation and (9) chemical toxicity (Carlton 1985, Galil & Hülsmann 1997, Lavoie et al. 1999).

Ballast-water exchange

The growing use of ballast-water exchange to reduce invasion risk presents a new filter in the ballast invasion pathway. During open-ocean exchange, coastal ballast water is replaced with ocean water while the vessel is underway (IMO 1991, Locke et al. 1993, NRC 1996). This replacement is believed to reduce invasion risk primarily by flushing out most coastal organisms; secondarily, remaining coastal organisms may be killed by the change in conditions. However, port surveys of arriving vessels show that exchanged tanks may contain live coastal plankton (Williams et al. 1988, Hallegraeff & Bolch 1991, Locke et al. 1993, Carlton et al. 1995, Smith et al. 1999). Exchange efficiency has been estimated directly on only 3 vessels (Rigby & Hallegraeff 1994, Ruiz & Hines 1997), and the effectiveness of exchange in removing live plankton remains unquantified during a commercial voyage. Further, of 2 primary methods of ballast exchange, flow-through and empty-refill (NRC 1996), only the former has been tested in vessel trials (Rigby & Hallegraeff 1994, Ruiz & Hines 1997).

In the present study, we compare survival of multiple taxa in 2 types of ballast tank during a transoceanic commercial voyage (ballast transport stage 2), and evaluate support for different causes of mortality during transport. We also test the ability of ballasted taxa to survive in harbor water of the recipient port at the end of the voyage (stage 3). Finally, we test the effectiveness of empty-refill ballast exchange in removing and killing coastal taxa.

METHODS

We conducted all ballast sampling and exchange experiments aboard the coal-carrier MV ‘Leon’ during a 3 wk trans-Atlantic voyage (see Wonham et al. 1996 for details). The ‘Leon’ began ballasting on 1 June 1995 in Hadera, Israel, and deballasted in Baltimore, USA, from 22 to 23 June. During the voyage, we sampled ballast water from 7 ballast tanks: a cargo hold (CH), and 3 paired deck tanks (DT2, DT4, and DT5) (Fig. 1).

Ballast water sampling. We collected plankton with a standard 0.25 m diameter, 80 µm mesh plankton net. On each sampling day, we collected 3 vertical plankton tows in each tank, raising the net at approximately 1 m s⁻¹. Tows were 22 m deep in the cargo hold and 3 m (the depth of the top compartment) in the deck tanks. For all but the first day, tank openings were covered to minimize illumination during sample collection. After deballasting in Baltimore, we sampled low water and sediment at the bottom of the empty cargo hold for macrofauna. Plankton samples were examined briefly while alive, fixed in 10% formalin with Rose Bengal, and preserved in 75% ethanol before counting and identification.

One species, Oithona nana (Copepoda: Cyclopoida) was sufficiently abundant to examine for evidence of starvation. We collected them from the initial (n = 88
individuals) and final (n = 20 individuals) cargo-hold ballast-water samples and scored them for a full or empty gut.

Dissolved oxygen (DO), temperature, salinity and pH were measured in the cargo hold every 2 h throughout the voyage with a Hydrolab Datasonde 3 suspended at a depth of 10 m. In the deck tanks, DO and temperature were measured with a hand-held YSI meter at the water surface and at 3 m depth. No differences were observed between the depths, so only surface values are presented.

**Survivorship in Baltimore harbor.** At the end of the voyage, organisms were collected from the cargo hold immediately before deballasting and were placed in 100 ml dishes with water from the source cargo hold or from Baltimore harbor. Sample sizes were limited by the low density of living organisms to 2 dishes per treatment with 10 individuals per dish for *Parvocalanus crassirostris* (Copepoda: Calanoida), 3 dishes for *Oithona nana*, and 4 dishes for an unidentified larval spionid polychaete. Animals were maintained at room temperature and scored as alive or dead after 24 and 48 h.

**Open ocean exchange.** Three deck tanks (1 member of each pair of DT2, DT4 and DT5) were exchanged in the mid-Atlantic over 2 d, beginning approximately 400 nautical miles west of Gibraltar (24–25°N, 16–25°W). The effectiveness of open-ocean exchange in removing the original water mass was estimated from the change in ballast water salinity before and after exchange, relative to simultaneously collected ocean water (salinity measured on a YSI conductivity meter model no. 32). Plankton samples were collected less than 24 h before and after exchange in both exchanged and unexchanged tanks. Deck tank 2 was pressed up (i.e., water added) in mid-ocean prior to exchange, so only post-exchange plankton samples were used from this tank.

In the laboratory aboard ship, we measured the survivorship of ballasted organisms in ocean water. Zooplankters were collected from the cargo hold on 14 June, and transferred to 100 ml dishes containing filtered ballast water from either the cargo hold or the exchanged deck tanks. Ten organisms were placed in each of 5 dishes per treatment for the copepods *Parvocalanus crassirostris* and *Oithona nana*. Five organisms were placed in each of 3 dishes for *Euterpina acquifrons* (Copepoda: Harpacticoida) and for an unidentified juvenile spionid polychaete. Animals were maintained at room temperature and scored as alive or dead after 24 and 48 h.

**Statistical analysis.** Log-transformed plankton densities were analyzed using JMP version 3 (SAS Institute). All unplanned multiple comparisons following significant ANOVA results were tested using sequential Bonferroni corrected α-values for pairwise comparisons and a table-wide α-value of 0.05 (Rice 1989). To evaluate trends in plankton density over time, log-transformed plankton densities were fit with a linear regression model. For the cargo hold, densities of each taxon were fit with a linear model, where the slope approximates a net mortality rate. In the deck tanks, plankton densities were fit first with a linear model; in some cases, taxa with a significant linear slope appeared better fit with an exponential decay model. Unless otherwise indicated, figures and tables show means and errors based on n = 3 tows for the cargo hold, and n = 3 tanks (with 3 tows per tank) for the deck tank values.

**RESULTS**

We identified at least 50 live coastal species aboard the ‘Leon’ (Appendix 1). This is a conservative estimate since many taxa were identified only to class or phylum. Zooplankton consisted of 44 taxa in 12 phyla, and phytoplankton consisted of 3 dinoflagellates and 2 diatoms. In addition, we collected 1 crab species (n = 6 live juvenile portunids, *Liocarcinus holstatus*, carapace width 6 to 11 mm) in the deballasted cargo hold at the end of the voyage. Six zooplankton taxa were collected only following open-ocean exchange (Appendix 1).
Plankton densities decreased during the voyage by over 98% in all tanks (Table 1). Despite this high mortality, we estimate that over $1.4 \times 10^6$ live organisms arrived in Baltimore harbor aboard the ‘Leon’ (based on densities collected in an 80 μm net and assuming homogeneous plankton densities). Plankton survivorship and final densities differed among taxa and between tank types.

### Taxon comparisons

More taxa survived to the end of the voyage in the cargo hold than in the deck tanks (Table 1), so taxa are compared only within the cargo hold. Mortality in the deck tanks is reported under tank comparisons. In the cargo hold, zooplankton densities were significantly higher than phytoplankton densities throughout the voyage ($t = 6.39$, $p = 0.02$), and both groups declined at the same rate (Fig. 2a, Table 2).

Within these 2 groups, 12 taxa (9 zooplankton and 3 phytoplankton) survived in the cargo hold to the end of the voyage. Mortality rate, indicated by linear regression slope, was significant for 10 of the 12 taxa (Table 2). Only poecilrostomatoid copepods, which consisted primarily of *Oncaea* sp., and platyhelminths suffered no significant mortality during the voyage. Among these 9 zooplankton, a taxon’s initial density was a significant predictor of its subsequent mortality rate (Fig. 3a). However, a taxon’s final density, which is commonly measured in port-based ballast surveys, was a poor predictor of its preceding mortality rate (Fig. 3b). All 3 dinoflagellate genera declined significantly, at indistinguishable rates (Table 2).

Copepods were the most abundant taxon in our samples, representing 63 to 99% of all zooplankton individuals throughout the voyage. The rate of copepod decline, including nauplii, juveniles, and adults, was significantly greater than that of all other larval taxa together (Gastropoda, Bivalvia, Cirripedia, Polychaeta, Platynhelminthes) (linear contrasts $F = 60.30$, $p < 0.0001$; Fig. 4a).

Among copepods, the cyclopoids, calanoids and harpacticoids all declined significantly during the voyage, but poecilrostomatoid density remained constant (Fig. 4b, Table 2). Cyclopoids and harpacticoids de-

<table>
<thead>
<tr>
<th></th>
<th>Cargo hold</th>
<th>Deck tank 2</th>
<th>Deck tank 4</th>
<th>Deck tank 5</th>
<th>Statistic</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial density</td>
<td>8298 ± 1272$^a,b$</td>
<td>1536 ± 982$^c$</td>
<td>3438 ± 1018$^c$</td>
<td>26178 ± 2550$^a$</td>
<td>17.38</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Final density</td>
<td>84 ± 8$^a$</td>
<td>24 ± 15$^a,b$</td>
<td>4.7 ± 0.0$^b,c$</td>
<td>1.6 ± 1.9$^c$</td>
<td>11.83</td>
<td>&lt;0.001</td>
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<tr>
<td>% decrease</td>
<td>99.0</td>
<td>98.5</td>
<td>99.9</td>
<td>&gt;99.9</td>
<td></td>
<td></td>
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<tr>
<td>Initial taxa</td>
<td>28</td>
<td>17</td>
<td>19</td>
<td>21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Final taxa</td>
<td>12</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
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<td>% decrease</td>
<td>57.1$^a$</td>
<td>82.4$^b$</td>
<td>94.7$^b$</td>
<td>95.2$^b$</td>
<td>14.8</td>
<td>&lt;0.01</td>
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</tbody>
</table>

Table 1. Mean (±1 SE) initial and final plankton densities (no. m$^{-3}$) in cargo hold and 3 unexchanged deck tanks during voyage. N = 3 replicate tows per tank. Statistic, ANOVA $F$ for plankton densities; chi-square for number of taxa. Different superscript letters indicate statistically different values determined by pairwise post-hoc comparisons.
clined significantly faster than calanoids or poecilostomatoids. Poecilostomatoids declined significantly more slowly than all other orders (pairwise linear contrasts \( p < 0.05 \) in all cases), with a slope indistinguishable from zero (Fig. 4b, Table 2). No taxa or stages showed a net increase in density during the voyage.

Significantly more cyclopoid copepods, *Oithona nana*, had full guts at the beginning of the voyage (41/88) than at the end of the voyage (4/20) in the cargo hold (Fisher’s Exact Test, \( p = 0.043 \)).

### Tank comparisons

Plankton density and diversity varied among tanks. Initial plankton density was significantly higher in Deck Tank 5, which was ballasted last, than in Deck Tanks 2 and 4 (Table 1). Initial cargo hold density was intermediate. Final plankton density was significantly higher in the cargo hold than in the deck tanks (Table 1).

Initial taxon richness tended to be higher in the cargo hold than in the deck tanks (Table 1). This difference appears to be largely accounted for by the higher total tow volume of samples collected in the cargo hold (Fig. 5). In the deck tanks the cumulative total number of taxa collected in 3 tows of 0.15 m\(^3\) each is approximately the same as the number of taxa collected in the first of the cargo hold tows (1.1 m\(^3\)) (Fig. 5). Final taxon richness was significantly higher in the cargo hold than in the deck tanks. This difference appears greater than what might be expected simply from differences in sample volume (Fig. 5), and reflects the higher mortality suffered by taxa in the deck tanks during the voyage.

Twelve phytoplankton and zooplankton taxa were collected in the cargo hold at the end of voyage; of these, only 3 were collected in the final deck tank samples: *Euterpina acutifrons*, *Oncaea* sp., and the dinoflagellate *Ceratium* sp. The final densities of these 3 taxa did not vary significantly between tank types. The higher plankton density in the cargo hold at the end of the voyage was due therefore to the significantly higher number of taxa (Fig. 5), rather than to higher densities of these 3 taxa.

Total zooplankton proportional mortality was significantly greater in the deck tanks than in the cargo hold (linear contrasts \( F = 6.62, p = 0.015 \)) (Table 2). Slopes for phytoplankton were indistinguishable between tank types (Table 2). Total taxon richness also declined significantly faster than calanoids or poecilostomatoids. Poecilostomatoids declined significantly more slowly than all other orders (pairwise linear contrasts \( p < 0.05 \) in all cases), with a slope indistinguishable from zero (Fig. 4b, Table 2). No taxa or stages showed a net increase in density during the voyage.

<table>
<thead>
<tr>
<th>Taxa</th>
<th>R(^2)</th>
<th>Intercept</th>
<th>SE</th>
<th>Slope</th>
<th>SE</th>
<th>p</th>
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<tr>
<td><strong>(A) Cargo hold</strong></td>
<td></td>
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<tr>
<td>Zooplankton</td>
<td>0.96</td>
<td>4.00</td>
<td>0.05</td>
<td>–0.12</td>
<td>0.00</td>
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<td>0.94</td>
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<td>3.93</td>
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<td>&lt;0.001</td>
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<td>0.86</td>
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<td>–0.10</td>
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<td>&lt;0.001</td>
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<tr>
<td>Poecilostomatoida</td>
<td>0.94</td>
<td>2.96</td>
<td>0.08</td>
<td>–0.13</td>
<td>0.01</td>
<td>&lt;0.001</td>
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<td>Cirripedia</td>
<td>0.09</td>
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<td>0.18</td>
<td>–0.02</td>
<td>0.02</td>
<td>0.186</td>
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<td>Bivalvia</td>
<td>0.55</td>
<td>0.79</td>
<td>0.12</td>
<td>–0.05</td>
<td>0.01</td>
<td>&lt;0.001</td>
</tr>
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<td>Gastropoda</td>
<td>0.50</td>
<td>1.69</td>
<td>0.20</td>
<td>–0.04</td>
<td>0.01</td>
<td>&lt;0.001</td>
</tr>
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<td>Polychaeta</td>
<td>0.34</td>
<td>1.35</td>
<td>0.12</td>
<td>–0.02</td>
<td>0.01</td>
<td>0.102</td>
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<td>Platychelminthes</td>
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<td>0.66</td>
<td>0.12</td>
<td>–0.04</td>
<td>0.01</td>
<td>0.006</td>
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<td>Phytoplankton</td>
<td>0.79</td>
<td>3.60</td>
<td>0.15</td>
<td>–0.12</td>
<td>0.01</td>
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</tr>
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<td><em>Ceratocorys</em> sp.</td>
<td>0.62</td>
<td>3.16</td>
<td>0.19</td>
<td>–0.17</td>
<td>0.02</td>
<td>&lt;0.001</td>
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<td><em>Protoperidinium</em> sp.</td>
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<td>–0.11</td>
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<td><em>Ceratium</em> sp.</td>
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<td><strong>(B) Deck tanks</strong></td>
<td></td>
<td></td>
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<tr>
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<td>3.71</td>
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<td>Harpacticoida</td>
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<td>3.13</td>
<td>0.50</td>
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<td>Poecilostomatoida</td>
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<td>3.38</td>
<td>0.61</td>
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<td>Bivalvia</td>
<td>0.75</td>
<td>1.43</td>
<td>0.18</td>
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<tr>
<td>Polychaeta</td>
<td>0.63</td>
<td>1.73</td>
<td>0.34</td>
<td>–0.13</td>
<td>0.03</td>
<td>&lt;0.001</td>
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<tr>
<td>Phytoplankton</td>
<td>0.72</td>
<td>1.69</td>
<td>0.28</td>
<td>–0.13</td>
<td>0.03</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><em>Ceratium</em> sp.</td>
<td>0.27</td>
<td>1.05</td>
<td>0.33</td>
<td>–0.06</td>
<td>0.03</td>
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</table>
faster in the deck tanks than in the cargo hold (linear contrasts \( F = 9.16, p = 0.001 \)). However, both plankton densities and taxon richness in the deck tanks appeared to be fit better with an exponential model than with a linear model, suggesting that proportional loss of organisms and taxa in these tanks decreased over time (Fig. 2b,c). At the taxon level, log-densities of most taxa appeared better fit with an exponential model (Table 3) than with a linear model (Table 2).

**Physical conditions**

Mean daily dissolved oxygen level (±1 SD) was more than twice as high in the cargo hold (8.0 ± 0.2 mg l\(^{-1}\)) than in Deck Tanks 2, 4 and 5 (3.1 ± 0.3, 3.2 ± 0.6, 3.3 ± 1.0 mg l\(^{-1}\), respectively) (ANOVA, \(n = 4\) tanks, \(F = 5.82, p = 0.005\)). Mean daily water temperature in all tanks varied over only 2°C, from 23.8 (±1.2)°C in DT2 to 25.7 (±1.2)°C in DT5. Mean daily salinity remained constant in all tanks, over a range from 36.9 (±0.1) ppt in DT5 to 38.7 (±0.1) ppt in the cargo hold. Mean daily pH was 8.12 (±0.02) in the cargo hold, and was not measured in the deck tanks.

**Survivorship in Baltimore harbor water**

Organisms transferred to Baltimore harbor water at the end of the voyage suffered 100% mortality within 24 h. In contrast, organisms transferred to ballast water survived for more than 48 h. Survivorship at 48 h was significantly greater for the spionid (mean 97.9%; 95% CI = 82.7 – 98.1%; \(n = 4\) dishes), than for the 2 copepods Parvocalanus crassirostris (70.0 and 80.0%; \(n = 2\) dishes) and Oithona nana (mean 57.8%; CI = 41.6 – 73.2%; \(n = 3\) dishes) (ANOVA \(F = 125, p < 0.0001\); NB small sample sizes).

**Ballast exchange**

Mean salinity in the exchanged tanks decreased significantly following exchange (Student’s \(t\)-test \(p \leq 0.001\) in each tank) (Table 4). Bootstrapped estimates of mean exchange effectiveness using all 3 measures
of salinity from each tank ranged from 93.3% in Deck Tank 5 to 100% in Deck Tank 4 (Table 4). Water temperature and dissolved oxygen did not differ significantly between exchanged and unexchanged tanks, before or after exchange.

The mean density of organisms increased significantly following exchange and remained significantly higher at the end of the voyage (Repeated measures ANOVA $F = 32, df = 1, p < 0.0001$) (Fig. 6). The number of taxa also increased significantly following exchange ($\chi^2 = 4.27, p = 0.039$) (Fig. 6).

Some coastal taxa increased in density after exchange while others decreased. Since most taxa could not be identified to species level, it is unclear whether the organisms collected after exchange were members of the same or different species. Three coastal taxa decreased following exchange in 1 or more tanks: cyprids, gastropods and Euterpina acutifrons (Table 5). Mean estimates of exchange efficacy vary by taxon from 100% (cyprids in both tanks, gastropods in DT5, and E. acutifrons in DT4) to 80% (E. acutifrons in DT5) (Table 5). However, the variance in plankton density was so high relative to the mean densities that exchange efficacy is impossible to distinguish from 0% in most cases (Table 5). The density of gastropods decreased following exchange in DT5, but increased in DT4. Six new ocean taxa were collected following exchange (Appendix 1).

Ocean water was not lethal to coastal organisms. Zooplankton collected from the cargo hold at the time of exchange showed no significant difference in survivorship between ocean water and ballast tank water after 48 h (Fig. 6). Survivorship was significantly higher for the spionid polychaete and significantly lower for Euterpina acutifrons and Oithona nana than for Parvocalanus crassirostris (ANOVA $F = 29.9, p < 0.0001$) (Fig. 7).

### DISCUSSION

The density and richness of potential invaders decreased significantly during ballast-water transport. The plankton assemblage surviving to the end of the voyage represented less than 2% of the density and fewer than 50% of the taxa initially ballasted (Table 1, Fig. 2). These levels of mortality are comparable to those reported for select taxa over similar length voyages (Carlton et al. 1982, Rigby & Hallegraeff 1994, Gollasch et al. 2000, Smith et al. in press). Final total plankton density was approximately 84 organisms m$^{-3}$ in the cargo hold, somewhat lower than maximum densities reported in ballast surveys for organisms in this size range (Chu et al. 1997, Ruiz & Hines 1997, 2000).
Lavoie et al. 1999, Smith et al. 1999, Levings et al. 1999). Mortality rates were variable among taxa during transport, but uniformly high upon transfer to Baltimore harbor water.

**Predicting invasion success**

A successful invasion requires both an adequate density (e.g., Williamson 1996) and an adequate quality or condition of organisms (Pechenik 2000, Ruiz et al. 2000). Most studies of ballasted organisms, including ours, have focused on the density of taxa. The condition of organisms is more difficult to ascertain, but it seems likely that those taxa suffering high mortality during a voyage may be in poor condition by the end. Mortality rates may thus provide a proxy measure of a species’ condition, and therefore of its ability to invade. Interestingly, these 2 potential predictors of invasion success, high final density and low mortality rate, were not correlated with each other across taxa (Fig. 4b). In particular, copepods (excluding poecilostomatoids) were significantly more abundant, but suffered significantly higher mortality than larval taxa.

Copepods in general exhibit a striking disparity between delivery frequency and invasion frequency. They are typically the most abundant and frequently collected organisms in ballast water (Carlton 1985, Locke et al. 1993, Carlton & Geller 1993, Chu et al. 1997, Smith et al. 1999, Gollasch et al. 2000, present study), but, notwithstanding the dramatic effects of some of these invasions (e.g., Cordell & Morrison 1996, Orsi & Ohtsuka 1999), they represent fewer than 2% of introduced holo- and meroplanktonic species reported in North American and Australian coastal waters (Pollard & Hutchings 1990, Ruiz et al. 2000). This disparity between transport frequency and invasion frequency may be explained by poor condition, and asso-

Table 5. Mean density (no. m\(^{-3}\) ± 1 SE) before and after open-ocean exchange, for the 3 taxa that decreased in at least 1 tank following exchange: *Euterpina acutifrons* (Copepoda: Harpacticoida), *Cirripedia* (cyprid larvae) and *Gastropoda* (veliger larvae). Deck tank 4, exchanged 13 June; Deck tank 5, exchanged 14 June. Note the striking difference between tanks in post-exchange gastropod densities. The high variance associated with density measurements before exchange makes efficacy estimates only approximate. na: efficacy could not be calculated

<table>
<thead>
<tr>
<th></th>
<th>Deck Tank 4 Before exchange</th>
<th>Deck Tank 4 After exchange</th>
<th>Deck Tank 4 Efficacy (%)</th>
<th>Deck Tank 5 Before exchange</th>
<th>Deck Tank 5 After exchange</th>
<th>Deck Tank 5 Efficacy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Euterpina acutifrons</em></td>
<td>0.38 ± 0.46</td>
<td>0.75 ± 0.92</td>
<td>0.38 ± 0.46</td>
<td>0.00 ± 0.00</td>
<td>0.38 ± 0.46</td>
<td>0.00 ± 0.46</td>
</tr>
<tr>
<td><em>Cirripedia</em></td>
<td>0.00 ± 0.00</td>
<td>0.38 ± 0.46</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>0.38 ± 0.46</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td><em>Gastropoda</em></td>
<td>100 ± 0.00</td>
<td>80 ± 0.29</td>
<td>100 ± 0.00</td>
<td>7.56 ± 0.40</td>
<td>0.00 ± 0.00</td>
<td>100 ± 0.00</td>
</tr>
</tbody>
</table>

![Fig. 6](image-url). Mean density of organisms remained constant in unexchanged tanks (light bars) following exchange, but increased significantly in exchanged tanks (dark bars) and remained significantly higher in final samples at end of voyage. Bars, mean of \( n = 9 \) plankton tows (\( n = 6 \) for Before Exchange); with error bars = 1 SE. Numbers on bars indicate number of taxa. ***Significant difference between exchanged and unexchanged tanks determined by post-hoc t-tests at \( p < 0.0001 \)

![Fig. 7](image-url). Coastal ballast taxa survived equally well in the laboratory in ballast-tank (light bars) and open-ocean (dark bars) water after 48 h. Mean survival (± 1 SE) for \( n = 5 \) dishes with 10 individuals each (*P. crassirostris* and *O. nana*) and \( n = 3 \) dishes with 5 individuals each (*E. acutifrons* and spionid). Different letters indicate statistically different survivorship for different taxa from pairwise post-hoc comparisons (ANOVA \( F = 29.9, p < 0.001 \))
ciated high mortality rates, of copepods on ballast voy-
ages (Fig. 3a). Alternatively, frequent and dense bal-
last transport may have led to numerous successful
invasions in the past that go unrecognized today,
reflecting a lack of appropriate sampling (Ruiz et al.
2000). Overall, however, it is likely that the condition
of arriving propagules can substantially modify the
relationship between delivery and invasion success.

The outcome of ballast-water transport is also influ-
enced strongly by survival at the recipient port. In our
study, none of the taxa tested survived transfer to local
waters. This high mortality presumably resulted from
the considerable difference in salinity (approximately
35 ppt). While low salinity of Baltimore harbor (approx-
imately 5 to 10 ppt) may consistently reduce the risk of
invasion by organisms from high salinity ports (Smith
et al. 1999), this is a highly case-specific filter, and the
environmental mismatch will be less severe for many
other pairs of coastal ports. Thus, the relative impor-
tance of changes in plankton quantity and quality in
predicting invasion success will vary greatly among
ports.

Causes of mortality

Carlton (1985) suggested that in the dark ballast
tanks, phytoplankton production stops and herbivo-
rous zooplankton are food limited, whereas bacterial
grazers, detritivores, scavengers, non-feeding larvae,
or larvae with food reserves may survive better. We
found that significantly more individuals of the
cyclopoid copepod *Oithona nana* had empty guts at the
end of the voyage than at the beginning, suggesting
that this species, at least, was food-limited. We evalu-
ate support for several additional predictions sug-
gested by the food-limitation hypothesis.

Mortality would be higher for herbivorous than for
detrivorous and microbial grazer holoplankton

Chu et al. (1997) found that cyclopoid and calanoid,
but not harpacticoid copepod densities, were lower in
ships with older ballast water. They suggested that
harpacticoids, grazing on bacterial production, sur-
vived better than the herbivorous copepods. In con-
trast, we found that the densities of cyclopoids (pri-
marily the omnivorous mid-water feeder *Oithona
nana*) and harpacticoids (primarily the herbivorous
*Euterpinia acutifrons*) decreased fastest. Calanoids
(primarily the suspension feeding *Parvocalanus cras-
sirostris*) decreased at an intermediate rate. Poecilo-
ostomatoids (primarily the omnivorous *Oncaea*
sp.), showed no significant decrease in density during the
voyage. High survival of *Oncaea* sp., which has a
broad diet that includes scavenging midwater detri-
tus (e.g., Steinberg et al. 1994, Hwang & Turner 1995)
and may therefore be less dependent on phytoplank-
ton for food, is consistent with the hypothesis of light-
limitation and starvation. The surprisingly high mor-
tality of harpacticoids may reflect the particular diet
of the dominant harpacticoid *E. acutifrons*, which
appears to be primarily an algal grazer (Sautour &
Castel 1993).

Zooplankton mortality would lag behind
phytoplankton mortality

Our data do not indicate any lag in the zooplankton
decline relative to the phytoplankton, but we mea-
sured only the comparatively large phytoplankton
fraction filtered by an 80 µm net. Smaller mesh nets
would provide a more accurate picture of the overall
phytoplankton community including smaller dinofla-
gellates and diatoms (e.g., Hallegraeff & Bolch 1991,

Zooplankton mortality would be
density-dependent

Zooplankton competing for limited food could
exhibit density-dependent mortality. Among zoop-
lankton taxa, we found that higher mortality rates
were associated with higher initial densities. In addi-
tion, overall zooplankton mortality rate in the deck
tanks tended to decrease as zooplankton densities
decreased over time. However, this latter pattern prob-
ably does not represent a density-dependent response,
but rather reflects the rapid, early loss of more sensi-
tive taxa followed by the persistence of more robust
taxa.
Mortality rates would be higher for photosynthetic than for heterotrophic phytoplankton.

We found no significant difference in mortality rate between the heterotrophic dinoflagellate *Protophrydinium* sp. and the autotrophic dinoflagellate *Ceratium* sp.

In addition to these bottom-up trophic interactions, physical conditions may have contributed to plankton mortality. Dissolved-oxygen levels and plankton survivorship were lower in the deck tanks than in the cargo hold, suggesting that oxygen limitation in the deck tanks may have reduced plankton survivorship. However, 2 additional factors may explain the higher mortality rates in the deck tanks. The cargo hold sampling depth was 23 m, but the deck tank upper compartments were only 3 m deep. If plankton tended to sink at the same rate to the bottom of both tank types, organisms would disappear sooner from the deck tank samples than from the cargo hold samples. Further, since deck tanks are higher than the cargo hold above the ship’s center of gravity, they may experience heavier water movement during rough weather, a phenomenon that has been correlated with high mortality during other voyages (Carlton et al. 1982). Mortality has also been associated with large changes in ballast water temperature (Carlton et al. 1982, Gollasch et al. 2000), but on this voyage as on several others (Carlton et al. 1982, Rigby & Hallegraeff 1994, Gollasch et al. 2000), mortality was high despite relatively constant temperatures.

Predation by macrofauna is unlikely to have contributed to mortality on this voyage. We collected only 2 larval fish during the voyage, and found no fish and only 6 small portunid crabs in the deballasted cargo hold at the end of the voyage. Other factors such as physical damage during ballasting, or the possible release of chemicals such as zinc (from anti-corrosion anodes in the deck tanks) (Jelmert & van Leeuwen 2000) or other compounds were not evaluated.

**Effectiveness of open-ocean exchange**

Ballast-water exchange represents a new and fundamental alteration to an invasion pathway that has been in operation for decades. We found that empty-refill exchange replaced 96 to 100% of coastal water, a level consistent with salinity-based measures of exchange on 2 other vessels (Ruíz & Hines 1997). Exchange also replaced an estimated 80 to 100% of live coastal organisms, although the high variability in pre-exchange plankton densities makes these estimates only approximate. However, they are consistent with estimates based on dye concentrations and dead plankton densities on one other vessel (Rigby & Hallegraeff 1994).

Total plankton density and diversity increased following exchange. Since most taxa could not be identified to species, we do not know whether the post-exchange individuals in a given taxon were coastal individuals retained during exchange, new oceanic individuals of the same species, or new oceanic individuals of a new species in the same taxon.

Ocean water was not lethal to 4 originally ballasted coastal taxa. Unlike many coastal ports, however, the water in Hadera harbor is hypersaline (36 to 39 ppt), and close to open ocean salinities (~35 ppt). Mortality from the osmotic stress of salinity change would be more likely to affect coastal organisms from brackish or fresh water (Smith et al. 1999). Our sampling methods did not allow us to evaluate the effectiveness of exchange in removing smaller size fraction and encysted phytoplankton or bacteria, which are known to be transported in ballast water (Hallegraeff & Bolch 1991, Hallegraeff 1998, Subba Rao et al. 1994, Gall & Hülsman 1997).

The stochastic nature of the invasion process makes it difficult to predict the identity and timing of an individual invasion event (Carlton 1996). In the case of ballast-water transport, the problem is exacerbated by the difficulty of identifying many larval organisms to species (Carlton 1996, Smith et al. 1999). This stochasticity does not mean, however, that ballast transport of potential invaders does not exhibit predictable patterns or cannot effectively be reduced. Clearly, ballast transport selectively filters taxon density and diversity during a voyage. Interestingly, 2 potential predictors of invasion success, mortality during transport (which may, indicate body condition), and final organism density, were not correlated across taxa. Although the specific causes of mortality remain speculative, trends in survivorship point to the roles of light, food, and oxygen limitation on this voyage. Open-ocean exchange represents an additional selective filter in the ballast invasion pathway, that reduces but does not eliminate coastal taxa.

Acknowledgements. This research was supported by the U.S. Fish and Wildlife Service and the Compton Foundation. J. Carlton, D. Smith, A. Hines and C. Czarnecki provided logistic support and thoughtful discussion throughout the project. D. Friedmann cheerfully provided invaluable field and linguistic assistance during the voyage, and S. Godwin, D. Smith, L. McCann and P. Fofonoff provided field and laboratory assistance in Baltimore. D. Correll loaned equipment for salinity analysis. We are grateful for the taxonomic expertise of F. Ferrari (copepods), S. Godwin and A. Williams (decapods). For statistical advice, we are indebted to G. Gilchrist and E. Holmes. We thank El-Yam Ships Ltd., National Coal Supply Corporation Ltd., John S. Connor Shipping Agency, and Consolidation Coal Dock for their cooperation and assistance. This study would not have been possible without the enthusiastic hospitality and collaboration of Captain Reuider and the officers and crew of the MV ‘Leon’.
## Appendix 1. Taxa collected during the 16 d voyage from Israel to Baltimore, USA. Plankton collected in C, cargo hold; D, unexchanged or pre-exchange deck tanks; E, exchanged deck tanks following exchange

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Collection</th>
<th>Taxa</th>
<th>Collection</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Zooplankton</strong></td>
<td></td>
<td><strong>Harpacticoida (continued)</strong></td>
<td></td>
</tr>
<tr>
<td>Tintinnida</td>
<td>E</td>
<td>Canuellida</td>
<td>C,D</td>
</tr>
<tr>
<td>Foraminifera</td>
<td>C</td>
<td>Other cyclopoids</td>
<td>C,D</td>
</tr>
<tr>
<td>Cnidaria</td>
<td></td>
<td>Poecilostomatoida</td>
<td></td>
</tr>
<tr>
<td>Hydromedusa</td>
<td>C,D</td>
<td>Corycaeus sp.</td>
<td>C,D,E</td>
</tr>
<tr>
<td>Siphonophora</td>
<td>C</td>
<td>Oncaea sp.</td>
<td>C,D,E</td>
</tr>
<tr>
<td>Ctenophora</td>
<td>C</td>
<td>Other poecilostomatoids</td>
<td>C,D</td>
</tr>
<tr>
<td>Platyhelminthes</td>
<td>C,D</td>
<td>Copepod nauplii</td>
<td>C,D,E</td>
</tr>
<tr>
<td>Mollusca</td>
<td></td>
<td>Copepoda, unidentified</td>
<td>C,D,E</td>
</tr>
<tr>
<td>Bivalvia</td>
<td>C,D,E</td>
<td>Cladocera</td>
<td></td>
</tr>
<tr>
<td>Gastropoda</td>
<td>C,D,E</td>
<td>Evadne spinifer</td>
<td>E</td>
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<tr>
<td>Other molluscs</td>
<td>D</td>
<td>Cirripedia</td>
<td></td>
</tr>
<tr>
<td>Polychaeta</td>
<td></td>
<td>Caridea</td>
<td>C,D</td>
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<td>Spionidae</td>
<td>C,D</td>
<td>Euphausiaacea</td>
<td>C,D</td>
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<td>Chaetognatha</td>
<td>C,D,E</td>
<td>Mysidacea</td>
<td>C,D</td>
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<tr>
<td>Nematoda</td>
<td>D</td>
<td>Sergestidae</td>
<td>C,E</td>
</tr>
<tr>
<td>Crustacea</td>
<td></td>
<td>Shrimp (unidentified)</td>
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</tr>
<tr>
<td>Copepoda</td>
<td></td>
<td>Gammaridea</td>
<td>C</td>
</tr>
<tr>
<td>Calanoida</td>
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<td>C</td>
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<td>Centropages sp.</td>
<td>C,D</td>
<td>Zoae (unidentified)</td>
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</tr>
<tr>
<td>Clausocalanus sp.</td>
<td>C,D</td>
<td>Bryozoa</td>
<td>C</td>
</tr>
<tr>
<td>Labidocera sp.</td>
<td>D</td>
<td>Pisciscus</td>
<td>C,D</td>
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<tr>
<td>Paracalanus sp.</td>
<td>C,D,E</td>
<td>Eggs (unidentified)</td>
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</tr>
<tr>
<td>Parvocalanus crasirostris</td>
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<td><strong>Diatomacea</strong></td>
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<tr>
<td>Pseudocyclopia sp.</td>
<td></td>
<td>Chaetoceros sp.</td>
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<td>Temora sp.</td>
<td>C,D</td>
<td>Discoid diatom</td>
<td>C,D</td>
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<tr>
<td>Acartia sp.</td>
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<td><strong>Phytoplankton</strong></td>
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<td>Dinoflagellida</td>
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<td>Cyclopoida</td>
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<td>Ceratium sp.</td>
<td>C,D,E</td>
</tr>
<tr>
<td>Oithona nana</td>
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<td>Ceratocorys sp.</td>
<td>C,D,E</td>
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<td>Oithona sp. A</td>
<td>D</td>
<td>Protothecoides sp.</td>
<td>C,D,E</td>
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<td>Oithona sp. B</td>
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<td>Discoid diatom</td>
<td>C,D</td>
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<tr>
<td>Hemicyclops sp.</td>
<td>D</td>
<td><strong>Bryozoa</strong></td>
<td>C</td>
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<td>Harpacticoida</td>
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<td>C,D</td>
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<td>Ceratium sp.</td>
<td>C,D,E</td>
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<tr>
<td>Dinoflagellida</td>
<td></td>
<td>Ceratocorys sp.</td>
<td>C,D,E</td>
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<tr>
<td>Protophycocystis sp.</td>
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<td>Discoid diatom</td>
<td>C,D</td>
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<tr>
<td>Diatomaceae</td>
<td></td>
<td>Chaetoceros sp.</td>
<td>D</td>
</tr>
<tr>
<td>Bryozoa</td>
<td>C</td>
<td>Discoid diatom</td>
<td>C,D</td>
</tr>
<tr>
<td>Pisciscus</td>
<td>C,D</td>
<td>Discoid diatom</td>
<td>C,D</td>
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</table>

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Editorial responsibility: Otto Kinne (Editor), Oldendorf/Luhe, Germany

Submitted: April 14, 2000; Accepted: September 29, 2000
Proofs received from author(s): April 10, 2001

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