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BIODIVERSITY RESEARCH

Assessing invasion risk across taxa and habitats: life stage as a determinant of invasion success

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ABSTRACT

Aim Many aquatic invertebrates produce dormant life-history stages as a means to endure inhospitable environments and to facilitate natural long-distance dispersal, yet we have little understanding of the role of dormant stages as a mechanism for human-mediated introductions of non-indigenous species. We explore the survival of invertebrate dormant eggs in collected ships' ballast sediment over a 1-year period to determine relative invasion potential across taxa (i.e. rotifers, copepods, cladocerans and bryozoans) and different habitats (freshwater, marine).

Location Canadian Atlantic and Pacific coasts and Laurentian Great Lakes.

Methods During 2007 and 2008, 19 ballast samples were collected as a part of a larger study. The degradation rate of dormant eggs was assessed by enumerating dormant eggs and by conducting viability hatching experiments.

Results Taxa examined included rotifers, copepods, anomopods, onychopods and bryozoans. Dormant eggs of rotifers degraded at the highest rate of all taxa examined, with no viable eggs remaining within 10 months. Copepods showed a less rapid degradation rate than rotifers. The degradation rate of anomopod dormant eggs was significantly slower than that of both rotifers and copepods. Onychopods and bryozoans did not visibly degrade at all over 12 months. Viability hatching experiments were successful for rotifers, copepods, and anomopods. Onychopods and bryozoans did not hatch during any of the three hatching trials.

Main conclusions Dormancy is not equally beneficial to all invertebrate taxa. Our results indicate that dormant eggs of rotifers and copepods degrade at a rapid rate and may not pose high invasion risk. In contrast, the slow degradation rate of anomopod dormant eggs and the lack of degradation of onychopod and bryozoan dormant eggs could result in high invasion risk because of their accumulation in ballast tanks. Species having resistant dormant eggs mostly belong to freshwater taxa making freshwater habitats at higher invasion risk by dormant invertebrates than marine habitats.

Keywords

Ballast sediment, biological invasions, degradation rate, habitat, invasion potential, invertebrate dormant eggs.

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INTRODUCTION

Humans have traded and transported species for centuries. In recent decades, however, increases in transport networks and demand for commodities have accelerated the unintentional introduction of non-indigenous species (NIS) beyond their native ranges in both terrestrial and aquatic environments world-wide (Ruiz & Carlton, 2003; Wonham & Carlton, 2005; Ricciardi, 2006; Hulme, 2009). To successfully establish in a new area, potential NIS first have to be taken into a transport

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vector (e.g. ballast water), survive the voyage, and be released into a new area (e.g. Kolar & Lodge, 2001; Colautti & MacIsaac, 2004). The invasion process can be viewed as a series of stages that starts at the transport stage, followed by introduction, and finally, establishment. Successful transition between stages depends on three primary factors: propagule pressure (i.e. number of individuals introduced), physiological tolerance to physical and chemical conditions and integration into the biological community, with most invasions failing at one of the stages (Kolar & Lodge, 2001; Colautti & MacIsaac, 2004).

Diversified life history traits, such as asexual reproduction, high fecundity or broad diet, may enable species to overcome harsh conditions encountered during the invasion process (Kolar & Lodge, 2001). The production of dormant eggs, diapausing eggs, resting eggs, cysts or statoblasts is a life history strategy involving some type of metabolic and/or developmental depression (Cáceres, 1997; Bailey *et al.*, 2005a; Duggan *et al.*, 2005). A unique feature of dormant stages is that they are highly resistant to harsh environments (Cáceres, 1997; Schröder, 2005) and may facilitate dispersal and colonization of new areas (Cáceres, 1997). Thus, dormancy may play an important role in human-mediated dispersal of NIS. Dormancy has evolved in many bacterial, fungal, protist, plant and animal species: while some taxa remain dormant only as long as environmental conditions are unfavourable, others can remain dormant from decades to several centuries (Hairston *et al.*, 1995, 1999; Hairston, 1996). For simplicity, the term 'dormant egg' is used in a broad sense in this paper and includes all of the dormant stages mentioned earlier.

Commercial vessels transport over 90% of the world's trade (Hulme, 2009) and are a leading mechanism for spread of NIS globally (Holeck *et al.*, 2004; Ricciardi, 2006; Molnar *et al.*, 2008). Ballast water, typically utilized to control the trim and stability of a vessel when not fully loaded with cargo, can contain significant amounts of suspended sediment that later settles to the bottom of the tank (Carlton, 1985; Gollasch & Leppäkoski, 1999). Sediments carried in ballast tanks can contain large numbers of active invertebrates as well as their viable dormant eggs that could pose an invasion risk if they are discharged along with ballast water, or if they hatch during a voyage and are subsequently discharged (Bailey *et al.*, 2005a, 2007; Duggan *et al.*, 2005; Drake & Lodge, 2007; Briski *et al.*, 2010; Kipp *et al.*, 2010).

Here, we explore long-term survivorship of dormant eggs collected from ballast tanks to determine the role of dormancy and relative invasion potential of different invertebrate taxa. Using sediment samples containing high densities of dormant eggs, we determined degradation rates and constructed degradation curves for dormant eggs of a variety of invertebrates over the 12-month period and examined relative invasion potential across different taxa (i.e. rotifers vs. copepods vs. cladocerans vs. bryozoans) and different habitats (freshwater vs. marine). We tested the hypotheses that the abundance and viability of dormant eggs will not vary across taxa or habitats over the 1-year period.

METHODS

Nineteen ballast samples, collected as part of a larger study, were selected for examination because they contained high densities of dormant eggs. Samples were collected from vessels visiting Atlantic or Pacific coast ports in Canada or American ports on the Great Lakes. Every sample was collected from a single tank from a single ship (Appendix S1 in Supporting Information). Approximately 6 kg of sediment was collected from each ship and thoroughly homogenized before being stored in dark conditions at 4°C (see Briski *et al.*, 2010). Sediment was stored in covered buckets to prevent desiccation. While we acknowledge that conditions inside ballast tanks are less stable than in the laboratory, often with pronounced variability in temperature, salinity and concentration of different atmospheric gasses over time (Reid *et al.*, 2007; Sutherland *et al.*, 2009), our methodology will likely provide a conservative assessment of degradation rate within ballast tanks because variable conditions are expected to increase degradation rate (Keller & Pitblado, 1984; Onbé, 1985; Hairston *et al.*, 1999; Brendonck & De Meester, 2003; Briski *et al.*, 2010; Gray & MacIsaac, 2010).

All selected sediments contained at least 40 dormant eggs of at least one species per 40 g of sediment. In addition, one sample containing a lower density of dormant eggs was included as it contained species of rotifers and copepods not found in the other samples (Appendix S1). In total, we investigated three species of rotifers, at least eight species of copepods (copepods from six samples were not identified to species level), five species of cladocerans (here treated as infraorders Anomopoda and Onychopoda), and two species of bryozoans. Of these, one rotifer, three copepods, three cladocerans and one bryozoan were freshwater species, one copepod was estuarine, and one rotifer, five copepods and two cladocerans were marine species. One rotifer, two copepods and one bryozoan were not defined as either a freshwater or marine species because they were not identified to species level or hatched in the laboratory experiments.

To examine the rate of visible degradation, dormant eggs were enumerated every 2 months, for 12 months. At each time point, each sediment sample was fully mixed and four 40 g subsamples were removed using a stratified random subsampling design. Each subsample was washed on a 45-μm sieve to remove fine sediment. Dormant eggs were separated from the retained sediment using the colloidal silica Ludox® HS 40 (Burgess, 2001) and enumerated under a dissecting microscope. For each sediment sample, eggs that appeared completely intact (potentially viable) were counted and grouped based on size and morphology, and a maximum of 20 dormant eggs per morphological group were taken for molecular identification following the methods of Briski *et al.* (2011). Eggs that appeared to be physically damaged such as having a cracked shell or missing egg content were not counted and were assumed non-viable (Dharani & Altaff, 2004; Briski *et al.*, 2011).

Further, to assess actual viability of dormant eggs over time, we conducted hatching experiments 1, 6 and 12 months after collection of each sediment sample. We used the number of hatched eggs as a proxy measure of actual egg viability, although we acknowledge that eggs that did not hatch may have been viable but did not receive appropriate hatching cues (Schwartz & Hebert, 1987). At each time point, dormant eggs were isolated from 40 g sediment subsamples using a sugar flotation method (Hairston, 1996; Bailey *et al.*, 2003; Briski *et al.*, 2010; 2011). Extracted dormant eggs were placed in vials containing sterile synthetic pond water (0 parts per thousand (‰) salinity; Hebert & Crease, 1980) or a sterile seawater medium with salinity of 15 or 30‰ using a stratified random design (see Briski *et al.*, 2010). The seawater medium was prepared using ballast water of open ocean origin, filtered through 2.5-μm Whatman filter paper and diluted to 15 or 30‰ with sterile, synthetic pond water. Different salinities were used in an attempt to provide unknown species with optimum fresh-, brackish- or salt-water habitat and to maximize hatching success. Four replicates were placed in each of the 0, 15 and 30‰ treatments at 20°C. To monitor for introduction of organisms from the environment, controls containing only hatching media were kept in each treatment group. Hatching percentage (H%) was calculated by dividing the total number of individuals hatched by the total number of dormant eggs isolated for hatching, multiplied by 100.

Statistical analysis

After enumerating and identifying dormant eggs throughout the year, we tested for differences in the onset and rate of visible degradation (i.e. estimated by visual assessment using above criteria) between dormant eggs within species on different ships, between species that belonged to the same taxonomic group (i.e. copepods, rotifers and anomopods) and between different taxonomic groups. To test for differences within species, we constructed a degradation curve for each species for each sediment sample, using data from all four replicates, described by the equation:

$$y = 100/1 + e^{-Z(t-Q)} \quad (1)$$

where t is time, Z is the rate of degradation and Q is the onset of degradation. The model was expanded to compare the rate and the onset of degradation between two curves using the equation:

$$y = 100/1 + e^{(-Z_1+Z_2)(t-Q_1-Q_2)} \quad (2)$$

where Z_1 and Z_2 are the rates of degradation, and Q_1 and Q_2 are the points of onset of degradation, for the first and second curves, respectively. All possible combinations of curve pairs were compared statistically by the Fit Nonlinear Model using Generalized Least Squares (S-PLUS® 6.1, 2002; Insightful Corp., Seattle, WA, USA). Further, to test for differences between species that belonged to the same taxonomic group, degradation curves were constructed as explained earlier, using data for each species combined across ships. Again, statistical comparisons were conducted using all possible combinations of species pairs within each taxonomic group using the nonlinear model. Finally, to test for differences between different taxonomic groups, data for each taxonomic group was combined across all ships for comparison as earlier. Significance levels for statistical comparisons of estimated parameters Z_1 and Z_2, and Q_1 and Q_2, were adjusted for multiple pairwise comparisons by Bonferroni-type correction to guard against inflating the type I error rate. The family-wise error rate of 0.05 was used. All tests were performed using the computer program S-PLUS 6.1 (S-PLUS® 6.1, 2002; Insightful Corp.). Dormant eggs of two taxonomic groups (i.e. bryozoans and onychopods) did not visibly degrade at all and were excluded from statistical analyses.

Given that viability hatching assessments were conducted by repeated sediment subsampling over time, one-way analysis of variance with repeated measures (ANOVA) and post hoc Bonferroni tests were used to test for differences in absolute numbers of eggs hatched among three hatching experiments (i.e. three time points). The tests were performed separately for rotifers, copepods and anomopods (SPSS 11.5.0, SPSS Inc., 1989–2002; Chicago, IL, USA). A logarithmic transformation was applied to all datasets to meet assumptions of parametric tests. Greenhouse–Geisser corrections were used when sphericity was violated. A significance level of 95% was used for within subject effect statistical analyses, while Bonferroni-type protection to guard against inflating the type I error rate and family-wise error rate of 0.05 were used for pairwise comparisons.

RESULTS

Visible degradation and viability of dormant eggs of 18 taxa were examined over a 12-month period. Taxa examined included three rotifers, at least eight copepods, three anomopods, two onychopods and two bryozoans (Table 1, Appendix S1). Eighty-two per cent of dormant eggs of rotifers, with a mean density of 11 eggs per 40 g of sediment at T_0, completely degraded in 6 months (Table 1; Figs. 1 and 2), with all eggs degraded within 10 months. Dormant eggs of copepods, with a mean density of 251 eggs per 40 g of sediment at T_0, degraded to a mean density of 68 eggs per 40 g of sediment at T_6, and finally to a mean density of 14 eggs per 40 g at T_{12}. On average, 73% of dormant eggs of copepods degraded in the first 6 months, while an additional 21% degraded thereafter (Table 1; Figs. 1 and 2). The degradation rate of anomopod dormant eggs was significantly slower than that of rotifer and copepod eggs ($P < 0.05$, Tables 1 and 2, Fig. 2), with only 20% of eggs degrading within the first 6 months and 34% degrading through the entire year. Dormant eggs of onychopods and bryozoans, with respective mean densities of 15 and 5 eggs per 40 g of sediment at T_0, did not visibly degrade at all during the experiment.

Seven species (*Brachionus plicatilis*, *Brachionus* sp., *Eurytemora affinis*, *Calanus euxinus*, *Daphnia magna, Evadne*

Table 1 Mean and standard error (SE) of dormant egg density, by species, counted initially (T_0), after 6 months (T_6) and 1 year later (T_{12}). Number of samples indicates the number of tanks from which the species was recorded at each time point. The number of samples declines over time because most species were not detected in all samples at later time points. Mean and SE of dormant eggs were calculated considering only those samples with densities > 0. Note the increase in abundance for a few cases at T_6 and T_{12} is the result of sample variability.

Higher taxa level	Species level	Number of samples T_0	Mean density of eggs T_0	SE T_0	Number of samples T_6	Mean density of eggs T_6	SE T_6	Number of samples T_{12}	Mean density of eggs T_{12}	SE T_{12}
Rotifer	*Brachionus plicatilis*	6	12.5	1.7	4	2.1	0.4	0	0	0
Rotifer	*Brachionus calyciflorus*	1	11.2	0.3	1	1.7	1	0	0	0
Rotifer	*Brachionus* sp.	2	9.7	2.6	0	0	0	0	0	0
Rotifer	All species	9	11.7	1.2	5	2.1	0.4	0	0	0
Copepod	*Eurytemora affinis*	5	347.4	66.3	5	113.9	21.4	5	10	3.5
Copepod	*Calanus euxinus*	3	169.9	14.5	3	29.1	2.4	3	7.7	1.1
Copepod	*Leptodiaptomus siciloides*	1	5	0.7	1	1	0	0	0	0
Copepod	*Acartia pacifica*	1	116.7	8.2	1	13.7	1.2	0	0	0
Copepod	Calanoid copepod	5	402.4	64.3	5	108.3	22.2	5	18.2	5.3
Copepod	*Diacyclops thomasi*	1	32.2	0.7	1	5.2	0.2	0	0	0
Copepod	Harpacticoid copepod	1	69.5	7.7	1	11.5	1.5	1	1.7	1.1
Copepod	Unidentified copepod	1	46.2	8.3	1	6.7	0.2	1	0.2	0.2
Copepod	All species	18	251.6	30.6	17	68.7	10.1	15	14.3	2.7
Anomopod	*Daphnia magna*	3	15.1	1.4	3	12.9	1.2	3	11.6	1.1
Anomopod	*Ceriodaphnia dubia*	1	46.7	4.9	1	34.5	3.9	1	29.2	1.2
Anomopod	*Moina* sp.	1	70.2	4.3	1	58	2.9	1	44.5	3.5
Anomopod	All species	5	32.5	5.4	5	26.3	4.3	5	21.7	3.2
Onychopod	*Evadne nordmanni*	6	14.3	2.2	6	14.7	2.2	6	14.9	2.0
Onychopod	*Pleopis polyphemoides*	1	9.7	0.2	1	10	0.4	1	10.5	0.6
Onychopod	All species	7	15.3	1.9	7	14.1	1.9	7	16.6	1.8
Bryozoan	*Plumatella emarginata*	12	4.2	0.5	12	4.3	0.5	12	4.3	0.5
Bryozoan	Unidentified bryozoan	1	7.7	0.4	1	7	0.4	1	7.5	0.6
Bryozoan	All species	13	4.7	0.5	13	4.9	0.5	13	5.4	0.6

nordmanni and *Plumatella emarginata*) occurred in multiple sediment samples (Table 1, Appendix S1). Thirty-one paired statistical comparisons of degradation curves within species across ships exhibited significant differences in neither the onset nor the rate of degradation ($P > 0.05$). Further, three, 28 and three statistical comparisons were applied to pairs of species within rotifer, copepod and anomopod taxonomic groups, respectively. Degradation curves for species within rotifer and anomopod groupings did not differ significantly from each other; however, variability was observed among some species within the copepod group ($P < 0.05$, Table 2, Fig. 1). Onset of egg degradation began significantly later for *E. affinis* than for all other copepod species. Indeed, *E. affinis* was the only species whose eggs degraded significantly slower than those of *Acartia pacifica*. Other copepod species pairs exhibited significant differences only for the onset of the degradation ($P < 0.05$, Table 2, Fig. 1) or not at all. Further, it is interesting to point out that dormant eggs of freshwater and estuarine copepods (i.e. *Diacyclops thomasi*, *Leptodiaptomus siciloides* and *E. affinis*) started to degrade later than marine species (i.e. *A. pacifica, C. euxinus* and an unidentified harpacticoid copepod hatched only at 30‰) (Fig. 1). When all copepod data were entered in the model, the constructed curve was significantly different from that of rotifers (Table 2, Fig. 2). However, when curves of copepod species were individually compared to those of rotifers, curves for *A. pacifica*, *C. euxinus*, *D. thomasi* and the unidentified harpacticoid copepod were not significantly different from those of the rotifers. Eggs of anomopods degraded very slowly, with the onset and degradation rate significantly different from both rotifers and copepods: after 12 months, < 50% of anomopod eggs were degraded (Table 2, Fig. 2).

Brachionus plicatilis was the only rotifer hatched in viability experiments, with a mean hatch rate of 64% (Table 3, Appendix S2). Six months later, the same species hatched from only one sample. While the total number of hatched individuals was lower than in T_1, eggs that remained were nearly all viable (95%). The number of rotifers hatched at T_1 was significantly higher than at T_6 and T_{12} (ANOVA; $P < 0.05$; Table 4). Five copepod taxa hatched from eight samples at T_1, with hatch rate varying from 6 to 54% (Table 3, Appendix S2). Six months later, only two taxa hatched from five samples, with a 100% success rate for both taxa. While the absolute number of individuals of the two taxa (unidentified calanoid and harpacticoid copepods) hatched at T_6 were higher than T_1, the difference was not statistically significant (ANOVA;

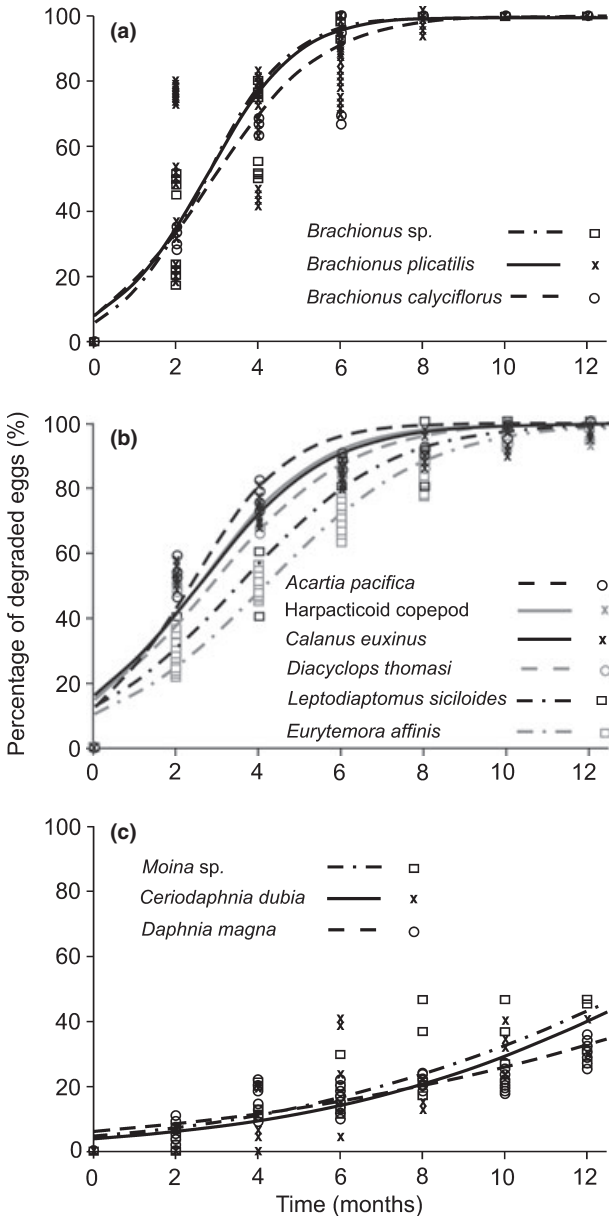

Figure 1 Degradation rates for dormant eggs of rotifer (a), copepod (b) and anomopod (c) species. The curves were constructed using data from all four replicates from all ships where a particular species was found.

$P > 0.05$; Tables 3 and 4). After 12 months, copepods were the only taxonomic group that hatched, though only from two samples. The absolute number of copepods hatched in T_{12} was significantly lower than in T_1 and T_6 (ANOVA; $P < 0.05$; Tables 3 and 4). In the anomopod taxonomic group, *Moina* sp. hatched from only one sample at T_1 (Table 3, Appendix S2). No other anomopod species hatched at T_1. *D. magna* hatched after 6 months, even though it did not hatch at T_1 (Table 3, Appendix S2). There was no significant difference in the number of individuals hatched between time points for the anomopods ($P > 0.05$; Table 4). Dormant eggs of onychopods and bryozoans did not hatch in any of the three hatching trials.

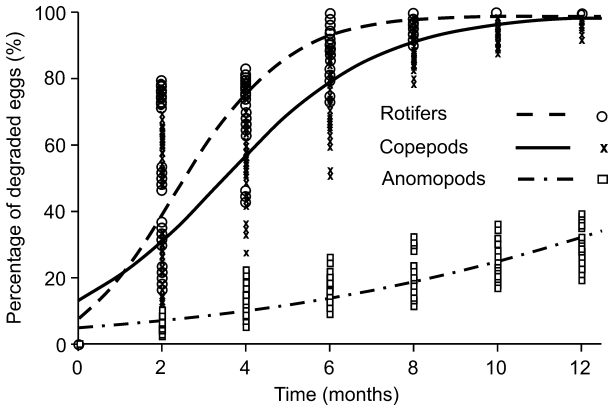

Figure 2 Degradation rates for dormant eggs of rotifer, copepod and anomopod taxonomic groups. Curves were constructed using data from all four replicates from all ships where a particular taxonomic group was found. Degradation rates of onychopods and bryozoans, having poor fit to the model, are not shown.

DISCUSSION

Invasion potential of invertebrate taxa

A large number of studies have addressed questions regarding different life history traits that predispose species to become successful invaders (O'Connor, 1986; Scott & Panetta, 1993; Duncan *et al.*, 1999; Kolar & Lodge, 2001), but no studies have explored the role of dormancy in invasion success. This study highlighted key interspecific differences in the survival of dormant eggs retained in ballast sediment – differences that could profoundly influence colonization potential of different taxonomic groups via ships' ballast sediment. We found that dormant eggs of rotifers degraded at the highest rate of all taxa examined, and consequently they likely represent very low risk of invasion. Copepod egg degradation rate was quite high, but lower than those of rotifers. Freshwater copepods appear to pose a higher invasion risk than marine species. In contrast, dormant eggs of anomopods, onychopods and bryozoans did not degrade in ballast sediments for at least 1 year, suggesting their invasion potential is the highest of all taxa examined. Rotifer, copepod, cladoceran and bryozoan invasions are likely vastly underreported for almost all freshwater and marine systems owing to a lack of historical work on biodiversity, biogeography and, especially, taxonomic limitations (Carlton, 2009).

Degradation rates and relative invasion threat to different habitats

While we found that degradation rates among different species of rotifers and anomopods were fairly consistent, we caution that sample size may have been insufficient to detect differences. Inconsistencies in the onset of degradation in dormant eggs of different copepod species could not be accounted for by variation among ships, as we did not find differences in the onset or rate of degradation within species sampled from

Table 2 Significance levels for statistical comparisons of parameters between pairs of fitted curves, which showed significant difference in the onset or rate of degradation, or both. The *t*-test incorporated in the Fit Nonlinear Model using Generalized Least Squares was used to test for differences between estimated parameters Z_1 and Z_2, and Q_1 and Q_2. Significant *P*-values are presented in bold. Bonferroni-type protection to guard against inflating the type I error rate and family-wise error rate of 0.05 were used for pairwise statistical comparisons. Results that were not significantly different are not shown.

Level of comparison	Pairs compared	The onset of degradation (*P*-value)	The rate of degradation (*P*-value)
Within copepods			
	Eurytemora affinis–Calanus euxinus	< **0.001**	0.1063
	Eurytemora affinis–harpacticoid copepod	< **0.001**	0.2320
	Eurytemora affinis–Diacyclops thomasi	< **0.001**	0.1771
	Eurytemora affinis–Acartia pacifica	< **0.001**	< **0.001**
	Eurytemora affinis–Leptodiaptomus siciloides	< **0.001**	0.585
	Harpacticoid copepod–*Leptodiaptomus siciloides*	< **0.001**	0.1499
	Acartia pacifica–Leptodiaptomus siciloides	< **0.001**	0.1323
Different taxonomic groups			
	Rotifers–copepods	< **0.001**	< **0.001**
	Rotifers–anomopods	< **0.001**	< **0.001**
	Copepods–anomopods	< **0.001**	< **0.001**

Table 3 Mean (± standard error) number of dormant eggs hatched, by species, at 1 month (T_1), 6 months (T_6) and 12 months (T_{12}) after collection of sediment. The number of samples each particular species hatched from at T_1, T_6 and T_{12} and mean hatching percentages (H%), are included. Mean, SE of hatched dormant eggs and H% were calculated considering only those samples with hatching > 0. * denotes cases where more dormant eggs hatched after 6 months than after 1 month.

Higher taxa level	Species level	Number of samples T_1	Mean number of viable eggs T_1 (± SE)	H% T_1	Number of samples T_6	Mean number of viable eggs T_6 (± SE)	H% T_6	Number of samples T_{12}	Mean number of viable eggs T_{12} (± SE)	H% T_{12}
Rotifer	*Brachionus plicatilis*	3	8 (5.7)	64	1	2 (1.2)	95	0	0	0
Copepod	*Eurytemora affinis*	1	22.4 (19.2)	6.4	0	0	0	0	0	0
Copepod	*Calanus euxinus*	2	92.6 (47.4)	54.5	0	0	0	0	0	0
Copepod	Calanoid copepod	3	81.2 (50.3)	20.1	4	109* (81.9)	100	1	9.8 (7.2)	53.8
Copepod	Harpacticoid copepod	1	31 (19.1)	44.6	1	11.5* (29.1)	100	1	2 (4.7)	100
Copepod	Unidentified copepod	1	10.6 (3.2)	22.9	0	0	0	0	0	0
Anomopod	*Daphnia magna*	0	0	0	1	0.1* (0.1)	0.7	0	0	0
Anomopod	*Moina* sp.	1	9 (9)	12.8	0	0	0	0	0	0

Table 4 Statistical comparisons of hatching data among three time points tested by repeated measures ANOVA and Bonferroni tests. Significant *P*-values are presented in bold.

Taxa	Within subject effects (*P*)	Pairwise comparisons (*P*)		
		T_1–T_6	T_1–T_{12}	T_6–T_{12}
Rotifers	**0.036**	**0.009**	**0.011**	0.992
Copepods	**0.002**	0.247	**0.002**	**0.014**
Anomopods	0.392	1	0.992	0.992

Within subject *P*-values for anomopods are with Greenhouse–Geisser corrections.

different ships in any paired comparison. One reason for the high mortality rate and inconsistent onset of degradation of eggs of different copepod species in our samples could be that some species produce mostly subitaneous (i.e. eggs with a thin shell layer, which hatch soon after they are produced) rather than dormant eggs (Marcus, 1996). It is very difficult to distinguish between dormant eggs and subitaneous eggs of copepods without using an electron microscope (Dharani & Altaff, 2004). The observed differences also may reflect evolutionary history since the onset of degradation for eggs of freshwater species was later than that of marine taxa, possibly resulting in higher invasion potential for the former group. Additionally, all freshwater species tested in this study had highly resistant dormant eggs, supporting the view that they may pose a greater invasion threat.

Dormant egg viability

Although onychopod and bryozoan dormant eggs did not hatch in our viability experiments, insufficient sample sizes

preclude us from concluding that the eggs are not viable. The physiology of dormant eggs is very complex, and hatching success depends on the degree of diapause termination, energy content of the eggs and hatching conditions, with low hatching success a common result (Schwartz & Hebert, 1987; Lavens & Sorgeloos, 1996; Hairston *et al.*, 1999; Bailey *et al.*, 2003; Simm & Ojaveer, 2006; Sopanen, 2008). Successful hatching of onychopod species was reported after incubation at 10°C (Bailey *et al.*, 2005a; Simm & Ojaveer, 2006; Sopanen, 2008); however, incubation at 18°C exceeds limits for development and hatching of *Cercopagis pengoi* (Sopanen, 2008). Considering that our hatching trials were conducted at 20°C, that there was no visible degradation of eggs, that DNA extraction was equally successful at the beginning and end of the year, and that low numbers of eggs were used for hatching in our hatching trials, we argue that the onychopod eggs were likely viable and could pose an invasion risk. Similarly, demonstrated resistance by bryozoan eggs to all kinds of extreme environmental conditions (Bushnell & Rao, 1974; Wood, 2005; Kipp *et al.*, 2010) leads us to conclude that these eggs were likely viable and could pose an invasion risk.

Comparison to natural habitats

Dormant eggs of rotifers and copepods have been reported as viable for decades or even centuries (Marcus *et al.*, 1994; Hairston *et al.*, 1995; Hairston, 1996; Hairston & Kearns, 1996; Cáceres, 1997); however, most research indicates loss of viability within 12–24 months (Chittapun *et al.*, 2005; De Stasio, 2007). Viability of dormant rotifer eggs is reduced by exposure to severe drought (Schröder, 2005), bacterial infection (De Stasio, 2007) or high amounts of organic matter (Hagiwara *et al.*, 1997). Similarly, environmental pollutants including heavy metals, acidic water, low oxygen, elevated hydrogen sulphide, high temperature and parasitism may reduce viability of dormant eggs of copepods (Keller & Pitblado, 1984; Kerfoot *et al.*, 1999; Brendonck & De Meester, 2003). Such adverse conditions are often found in ballast tanks (Wagner *et al.*, 1996; Reid *et al.*, 2007; Ago *et al.*, 2008; Sutherland *et al.*, 2009; E. Briski, personal observation, relative to low oxygen level); thus, it is not surprising that dormant rotifer and copepod eggs degraded rapidly in our study. It is noteworthy, however, that dormant eggs of both of these groups degraded in half the time reported in nature.

Viability of dormant eggs of cladocerans could be reduced by exposure to the aforementioned conditions for rotifers and copepods (Keller & Pitblado, 1984; Onbé, 1985; Hairston *et al.*, 1999). In our study, cladocerans exhibited two patterns of dormant egg degradation: anomopods degraded at a very slow rate, while onychopods did not degrade at all. While anomopod degradation rate was similar to that reported in nature by Hairston *et al.* (1999), the lack of degradation of onychopod eggs was surprising as Onbé (1985) reported degradation of onychopod dormant eggs following exposure to environmental pollutants. The last taxonomic group explored in our study were bryozoans, whose dormant stages (i.e. statoblasts) are reported to be resistant to extreme temperature, drought, heavy metals, toxins, freezing and desiccation (Bushnell & Rao, 1974; Kipp *et al.*, 2010) and can remain viable from 2 to 4 years (Cáceres, 1997; Wood, 2005). Our findings for bryozoans are consistent with reports from natural habitats.

Dormant eggs in ships' ballast tanks

This study indicates that variability in survival and longevity of dormant eggs of different invertebrate taxa produces at least two general patterns of invasion risk. Dormant eggs of rotifers and copepods pose high invasion risk only ephemerally during reproductive pulses, while anomopod, onychopod and bryozoan dormant eggs pose a high invasion risk throughout the year. The rapid degradation rate observed across species of rotifers and copepods, even though samples were collected from different tanks and at different times, suggests that eggs of these taxa entered tanks not long before samples were collected. The low number of ships carrying a high abundance of dormant eggs (Bailey *et al.*, 2005a; Duggan *et al.*, 2005; Briski *et al.*, 2010) supports the conclusion that dormant eggs of rotifers and copepods do not continually accumulate inside ballast tanks.

Further, we acknowledge two major limitations in this study. First, we do not know the history of eggs in different ballast sediments, including their age, geographical source or conditions to which they were exposed (see Appendix S3 for ship particulars). Second, environmental conditions inside ballast tanks are less stable than storage in the laboratory. Nevertheless, our study still provides robust insight into the survivorship and viability of dormant eggs.

Invasion risk of active vs. dormant invertebrate stages

This study provides empirical support for the hypothesis that different life history traits may be helpful during various stages of the invasion process. However, dormancy does not provide equal benefit to all invertebrate taxa. Our results suggest that rotifers and copepods are less likely to be transported as dormant eggs in ships' ballast sediment than as active planktonic adults in ballast water (see also Bailey *et al.*, 2005b; Duggan *et al.*, 2005). Nevertheless, transport is only the first stage of the invasion process, and environmental and demographic stochasticity further reduce the number of individuals successfully transitioning from the introduction stage to the establishment stage. If dormant eggs, rather than active individuals, are released from the transport vector, dormancy could facilitate survival of invertebrate taxa through environmental stochasticity because dormant eggs often remain inactive until environmental conditions are favourable (Hairston *et al.*, 1995, 1999; Hairston, 1996). All of the aforementioned factors indicate that invertebrates producing dormant eggs may pose higher invasion risk than those without, and as our data discriminated between more

and less resistant dormant eggs, and assuming all other factors being equal (e.g. propagule pressure, habitat characteristics), this work indicates that anomopods, onychopods and bryozoans may be the most successful invertebrate invaders.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Mean and standard error (SE) of egg density out of four replicates counted at the beginning of the experiment (T_0), after 6 months (T_6) and 1 year later (T_{12}).

Appendix S2 Mean and standard error (SE) number of eggs hatched out of four replicates set for hatching experiments: after 1 month (T_1), 6 months (T_6) and 12 months (T_{12}) from the collection of the sediment.

Appendix S3 Data for nineteen ships sampled.

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BIOSKETCH

Elizabeta Briski completed her PhD at the Great Lakes Institute for Environmental Research at the University of Windsor. Her dissertation focused on the invasion risk of invertebrates and their dormant stages in sediment of ballasted ships entering Canadian Great Lakes and marine ports. All authors are interested in invasion ecology, particularly in vectors and pathways by which non-indigenous species are introduced.

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