

# Salinity tolerance of diapausing eggs of freshwater zooplankton

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## SUMMARY

1. Many freshwater zooplankton produce diapausing eggs capable of withstanding periods of adverse environmental conditions, such as anoxia, drought and extreme temperature. These eggs may also allow oligostenohaline species to survive increased salinity during periods of tidal flux or evaporation, and here we test the ability of diapause eggs to withstand such conditions.

2. Salinity tolerance may also enable organisms to invade new environments. The increased rate of introduction of non-indigenous species to the Laurentian Great Lakes since 1989, when ballast water exchange regulations (to replace fresh/brackish water at sea with full seawater) were first implemented for transoceanic vessels, has stimulated studies that explore mechanisms of introduction, other than of active animals, in ballast water.

One hypothesis proposes that freshwater organisms transported in ballast tanks as diapausing eggs may be partially responsible for the increased rate of species introduction, as these eggs may tolerate a wide array of adverse environmental conditions, including exposure to saline water.

3. We collected ballast sediments from transoceanic vessels entering the Great Lakes, isolated diapausing eggs of three species (*Bosmina liederi*, *Daphnia longiremis* and *Brachionus calyciflorus*), and measured the effect of salinity on hatching rate. In general, exposure to salinity significantly reduced the hatching rate of diapausing eggs. However, as non-indigenous species can establish from a small founding population, it is unclear whether salinity exposure will be effective as a management tool.

*Keywords:* ballast water exchange, biological invasion, hatching rates, resting eggs, salinity tolerance

## Introduction

The introduction of non-indigenous species is a potent agent of biodiversity change, particularly for lake ecosystems (Sala *et al.*, 2000) and measures are urgently needed to identify and eliminate the vectors that transport them. Ballast water is recognised as the single most important vector for species introduction to aquatic habitats. Approximately 10 billion tonnes of

ballast water (and its associated biota) are transferred annually between global ports, providing the primary means of transport and introduction of non-indigenous aquatic biota to ecosystems, including bacteria, dinoflagellates, phytoplankton, zooplankton and fish (Rigby, Hallegraeff & Sutton, 1999; Ruiz *et al.*, 2000). Transoceanic shipping accounts for 77% of the species introduced to the Laurentian Great Lakes since 1970 (Ricciardi, 2001). To reduce this threat, voluntary regulations were enacted in 1989, and mandated in 1993, that effectively require inbound vessels to exchange fresh or brackish ballast water with open-ocean saltwater if that water is to be discharged in the

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Great Lakes (United States Coast Guard, 1993). Despite these regulations, the rate at which new species have been recorded in the Great Lakes tripled between 1989 and 1999 compared with the preceding 40 years (Grigorovich *et al.*, 2003). Increased sampling effort and time lags between establishment and discovery of non-indigenous species may partially account for this pattern (Costello & Solow, 2003; Grigorovich *et al.*, 2003), although modelling exercises indicated that ballast water exchange offers only incomplete protection and is least successful for species with benthic or dormant stages contained within ballast sediments (MacIsaac, Robbins & Lewis, 2002). Alternatively, the recent surge in non-indigenous species may be because of the presence of live or dormant organisms contained in the residual ballast of ships declaring 'no ballast on board', which are exempt from the regulations (MacIsaac *et al.*, 2002; Bailey *et al.*, 2003). These vessels carry tonnes of residual sediment and ballast water, and dominate trade inbound to the Great Lakes (Colautti *et al.*, 2003).

Many freshwater zooplankton, including copepods, cladocerans and rotifers, produce diapausing or 'resting' eggs during annual population cycles. These dormant stages probably evolved as an adaptation to periods of adverse environmental conditions, including anoxia, drought and extremely low or high temperature (Gilbert, 1974; Hairston, 1996; Hairston & Cáceres, 1996; Williams, 1998). These eggs could also provide temporal escape from unfavourable salinity, facilitating the intercontinental transfer of freshwater species in sediments of ballast tanks, even those subjected to ballast water exchange. Some of the species recently recorded in the Great Lakes are euryhaline endemics of the Ponto-Caspian region of southeast Europe, which may have been transported as resting stages in ballast water and/or sediments (Ricciardi & MacIsaac, 2000; Reid & Orlova, 2002). While the salinity tolerance of some juvenile and adult freshwater cladocerans and rotifers has been examined (e.g. Miracle & Serra, 1989; Teschner, 1995; Hall & Burns, 2002), very little is known of the salinity tolerance of the diapausing eggs of freshwater taxa. Consequently, it is difficult to infer whether invertebrates capable of producing diapausing eggs could circumvent the salinity 'filter' imposed on potential Great Lakes invaders by ballast water exchange.

In this study, we examine the effect of salinity on the hatching rate of diapausing eggs of the cladocerans *Bosmina liederi* De Melo & Hebert and *Daphnia longiremis* Sars, and the rotifer *Brachionus calyciflorus* Pallas, common inhabitants of ballast sediments of transoceanic vessels entering the Great Lakes. While these three species are native to the Great Lakes, their presence in residual ballast sediments suggests that they are representative of the types of organisms that pose a potential risk of invasion. Bailey *et al.* (2003) tested the viability of diapausing eggs recovered from ballast sediments and noted a tendency for reduced viability with high pore water salinity, although this relationship was not tested directly. Here, we test the hypothesis that diapausing eggs of freshwater zooplankton will be destroyed by exposure to saline water.

## Methods

### Sample collection

Residual sediments were collected from five transoceanic vessels entering the Great Lakes in 'no ballast on board' status in December 2000, May, August and December 2001, and in June 2002. Ships were sampled at the ports of Hamilton, Thorold and Toronto, Ontario, Canada, and Cleveland, OH, U.S.A. Residual sediment was collected from at least one ballast tank per ship, with additional tanks sampled depending upon availability and the ease and safety of access. Sediments were collected along longitudinal shell frames that trapped sediment in areas away from drainage flows. Approximately 4 kg of sediment (in total) were collected from at least five areas within each tank and placed in a single container. These composite samples were stored in the dark at 4 °C until experimentation. The salinity of residual sediment pore water, separated from sediment by centrifugation at approximately 3300 g (approximately  $32\,360\text{ m s}^{-2}$ ) for 15 min, was measured using an optical refractometer (F. Dobbs, Old Dominion University, Norfolk, VA, U.S.A.).

### Egg density counts

After thorough mixing, four 40-g subsamples (wet weight) were taken from each sample and preserved in 95% ethanol. Subsamples were each washed

through a 45 µm sieve to remove fine sediment. Diapausing eggs were subsequently separated from the coarse sediment using the colloidal silica Ludox® HS 40 (Burgess, 2001) and counted under a dissecting microscope.

#### Hatching experiments

Sediments were stored in plastic containers in the dark at 4 °C for at least 4 weeks to allow a refractory period before hatching experiments commenced (see Grice & Marcus, 1981; Schwartz & Hebert, 1987). After this time, diapausing eggs were removed from sediment using a sugar flotation method (Bailey *et al.*, 2003). Briefly, sediment was processed through a 45 µm sieve and washed into centrifuge tubes using a 1 : 1 (w : v) mixture of sucrose and water. After centrifugation (5 min at approximately ~27 g) the supernatant was decanted and rinsed thoroughly with water through 45 µm mesh before being transferred to a counting dish. Diapausing eggs were immediately recovered from the supernatant and sorted by size and gross morphology under a dissecting microscope, selecting only fully intact, apparently

healthy eggs. A single, replicated experiment was conducted on the most abundant egg type (*Brachionus* or *Daphnia* species) for each of five tanks. For sediment from ship 1, in which *Brachionus budapestinensis* Daday eggs dominated (see Table 1), experiments were attempted using *B. budapestinensis*, but were abandoned owing to loss of eggs over time because of their extremely small size. Experiments were therefore conducted on a subdominant species, *B. liederii*. All other eggs were incubated at 0‰ (parts per thousand salinity) for identification purposes only. Occasionally, two or three species hatched during a single trial (see Table 1). These secondary species always contributed <1% of the total number of hatchlings. In total, 11 species hatched, although only three were used in the replicated experiments. Four trials were conducted with the rotifer *B. calyciflorus* and one trial each for the cladocerans *D. longiremis* and *B. liederii*.

Eggs used in the experiments were separated into 20 replicates of 20 eggs each, and placed into vials containing 15 mL of sterile medium (0, 8, 16, or 32‰) representing incremental efficiencies of ballast exchange. Five replicates were placed into each

Ship	No. of replicates	Species	No. eggs per 40 g	Pore water salinity (‰)
1 (FP)	N/A	<i>Brachionus budapestinensis</i>	92	2
	5	<i>Bosmina liederii</i>	56	
	N/A	<i>Brachionus calyciflorus</i>	52	
	N/A	<i>Daphnia longiremis</i>	6.3	
	N/A	<i>Daphnia ambigua</i> Scourfield*		
2 (DB)	5	<i>Daphnia longiremis</i>	391	10
	N/A	<i>Daphnia ambigua</i> *		
3 (DB)	5	<i>Brachionus calyciflorus</i>	100	35
	N/A	<i>Brachionus quadridentatus</i> f. <i>rhenanus</i> (Lauterborn)*		
	N/A	<i>Brachionus urceolaris</i> Müller*		
	N/A	<i>Brachionus budapestinensis</i>	56.5	
	N/A	<i>Brachionus angularis</i> Gosse*		
4 (DB)	5	<i>Brachionus calyciflorus</i>	57.8	4
	N/A	<i>Daphnia magna</i> Straus	1.5	
	N/A	<i>Diaphanosoma</i> sp.	<1	
4 (FP)	5	<i>Brachionus calyciflorus</i>	119.5	20
5 (DB)	5	<i>Brachionus calyciflorus</i>	187.8	10
	N/A	<i>Asplanchna brightwelli</i> Gosse	1.5	

**Table 1** List of species hatched from ballast sediments through quantitative and qualitative hatching studies

Species with N/A replicates were not used during experimentation, and were hatched only for identification purposes. Ship tanks are identified by type: FP, forepeak tank; DB, double-bottom tank.

\*Denotes secondary species hatched from single morphological egg type listed immediately above.

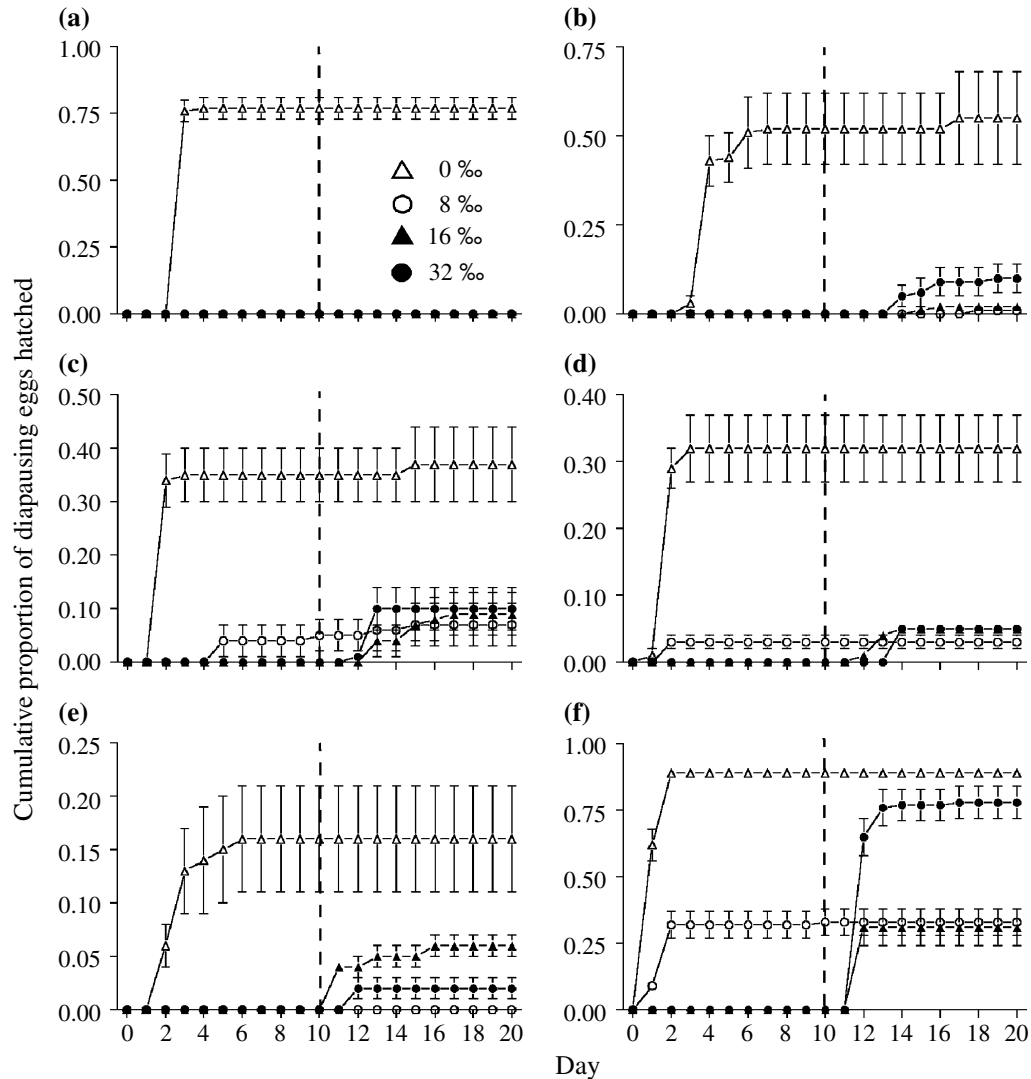
salinity treatment at 20 °C (photoperiod 16 h light : 8 h dark), resulting in an experimental design using 400 eggs per trial. The 0‰ treatment was considered a control to assess maximum viability for these freshwater species. Synthetic pond water (Hebert & Crease, 1980) or diluted, filtered, natural seawater (collected from a vessel transiting the Great Lakes loaded with ocean water ballast, filtered through 0.2 µm Whatman number 5 paper filter) were used as hatching media. Vials were checked for emergence every 24 h for 10 days, with all hatched individuals removed daily. Media were refreshed on day 5. On day 10 all remaining eggs were transferred to synthetic freshwater media by pipette to examine hatching rates after salinity exposure. Again, the number of hatchlings was recorded daily. Negative controls containing only treatment media were kept in each treatment group to detect any introductions of organisms from the environment. We chose the 10 day hatching period after exposure for two reasons. First, previous experiments indicated that 96% of hatching occurs within the first 10 days of trials run for 20 or 30 days in the manner described above (Bailey *et al.*, 2003; unpublished data). Secondly, the typical transit time of a 'no ballast on board' vessel carrying Great Lakes water within the lake system is 7–10 days. If the uptake of Great Lakes water does induce diapausing eggs contained in ballast sediments to hatch (as suggested by Bailey *et al.*, 2003), this is the period of greatest risk.

Variation in the cumulative proportion of diapausing eggs hatched between treatments was analysed using a one-way ANOVA with repeated measures using Systat 8.0 (SPSS, 1998; SPSS, Chicago, IL, U.S.A.). Tukey's multiple comparison test was performed on the total proportion of eggs hatched to determine the impact of salinity on hatching rate. As emergence was inhibited at higher salinities, analyses were conducted on the 10-day hatching segment for each treatment, depending on the timing of emergence (i.e. days 0–10 for 0 and 8‰ treatments were compared with days 10–20 for 16 and 32‰; if no hatching occurred during days 0–10 for the 8‰ treatment, then days 10–20 were used). Only days when hatching occurred in at least one of the replicates were analysed. The proportion of eggs hatched was normalised using an arcsine square root transformation before analysis.

## Results

Hatching experiments were conducted on zooplankton diapausing eggs isolated from residual ballast sediment collected from six tanks on five vessels. Salinity of sediment pore water varied from 2 to 35‰ (Table 1). Diapausing egg densities of dominant taxa were high (>50 eggs per 40 g sediment, Table 1). Eggs were induced to hatch in all experiments, with hatching rates ranging from 16 to 89% in the 0‰ treatment (Fig. 1). In each experiment the proportion hatching declined with increasing salinity. No organisms were recorded in the negative control vials. *Brachionus calyciflorus* generally began to hatch within 24 h of incubation at 0‰, while for the cladocerans *B. liederi* and *D. longiremis* emergence began at day 3 (Fig. 1). Development also began promptly in the 8‰ treatments, with *B. calyciflorus* hatching in three out of four trials by day 5. In addition, development of eye-stage embryos was recorded by day 5 in the 8‰ treatments for 50 and 90% of *Daphnia* and *Bosmina* eggs, respectively (see Fig. 2a). Apparently, these species could not tolerate emergence into brackish water, as development always stopped before emergence from eggs was complete. None of the eye-stage embryos recorded in 8‰ treatment continued development after the medium was replaced with 0‰ water on day 10. Conversely, no organisms hatched or completed significant development during the 10 days of exposure at either of the two higher salinities (i.e. 16 and 32‰, Fig. 2b,c), although some lipid accumulation was noted in the 16‰ treatment. Rather, emergence occurred in these treatments only after brackish or saltwater media were exchanged for 0‰ water (Fig. 1). After exchange, hatching rates among experiments varied between 0–31 and 0–78% for the 16 and 32‰ treatments, respectively. *Bosmina liederi* was the only species tested for which no hatching occurred after exposure to any of the salinity (>0‰) treatments.

The difference in hatching rate between treatments was highly significant for all trials ( $P < 0.05$ , ANOVA, Table 2). All trials exhibited divergence of hatching rates over time, as time × treatment interaction terms were significant ( $P < 0.0001$ , ANOVA, Table 2). The proportion of eggs hatched was higher in the 0‰ treatment for eggs recovered from ships 1–4 (Fig. 1a–e;  $P < 0.05$ , Tukey *post hoc* test). The hatching rates of *B. calyciflorus* for the 0 and 32‰ treatments for ship 5



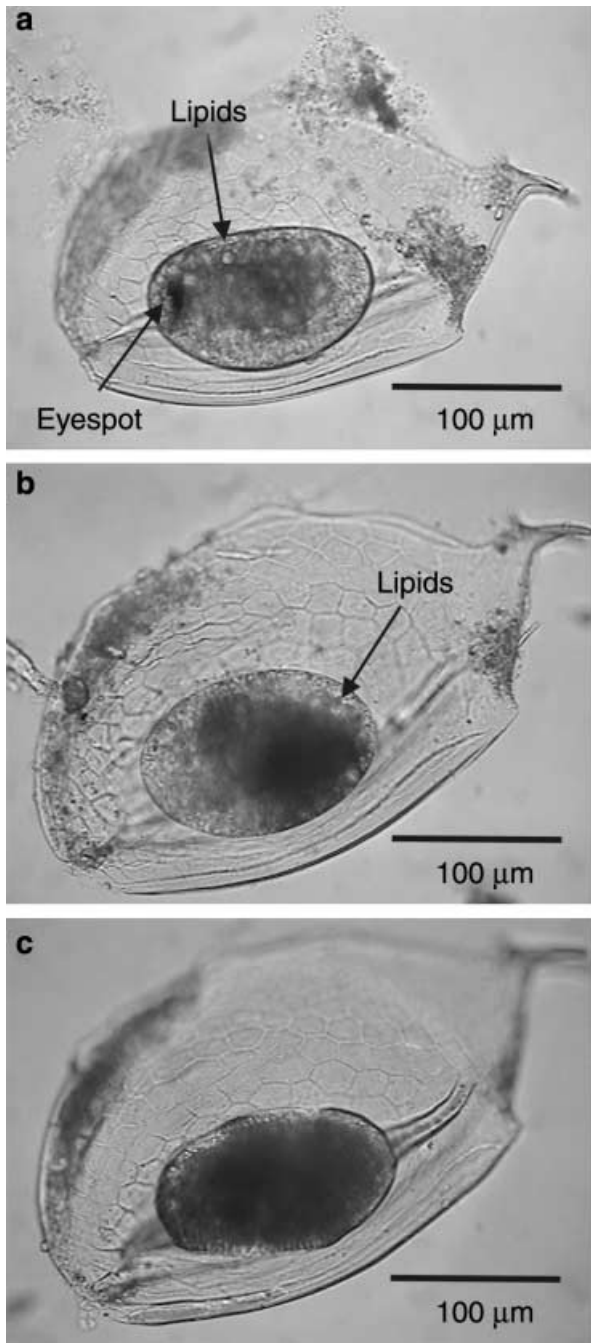
**Fig. 1** Mean ( $\pm$ SE) cumulative proportion of diapausing eggs hatched under salinity treatments, by species. (a) *Bosmina liederii* (ship 1), (b) *Daphnia longiremis* (ship 2), (c) *Brachionus calyciflorus* (ship 3), (d) *B. calyciflorus* (ship 4-DB), (e) *B. calyciflorus* (ship 4-FP) and (f) *B. calyciflorus* (ship 5). After 10 days (dotted vertical line) all unhatched eggs in each treatment group were transferred to 0‰ media. Note scale difference for each ordinate. Error bars  $<0.03$  are hidden by graph symbol.

were significantly higher than for the other two treatments (Fig. 1f;  $P < 0.001$ , Tukey *post hoc* test).

## Discussion

To date, investigations of the salinity tolerance of freshwater zooplankton have been limited to measuring direct effects on growth and survival (e.g. Miracle & Serra, 1989; Teschner, 1995; Hall & Burns, 2002), or examining species richness and composition in waterbodies of varying salinity (Frey, 1993; Brain, Fourie & Shiel, 1995). These approaches have

not considered diapausing egg stages, probably resulting in an underestimate of the range of salinities a particular taxon can tolerate, particularly in instances where salinity varies temporally. In this study, we have demonstrated that the hatching rate of diapausing eggs is reduced by exposure to saline conditions. The ability of diapausing eggs to tolerate fluctuations in salinity may stem from an evolutionary history in temporary habitats, which generally fluctuate more in their physical and chemical environment than adjacent, permanent ones (Williams, 1998).



**Fig. 2** Condition of diapausing eggs of *Bosmina liederii* after 10 days at each treatment. (a) 8‰, aborted eyed-embryo, (b) 16‰, little differentiation and (c) 32‰, no change. Scale bars (100 µm) are included on each image.

Of the species examined here, diapausing eggs of *B. liederii* appear to exhibit the lowest salinity tolerance with no hatching after exposure to saltwater. *Daphnia longiremis* exhibited a modest degree of salinity

tolerance, as a small proportion of diapausing eggs hatched following saltwater exposure. *Brachionus calyciflorus* demonstrated the widest tolerance, with up to 78% eggs hatching after saltwater exposure. Interestingly, *B. calyciflorus* also displayed a wider salinity tolerance as adults than either *B. liederii* or *D. longiremis*. Although typically considered to 'prefer' freshwater, *B. calyciflorus* is frequently observed in brackish waters (e.g. Brain *et al.*, 1995; Park & Marshall, 2000). It is also the only species in these trials that successfully hatched into 8‰ salinity during days 0–10, albeit at rates significantly lower than in freshwater. This finding is consistent with observations of Snell *et al.* (1991), who reported an approximately 40% reduction in the hatching rate of *B. calyciflorus* at 8‰ as compared with 2‰ growth media.

The 'maximum' hatching rates observed at 0‰ ranged from 16–89%. Bailey *et al.* (2003) suggested that pore water salinity might be negatively correlated with hatching success. Our study also found a low hatching rate for eggs recovered from sediments with a pore water salinity of 20‰ or higher (16 and 37%). However, lower pore water salinity ( $\leq 10$ ‰) did not guarantee a high hatching rate (i.e. 32% hatch for 4‰), so other factors (such as age of eggs, duration of diapause or hatching cues) are probably involved. In addition, the effects seen in both studies may be impacted by the wide salinity tolerance of *B. calyciflorus* (i.e. 10‰ is only slightly above the natural range for this species, resulting in a high hatching rate at intermediate salinity). Alternatively, pore water salinity measured at the time of collection may not be a good indicator of egg history; eggs may have been retained in ballast sediments for years, experiencing widely varying salinity, of which only the most recent may be reflected by pore water salinity. Nevertheless, while it is possible that the 'maximum' hatching rate (and subsequent reductions in hatching rate) measured in this study may be affected by previous exposures, our results do indicate the effectiveness of ballast water exchange because the sediments carried in transoceanic vessels originate from ports of varying salinity.

Hatching during days 10–20, following transfer to 0‰ medium, occurred mainly in the 16 and 32‰ treatments. Very little hatching occurred during this period in either the 0 or 8‰ treatments, with individuals emerging only from eggs that had not visibly developed during the first 10 days. This suggests that

Ship	Organism	ANOVA effects [F value (d.f.)]		
		Treatment	Time	Time × treatment
1 (FP)	<i>Bosmina liederii</i>	32.56 (3,16)***	43.48 (3,48)***	16.98 (9,48)***
2 (DB)	<i>Daphnia longiremis</i>	368.97 (3,16)***	353.05 (9,144)***	350.90 (27,144)***
3 (DB)	<i>Brachionus calyciflorus</i>	19.78 (3,17)***	41.08 (7,119)***	10.39 (21,119)***
4 (DB)	<i>Brachionus calyciflorus</i>	36.60 (3,16)***	47.26 (4,64)***	15.10 (12,64)***
4 (FP)	<i>Brachionus calyciflorus</i>	6.15 (3,16)*	14.97 (6,96)***	6.54 (18,96)***
5 (DB)	<i>Brachionus calyciflorus</i>	35.25 (3,16)***	322.04 (4,64)***	22.47 (12,64)***

Data were arcsine square root transformed prior to analysis. Significance levels for F-values: \* $P < 0.05$ ; \*\*\* $P < 0.0001$ . Ship tanks are identified by type: FP, forepeak tank; DB, double-bottom tank.

the *Bosmina* and *Daphnia* eye-stage embryos that developed by day 5 at 8‰ were no longer viable. It is possible that a salinity of 8‰ is sufficiently low for the initiation of egg development in freshwater species, but too high for complete development and emergence to occur. In contrast, no development in these genera was apparent in eggs exposed to 32‰ water, thus there remained a 'bank' of viable embryos left to emerge following transfer to freshwater media. Although both *B. liederii* and *D. longiremis* displayed this trend, we cannot explain why only *Daphnia* eggs hatched after exposure to 32‰. However, this trend was also observed for *B. calyciflorus*, with a higher emergence rate after exposure to higher rather than to lower salinity during the latter half of the experiment, particularly for eggs from ship 5. A similar phenomenon was observed by Lutz, Marcus & Chanton (1994), who exposed copepod resting eggs to variable oxygen conditions. They noted that low oxygen concentrations were more detrimental to egg viability than total anoxia because metabolism was completely shut down during anoxia but not under low oxygen conditions. Thus, there appears to be greater interaction between the embryo and the environment under nearly favourable conditions than under extreme conditions. However, it is also possible that the transfer of eggs from 32 to 0‰ acted as a stronger hatching cue than the transfer of eggs from 8 or 16 to 0‰. If this is the case, then subjecting diapause eggs to ballast water of 32‰ may actually promote mass hatching once the eggs are returned to freshwater conditions.

Charmantier & Charmantier-Daures (2001) suggested that rehydrated *Artemia* embryos are protected from high salinity by the cyst envelope that is permeable to water but impermeable to ions. How-

ever, salinity and temperature are known to interact in their effects on tolerance, with temperature affecting metabolic rate, ion uptake rate, and membrane permeability (Lee & Bell, 1999). Our experiments explored salinity tolerance at 20 °C, arguably a more challenging environment than exposure at a lower temperature for temperate species. It will be necessary to conduct future trials at a variety of temperatures to deduce the interaction between temperature and salinity on diapausing egg viability.

The variation in hatching rate seen among *B. calyciflorus* trials after exposure (day 10–20) may have resulted from the disparate histories of the populations tested, as indicated by pore water salinity of ballast sediments. Of particular interest was the hatching rate of *B. calyciflorus* collected from ship 5, as 78% eggs hatched successfully after exposure to salinities up to open-ocean levels (i.e. 32‰). In contrast, hatching rates of the other three *B. calyciflorus* populations were significantly reduced after similar exposure (<10%). It is possible that salinity experienced during diapause egg formation may influence the range of salinities eggs can survive while dormant, much like it affects the optimal salinity for the initiation of hatching for the euryhaline rotifer *Brachionus plicatilis* Müller (Gilbert, 1974). We were unable to explore this hypothesis, as the origins of the diapausing eggs in this study are unknown. Future studies using clonal populations from both permanent and temporary habitats may help clarify this possibility.

In general, <10% of *Daphnia* and *Brachionus* eggs hatched after salinity exposure in our experiments. Nevertheless, considering the high egg density in ballast sediments ( $10^4$ – $10^5$  eggs  $m^{-2}$  using  $1.6 \text{ g cm}^{-3}$

**Table 2** Analysis of variance with repeated measures demonstrating the effect of salinity treatment on the hatching rate of diapausing eggs

conversion factor for wet sediment), large populations of viable zooplankton eggs may remain after salinity exposure. While it is possible that a longer exposure may have reduced egg viability further, the length of transoceanic crossings will generally not permit longer exposure regimes. As only a small 'seed population' is necessary to establish a cohort of reproductive individuals, and given that the maximum density of diapausing eggs in natural populations ranges between  $10^3$  and  $10^6$  eggs  $m^{-2}$  (Hairston, 1996), new populations could establish when salinity returns to favourable values (i.e. when the vessel subsequently loads freshwater, or if the eggs get flushed into a freshwater environment). Hall & Burns (2001) suggest that resting eggs of *Boeckella hamata* Brehm, a freshwater copepod, are responsible for the recolonisation of the tidally-influenced Lake Waiholo, New Zealand, after seasonal salinisation up to 4.8‰. The average hatching rate for resting eggs of *B. hamata* was only 2.3% under optimal conditions in the laboratory. Therefore, while ballast water exchange may reduce the viability of diapausing eggs by as much as 90% for some taxa, it apparently does not offer complete protection against non-indigenous species entering the Great Lakes by this mechanism. Interestingly, our study indicates that ballast water exchange using brackish water (e.g. 8‰) may have a larger impact on diapausing egg viability than 32‰; however, this effect would have to be weighed against the possibility of introducing live euryhaline species in water of lower salinity, for which ballast water exchange of 32‰ is decidedly more effective (Locke *et al.*, 1993; MacIsaac *et al.*, 2002).

Furthermore, most transoceanic vessels currently trading on the Great Lakes declare 'no ballast on board' status (Colautti *et al.*, 2003), and thus are exempt from ballast water exchange regulations (United States Coast Guard, 1993). MacIsaac *et al.* (2002) suggested that these vessels, collectively, may pose a higher invasion risk than vessels entering the system with saline ballast water owing to the abundance of viable diapausing eggs contained within residual sediments. Our results suggest that the risk posed by diapausing eggs present in sediments of these vessels could be reduced, but not eliminated, by introducing a lens of saltwater into the 'empty' ballast tanks similar to ballast exchange.

Sala *et al.* (2000) suggested that lakes will experience very steep declines in biodiversity this century owing to biotic exchange, land use change and climate change. The salinity of endorheic freshwater habitats is likely to increase during summer months as water inputs decline and evaporation increases (Schindler, 1997, 2001). In addition, coastal lakes and freshwater habitats upstream from tidal estuaries may suffer periodic salinisation as pulsing surges of saltwater seep inland owing to evaporation and anthropogenic diversion of freshwater (Jones, 1994; Hall & Burns, 2003). The persistence of populations through salinity fluctuations by means of diapausing eggs could have profound implications on the extent of biodiversity loss during habitat change. Species incapable of tolerating changing salinity could be replaced by taxa tolerant of brackish or saline conditions (Schindler, 1997); however, this study demonstrates that some populations may be capable of tolerating enhanced fluctuations in habitat salinity, providing a mechanism for enriching biodiversity if the habitat returns to freshwater conditions.

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