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Research Article

Effect of osmotic shock as a management strategy to reduce transfers of non-indigenous species among low-salinity ports by ships

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Abstract

Open-ocean ballast-water exchange (BWE) is currently the most common treatment used to reduce the ballast transfer of organisms and the subsequent risk of invasions among coastal ecosystems. Freshwater or estuarine organisms remaining after BWE often experience high mortality, due to osmotic shock caused by high-salinity exposure. We conducted 70 salinity tolerance experiments on 54 different taxa to measure mortality rates of freshwater and estuarine organisms after exposure to oceanic seawater (34 psu), simulating both flow-through (F-T) and empty-refill (E-R) BWE methods. We focused especially on larval and adult crustaceans from freshwater and mesohaline habitats adjacent to ports of the Baltic Sea, North Sea, Great Lakes, Chesapeake Bay, and San Francisco Bay. Animals from oligohaline habitats (0-2 psu) experienced the highest mortality: all individuals died in 82% of the F-T treatments and 88% of the E-R treatments. The effectiveness of both treatment types decreased with animals from low-salinity (2-5 psu, 100% mortality in 27% of F-T and 46% of E-R treatments) and mesohaline habitats (5-18 psu, 100% mortality in 40% of F-T and 52% of E-R treatments). In 43% of cases among all salinity categories, empty-refill treatments required less exposure time to cause significant mortality than flow-through treatments. Invertebrates that exhibited significant survivorship were most often peracarid crustaceans including widely introduced species of mysid shrimps and amphipods. Although salinity shock does not completely prevent the transfer of all low-salinity biota, BWE provides a useful management tool to reduce species transfers, especially considering the combined effects of removal and mortality.

Key words: ballast-water exchange, Baltic Sea, estuaries, Great Lakes, non-native species, salinity tolerance, zooplankton

Introduction

Invasions of coastal ecosystems by non-indigenous species are having significant ecological, economic, and human health impacts (Ruiz et al. 1997; Pimentel 2005). Recent analyses indicate that the rate of discovery for new invasions has increased in many global regions over the past 50 years (Costello and Solow 2003; Ruiz et al. 2000; Ricciardi 2001; Hewitt et al. 2004). These analyses also underscore the importance of shipping as a major transfer mechanism (vector), contributing a high proportion of newly detected invasions.

Commercial shipping results in the unintentional transfer of freshwater and marine

organisms by two dominant mechanisms. First, organisms that occur on the exposed underwater surfaces of vessels can be transferred among ports. This assemblage can include sessile invertebrates and algae, as well as mobile biota. For the latter, areas of low flow may be especially important, including water intakes and seachests (Coutts et al. 2003; Golasch 2006). Second, ships take on surrounding coastal and oceanic water into ballast tanks, using this for trim and stability. This process often entrains a diverse community of planktonic organisms, which are later discharged at subsequent ports of call during cargo operations (Carlton and Geller 1993; Smith et al. 1999, Levings et al. 2004).

Efforts to reduce the risk of ship-mediated invasions have focused primarily on ballast water management. Currently, several countries now require ships from overseas to treat their ballast water before discharge to reduce the concentration of viable organisms in their ballast water, and thus minimizing the risk of introducing non-native species (US Fed register; NZ law; Aus law). In addition, the International Maritime Organization has adopted a convention on ballast-water treatment that is pending ratification by member countries (IMO 2005). Under these various management strategies, ships are required to undertake ballast-water exchange (BWE*) or use an alternative approved treatment. Although considerable effort is underway to advance alternative treatments, several are still being developed and tested, and none is currently approved for general use. Thus, BWE is a treatment method that will likely persist for several years.

The intent of BWE is to flush coastal organisms out of the ballast tanks and into the open ocean, where they are considered unlikely to survive. While BWE can be highly effective in reducing the concentration of coastal organisms by physical removal (Ruiz and Smith 2005), it is clear that residual coastal organisms remain, sometimes in relatively high numbers, in ballast tanks following BWE (Duggan et al. 2005; Duggan et al. 2006; Minton et al. 2006; Drake and Lodge 2007). In addition to removing coastal organisms, BWE may also cause mortality of any remaining coastal organisms in ballast tanks, due to changes in environmental conditions such as salinity or temperature. This mortality can further augment the effects of BWE and may be especially relevant fororganisms from oligohaline habitats that may experience lethal osmotic stress if exposed to oceanic water. However, a robust examination of the mortality of organisms remaining in ballast tanks after BWE caused by exposure to open-ocean water is still lacking. Such mortality may greatly enhance the efficacy of BWE, especially for ballast tanks with freshwater or stenohaline organisms that cannot tolerate higher salinities. For example, many recent invasions to the Laurentian Great Lakes have been linked to ballast-mediated transfers from low-salinity ports in northern Europe (Leppakoski et al. 2002; MacIssac et al. 2002; Reid and Orolva 2002). Likewise, many recent invasions to San Francisco Bay and the Columbia River in western North America have occurred in low-salinity habitats and are attributed to ships' ballast water (Bollens et al. 2002). It is further noteworthy that many of these invasions predate the use of BWE, which had it been in place, may have been an effective barrier against the transfer of coastal organisms from low-salinity habitats.

Our goal was to provide guidelines for minimum salinity concentrations and exposure times necessary to cause 100% mortality in a wide range of invertebrate taxa. Here, we measured the effect of high-salinity exposure on mortality rates of freshwater and brackish-water organisms, using laboratory experiments to simulate exposure times and conditions associated with BWE. Our initial experiments focused on invertebrate species from the Baltic Sea, a known source region for vessels and low-salinity organisms arriving to the Great Lakes (Leppakoski et al. 2002), and expanded the geographic scope to include biota from freshwater and mesohaline ports in North America. Our experiments demonstrate that organisms from freshwater and oligohaline habitats experience high mortality rates when exposed to euhaline seawater (34 psu) during BWE and salt-water flushing, enhancing the effect of these treatments. Interestingly, several of the animals that exhibited significant survivorship in our experiments were widely introduced species of peracarid crustaceans. We propose that our simple experimental protocol

* **Abbreviations :** Ballast-Water Exchange (BWE); Chesapeake Bay, U.S.A. (CB); Curonian Lagoon, Lithuania (CLL); Empty-Refill (E-R); Flow-Through (F-T); Lake Huron, Alpena, Michigan, U. S. A. (LH); Lake Erie, Toledo, Ohio, U.S.A. (OH); Oder River, Poland (ORP); Port of Rotterdam, The Netherlands (PRN); San Francisco Bay, California, U.S.A. (SFB); Lake Michigan, Traverse City, Michigan, U.S.A. (TC); Vistula River, Poland (VRP)

also provides a useful management tool for identifying species posing a greater risk of dispersal via the operations of commercial ships.

Materials and methods

Animal Collection and Sites

We chose to experiment with a broad range of native and non-native invertebrate taxa from freshwater and brackish-water habitats known to be source regions of introduced species (Baltic and North Seas) transferred via ships to the Great Lakes and estuarine ports of the United States. Experiments focused initially on organisms from Curonian Lagoon, Lithuania, being a low-salinity system with the third largest commercial port in the Baltic Sea (see Gasiūnaite 2000 for details on local conditions and biota).

Additional experiments were conducted in Europe (The Vistula and Oder Rivers, Poland; Rotterdam, Netherlands), eastern United States (Chesapeake Bay, Maryland ; Lake Erie, Ohio; Lake Michigan, Michigan; Lake Huron, Michigan), and western United States (San Francisco Bay, California). Experiments at these sites were intended to broaden the general scope of analysis by including additional taxa and geographic regions. Collectively, these sites have commercial ports that range between freshwater and mesohaline conditions and have also been sites of ship-mediated invasions (Santagata and Ruiz 2007).

At each site, we collected as many native and non-native planktonic invertebrates as practical. Experiments were conducted using organisms collected in sufficient numbers to achieve a minimum sample size needed for the experiments (see below). Most animals were collected directly from the field, using a variety of sampling methods (plankton net, light trap, or seine net), sorted quickly (within 1-4 hours) in the laboratory, and used soon thereafter (within 12 hours) in laboratory experiments outlined below. A small subset of species were collected from animals brooding embryos, which were held for a short time (days) in the laboratory until they developed to planktonic stages and/or juveniles for experiments. During the course of experiments it was clear that particular taxonomic groups and life stages exhibited higher survivorship than others despite occurring in similar salinity ranges. For these reasons, we focused a series of experiments on widely introduced species of crustaceans especially amphipods and early larval stages of the crab, *Rhithropanopeus harrisii* (Gould, 1841).

Salinity-Tolerance Experiments

We designed salinity-tolerance experiments to explicitly test the effects of the two methods of BWE that are commonly used, the empty-refill (E-R) and flow-through (F-T) methods. For E-R BWE, a ballast tank is deballasted and then refilled with oceanic seawater surrounding the ship. Some (generally < 10%, see Ruiz and Smith 2005, Gray et al. 2007) of the original water and organisms remain in the tank, and these organisms are exposed immediately to oceanic salinities upon refill. In contrast, during F-T BWE ambient seawater is pumped water into a ballast tank, displacing (often by overflow from the top of the tank) the original water and organisms. The F-T method is less efficient in the exchange of original ballast water from the tank than the E-R method (Ruiz and Smith 2005), and the transition to open-ocean salinities can take several hours.

Experiments were conducted in the laboratory, with treatments simulating these two types of BWE. Animals were collected and assigned randomly to one of three treatments: (1) Control - animals were kept at the salinity and temperature observed in their habitat at the time of collection; (2) Empty-Refill - animals experienced an instantaneous shift to full-strength seawater (34 psu); and (3) Flow-Through - animals experienced a stepwise increase in salinity from ambient conditions to 14, 24, and 34 psu seawater over a total period of three hours (i.e., one hour per step). Each experiment included four replicates for each BWE method and control treatment. Each replicate contained ten individuals maintained at the specified salinity treatment (as above) in a 100 ml glass bowl. Each bowl was 4 cm in height and 8 cm in diameter, with a high surface to volume ratio to sustain high dissolved oxygen concentrations. Saline solutions were made from filtered water (0.2 μm) from the collection site and adjusted to the target salinity with artificial sea salt.

Experiments had a duration of 48 hours, which was selected to include plausible exposure times for ships conducting BWE. All treatments were maintained in temperature-controlled incubators set at the ambient water temperatures measured

during field collections. The water in the F-T treatment was changed at one, two, and three hours, adjusting the salinity upward at each time point (as above). Water in all treatments was changed at 24 hours to limit any buildup of metabolites that might stress the organisms. No food was provided for these short-duration experiments.

Survivorship was recorded for all treatments after exposure times of one hour (T1), two hours total (T2), three hours total (T3), 24 hours total (T4), and 48 hours total (T5). If 100% mortality occurred in all replicates of a particular treatment (F-T or E-R), further observations at any subsequent time points were usually discontinued for that treatment. In all cases, survivorship was assessed visually, using a Wild dissecting microscope (10-60x), to detect any muscular contractions or respiratory currents produced by the animals. In the absence of any noticeable movement, even after contact with a probe, an animal was considered dead. At this point the tissues of most animals were compromised and lost their natural pigment. In a subset of experiments, when all individuals within a given treatment appeared dead or when 48 hours was reached, animals were transferred back to their ambient water to assess whether any recovery occurred during the next 1-24 hours. This latter step was particularly important for determining the survivorship of copepods and peracarid crustaceans.

Data Analyses

We assessed the proportion of species from low-salinity habitats that could withstand full-salinity conditions. For each independent experiment (i.e., species x location), we report the critical time point at which 0% survivorship occurred in the F-T and E-R treatments or the survivorship at 48 hours if live organisms were present in either treatment. This result is compared directly to survivorship of the control treatment for the same experiment and time period. In this format, the mean survivorship (± standard deviation) is estimated and compared among treatments. We considered any survivorship in either of the experimental treatments after 48 hours to be significant with respect to the potential risk of geographic spread of a species. The effectiveness of salinity treatments among all species was compared by the relative mortality observed within major habitat salinity categories (freshwater to mesohaline conditions). Together, these data were used to make recommendations for the minimum salinity levels and exposure times necessary to cause 100% mortality in numerous taxa potentially dispersed among low-salinity ports via ships.

Results

Curonian Lagoon

Nine of the 14 species tested from the Curonian Lagoon were intolerant of full-strength salinity (Figure 1, Annex 1). Cladocerans and copepods were the dominant taxonomic groups tested, reflecting their relative abundance at this location, and, in general, these organisms exhibited relatively poor survivorship as compared to that of other taxa. In the F-T trials, the cladocerans *Chydorus sphaericus* (O. F. Mueller, 1776) and *Daphnia longispina* O. F. Mueller, 1785 as well as the copepod *Thermocyclops dybowskii* (Landé, 1890) were all eliminated with exposure to 14 psu seawater for one hour. The cladocerans *Bosmina* (*Eubosmina*) *coregoni maritima* (P. E. Müller, 1867), *Diaphanosoma brachyurum* (Liévin, 1848), *Leptodora kindtii* (Focke, 1844) and the copepods *Eudiaptomus graciloides* (Lilljeborg, 1888) and *Mesocyclops leuckarti* (Claus, 1857) and the mysid shrimp *Paramysis lacustris* (Czerniavsky, 1882) were slightly more tolerant, surviving up to 24 psu. The copepods *Acartia bifilosa* (Giesbrecht, 1881) and *Eurytemora hirundoides* = *affinis* (Poppe, 1880) were able to survive for longer periods, tolerating salinities up to 34 psu when acclimated in stepwise fashion (F-T treatment), but these two species died immediately when abruptly exposed to 34 psu seawater in the E-R treatment.

In contrast, two species of mysid shrimps, and the amphipod *Gammarus tigrinus* Sexton, 1939 exhibited significant survivorship in both experimental treatments (> 50 %). Nauplii of the barnacle *Balanus improvisus* Darwin, 1854 were unaffected by the F-T treatment, but experienced significant mortality (> 40%) in the E-R treatment. Comparing habitat salinities among species, only one taxon (of nine) collected from < 1 psu source water exhibited any survivorship, and this was the mysid shrimp, *Limnomysis benedeni* Czerniavsky, 1882. In contrast, three of eight species collected from 1-7 psu source water exhibited some survivorship (60-93%) after 48 hours of full-salinity exposure.

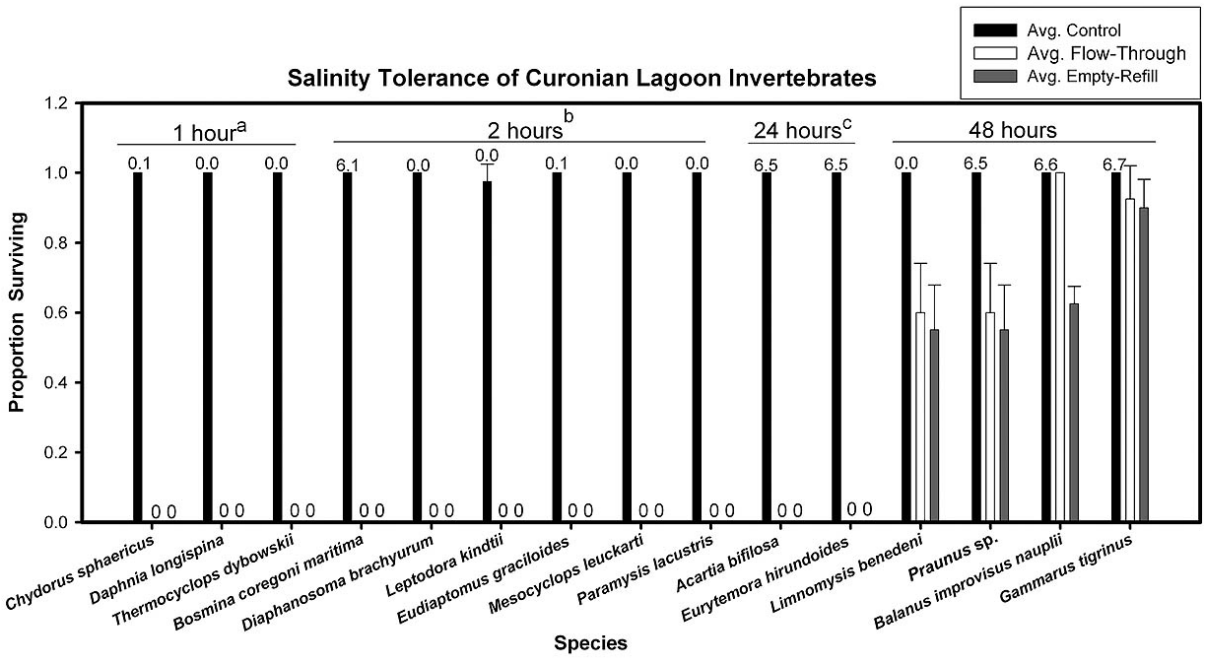

Figure 1. Salinity tolerance of Curonian Lagoon invertebrates. Habitat salinities are listed above each control (black bars). Error bars equal one standard deviation. Species are grouped from left to right by their relative survivorship according to the time and salinity required for maximum mortality in the flow-through treatments (white bars). Empty-refill treatments (gray bars) required less exposure time to cause 100% mortality for several species. a - Minimum salinities and times required to cause 100% mortality in the flow-through treatments for these three species were all 14 psu for 1 hour, respectively; b - Minimum times and final salinities required to cause 100% mortality in the flow-through treatments for these six species were all two hours and 24 psu, respectively; c – 100% mortality occurred at 24 hours in flow-through treatments and at 1 hour in empty-refill treatments for both of these species.

Additional Sites

Similar to the results for the stenohaline taxa of Curonian Lagoon, species of cladocerans and copepods (n = 9) from sites in the Great Lakes were eliminated within one or two hours by either the 14 or 24 psu treatments, respectively, in F-T trials (Annex 1). Only four of 15 species or taxa from the Chesapeake Bay were eliminated by both the F-T and E-R treatments (Annex 1), and three of these were collected from source water < 1 psu. Four taxa exhibited more survivorship in the F-T treatments compared to the results from the E-R treatments. The remaining taxa were collected from habitats with salinities ranging from 4.5 to 12.2 psu and exhibited intermediate to high levels of average survivorship (45-93%).

Similar observations were made for selected invertebrates from San Francisco Bay. Two introduced species of copepods from Asia, *Sinocalanus doerrii* (Brehm, 1909) and *Tortanus dextrilobatus*, Chen and Zhang, 1965 were eliminated when exposed to 34 psu seawater (24 hours for F-T treatment and 1 hour for E-R treatment; Annex 1). *Balanus improvisus* larvae and the copepods *Limnoithona tetraspina* Zhang and Li, 1976 and *Eurytemora affinis* all underwent 100% mortality over different periods of time after an abrupt exposure to full-strength seawater, but did survive when the salinity was gradually increased in the F-T treatments. Two of the most salinity-tolerant organisms, the introduced cumacean, *Nippoleucon hinumensis* (Gâmo, 1967), and the native isopod, *Gnorimosphaeroma insulare* (Van Name, 1940) did not experience any significant mortality in either of the experimental treatments. Interestingly, five of the six introduced species in our San Francisco Bay experiments (*S. doerrii*, *T. dextrilobatus*, *Rhithropanopeus harrisii*, *B. improvisus*, and *L. tetraspina*) were eliminated by the E-R treatments.

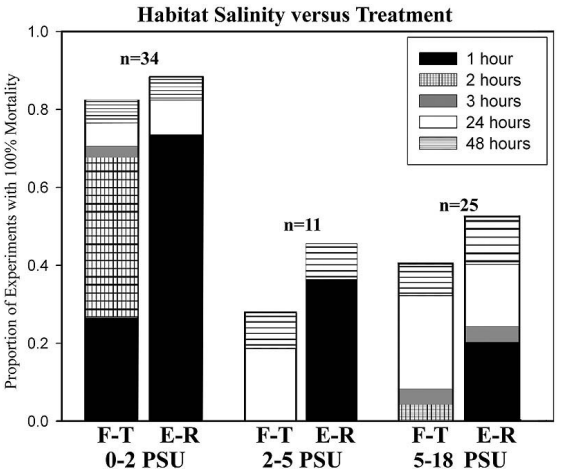

Figure 2. Effectiveness of the flow-through (F-T) and empty-refill (E-R) salinity treatments grouped by the species' habitat salinity observed at the time of collection. Habitat salinity categories are based on the physiological preferences between freshwater and brackish-water invertebrates. The number of experiments (n) is listed above each salinity range. Bars represent the proportion of experiments within a given salinity category that yielded 100% mortality by treatment. Each bar is divided into groups representing the amount of exposure time required. Although flow-through and empty-refill treatments were equally effective against species from different salinity ranges, empty-refill treatments required significantly less exposure time in 43% of all cases. Overall, there were significant differences in treatment effects among the three habitat salinity ranges (Chi-square for F-T = 15.9 and E-R = 12.0, both have a $p < 0.01$).

Table 1. Summary of salinity levels necessary to eliminate the numbers of species from different taxonomic groups within our experiments.

Taxonomic Group(s)	14 psu	14 - 24 psu	24 - 34 psu	>34 psu
Cladocerans	5	9	0	0
Copepods	1	3	9	2
Amphipods, Isopods, Cumaceans, or Mysids	0	0	6	11
Larvae of crabs, shrimps, barnacles, or bivalves	0	0	2	6

Salinity Tolerance: All Sites

Results from our experiments (n = 70) for all sites demonstrated strong treatment effects that differed as a function of the salinity at the habitat from which the organisms were collected (Annex 1 and Figure 2). Both BWE treatments were effective in nearly all of the experiments on animals collected from oligohaline (0-2 psu) habitats, causing 100% mortality in 82 % of the F-T experiments and 88% of the E-R experiments. The effectiveness of both treatment types decreased with animals from low-salinity (2-5 psu, 100% mortality was observed in 27% of F-T experiments and 46% of E-R experiments, n = 11 cases) and mesohaline habitats (5-18 psu, 100% mortality was observed in 40% of F-T experiments and 52% of E-R experiments, n = 25 cases, see Figure 2). Among all experiments there were several cases where the mortality was greater than 90% for both F-T and E-R treatments (3 and 4 cases, respectively, see Annex 1). Of the remaining experiments in which there was significant survivorship (25 cases for F-T and 20 cases for E-R), the average mortality was 31 and 39% for the F-T and E-R treatments, respectively. Although there was a small difference in the proportion of experiments (and species) that were eliminated by the E-R treatment and not the F-T treatment within the oligohaline category (6%), the differences between treatments were more pronounced within the higher salinity categories (19 and 12%). Also, in 43% of the E-R experiments, significantly less time was needed to achieve 100% mortality among all salinity categories (Annex 1). Most mortality occurred within one hour in the E-R treatments, but this was not the case with the F-T treatments (black-shaded area in bars of Figure 2). Overall, there were significant differences in these treatment effects among the three habitat salinity ranges (Chi-square for F-T = 15.9 and E-R = 12.0, both have a $p < 0.01$). However, when results from the oligohaline habitats (0-2 psu) are removed there are no significant differences between the two remaining habitat salinity categories.

Salinity Tolerance of Different Taxonomic Groups

Table 1 summarizes our experimental results for different taxonomic groups. All of the cladocerans (14 species) in our experiments were eliminated by either 14 or 24 psu seawater. The majority of copepod species (13 of 15) in our experiments did not survive in full-strength seawater. The larvae of crabs, shrimps, barnacles, and bivalves as well as adult peracarid crustaceans (amphipods, isopods, cumaceans, and mysids) were generally tolerant of full-strength seawater, with individuals from 11 of 17 species surviving in 34 psu seawater at the end

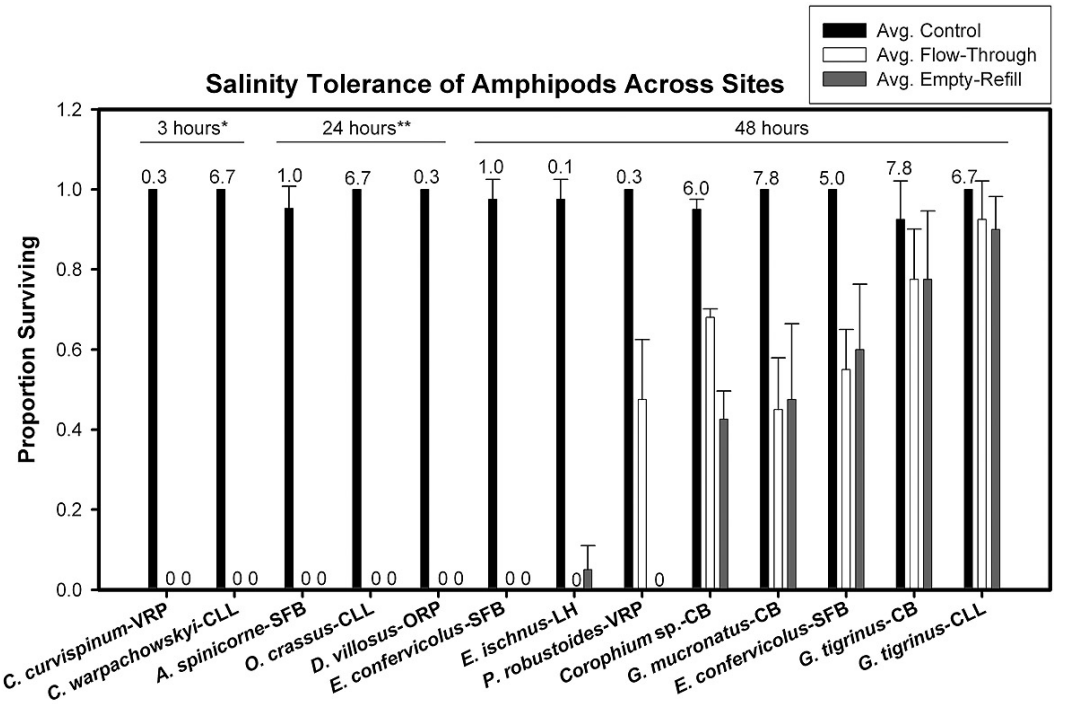

Figure 3. Salinity tolerance of amphipod species across sites. Habitat salinities are listed above each control (black bars). Error bars equal one standard deviation. Species are grouped from left to right by their relative survivorship according to the time and salinity required for maximum mortality in the flow-through treatments (white bars). Empty-refill treatments (gray bars) required less exposure time in a few species as indicated. * - *Chaetogammarus warpachowskyi* and *Chelicorophium curvispinum* both died at 1 hour in the E-R treatment. ** - *Obesogammarus crassus* died at 3 hours in the E-R treatment. Chesapeake Bay, U. S. A. (CB); Lake Huron, Michigan, U. S. A.(LH); San Francisco Bay, U. S. A. (SFB); Curonian Lagoon, Lithuania (CLL); Oder River, Poland (ORP); and Vistula River, Poland (VRP).

of 48 hours. Below, we report on the salinity tolerance of peracarids and decapod zoeae in more detail.

Salinity Tolerance of Amphipods Across Sites

Of the 11 species of amphipods used in experiments, some individuals from six species survived a 48-hour exposure to full-strength seawater in one or both treatments, with survivorships ranging from 5 to 93% (Figure 3). The five species that experienced 100% mortality included *Chelicorophium curvispinum* (G. O. Sars, 1895), *Chaetogammarus warpachowskyi* (G. O. Sars, 1894), *Americorophium spinicorne* (Stimpson, 1857), *Obesogammarus crassus* (G. O. Sars, 1894), and *Dikerogammarus villosus* (Sowinsky, 1894). Based on time to 100% mortality, the least tolerant species were *C. curvispinum* and *C. warpachowskyi* from the Baltic Sea, as both species died in full-strength seawater within one hour (E-R). Slightly more tolerant species were *A. spinicorne* from San Francisco Bay and *O. crassus* and *D. villosus*, two species from the Baltic Sea, which survived up to 24 hours.

Interestingly, *Eogammarus confervicolus* (Stimpson, 1856), an amphipod species from San Francisco Bay, had varying survivorship in two experiments. Experiments with this species run during April, 2004 with animals that were collected from water with a salinity of 5 psu survived in both the F-T and E-R treatments (>50 %). However, animals that were reared at a salinity of 1 psu during June, 2004 were completely eliminated by both the F-T and E-R treatments. Differences were also observed between the closely related species *Pontogammarus robustoides* (G. O. Sars, 1894) and *Obesogammarus crassu*s that have overlapping ranges in the Baltic Sea. *P. robustoides* was able to survive in full-strength seawater within the

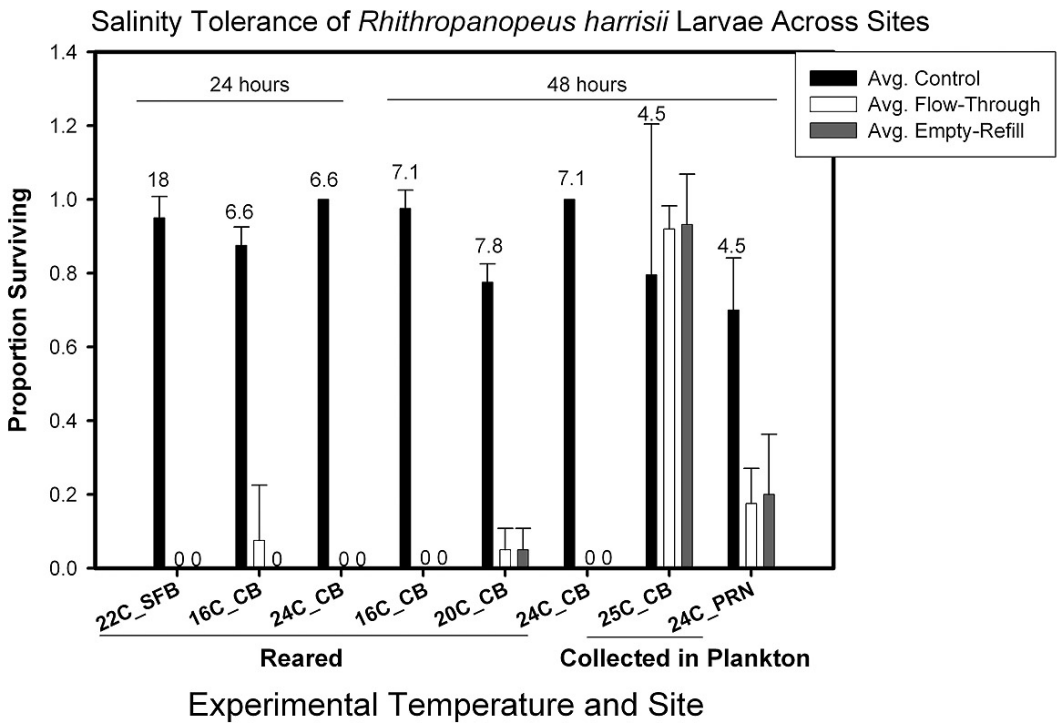

Figure 4. Salinity tolerance of *Rhithropanopeus harrisii* larvae across sites. Habitat salinities are listed above each control (black bars). Error bars equal one standard deviation. The temperature at which the experiment was run and the habitat sites are listed along the bottom axis. Experimental results are grouped from left to right by their relative survivorship according to the time required for maximum mortality and the source (site and collection method) of the larvae. Overall, experiments using larvae collected from the plankton (regardless of site and temperature) from the Chesapeake Bay, MD, U. S. A. (CB) and the port of Rotterdam, The Netherlands (PRN) had greater survivorship than larvae hatched from the broods of adult female crabs collected from the Chesapeake Bay and San Francisco Bay, CA, U. S. A. (SFB).

F- T treatment but *O. crassus* did not. For *Echinogammarus ischnus* (Stebbing, 1899), an introduced species in the Great Lakes, a small percentage (5%) survived the E-R treatment, but not the F-T treatment. Two native gammarid species from the Chesapeake Bay, *Gammarus tigrinus* and *Gammarus mucronatus* Say, 1818, had wide salinity tolerances, and a significant proportion of both species survived in the experimental treatments for 48 hours. As a final check of survivorship, individuals of both gammarid species used in the experimental treatments were placed directly back into mesohaline water from the collection site and survived for another 24 hours. *G. tigrinus* adults collected from Curonian Lagoon were similarly tolerant, surviving two days of exposure to full-strength seawater, and an additional (abrupt) switch into mesohaline water from the collection site.

Rhithropanopeus Larvae Across Sites

Figure 4 shows results for larvae of the crab *Rhithropanopeous harrisii* among different experiments, varying in source region, ambient temperature, and ambient salinity. All or nearly all (> 90%) of larvae reared from ovigerous female crabs collected from San Francisco Bay and Chesapeake Bay were eliminated in experimental treatments at temperatures below 25°C. These particular experiments were conducted on newly hatched zoeae (stage I). Interestingly, zoeae reared in similar fashion from adult broods of animals from the San Francisco Bay at 18 psu had similar mortality rates to those of zoeae reared at lower salinities from the Chesapeake Bay.

In contrast to the results from hatched zoeae, *Rhithropanopeous harrisii* larvae collected from the plankton of the Chesapeake Bay and the port

of Rotterdam were more tolerant of full-strength seawater (mean survivorship of 92% and 19% for Chesapeake and Rotterdam, respectively), than zoeae reared from adult broods. The Rotterdam experiments also used stage I zoeae, whereas the wild-collected zoeae from Chesapeake were not staged. In both experiments, the ambient temperature was 24-25°C and the ambient salinity was 4.5 psu.

Discussion

Exposure to the average salinity of the open-ocean (34 psu) provides a significant barrier to the ballast-mediated transfer of many organisms from freshwater and oligohaline ports. Although most previous studies of the efficacy of BWE have focused on the effects of physical removal of coastal organisms, the survivorship of any remaining organisms in ballast tanks is also affected by changing environmental conditions. Our experiments demonstrate that organisms from freshwater and oligohaline habitats experience high mortality rates when exposed to full-strength seawater during BWE or saltwater flushing. The additional effect of osmotic shock in concert with the physical removal of organisms during BWE enhances the overall effectiveness of BWE-related treatments.

The potential importance of environmental conditions on survivorship of ballasted organisms is recognized (Hamer 1998; MacIsaac et al. 2002; Bailey et al. 2006); especially important are changes in salinity and temperature, but their effects have remained largely untested to date. Some past experiments on ships have examined the effect of BWE on the concentration of coastal organisms (Levings et al. 2004; Ruiz and Smith 2005; Choi et al. 2005), including those from low-salinity habitats (Locke et al. 1993), but the relative importance of removal versus the survivorship of remaining organisms was not evaluated (but see Gray et al. 2007). Several laboratory experiments have examined the short-term survivorship or viability of organisms from ballast tanks after discharge, and some of these have examined the effects of salinity on survivorship (Smith et al. 1999; Hulsmann and Galil 2001). However, most of the latter experiments have focused primarily on survival of organisms from high-salinity ballast (source) waters in low-salinity recipient waters.

Our results greatly expand the taxonomic and geographic scope of these studies, documenting effects of salinity shock on mortality of organisms from numerous habitats and source regions. The tolerances of freshwater and estuarine organisms were generally correlated with the preferred salinity ranges in which they occurred. For example, species of rotifers and cladocerans that are generally limited to freshwater or oligohaline habitats were quickly killed by the 14 or 24 psu salinity treatments. There are marine cladocerans such as species of *Podon*, *Pseudoevadne*, *Evadne*, *Penilia*, and *Pleopsis* that can survive in salinities greater than 24 psu. However, these species are rarely found in freshwater habitats or cannot survive in constant freshwater systems (Frey 1993). Brackish-water invertebrates and their larval forms often experience wide fluctuations in salinity and temperature (Lockwood 1976), and although these organisms exhibited significantly greater survivorship in our experiments than oligohaline biota, E-R treatments still caused 100% mortality in approximately half of our experiments using invertebrates collected from mesohaline habitats (2-18 psu).

Salinity Tolerance of Native and Introduced Amphipods

These data suggest that some amphipod species common to both fresh- and brackish-water habitats are well adapted for dispersal via ships. Seven of the 11 amphipod species tested were introduced species with wide geographic distributions. The majority of these species (*Chelicorophium curvispinum*, *Chaetogammarus warpachowskyi*, *Obesogammarus crassus*, *Echinogammarus ischnus*, *Dikerogammarus villosus*, and *Pontogammarus robustoides*) originated from the Ponto-Caspian region and have spread widely and rapidly within the Baltic and North Seas (Grabowski et al. 2007). *Gammarus tigrinus* is native to the coast of the eastern United States; however introduced populations are particularly widespread in the Baltic and North Seas (Kukert 1984; Daunys and Zettler 2006). *G. tigrinus* and *E. ischnus* have also been introduced to the Great Lakes (Witt et al. 1997; Grigorovich et al. 2005).

In general, amphipod species included in our study had a wider range of salinity tolerances relative to other taxonomic groups. Full-strength seawater was the minimum concentration required to cause significant mortality in any amphipod species, but exposure time differed among species. Species that died within 24 hours

such as *Chaetogammarus warpachowskyi*, *Obesogammarus crassus*, and *Dikerogammarus villosus* have reported salinity tolerances between 0 and 20 psu (Bruijs et al. 2001; Paavola et al. 2005). However, the most tolerant amphipod species (including two species of *Gammarus*) tended to prefer mesohaline habitats and have reported salinity tolerances up to 25 psu (Dorgelo 1974). *G. tigrinus* was one of the more hardy species in all of our experiments. Although a wide range of salinity preferences have been reported for this species across its entire range (Dorgelo 1974; Savage 1982), survivorships from the Baltic Sea and Chesapeake Bay populations were identical in our experiments.

Considering that some species of amphipods are relatively unaffected by salinity shock, combined with earlier studies documenting the introduction and spread of non-native species in the Baltic Sea, suggest that several amphipod species are potential colonists of North America. Based on our experimental data, *Pontogammarus robustoides* may be more likely to be introduced to freshwater and estuarine habitats of the eastern United States than other amphipod species found in northern Europe. This species is native to the Ponto-Caspian region (Dediu 1980; Jazdzewski 1980) and is now abundant in estuarine regions of the Baltic Sea (Gruszka 1999; Jazdzewski et al. 2005). Its high abundance in these areas may be partially explained by its ability to acclimate to a variety of habitat salinities.

Variation in Survivorship

The salinity-tolerance range for a species may be influenced by both temperature and local salinity conditions (Laughlin and French 1989; Fockedey et al. 2005). However, the effect of temperature is less pronounced and does not extend the salinity range of stenohaline biota enough to permit them to withstand exposure to full-strength seawater. The effect of temperature would also be less important for euryhaline species since these organisms normally tolerate full-strength salinity at some point in their life histories. Our experiments also demonstrate that some species are more tolerant of full-strength seawater under gradually increasing conditions (F-T) rather than abrupt exposures (E-R), but may still be eliminated if exposure time is increased. For these reasons, we regard the effect of temperature on the salinity tolerance of a species to be a less important factor influencing species survivorship during BWE as compared to the minimum salinity level reached, exchange rate of ballast water, and exposure duration.

Many euryhaline organisms have lower salinity limits near 1 to 2 psu, but cannot survive in freshwater habitats (<1 psu). Thus, increased survivorship for estuarine taxa during ballast-water exchange may be of less concern for introductions to freshwater habitats such as the Great Lakes. Greater risk instead lies with brackish-water species capable of surviving the osmotic effects of BWE and subsequently able to reproduce in freshwater habitats. Several of the peracarid crustaceans included within our experiments fit these latter criteria. However, this is not the case for the invasive cladoceran, *Cercopagis pengoi* (Ostroumov, 1891). Adult stages of this species are intolerant of high salinity. Establishment of this species in the Great Lakes may have been due to several factors such as (1) poor ballast-water exchange practices before the implementation of BWE regulations, (2) introduction via the discharge of residual low-salinity ballast-water from vessels after BWE regulations were implemented, and/or (3) introduction via resting stages having greater physiological tolerances than those of the adults. Experiments designed to test the efficacy of ballast-water exchange on the hatching success of resting stages of some other species of cladocerans from the Great Lakes have yielded mixed results, but overall the viability of diapausing eggs of several freshwater cladoceran species was most reduced by exposure to 8 psu seawater at 20°C rather than other salinity-temperature combinations (Bailey et al. 2006).

In this study, *Rhithropanopeus harrisii* larvae collected from Chesapeake Bay and the port of Rotterdam were more tolerant of full-strength seawater than were larvae reared from adult broods. The larval stages of *R. harrisii* have a developmental salinity range between 2 and 30 psu, and larval development in geographically distant populations shows some adaptation to local environmental conditions (Laughlin and French 1989). Similar results were observed in our experiments using reared and field-collected specimens of the amphipod, *Eogammarus confervicolus*. Another example of significant within-species variation was observed for the mysid, *Limnomysis benedeni* that survived in both of our salinity treatments. However, Ovčarenko et al. (2006) reported that exposing this species to full-strength seawater (34 psu) for

only 24 hours caused 100% mortality. Survivorship differences within a species may be due to differences in cohort quality or genetic differences among animals, but the underlying causes remain to be determined.

Although wide reaction norms (phenotypic plasticity) have been implicated many times as a reason for invasion success (Sexton et al. 2002; Lee et al. 2003; Richards et al. 2006), several empirical studies have concluded that fluctuating environmental factors act as a selection force on natural and (possibly) ballast-water populations, favoring low-frequency genotypes that survive and propagate in the new environmental conditions (Lee 2002; Dybdahl and Kane 2005). The low proportion of genotypes that survive under physiologically-stressed conditions may be one reason why propagule pressure is important (Ruiz et al. 2000). In particular, for a given species, salinity tolerance is variable within a population, and these physiological differences can be heritable (Lee et al. 2003). Overall, a significant factor for the introduction potential of freshwater and estuarine species may be low-frequency genotypes with wide environmental tolerances from a few or several source populations. Evidence based on the molecular phylogeography of a few recent ballast-water invaders in the Great Lakes support this hypothesis, as their invasive populations are the result of several introduction events from multiple source populations (Cristescu et al. 2001; Colautti et al. 2005; Stepien et al. 2005; Kelly et al. 2006).

Conclusions

Although not a complete barrier against all exotic species, taxa that originate from oligohaline ports can often be eradicated from ballast tanks relatively quickly through exposure to full-strength seawater (34 psu). More broadly, these results indicate that the current management practices of BWE and saltwater flushing serve to reduce the ship-mediated transfer and subsequent risk of introduction of non-indigenous species to the Great Lakes and other low-salinity recipient systems. Based on our study, we would recommend that transoceanic ships undergo BWE as soon as oceanic water is reached after leaving the home port. This would maximize the exposure time of the salinity treatment increasing the probability of causing significant mortality, and also leave expelled organisms far from the recipient port in an open-ocean environment where they would be less likely to survive than if released in a coastal area.

Salinity-tolerant taxa identified by our experiments were often peracarid crustaceans including several species of widely introduced mysid shrimps and amphipods. Members of these taxonomic groups often experience dramatic fluctuations in salinity and temperature as part of their normal life histories, and these factors have contributed to their ability to invade estuarine habitats (Lockwood 1976, Wittmann and Ariani 2000, Bruijs et al. 2001). Among these brackish-water peracarid species, some are also capable of surviving and reproducing in a freshwater habitat such as the Great Lakes. These latter species pose a greater risk of introduction to both freshwater and low-salinity habitats, and future studies should determine alternative treatments capable of preventing their dispersal via the operations of commercial ships.

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Annex 1. Species and site details for the salinity-tolerance experiments. Species are grouped by site of collection, taxonomic group, and arranged in order of their tolerance level within the flow-through (F-T) treatment. * - Indicates where there was a significant difference in the amount of time required to kill the majority of animals between F-T and empty-refill (E-R) treatments. a – Denotes where there is a significant difference in the survivorship between the F-T and E-R treatments. Abbreviations: Chesapeake Bay, Maryland, U.S.A. (CB), Curonian Lagoon, Lithuania (CLL), Lake Huron, Alpena, Michigan, U. S. A. (LH), Lake Erie, Toledo, Ohio, U.S.A. (OH), Oder River, Poland (ORP), Port of Rotterdam, The Netherlands (PRN), San Francisco Bay, California, U.S.A. (SFB), Lake Michigan, Traverse City, Michigan, U.S.A. (TC), and Vistula River, Poland (VRP)

Site	Species	Taxonomic Group	Date	°C	PSU	F-T ±SD	Time	E-R ±SD	Time	Control ±SD
CLL	*Chydorus sphaericus*	Cladocera	July.12.05	22	0.1	0±0	1 hr	0±0	1 hr	1±0.0
CLL	*Daphnia longispina*	Cladocera	Aug.23.05	19.5	0	0±0	1 hr	0±0	1 hr	1±0.0
CLL	*Thermocyclops dybowskii*	Copepoda	July.13.05	22	0	0±0	1 hr	0±0	1 hr	1±0.0
CLL	*Bosmina coregoni maritima*	Cladocera	Sept.22.04	15	6.1	0±0	2 hr	0±0	1 hr*	1±0.0
CLL	*Diaphanosoma brachyurum*	Cladocera	July.13.05	22	0	0±0	2 hr	0±0	1 hr*	1±0.0
CLL	*Leptodora kindtii*	Cladocera	Aug.23.05	19.5	0	0±0	2 hr	0±0	1 hr*	0.98±0.05
CLL	*Eudiaptomus graciloides*	Copepoda	July.12.05	22	0.1	0±0	2 hr	0±0	1 hr*	1±0.0
CLL	*Mesocyclops leuckarti*	Copepoda	Aug.23.05	19.5	0	0±0	2 hr	0±0	1 hr*	1±0.0
CLL	*Paramysis lacustris*	Mysidacea	Aug.20.04	20	0	0±0	2 hr	0±0	1 hr*	1±0.0
CLL	*Chaetogammarus warpachowskyi*	Amphipoda	July.08.06	20	6.7	0±0	3 hr	0±0	1 hr*	1±0.0
CLL	*Acartia bifilosa*	Copepoda	July.20.05	21	6.5	0±0	24 hr	0±0	1 hr*	1±0.0
CLL	*Eurytemora hirundoides*	Copepoda	July.20.05	21	6.5	0±0	24 hr	0±0	1 hr*	1±0.0
CLL	*Obesogammarus crassus*	Amphipoda	July.08.06	20	6.7	0±0	24 hr	0±0	3 hr*	1±0.0
CLL	*Limnomysis benedeni*	Mysidae	Aug.20.04	20	0	0.6±0.14	48 hr	0.55±0.13	48 hr	1±0.0
CLL	*Praunus* sp.	Mysidae	Aug.24.04	14.9	6.5	0.6±0.14	48 hr	0.55±0.13	48 hr	1±0.0
CLL	*Gammarus tigrinus*	Amphipoda	July.08.06	20	6.7	0.93±0.10	48 hr	0.9±0.08	48 hr	1±0.0
CLL	*Balanus improvisus* nauplii	Cirripedia	Aug.09.05	18	6.6	1±0.0	48 hr	0.63±0.05	48 hr[a]	1±0.0
VRP	*Chelicorophium curvispinum*	Amphipoda	Nov.29.06	8.0	0.3	0±0	3 hr	0±0	1 hr*	1±0.0
ORP	*Dikerogammarus villosus*	Amphipoda	June.25.06	17.6	0.3	0±0	24 hr	0±0	24 hr	1±0.0
VRP	*Pontogammarus robustoides*	Amphipoda	June.25.06	14	0.3	0.48±0.15	48 hr	0±0	48 hr[a]	1±0.0
PRN	*Daphnia galeata galeata*	Cladocera	July.17.06	24	1.7	0±0	1 hr	0±0	1 hr	1±0.0
PRN	*Rhithropanopeus harrisii* zoeae	Decapoda	July.19.06	24	4.5	0.18±0.1	48 hr	0.2±0.16	48 hr	0.7±0.14
PRN	*Neomysis integer*	Mysidae	July.25.06	24	1.8	0.05±0.06	48 hr	0.05±0.06	48 hr	0.3±0.08

Annex 1 (continued)

Site	Species	Taxonomic Group	Date	°C	PSU	F-T ±SD	Time	E-R ±SD	Time	Control ±SD
CB	Rotifera	Rotifera	June.08.04	21	0.1	0±0	1 hr	0±0	1 hr	0.98±0.04
CB	Cladocera	Cladocera	June.08.04	21	0.1	0±0	2 hr	0±0	1 hr*	1±0.0
CB	*Eurytemora* sp.	Copepoda	Aug.10.04	26.5	0.2	0±0	3 hr	0±0	1 hr*	0.91±0.1
CB	*Acartia* sp.	Copepoda	July.13.04	28	5.9	0±0	24 hr	0±0	1 hr*	0.87±0.07
CB	*Rhithropanopeus harrisii* zoeae	Decapoda	July.10.06	16	6.6	0.08±0.15	24 hr	0±0	24 hr	0.88±0.05
CB	*Rhithropanopeus harrisii* zoeae	Decapoda	July.10.06	24	6.6	0±0	24 hr	0±0	24 hr	1±0.0
CB	*Platyhelminthes*	Platyhel-minthes	Aug.31.04	27.6	6.8	0.72±0.09	48 hr	0.79±0.04	1 hr*	0.98±0.06
CB	*Corophium* sp.	Amphipoda	May.11.04	22.5	6	0.68±0.02	48 hr	0.43±0.07	48 hr	0.95±0.03
CB	*Gammarus mucronatus*	Amphipoda	July.08.06	20	7.8	0.45±0.13	48 hr	0.48±0.19	48 hr	1.0±0.0
CB	*Gammarus tigrinus*	Amphipoda	July.08.06	20	7.8	0.78±0.13	48 hr	0.78±0.17	48 hr	0.93±0.1
CB	Barnacle nauplii	Cirripedia	Aug.18.04	26	7.2	0.54±0.21	48 hr	0.57±0.09	48 hr	0.83±0.13
CB	Harpacticoida sp.	Copepoda	May.18.04	25.2	4.5	0.95±0.05	48 hr	0.32±0.04	48 hr	0.85±0.04
CB	*Leptinogaster major*	Copepoda	Sept.20.04	23.7	4.9	1±0	48 hr	0.32±0.09	48 hr[a]	0.92±0.10
CB	*Palaemonetes pugio* zoeae	Decapoda	June.22.06	12.2	12.2	0.48±0.10	48 hr	0.93±0.05	48 hr	1.0±0.0
CB	*Rhithropanopeus harrisii* zoeae	Decapoda	July.27.06	16	7.1	0±0	48 hr	0±0	48 hr	0.98±0.05
CB	*Rhithropanopeus harrisii* zoeae	Decapoda	July.31.06	20	7.8	0.05±0.06	48 hr	0.05±0.06	48 hr	0.78±0.05
CB	*Rhithropanopeus harrisii* zoeae	Decapoda	July.27.06	24	7.1	0±0	48 hr	0±0	48 hr	1.0±0.0
CB	*Rhithropanopeus harrisii* zoeae	Decapoda	May.18.04	25.2	4.5	0.92±0.06	48 hr	0.93±0.14	48 hr	0.80±0.41
CB	Bivalve veligers: mixed species	Mollsca	Aug.03.04	29	7.9	0.68±0.04	48 hr	0.4±0.14	48 hr[a]	0.98±0.23
CB	Polychaetes: mixed species	Polychaeta	July.13.04	28	9	0.93±0.02	48 hr	0±0	48 hr	0.88±0.03
CB	Spionid polychaetes: mixed species	Polychaeta	Sept.27.04	23	9.7	0.73±0.11	48 hr	0.20±0.11	48 hr[a]	1.0±0.0
SFB	*Sinocalanus doerrii*	Copepoda	April.20.04	17	4	0±0	24 hr	0±0	1 hr*	0.92±0.06
SFB	*Tortanus dextrilobatus*	Copepoda	April.25.04	19	5	0±0	24 hr	0±0	1 hr*	0.7±0.17
SFB	*Americorophium spinicorne*	Amphipoda	June.21.04	28.2	1	0±0	24 hr	0±0	24 hr	0.95±0.06
SFB	*Rhithropanopeus harrisii* zoeae	Decapoda	Aug.31.04	22.4	18	0±0	24 hr	0±0	24 hr	0.95±0.06
SFB	*Balanus improvisus* nauplii	Cirripedia	April.26.04	20	4	0.62±0.41	48 hr	0±0	1 hr*[a]	0.68±0.43
SFB	*Limnoithona tetraspina*	Copepoda	April.26.04	20	4	0.05±0.07	48 hr	0±0	1 hr*	0.93±0.03
SFB	*Acartia (Acartiura)* sp.	Copepoda	April.20.04	16	18	0.35±0.16	48 hr	0±0	24 hr*[a]	0.88±0.09

Annex 1 (continued)

Site	Species	Taxonomic Group	Date	°C	PSU	F-T ±SD	Time	E-R ±SD	Time	Control ±SD
SFB	*Eurytemora affinis*	Copepoda	April.21.04	18	1	0.4±0.29	48 hr	0±0	24 hr*	0.89±0.09
SFB	*Eogammarus confervicolus*	Amphipoda	April.23.04	15	5	0.55±0.1	48 hr	0.6±0.16	48 hr	1.0±0.0
SFB	*Eogammarus confervicolus*	Amphipoda	June.21.04	20.8	1	0±0	48 hr	0±0	48 hr	0.98±0.05
SFB	*Gnorimosphaeroma insulare*	Isopoda	April.23.04	15	5	1±0.0	48 hr	0.95±0.1	48 hr	1±0.0
SFB	*Nippoleucon hinumensis*	Cumacea	April.25.04	19	3	1±0.0	48 hr	0.82±0.24	48 hr	0.92±0.11
OH	*Bosmina longirostris*	Cladocera	June.05.06	21	0.1	0±0	1 hr	0±0	1 hr	1±0.0
OH	*Daphnia retrocurva*	Cladocera	June.01.06	21	0.1	0±0	1 hr	0±0	1 hr	1±0.0
OH	*Leptodora kindtii*	Cladocera	June.02.06	21	0.1	0±0	1 hr	0±0	1 hr	1±0.0
OH	Quagga and Zebra mussel larvae	Mollusca	June.01.06	21	0.1	0.63±0.17	48 hr	0.88±0.15	48 hr	1±0.0
TC	*Asplanchna priodonta*	Rotifera	Aug.04.06	23	0.1	0±0	1 hr	0±0	1 hr	1±0.0
TC	*Alona quadrangularis*	Cladocera	Aug.05.06	23	0.1	0.03±0.05	2 hr	0±0	1 hr*	1±0.0
TC	*Bythotrephes longimanus*	Cladocera	Aug.04.06	23	0.1	0±0	2 hr	0±0	1 hr*	1±0.0
TC	*Bythotrephes longimanus*	Cladocera	Aug.08.06	23	0.1	0±0	2 hr	0±0	1 hr*	1±0.0
TC	*Cercopagis pengoi*	Cladocera	Aug.06.06	23	0.1	0±0	2 hr	0±0	1 hr*	0.9±0.08
TC	*Eurycercus lamellatus*	Cladocera	Aug.05.06	23	0.1	0±0	2 hr	0±0	1 hr*	1±0.0
TC	*Polyphemus pediculus*	Cladocera	Aug.07.06	23	0.1	0±0	2 hr	0±0	1 hr*	0.93±0.1
LH	*Chydorus sphaericus*	Cladocera	May.02.07	10	0.1	0±0	2 hr	0±0	1 hr*	1±0.0
LH	*Eucyclops speratus*	Copepoda	May.03.07	10	0.1	0±0	2 hr	0±0	1 hr*	1±0.0
LH	*Eurytemora affinis*	Copepoda	April.30.07	5	0.1	0±0	2 hr	0±0	1 hr*	1±0.0
LH	*Echinogammarus ischnus*	Amphipoda	April.30.07	5	0.1	0±0	48 hr	0.05±0.06	48 hr	0.98±0.05