# Invertebrate resting stages in residual ballast sediment of transoceanic ships

**Sarah A. Bailey, Ian C. Duggan, Philip T. Jenkins, and Hugh J. MacIsaac**

**Abstract:** Ballast water has been the primary vector of nonindigenous species (NIS) to the Laurentian Great Lakes over the past 45 years. Although ballast water exchange regulations were implemented in 1993 to reduce propagule loads, new NIS continue to be discovered. A possible explanation for this trend is the importance of alternative vectors, such as residual ballast of ships claiming "no ballast on board". We investigate resting stages of invertebrates in residual ballast sediments of transoceanic ships as a possible vector of NIS to the Great Lakes. To model the introduction effort potentially associated with this vector, we collected sediment samples from 39 ships entering the Great Lakes and measured the density, viability, and species richness of resting stages contained therein. Viable resting stages of NIS were found in 32% of ships, at a mean density of $3.0 \times 10^5 \cdot \text{ship}^{-1}$. Temperature, salinity, and removal of eggs from sediment during incubation had a significant impact on total abundance and species richness of hatched taxa. A total of 21 NIS were identified, consisting exclusively of rotifers and cladocerans. Salinity of residual ballast water and geographic region of ballast uptake were predictive variables for profiling invasion risk due to ships, although explained variability was low.

**Résumé :** L'eau de ballastage a été le vecteur principal d'introduction des espèces non indigènes (NIS) dans les Grands Lacs laurentiens au cours des 45 dernières années. Bien que des règlements concernant l'échange des eaux de ballastage en vigueur depuis 1993 visent à réduire les charges de propagules, de nouvelles NIS continuent d'être découvertes. Une explication possible de cette tendance est l'importance des vecteurs secondaires, tels que l'eau résiduelle de ballastage dans les navires qu'on déclare avoir les ballasts vides. Nous avons étudié les stades de repos d'invertébrés dans les sédiments des eaux résiduelles des ballasts de navires transocéaniques comme vecteurs possibles des NIS vers les Grands Lacs. Afin de faire un modèle du potentiel d'introduction associé à ce vecteur, nous avons récolté des sédiments dans 39 navires à leur arrivée dans les Grands Lacs et nous avons mesuré la densité, la viabilité et la richesse spécifique des stades de repos présents. Nous avons trouvé des stades de repos viables de NIS dans 32 % des navires, à une densité moyenne de $3,0 \times 10^5 \cdot \text{navire}^{-1}$. La température, la salinité et la récolte des oeufs dans les sédiments durant l'incubation ont un impact significatif sur l'abondance totale et la richesse spécifique des taxons éclos. Nous avons identifié 21 NIS en tout, toutes des rotifères et des cladocères. La salinité de l'eau résiduelle de ballastage et la provenance géographique de l'eau sont des variables prédictives pour évaluer le risque d'invasion présenté par le navire, bien que la variabilité qu'elles expliquent soit faible.

[Traduit par la Rédaction]

## Introduction

The introduction of nonindigenous species (NIS) beyond their native ranges is a major threat to global biodiversity, particularly for lake ecosystems (Brönmark and Hansson 2002; Rahel 2002). Because economic and ecological costs associated with NIS are significant (e.g., Pimentel et al. 2000; Colautti et al. 2005), predicting and preventing invasions is an increasingly important global priority (Kolar and Lodge 2001). Predicting invasion success requires knowledge of all of the stages inherent in the invasion process, in-

cluding uptake, transportation, release, and establishment (Kolar and Lodge 2001; Sakai et al. 2001). The starting point, and arguably the most important aspect of an invasion, is the introduction effort or propagule pressure associated with transfer of NIS to new areas (Kolar and Lodge 2001; Duncan et al. 2003; Colautti and MacIsaac 2004).

Vectors associated with transoceanic shipping (e.g., ballast water, hull fouling) are recognized as the largest source of aquatic NIS introductions globally (e.g., Ruiz et al. 2000; Leppäkoski et al. 2002; Lewis et al. 2003), and account for 67% of the species introduced to the Laurentian Great Lakes

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since 1959 (Grigorovich et al. 2003). Changes in the type of NIS associated with shipping have occurred concomitantly with a transformation in the nature of ships' ballast. Initial invaders of the Great Lakes were predominantly plants, transported as seeds in solid ballast; this pattern shifted during the 20th century to dominance by algae and invertebrates carried by liquid ballast (Ricciardi and MacIsaac 2000; Colautti et al. 2003). A number of invertebrate species that inhabit the sediment–water interface have been reported since 1988 (Holeck et al. 2004), possibly reflecting another shift as the relative importance of ships claiming "no ballast on board" (NOBOB) status increased after ballast water exchange regulations were introduced (Colautti et al. 2003).

NOBOB ships are exempt from current ballast water exchange legislation because their ballast tanks are considered empty. However, NOBOB ships carry an average of 60 t of residual water and sediments while operating on the Great Lakes (Bailey et al. 2003). NOBOB ships could pose a risk of introduction of NIS to the Great Lakes because they theoretically carry more live freshwater individuals and participate in a greater number of inoculation events than do ballasted vessels (MacIsaac et al. 2002; Colautti et al. 2003). Thus, dormant resting stages in residual sediments are a potentially important contributor to the number of propagules carried by NOBOB ships (Bailey et al. 2003); however, this mechanism has not been thoroughly quantified.

Dormant resting stages are produced by many invertebrates, particularly freshwater taxa, and occur in various forms at different life-history stages (e.g., diapausing eggs, dormant buds, quiescent juveniles, and anhydrobiotic adults; for a review see Cáceres 1997). While distributions of many rotifer and cladoceran taxa capable of producing resting stages have been considered cosmopolitan, owing to widespread passive dispersal, recent work has shown a greater prevalence of endemism between continents (Dodson and Frey 2001; Wallace and Snell 2001). As transfer of resting stages in relocated sediments has been previously implicated as a vector for zooplankton introductions (Schrimpf and Steinberg 1982; Koste and Shiel 1989; Hairston et al. 1999), and considering that over one hundred million tonnes are being transported internationally by ships annually (Endresen et al. 2003; assuming that sediment constitutes <10% of ballast water), ballast sediments have emerged as a potentially important source of NIS.

We carried out a preliminary study of diapausing eggs in ballast sediments of ships trading on the Great Lakes, involving an examination of the viability of eggs from a few dominant species from nine ships (Bailey et al. 2003). Studies of ballast sediments in ships in other trade areas have also been limited, focusing primarily on marine phytoplankton (e.g., Hallegraeff and Bolch 1992; Kelly 1993; Hamer et al. 2000). In this study we test the hypothesis that NOBOB ships are transport vectors of NIS to the Great Lakes. We sampled transoceanic ships entering the system during a 2-year period to quantitatively characterize the density, viability, and species richness of dormant propagules carried in residual ballast sediments. We use these results to construct a heuristic model of the propagule pressure associated with sediments of NOBOB ships, and to predict the number of NIS carried by NOBOB ships to the Great Lakes annually. Finally, we test the hypothesis that ballast-history

parameters can be used to predict which ships present a "high risk" of causing new invasions.

## Materials and methods

Thirty-nine transoceanic ships of NOBOB status were boarded for collection of residual sediments from 69 ballast tanks between December 2000 and December 2002 inclusive. This sample size represents approximately 16% of the annual "multiport" NOBOB traffic in the Great Lakes (i.e., NOBOB ships that fill and empty ballast tanks within the Great Lakes). Collection methods are described in detail in Bailey et al. (2003). Salinity of residual ballast water, if any was present, was measured at the time of sediment collection, using an optical refractometer. Information regarding ships' ballast histories and other physical parameters was recorded at the time of sampling (see Analysis of ballast history). Four ships were sampled twice during the sampling period, with each independent trip into the Great Lakes considered a unique ship sample, since new ballast had been held in the tanks between sampling periods.

We calculated Sorensen's coefficient of similarity (Krebs 1999), based on presence/absence of species in each sample, for pairs of tanks within and between ships to analyze both the spatial and the temporal variation in community composition. Sorensen's coefficient typically ranges from 0 to 1, with higher values indicating greater similarity of samples. First, to determine whether tanks from the same ships were biologically more similar than randomly drawn pairs of tanks from different ships, we contrasted Sorensen's coefficients for all pairs of tanks within ships ($n = 17$) against those for 1000 randomly drawn pairs of tanks between ships using a Mann–Whitney $U$ test (SYSTAT® version 8.0; SPSS Inc. 1998). Secondly, to confirm that the four ships sampled on two occasions should be treated as independent samples, we contrasted Sorensen's coefficients for all pairs of tanks on ships sampled repeatedly ($n = 10$) against those for the same 1000 randomly drawn pairs of tanks between ships, using a Mann–Whitney $U$ test. Only 1 of the 10 pairs of tanks was a true temporally replicated sample, with the same tank sampled at both time periods. The other "pairs" were independent tanks that had each been sampled once, with one tank sampled during the first visit and a different tank sampled on the second visit. Since the spatial analysis determined that tanks within ships at a single time point are more similar than random pairs, we included these "pairs" as replicates for investigation of temporal trends. Species lists generated from maximum-diversity experiments conducted in 0‰ medium at 20 °C (described below) for 47 tanks on 29 ships were used to calculate all Sorensen's coefficients.

### Resting stage density counts

After thorough mixing, four 40-g sediment subsamples (wet weight) were taken from each ballast-tank sample and preserved in 95% ethanol. Resting stages were enumerated under a dissecting microscope after separation from coarse sediment using the colloidal silica Ludox® HS 40 (Burgess 2001). Average density calculated from the four subsamples was subsequently converted to density of resting stages per tonne of sediment.

## Hatching experiments

Unprocessed 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 and Marcus 1981; Schwartz and Hebert 1987). After this time, sediments were stirred manually and 40-g subsamples were removed in four 10-g allotments. Synthetic pond water of 0‰ salinity (Hebert and Crease 1980) or serial dilutions (8‰, 16‰, or 32‰) of filtered natural seawater were used as hatching media. Natural seawater for these experiments was collected from a ship loaded with ocean-water ballast, filtered through a 2.5-µm Whatman paper filter, and diluted using the same synthetic pond water. All experiments were conducted using a cycle of 16 h light : 8 h dark. We conducted two types of experiments: maximum diversity and whole sediment.

Maximum-diversity experiments were designed to promote maximum hatching abundance of the dormant taxa in the sediment community to assess species richness and abundance across ships. Resting stages were separated from sediments collected from five tanks (four ships) — selected for high density of resting stages — using a sugar flotation method (Bailey et al. 2003). Briefly, four 40-g subsamples of each tank sediment were processed through a 45 µm mesh sieve to remove fine sediment before being washed into centrifuge tubes using a 1:1 (*w/v*) mixture of sucrose and water. After centrifugation for 5 min at 27000*g* (~7.7 m·s$^{-2}$), the supernatant was decanted and rinsed thoroughly with water through 45 µm mesh. The supernatant from each subsample was subsequently transferred to a 9-cm$^2$ petri dish containing 40 mL of sterile medium. Four replicates were incubated in each of four treatments: 0‰ and 8‰ media at each of 10 and 20 °C. Dishes were checked for emergence every 24 h for the first 10 days and every 48 h for the subsequent 10 days. All hatched individuals were immediately removed for enumeration and identification. Controls containing blank growth medium were kept in each treatment group to monitor for introduction of organisms from the environment. Sediments from an additional two tanks were incubated only in 0‰ medium at 20 °C for 10 days. Variation in total abundance and species richness of hatched organisms between salinity and temperature treatments was analyzed using two-way multivariate analysis of variance (MANOVA) (SYSTAT® version 8.0; SPSS Inc. 1998). For consistency, the two 10-day experiments were excluded from analyses. If a significant multivariate effect was observed, univariate analysis of variance (ANOVA) was performed to discern the effect of salinity and temperature on each dependent variable. Both total abundance and species richness were transformed to improve normality before analysis. As these experiments were extremely labour-intensive, an unreplicated 40-g sediment sample was prepared in the same way and incubated in 0‰ medium at 20 °C for 20 days, for all remaining sediments (50 tanks from 28 ships). In this manner, we could collect information on richness and abundance of common species from a broad array of ships with reduced overall effort.

Whole-sediment experiments were designed to give a more realistic estimate of hatching abundance and species richness in situ. The protocol used was modified from that of May (1986) and Duggan et al. (2002). Four 40-g subsamples were removed from each of 19 tank sediments (16 ships) and placed in 500-mL glass vessels. One hundred and fifty millilitres of 0‰ medium was added to each vessel before incubation at 20 °C. Vessels were swirled by hand to mix the sediment with the medium. Vessels were examined for emergence of invertebrates every 48 h for 20 days by carefully decanting the medium through a 45-µm mesh screen. All material retained on the mesh was washed into a counting tray for enumeration and identification. Growth medium was immediately returned to each vessel. Vessels were examined at time 0 to ensure that any active copepods present in the sediment would not be mistaken later for those awakening from quiescent stages; one taxon in one experiment was disregarded for this reason. Controls containing autoclaved sediment were kept in each treatment group to monitor for introduction of organisms from the environment.

Three additional salinity treatments, 8‰, 16‰, and 32‰, were added for a subset of the whole-sediment experiments (10 tanks from eight ships) to determine whether brackish- or salt-water taxa were also present in the sediment egg bank. Variation in total abundance and species richness of organisms hatched between salinity treatments was analyzed using one-way MANOVA. Both total abundance and species richness were transformed to improve normality before analysis. Sediments analyzed for 20 days in both the replicated maximum-diversity and whole-sediment experiments in 0‰ medium at 20 °C were analyzed by one-way MANOVA to determine if total abundance and species richness of hatched organisms differed according to the experimental method used (*n* = 5). Again, all significant MANOVA results were subsequently investigated using ANOVA and Bonferroni post-hoc tests were used to determine differences between the four salinity levels on each dependent variable.

For all experiments conducted in the laboratory, hatched individuals were removed to separate vials and raised to maturity, when possible, to aid in identification. Taxa were identified using standard taxonomic keys. No individuals were recovered from control vials at any time. All waste generated during the experiments was autoclaved prior to disposal to minimize the possibility of environmental contamination.

## Analysis of ballast history

We recorded ballast-history information, obtained from each ship's crew, to determine whether risk identified from hatching trials was related to each ship's activities. Information collected included total ballast capacity, previous dates and locations of ballast uptake, and prevalence of salt-water exchange or tank flushing. As some ships did not have records beyond the last change of crew, management, and (or) ownership, we could only obtain an adequate set of records for the two most recent ports of ballast uptake. Locations of ballast uptake were assigned to one of seven broad geographic regions: "Baltic Sea", "Great Lakes basin" (including the St. Lawrence Seaway), "Mediterranean and Black Seas", "North Sea", "northwest Pacific Ocean", "west-central Atlantic Ocean", and "other". In addition, we used current and historical records of tank residuals obtained from ships' crews, as well as personal observations made while inside ballast tanks, to estimate the amount of residual sediment aboard each ship.

Most tanks on the same ship have identical ballast histories, thus we chose to average data for these tanks to avoid pseudoreplication problems. However, we excluded data collected from six tanks, each from a different ship, because the salinity of the residual ballast water in those tanks could not be explained by the ballast history provided by the crew. Averaging tanks for this truncated data set was further justified by separate paired $t$ tests of residual-water salinity and resting-stage density, each of which showed no significant difference between paired tanks within ships. Stepwise multiple regression was used to determine if any of the continuous ballast history variables (i.e., residual-water salinity, total ballast capacity, volume of residual sediment, and month of last ballast uptake) were important predictor variables of resting-stage density or total abundance of hatched invertebrates. Subsequently, analysis of covariance was used to investigate the relationships between both egg density and total abundance hatched and previous regions of ballast uptake. Again, only total abundances of hatched invertebrates in 0‰ medium at 20 °C during maximum-diversity experiments were used for analysis, as this was our most comprehensive data set. Dependent and independent variables were transformed when necessary to improve normality prior to analysis.

**Estimation of propagule pressure**

We calculated the number of viable dormant propagules, $\pi_\varphi$ carried by any ship as

(1)     $\pi_\varphi = \delta\,\varphi\,\tau$

where $\delta$ is the density of resting stages per tonne of sediment for that vessel, $\varphi$ is the proportion of resting stages that are viable, and $\tau$ is the amount of sediment in tonnes aboard the vessel. We calculated propagule pressure for the 34 ships analyzed in the maximum-diversity experiments above, using parameter values generated in 0‰ medium at 20 °C. To deduce the number of viable NIS propagules carried, $\pi_v$, we added a term, $\nu$, to indicate the proportion of viable propagules that are considered nonindigenous to the receiving area:

(2)     $\pi_\mu = \delta\,\varphi\,\tau\,\nu$

where $\nu$ is the product of the number of nonindigenous individuals divided by the total number of individuals. The method of maximum likelihood coupled with parametric bootstrapping techniques (Efron and Tibshirani 1998) was used to estimate the mean and confidence limits for the number of resting stages of NIS carried by NOBOB ships ($X$), when present, using a log-likelihood function for an exponential distribution (eq. 3), after removal of one outlier point (see Discussion):

(3)     $l(\omega|X) = \sum_i^n (\ln\omega - \omega x_i)$

where $\omega = 3.39064 \times 10^{-6}$ is the estimate when eq. 3 is maximized.

To determine the total number of species associated with NOBOB ships deballasting in the Great Lakes annually, we conducted a Monte Carlo simulation of the cumulative number of non-redundant species (i.e., not identified from a previously selected ship) as a function of the number of ships sampled, using data generated from both maximum-diversity and whole-sediment experiments. We randomly selected samples of incremental size, from 1 to 36 ships, without replacement. This procedure was repeated for 500 bootstrap iterations, with the average and standard error of the cumulative number of non-redundant species calculated. A species–area curve was fitted as an asymptotic (i.e., hyperbolic) curve to the average of the iterations using STATISTICA® version 5.5 (StatSoft Inc. 2000) after preliminary inspection of the data. We used least-squares loss functions and Hooke–Jeeves/quasi-Newton root-solving methods (Hooke and Jeeves 1961) to build the nonlinear model describing the number of non-redundant species as

(4)     $y = \dfrac{\alpha\beta x^{\theta}}{1 + \beta x^{\theta}}$

where $\gamma$ is the mean cumulative number of non-redundant species, $x$ is the number of ships sampled, and $\alpha$, $\beta$, and $\theta$ are estimated parameters. Species–area curves were developed separately for species considered indigenous and nonindigenous to the Great Lakes. Although most concern currently centres on the latter group, it is possible that in the case of some species in the former group, intraspecific genetic invasions may occur. Finally, we extrapolated the species–area curves to 250 ships, which is typical of the number of multiport NOBOB ships deballasting in the Great Lakes in any given year (Colautti et al. 2003).

## Results

The amount of residual ballast sediment ranged from <1 to 65 t per ship, with an average of 14 t. Spatial variation in community composition in tanks within ships was significantly lower than that in tanks between ships, with mean Sorensen's coefficients of 0.35 and 0.25, respectively (Mann-Whitney $U$ test, $p < 0.05$; Fig. 1). In contrast, ships sampled on two occasions showed significant temporal variation in community composition, as Sorensen's coefficients for tanks on repeatedly sampled ships were not significantly different from those of 1000 randomly selected pairs of tanks between ships (Mann–Whitney $U$ test, $p > 0.05$; see Fig. 1).

The density of invertebrate resting stages in ship sediments had a lognormal distribution, ranging from $4.0 \times 10^4$ to $9.1 \times 10^7$ resting stages·$t^{-1}$ (median and mean values of $7.2 \times 10^5 \cdot t^{-1}$ and $3.6 \times 10^6 \cdot t^{-1}$, respectively). Taxonomic identifications, based upon resting-stage morphology, were made for 12 groups in the sediments (see Table 1). Clearly, total species richness was underestimated, as many resting stages could not be differentiated beyond genus, while other, smaller taxa (e.g., Dicranophoridae) may have been overlooked. Diapausing eggs of rotifers, particularly *Brachionus* species, dominated (77.9%) resting-stage abundance. This pattern was influenced by one ship with an extremely high density of diapausing eggs of *Brachionus* spp. (65.3% of resting-stage abundance for all ships), although the general pattern was the same even if that ship was excluded.

Sufficient quantities of sediment for laboratory experiments were lacking for three ships, limiting assessments to

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**Fig. 1.** Sorensen's coefficients of similarity (mean ± standard error) for tanks within ships ($n = 17$), tanks on ships sampled repeatedly ($n = 10$), and tanks between ships ($n = 1000$). Between-ship pairs were selected randomly from a possible 47 tanks on 29 ships.
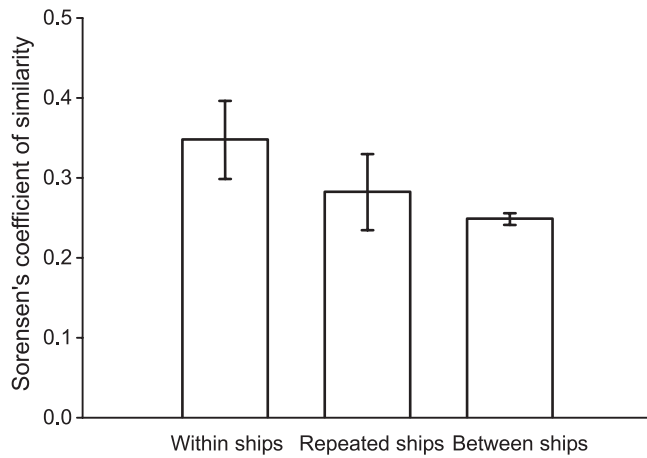


**Table 1.** Percent occurrence and abundance of resting stages collected from 39 ships by taxon, arranged phylogenetically.

|  | Percent occurrence | Percent abundance |
|---|---|---|
| **Rotifera** | **100** | **77.9** |
| *Asplanchna* spp. | 66.7 | 1.0 |
| *Brachionus* spp. | 97.4 | 76.2 |
| *Conochilus* spp. | 5.1 | <1 |
| *Filinia* spp. | 48.2 | <1 |
| *Synchaeta* spp. | 5.1 | <1 |
| **Bryozoa** | **61.5** | **<1** |
| **Anomopoda** | **76.9** | **9.3** |
| *Bosmina* spp. | 51.3 | <1 |
| Chydoridae | 5.1 | <1 |
| *Daphnia* spp. | 46.2 | 7.9 |
| *Moina* spp. | 25.6 | <1 |
| **Ctenopoda** | **2.6** | **<1** |
| *Diaphanosoma* spp. | 2.6 | <1 |
| **Copepoda** | **76.9** | **2.6** |
| **Indeterminate** | **100** | **9.8** |

**Note:** Values in boldface type are total values by taxon.

**Table 2.** Species hatched from diapausing eggs in residual ballast sediment that are considered nonindigenous to the Great Lakes.

|  | Occurrence[a] | Abundance[b] | Habitat match?[c] |
|---|---|---|---|
| *Daphnia magna*[d] | 4 | 6 | Yes |
| *Filinia passa*[d] | 4 | 3.5 | Yes |
| *Brachionus leydigi*[d] | 4 | 3 | Yes |
| *Filinia cornuta*[d] | 3 | 3 | Yes |
| *Asplanchna girodi*[d] | 2 | 1 | Yes |
| *Cephalodella sterea*[d] | 1 | 4.75 | Yes |
| *Bosmina maritima*[e] | 1 | 2 | Yes |
| *Diaphanosoma orghidani* | 1 | 1.25 | Yes |
| *Brachionus forficula* | 1 | 1 | Yes |
| *Brachionus nilsoni*[d] | 1 | 1 | Yes |
| *Conochilus coenobasis*[d] | 1 | 0.5 | Yes |
| *Diaphanosoma mongolianum* | 1 | 0.5 | Yes |
| *Cephalodella* cf. *stenroosi* | 1 | 0.3 | Yes |
| *Alona rustica* | 1 | 0.25 | Yes |
| *Brachionus bennini*[d] | 1 | 0.25 | Yes |
| *Brachionus diversicornis* | 1 | 0.25 | Yes |
| *Diaphanosoma sarsi* | 1 | 0.25 | Yes |
| *Hexarthra intermedia*[d] | 1 | 0.25 | Yes |
| *Moina affinis*[f] | 1 | na | Yes |
| *Synchaeta baltica* | 1 | 2.75 | No |
| *Synchaeta bacillifera* | 1 | 2.25 | No |
| *Evadne nordmanni*[d] | 1 | 0.5 | No |
| *Pleopis polyphemoides*[g] | 1 | na | No |

**Note:** Species are listed in order of decreasing risk, according to propagule pressure and suitability of habitat.

[a]The number of ships from which the species was collected (out of a possible 35).

[b]The cumulative mean number of individuals that emerged from 40-g sediment samples from all ships on which that species was found (na, not applicable).

[c]Species hatched in 0‰ medium during laboratory experiments were considered a match for the habitat in the Great Lakes.

[d]Species with a broad geographic distribution.

[e]Species already established in the Great Lakes.

[f]From Bailey et al. (2003).

[g]S. Bailey, unpublished data.
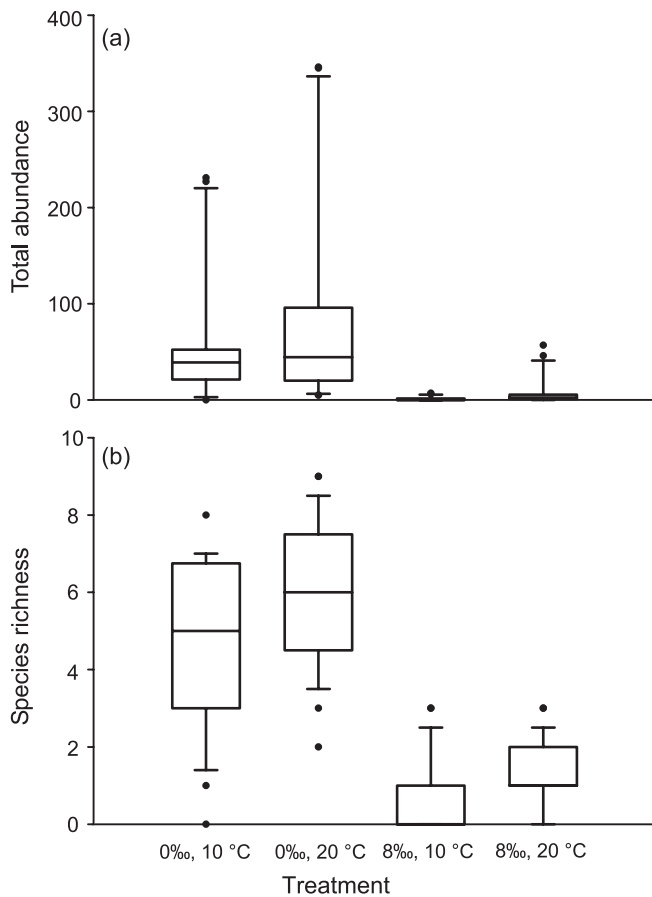
density of resting stages. For the remaining 36 ships, we hatched 76 distinct taxa. Twenty-one NIS were identified, consisting of 14 rotifers and 7 cladocerans (Table 2). One NIS identified in this study, *Bosmina maritima*, has already become established in the Great Lakes. Both the total abundance and the frequency of occurrence of NIS were low compared with species considered native to the Great Lakes (see Appendix A).

In the maximum-diversity experiments, 59 taxa were hatched in the replicated trials, although this number may be conservative, owing to the presence of unidentifiable juvenile invertebrates. Species richness ranged from 0 to 20 taxa per sediment, with a median value of 3. Thirteen additional unique taxa, of 45 in total, were identified from the 50 unreplicated maximum-diversity trials. All taxa were hatched from true diapausing stages; no quiescent copepodids were recovered by this method. The rotifer *Synchaeta bacillifera* and the cladoceran *Evadne nordmanni* were the only organisms that hatched exclusively in brackish water. Rotifers were the most species-rich group, comprising 75% of all species hatched. Cladocerans were the second richest taxon, representing 23% of hatched species. Copepod nauplii were hatched from 14 sediments, though they could not be identified to the species level and were considered one taxon in
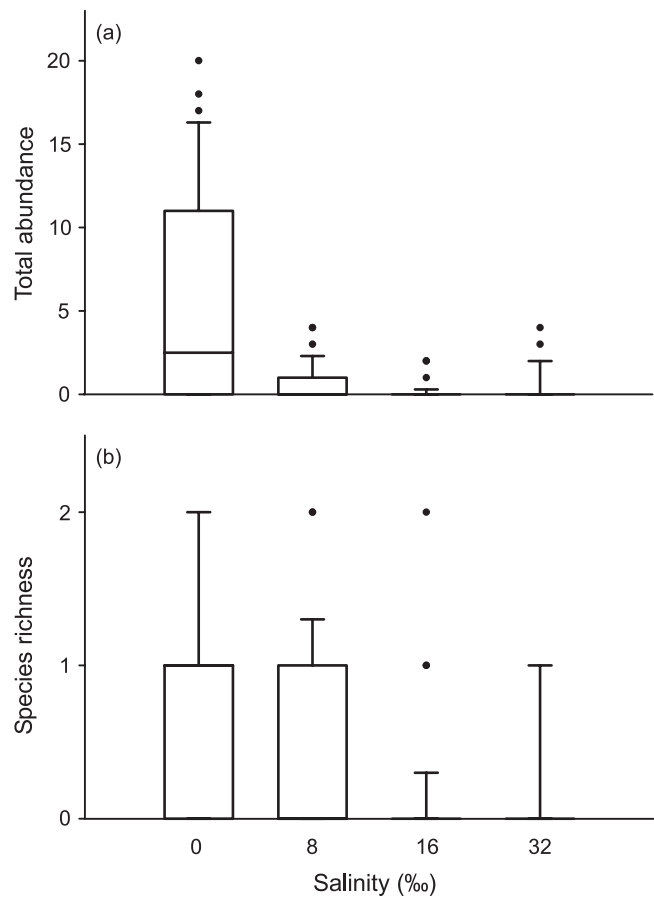
consequence. Of the organisms hatched, the 0‰, 20 °C treatment group had both the highest abundance and greatest species richness, followed by the 0‰, 10 °C treatment group (Fig. 2). Both total abundance and species richness were significantly affected by experimental temperature and salinity (MANOVA, $p < 0.01$; Table 3). Univariate analyses indicated that higher salinity and lower temperature each suppressed total abundance and species richness of hatched taxa independently, and that there was no interaction effect for salinity × temperature on either variable (ANOVA, $p < 0.05$; Table 3).

In the whole-sediment experiments, 21 taxa hatched, although for six sediments, no animals emerged under any treatment regime. Three taxa, *Acanthocyclops robustus*, *Nitocra lacustris*, and an unidentified juvenile cyclopoid, were found as quiescent copepodids. All remaining taxa were hatched from diapausing eggs. Species richness ranged from 0 to 13 taxa per sediment, with a median value of 2. The rotifers *Synchaeta baltica* and an unidentified *Synchaeta* species and copepod nauplii were the only taxa that hatched exclusively in salt water. Rotifers and copepods were the most species-rich groups, comprising 76% and 14% of all species hatched from whole sediments, respectively. Copepod nauplii hatched from six sediments, and were again

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**Table 3.** Results of two-way multivariate analysis of variance addressing the effect of experimental temperature and salinity treatment on total abundance and species richness of the invertebrates hatched during the maximum-diversity experiments.

| Variable | Sum of squares | df | Mean square | $F$ | $p$ |
|---|---|---|---|---|---|
| **Salinity** | | | | | |
| Univariate $F$ tests | | | | | |
|   Total abundance | 26.30 | 1 | 26.30 | 92.50 | <0.001 |
|     Error | 21.32 | 75 | 0.28 | | |
|   Species richness | 5.07 | 1 | 5.07 | 125.89 | <0.001 |
|     Error | 3.02 | 75 | 0.04 | | |
| Multivariate test (Wilks' λ = 0.36) | | 2,74 | | 64.54 | <0.001 |
| **Temperature** | | | | | |
| Univariate $F$ tests | | | | | |
|   Total abundance | 1.76 | 1 | 1.76 | 6.20 | 0.015 |
|     Error | 21.32 | 75 | 0.28 | | |
|   Species richness | 0.45 | 1 | 0.45 | 11.23 | 0.001 |
|     Error | 3.02 | 75 | 0.04 | | |
| Multivariate test (Wilks' λ = 0.87) | | 2,74 | | 5.55 | <0.01 |
| **Interaction** | | | | | |
|   Univariate $F$ tests | | | | | |
|   Total abundance | 0.33 | 1 | 0.33 | 1.18 | 0.28 |
|     Error | 21.32 | 75 | 0.28 | | |
|   Species richness | 0.002 | 1 | 0.002 | 0.06 | 0.82 |
|     Error | 3.02 | 75 | 0.04 | | |
| Multivariate test (Wilks' λ = 0.98) | | 2,74 | | 0.89 | 0.42 |

**Table 4.** Results of multivariate analysis of variance addressing the effect of experimental salinity treatment on total abundance and species richness of the invertebrates hatched during the whole-sediment experiments.

| Variable | Sum of squares | df | Mean square | $F$ | $p$ |
|---|---|---|---|---|---|
| Univariate $F$ tests | | | | | |
|   Total abundance | 3.20 | 3 | 1.07 | 21.29 | <0.001 |
|     Error | 6.22 | 124 | 0.05 | | |
|   Species richness | 2.81 | 3 | 0.94 | 13.00 | <0.001 |
|     Error | 8.94 | 124 | 0.07 | | |
| Multivariate test (Wilks' λ = 0.58) | | 6,244 | | 12.63 | <0.001 |

considered a single taxon. One cladoceran, *Daphnia magna*, hatched from ephippial eggs. The experimental salinity treatment had a significant influence on total abundance and species richness of hatched invertebrates (MANOVA, $p <$ 0.001; Table 4). Univariate analyses further revealed that increased salinity suppressed both total abundance and species richness (ANOVA, $p < 0.001$; Table 4). However, pairwise contrasts revealed that total abundance was significantly greater at 0‰ than for all other treatments (Bonferroni post-hoc test, $p < 0.001$), while species richness did not differ between the 0‰ and 8‰ treatments (Bonferroni post-hoc test, $p > 0.05$; see Fig. 3). Burial in sediment significantly decreased both total abundance and species richness of hatched taxa, with 0%–43% of individuals hatched from isolated resting stages also emerging from buried resting stages (MANOVA, $p < 0.001$; Table 5; Fig. 4). Again this effect was significant for both total abundance and species richness independently (ANOVA, $p < 0.001$; Table 5).

Of the geographic regions where the most recent site of ballast uptake was located, the two dominant ones were the North Sea ($n = 14$) and west-central Atlantic Ocean ($n = 8$). The Great Lakes basin was the most frequent penultimate
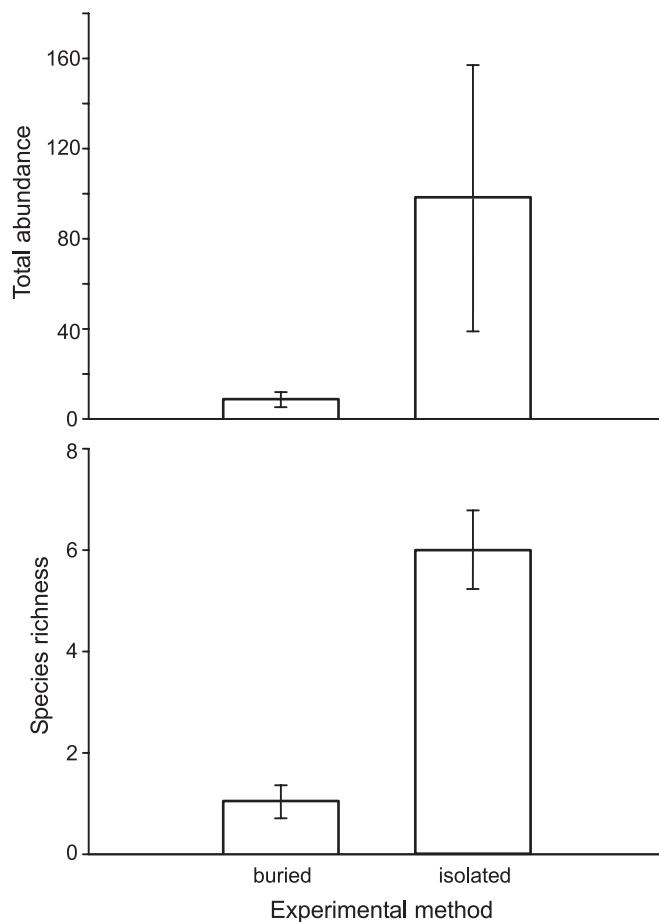
source of ballast ($n = 14$). Since the total abundance of hatched individuals was significantly related to the density of resting stages (linear regression, $r^2 = 0.49$, $p < 0.001$; Fig. 5), relationships to ballast-history variables are nearly identical and we only present results of the analysis of resting-stage density. Resting-stage density was weakly related to the salinity of residual ballast water (stepwise multiple regression, $r^2 = 0.195$, $p = 0.013$). All other continuous ballast history variables were found to be unimportant in relation to resting-stage density ($p > 0.05$). Furthermore, analysis of previous regions of ballast uptake showed that only the interaction between the most recent and penultimate sources of ballast was a significant determinant of resting-stage density after residual-water salinity was held as a covariate (analysis of covariance, $p < 0.05$).

Incorporation of experimental values for resting-stage density and viability and sediment tonnage into our propagule-pressure model (eq. 1) revealed that the distribution of the number of viable resting stages per ship is right-skewed (mean and median numbers $9.8 \times 10^6$ and $1.4 \times 10^6$, respectively; Fig. 6). Resting stages recovered from sediments of six ships (17.5%) could not be induced to hatch in the labo-
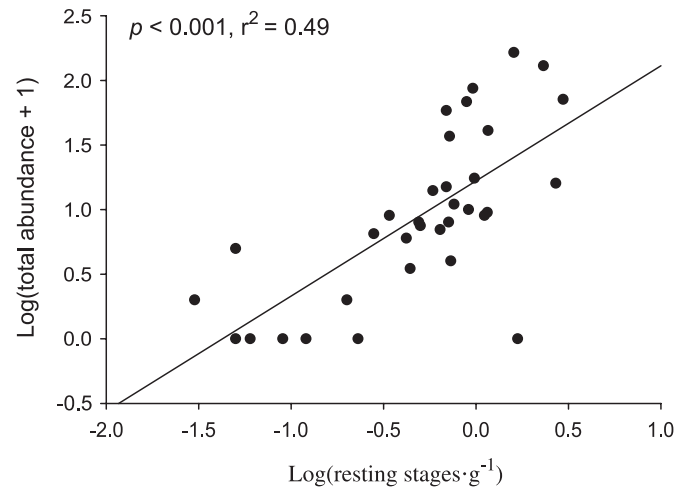
**Table 5.** Results of multivariate analysis of variance addressing the effect of experimental method on total abundance and species richness of the invertebrates hatched.

| Variable | Sum of squares | df | Mean square | $F$ | $p$ |
|---|---|---|---|---|---|
| Univariate $F$ tests | | | | | |
|    Total abundance | 6.22 | 1 | 6.22 | 29.10 | <0.001 |
|      Error | 8.97 | 42 | 0.21 | | |
|    Species richness | 15.07 | 1 | 15.07 | 128.40 | <0.001 |
|      Error | 4.93 | 42 | 0.12 | | |
| Multivariate test (Wilks' $\lambda$ = 0.23) | | 2,41 | | 69.71 | <0.001 |

**Note:** Maximum-diversity (isolated resting stages) and whole-sediment (buried resting stages) experiments are described in detail in Methods.

**Fig. 4.** (*a*) Total abundance and (*b*) species richness (mean ± standard error) of organisms hatched from residual sediments from five ballast tanks in whole-sediment (buried) and maximum-diversity (isolated) experiments. All replicates were incubated in 0‰ growth medium at 20 °C. Note the difference in scale for each *y* axis.



**Fig. 5.** Scatterplot of total abundance hatched versus density of resting stages. Both variables were log-transformed prior to analysis. The regression line is $y = 0.89x + 1.22$.



## Discussion

Propagule pressure is emerging as an important factor in the prediction of invasion success in both aquatic and terrestrial environments (e.g., Grevstad 1999; Forsyth and Duncan 2001; Drake and Lodge 2004). The propagule-pressure hypothesis states that invasion success is directly related to introduction effort. Our results indicate that the average NOBOB ship entering the Great Lakes carries about $9.8 \times 10^6$ viable, dormant propagules in residual sediment, one order of magnitude more than the number of live freshwater propagules estimated to be carried in residual ballast water (MacIsaac et al. 2002). In previous studies of ballast-water taxa, the proportion of propagules that are nonindigenous to the receiving area has not been described; we suggest that the actual risk posed by resting stages is much lower than the above numbers indicate, as only ~2.5% of viable resting stages are NIS (i.e., $2.2 \times 10^5$). However, considering that approximately 250 NOBOB ships engage in multiport activities on the Great Lakes each year (Colautti et al. 2003), the expected number of ships entering the system carrying dormant resting stages of NIS is 81 (standard deviation = 7.4), or $2.4 \times 10^7$ resting stages of NIS per year. Furthermore, this study recorded one extreme event, a single ship carrying $4.5 \times 10^6$ resting stages, which was excluded when calculating the population mean to allow for application of maximum-

ratory under any conditions, and were apparently nonviable. Thirty-two percent of ships sampled carried viable resting stages of NIS, at a mean density of $3.0 \times 10^5$, estimated from a fitted exponential distribution (95% confidence interval: $1.3 \times 10^5$ to $4.9 \times 10^5$; Fig. 6). The nonlinear model generated to predict the number of non-redundant species transported in residual sediments fit the data robustly ($r^2 = 0.99$). From species–area curves we predict that up to 150 invertebrate species, including 52 NIS, are transported as resting stages to the Great Lakes each year (Fig. 7).

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**Fig. 6.** Numbers of viable resting stages transported in sediments of 34 ships designated "no ballast on board" (NOBOB). Solid bars denote egg-density distribution for all taxa; open bars represent only those species considered non-indigenous to the Great Lakes.



**Fig. 7.** Monte Carlo simulations of the cumulative number (mean ± standard error) of non-redundant native (●) and non-indigenous (○) species by number of ships sampled. Results were bootstrapped 500 times.
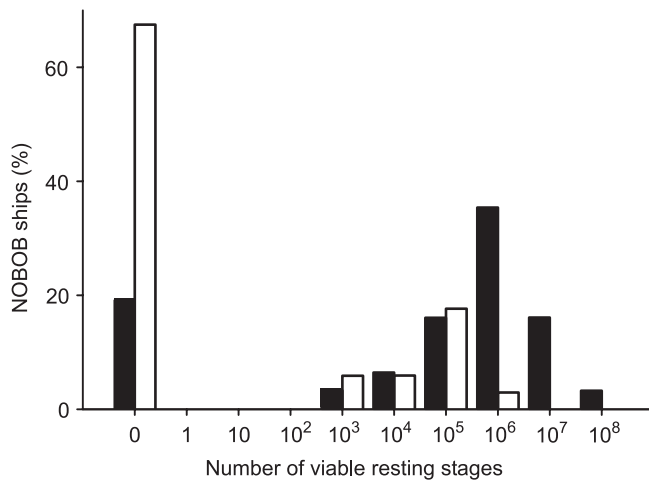


likelihood estimation. Rare events can be very important in biological invasions (e.g., Lewis 1997), so even though we did not use this value in our propagule-pressure calculations, its biological significance should not be overlooked. In a management context, identification of the very small number of vessels transporting large egg banks should be a priority.

Our Monte Carlo simulation indicates that resting stages of 150 species are likely transported to the Great Lakes each year in NOBOB sediments, and of these 52 are NIS. To date, 23 NIS have been recorded from residual ballast sediments. Four of these taxa (17%) are brackish-/salt-water species (*E. nordmanni*, *Pleopis polyphemoides*, *S. bacillifera*, and *S. baltica*) that probably would not survive if introduced to the Great Lakes. One freshwater species, *B. maritima*, is already established in the Great Lakes, possibly owing to transfer in freshwater ballast (De Melo and Hebert 1994). The remaining 18 taxa consist exclusively of rotifers (67%) and cladocerans (33%), and all appear capable of surviving abiotic conditions in the Great Lakes. According to the propagule-pressure hypothesis, *D. magna* should have the greatest opportunity to invade the Great Lakes considering that it had the highest frequency and abundance of propagules of NIS. This species has not been recorded in the Great Lakes to date despite having a broad global distribution. Its large body size may predispose it to size-selective predation by planktivorous fishes (Boersma et al. 1999), reducing its likelihood of successful establishment even if introduced to the lakes in large inocula. Interestingly, the next five highest risk NIS (*Brachionus leydigi*, *Filinia passa*, *Filinia cornuta*, *Asplanchna girodi*, and *Cephalodella sterea*), and indeed over half the list of NIS, also possess broad global distributions, yet none have been reported from the Great Lakes. The remaining species (~25%) are almost entirely restricted to the Palaearctic and Oriental biogeographic regions, possibly reflecting current trade patterns of NOBOB ships. To date, no NIS of rotifers have been recorded from the Great Lakes, although Gray et al. (2005) recorded one species from Lake Erie whose status remains uncertain. Our hatching studies suggest that rotifers should present the pre-

dominant invasion risk to the Great Lakes. Alternatively, the lack of reported invasions by this group may indicate that resting stages contained in residual sediments are a weak or emerging vector. It is not clear whether copepods also represent an invasion threat via NOBOB ships, as we were unable to ascertain the species of any of the naupliar stages that hatched from diapausing eggs contained in ballast sediments.
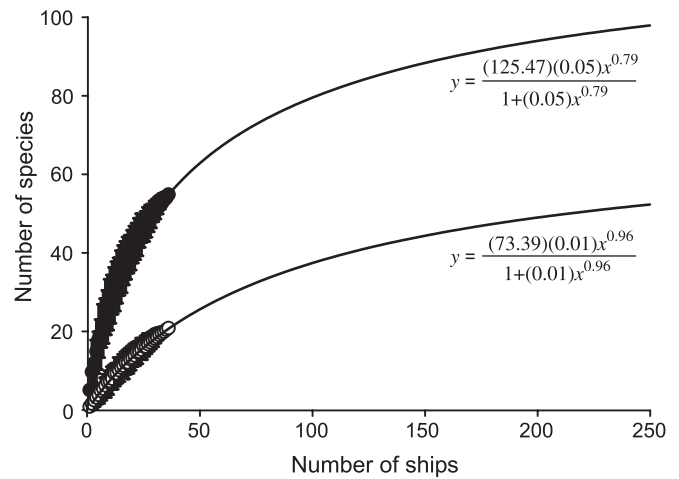
In total, we identified 76 distinct taxa from resting stages in residual sediment, with nearly the entire assemblage representing planktonic freshwater species, particularly rotifers (e.g., *Brachionus* spp., *Keratella* spp., *Polyarthra* spp.). This may be an indication that resting stages are deposited directly into ballast tanks by reproductive females drawn in with ballast water rather than being brought in with disturbed sediments. In addition, rotifers of the genus *Brachionus* were the most common and abundant species in this study. This could be because ballast was taken in areas — such as the lower Rhine River — where *Brachionus* species dominate the planktonic community (van Dijk and van Zanten 1995). However, the predominantly freshwater *Brachionus* species are minor components of other ballast-loading regions, such as the Baltic Sea (Viitasalo et al. 1995; M. Simm and A. Põllumäe, Estonian Marine Institute, Viljandi mnt. 18B, 11216 Tallinn, personal communication), and presumably also of coastal areas with high salinity. Thus, the ubiquitous occurrence of these species may be due to enhanced survivability within ballast tanks associated with a broad salinity tolerance (see Bailey et al. 2004). Although 73% of species encountered during this study are considered native to the Great Lakes and do not appear to represent an invasion risk, there is potential for cryptic invasions through genetic introgression if novel genotypes are introduced from ports in other parts of the world (e.g., Saltonstall 2002; Turon et al. 2003). Furthermore, the possibility exists that some Nearctic species (e.g., *Daphnia retrocurva*, *Brachionus havanaënsis*, and *Trichocerca multicrinis*) could be transferred from the Great Lakes to trading partners overseas.

It is important to note that our measure of propagule pressure is an estimate of the number of dormant individuals

transported by NOBOB vessels, rather than the number released into the system. Furthermore, heavily compacted ballast sediments may offer little opportunity for direct expulsion of resting stages from ballast tanks. Therefore, the potential for introduction of dormant NIS in residual sediments is greatest if resting stages are stimulated to hatch inside the ballast tanks of multiport NOBOB ships, prior to ballast discharge elsewhere on the Great Lakes (Bailey et al. 2003). Our measures of viability and species richness may not reflect actual hatch rates inside ballast tanks of operational ships, as all of these experiments were conducted in the laboratory under conditions designed to induce hatching. It is to be expected that a large proportion of resting stages will not hatch within ballast tanks because they do not receive the requisite cues for termination of diapause. Although ballast tanks are typically dark, hatching success should not be greatly impacted, as previous work has demonstrated that light is not always an essential cue for hatching (Bailey et al. 2003). Instead, the greatest impediment to hatching may come from burial. For example, fewer than 50% of resting stages hatched during our laboratory experiments when buried in sediment 2 cm thick, compared with an average of 20% of those isolated from sediment. Thus, the propagule pressure estimated in this study should be considered a maximum value, with the number of propagules available for introduction from resting stages being at least one order of magnitude lower than that being transported (i.e., ~4.2 × $10^4$ resting stages of NIS).

Even if only a small number of resting stages receive hatching cues, it is theoretically possible that these individuals could develop a substantial population within ballast tanks before ballast discharge occurs (see Bailey et al. 2003). All rotifers and cladocerans in this study are capable of parthenogenetic reproduction and all have short, multivoltine life cycles, both of which facilitate rapid population growth during warm summer months. Both hatch rate and subsequent population growth rate are affected by temperature (Ruttner-Kolisko 1974; Allan 1976). Thus, season could also influence the invasion risk posed by vessels, all else being equal. In terms of the multiport NOBOB ship on the Great Lakes, we expect that the invasion risk would be greatest during late summer, when temperatures are highest.

### Can risk be related to ballast history?

Our results suggest that the biological composition of tank contents varies through time, as Sorensen's coefficients for repeated ship samples were only marginally higher than that for pairs of tanks from different ships. Ballast-history information may be useful for determining differences in community composition. Although resting-stage density was significantly related to the salinity of residual ballast water, it could only explain ~20% of the variability and thus would not be a particularly informative tool for making management decisions; high salinities may simply reflect a low occurrence of freshwater taxa in the region of ballast uptake. However, there may be some predictive value in performing a risk analysis of ballast source regions. Although we could not deduce which geographic regions pose a "high risk" statistically, the significance of the interaction between previous areas of ballast uptake may be an indicator that sediment

is retained in ballast tanks from numerous ballast events. In terms of risk of NIS introduction to the Great Lakes, ships loading ballast at ports in the Mediterranean and Black seas, northwest Pacific and west-central Atlantic oceans, and regions classified as "other" appear to pose a minimal risk because these areas are predominantly saline. By contrast, ships loading ballast in the Baltic Sea or North Sea may pose a relatively higher risk, owing to the occurrence of freshwater ports in these regions. In our study, 7 of the 12 ships carrying NIS had last loaded ballast at ports in the North Sea, while 3 did so in the Baltic Sea, supporting the hypothesis that these two areas are important donors of NIS to the Great Lakes (Ricciardi and MacIsaac 2000; MacIsaac et al. 2001; Holeck et al. 2004).

While we have described the propagule supply of resting stages associated with NOBOB ships in the transportation stage, measurement error associated with any parameters in the propagule-pressure model could have significant consequences, and caution must be used in interpreting these data. Furthermore, we can make only preliminary estimations of the subsequent success of NIS carried as resting stages at the introduction and establishment stages of the invasion process. Ongoing in-situ studies should help further refine the risks associated with release of propagules from NOBOB vessels by assessing hatch rates of invertebrate resting stages from ballast sediments under operational conditions.

## Acknowledgements

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# Appendix A.

**Table A1.** List of invertebrate taxa hatched from resting stages during this study, arranged taxonomically.

| | Occurrence[a] | Abundance[b] | Experiment type[c] | |
| --- | --- | --- | --- | --- |
| | | | Maximum diversity | Whole sediment |
| **Gastrotricha** | | | | |
| Chaetonotidae, unidentified | 1 | 3 | × | |
| **Rotifera** | | | | |
| *Ascomorpha ecaudis* | 1 | 0.25 | × | |
| *Ascomorpha saltans* | 1 | 0.25 | × | |
| *Ascomorpha* sp. | 1 | 1 | × | |
| *Asplanchna brightwelli* | 4 | 0.25–1 (0.75) | × | × |
| *Asplanchna girodi* | 2 | 0.5 (0.5) | × | × |
| *Asplanchna priodonta* | 3 | 1–11.5 (3) | × | × |
| *Brachionus angularis* | 21 | 0.25–21.8 (4) | × | × |
| *Brachionus bennini* | 1 | 0.25 | × | |
| *Brachionus budapestinensis* | 15 | 0.75–341.5 (3) | × | × |
| *Brachionus calyciflorus* | 25 | 0.63–77.77 (3) | × | × |
| *Brachionus caudatus* | 2 | 0.5–2 (1.25) | × | |
| *Brachionus diversicornis* | 1 | 0.25 | × | |
| *Brachionus forficula* | 1 | 1 | × | |
| *Brachionus havanaënsis* | 2 | 1 (1) | × | |
| *Brachionus leydigi* | 4 | 0.25–1 (0.78) | × | × |
| *Brachionus nilsoni* | 1 | 1 | × | |
| *Brachionus quadridentatus* | 4 | 0.5–12.25 (1.25) | × | × |
| *Brachionus urceolaris* | 9 | 0.25–78 (1) | × | × |
| *Cephalodella catellina* | 2 | 0.25 (0.25) | × | |
| *Cephalodella forficula* | 1 | 0.25 | × | |
| *Cephalodella* cf. *stenroosi* | 1 | 0.3 | | × |
| *Cephalodella sterea* | 1 | 4.75 | × | |
| *Cephalodella* cf. *theodora* | 1 | 0.25 | × | |
| *Cephalodella* sp. | 1 | 1 | × | |
| *Conochilus coenobasis* | 1 | 0.5 | × | |
| *Conochilus dossuarius* | 1 | 1 | × | |
| *Conochilus hippocrepis* | 2 | 1 (1) | × | |
| *Conochilus* cf. *natans* | 1 | 0.25 | × | |
| *Conochilus unicornis* | 1 | 0.8 | | × |
| Dicranophoridae, unidentified | 1 | 83 | × | |
| *Euchlanis* cf. *dilatata* | 2 | 0.25–1 (0.63) | × | |
| *Filinia brachiata* | 1 | 0.25 | × | |
| *Filinia cornuta* | 3 | 0.5–1 (0.5) | × | |
| *Filinia longiseta* | 6 | 0.25–4 (1) | × | |
| *Filinia passa* | 4 | 0.25–1 (0.75) | × | |

**Table A1** (*continued*).

| | Occurrence[a] | Abundance[b] | Experiment type[c] | |
|---|---|---|---|---|
| | | | Maximum diversity | Whole sediment |
| *Filinia terminalis* | 5 | 0.38–2.5 (1) | × | |
| Flosculariidae, unidentified | 1 | 0.25 | × | |
| *Hexarthra intermedia* | 1 | 0.25 | × | |
| *Hexarthra mira* | 3 | 0.25–1 (1) | × | |
| *Keratella cochlearis* | 3 | 0.25–1 (1) | × | |
| *Keratella quadrata* | 5 | 0.25–4 (0.5) | × | × |
| *Keratella tropica* | 1 | 2 | × | |
| *Keratella* sp. | 1 | 1 | × | |
| *Lacinularia* sp. | 1 | 0.25 | × | |
| *Lecane closterocerca* | 2 | 0.3–0.5 (0.4) | × | × |
| *Lecane flexilis* | 1 | 0.25 | × | |
| *Lindia truncata* | 1 | 0.5 | × | |
| *Ploesoma truncatum* | 3 | 0.25–2 (2) | × | |
| *Polyarthra dolichoptera* | 9 | 0.5–5 (1) | × | |
| *Polyarthra vulgaris* | 6 | 0.25–21 (2) | × | |
| *Polyarthra* spp. | 2 | 0.25–1 (0.63) | × | |
| *Pompholyx sulcata* | 4 | 0.25–7 (3.5) | × | |
| *Synchaeta bacillifera* | 1 | 2.25 | × | |
| *Synchaeta baltica* | 1 | 2.75 | | × |
| *Synchaeta kitina* | 1 | 0.25 | × | |
| *Synchaeta oblonga* | 1 | 0.25 | × | |
| *Synchaeta stylata* | 4 | 0.25–1 (0.28) | × | × |
| *Synchaeta tremula* | 2 | 1–3.5 (1) | × | × |
| *Synchaeta* sp. | 1 | 0.25 | | × |
| *Trichocerca multicrinis* | 1 | 39 | × | |
| *Trichocerca pusilla* | 7 | 1–17.63 (1.25) | × | |
| *Trichocerca rattus* | 1 | 1 | × | |
| *Trichocerca similis* | 1 | 0.25 | × | |
| Monogonont, unidentified | 2 | 1 (1) | × | |
| **Bryozoa** | | | | |
| *Plumatella casmiana* | 2 | 0.25–1 (0.63) | × | |
| *Plumatella* sp. | 1 | 0.25 | × | |
| **Anomopoda** | | | | |
| *Alona rectangula* | 1 | 0.5 | × | |
| *Alona rustica* | 1 | 0.25 | × | |
| *Bosmina liederi* | 3 | 1–6 (1) | × | |
| *Bosmina maritima* | 1 | 2 | × | |
| *Bosmina* spp. | 2 | 1 (1) | × | |
| *Ceriodaphnia quadrangula* | 1 | 0.25 | × | |
| *Ceriodaphnia* sp. | 2 | 1 (1) | × | |
| *Daphnia longiremis* | 2 | 1 (1) | × | |
| *Daphnia magna* | 4 | 0.5–2 (1) | × | × |
| *Daphnia retrocurva* | 1 | 2 | × | |
| *Disparalona leei* | 1 | 0.25 | × | |
| *Moina micrura* | 2 | 1–47.88 (24.44) | × | |
| *Moina* sp. | 1 | 1 | × | |
| **Ctenopoda** | | | | |
| *Diaphanosoma birgei* | 2 | 0.75–6 (3.38) | × | |
| *Diaphanosoma brachyurum* | 1 | 0.25 | × | |
| *Diaphanosoma mongolianum* | 1 | 0.5 | × | |
| *Diaphanosoma orghidani* | 1 | 1.25 | × | |
| *Diaphanosoma sarsi* | 1 | 0.25 | × | |
| *Diaphanosoma* spp. | 6 | 1 (1) | × | |
| **Onychopoda** | | | | |
| *Evadne nordmanni* | 1 | 0.5 | × | |

**Table A1** (*concluded*).

| | Occurrence[a] | Abundance[b] | Experiment type[c] | |
| | | | Maximum diversity | Whole sediment |
|---|---|---|---|---|
| **Copepoda** | | | | |
| *Acanthocyclops robustus* | 1 | 0.8 | | × |
| Cyclopoida, unidentified | 3 | 0.25–1.25 (0.25) | | × |
| *Nitocra lacustris* | 1 | 1 | | × |
| Copepod nauplii, unidentified | 14 | 0.25–20 (3) | × | × |

[a]The number of ships on which the species was collected, of a possible 35.

[b]The range of numbers of individuals emerging from 40 g of sediment for all ships, with the median number in parentheses.

[c]The taxa present in maximum-diversity (isolated from sediment) and whole-sediment (buried in sediment) trials. All were able to hatch in 0‰ medium, except *S. bacillifera*, *Synchaeta* sp., and *E. nordmanni*, which hatched exclusively in 8‰, and *S. baltica*, which hatched exclusively in 32‰.