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Invertebrates and their dormant eggs transported in ballast sediments of ships arriving to the Canadian coasts and the Laurentian Great Lakes

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Abstract

The most effective strategy for managing nonindigenous species (NIS) is through prevention of their transport via regulation of introduction vectors. We sampled 135 ships arriving to three different regions of Canada to assess abundance and species richness of invertebrates and their dormant eggs transported in ballast sediments. By sampling ships that followed particular pathways, we were able to compare vector strength to different regions, the invasion risk of transoceanic vs. coastal vessels, and the effect of midocean exchange, length of voyage, and amount of sediment on the richness and abundance of species inside ballast tanks. Although standardized ballast management regulations have been implemented across Canada, the resulting invasion risk is not uniform across regions. Ships arriving to the Atlantic region carried a greater sediment load with correspondingly higher abundance and species richness than those arriving to the Pacific and Great Lakes regions. Abundance and species richness of invertebrates and their dormant eggs associated with transoceanic ships did not differ from that of ships operating along coastal areas of North America. Similarly, midocean exchange did not reduce either abundance or species richness of invertebrate dormant eggs in ships. Finally, the length of voyage did not influence taxonomic composition or abundance of invertebrate dormant eggs but was directly related to survival of active macroinvertebrates. Ballast sediments could introduce new NIS to some regions of Canada despite requirements to manage ships' ballast by midocean exchange. Minimizing sediment accumulation may be the only effective management option for this vector.

The introduction of species into habitats outside of their native ranges represents an enormous conservation and management challenge (Leppäkoski et al. 2002). Nonindigenous species (NIS) may act as predators, parasites, or pathogens on or as competitors with native species, and their introduction can result in significant ecosystem changes (Leppäkoski et al. 2002). Freshwater and marine invertebrates may disperse naturally by water currents, wind, or animal vectors (Bilton et al. 2001). However, current rates of human-mediated dispersal can be immensely greater than natural historical rates—an estimated 50,000 times greater for freshwater zooplankton to the Laurentian Great Lakes (Hebert and Cristescu 2002). The transport and release of ballast water has led to establishment of hundreds of NIS in freshwater, brackish, and marine ecosystems throughout Europe and North America (Mills et al. 1996; Bij de Vaate et al. 2002). While the invasion process can be divided into several stages, including introduction, establishment, and spread (Colautti and MacIsaac 2004), cost-effective management of NIS is most successfully conducted at the introduction stage through prevention efforts (Simberloff 2009*a*).

Ships' ballast water regulations have been enacted by the United States and Canada to manage the introduction of new NIS. First, midocean exchange (MOE) was recommended in 1989 and became mandatory in 1993 for vessels entering the Great Lakes with filled ballast tanks in an effort to reduce the number of species transported to the lakes (Canadian Coast Guard 1989; U.S. Coast Guard 1993). Beginning in 2006, this regulation was extended to the Pacific and Atlantic coasts of Canada and was further improved by including management of residual ballast water and accumulated sediments through mandatory saltwater flushing for vessels carrying only ballast residuals (Government of Canada 2006; Saint Lawrence Seaway Development Corporation [SLSDC] 2008). By purging fresh or coastal water, sediments, and taxa contained therein from tanks and by exposing taxa remaining in tanks to euhaline water, MOE and saltwater flushing should reduce both abundance and species richness inside tanks (MacIsaac et al. 2002; Briski et al. 2010).

Invertebrate species may be present in ballast tanks as active or dormant stages (e.g., dormant eggs, resting eggs, statoblasts, and cysts; Cáceres 1997; Bailey et al. 2005; Duggan et al. 2005). The effect of MOE and saltwater flushing on active and dormant stages of invertebrates has been assessed in a number of studies with varying results (Gray et al. 2007; Humphrey 2008; Briski et al. 2010). Humphrey (2008) compared the invasion risk posed by invertebrate active (i.e., planktonic) stages arriving in ballast water to different regions of Canada and found significantly higher total zooplankton density in ships arriving to the Atlantic region than in those arriving to the Pacific and Great Lakes regions. This work is a complementary study to Humphrey (2008), as it assessed the invasion risk posed by invertebrates and their dormant eggs in ballast sediment. No similar studies have been conducted for invertebrate dormant eggs in ballast sediment. As eggs buried in sediment are possibly protected from saltwater exposure during ballast management activities (Bailey et al. 2006; Briski et al. 2010), this vector may be more important than the transport of active invertebrates in water. In

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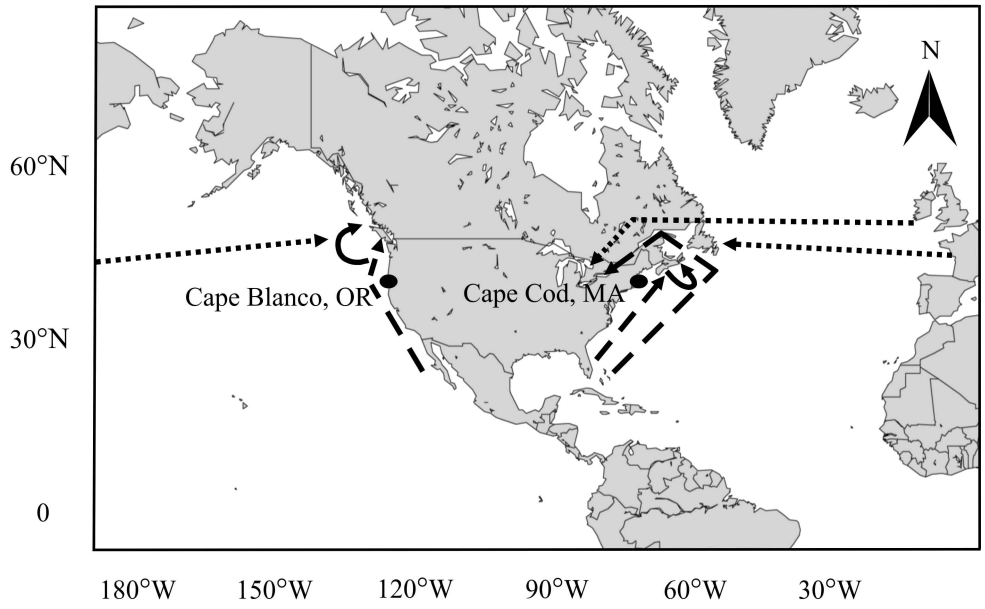

Fig. 1. Schematic representation of pathways utilized by ships arriving to the Pacific, Great Lakes, and Atlantic regions. Dotted lines represent transoceanic voyages with midocean exchange (TOE), dashed lines represent coastal voyages with midocean exchange (CE), and solid lines represent coastal voyages exempt from midocean exchange (CNE). Vessels arriving from ports north of Cape Blanco, Oregon (OR), or Cape Cod, Massachusetts (MA), are exempt from midocean exchange requirements if destined for specific Canadian ports on the Pacific or Atlantic coasts, respectively (Government of Canada 2006).

addition, ballast water studies have typically focused on transoceanic ships while neglecting intracoastal ship traffic (Rup et al. 2010). It is possible that intracoastal shipping could represent an important vector depending on the proportion of total ship traffic that it represents (Rup et al. 2010; 71% in the Great Lakes). Many intracoastal ship transits are exempt from mandatory flushing and exchange regulations on the presumption that movement of ballast water is occurring only within a single marine ecoregion and thus is viewed as nonrisky (Government of Canada 2006; SLSDC 2008). This assumption may not be valid if NIS established in one part of the region are transferred in ballast water to another location in the ecoregion where the NIS is not present (Rup et al. 2010). Such intracoastal transfers could be particularly effective at introducing NIS since these voyages typically are of relatively short duration, with relatively high survivorship of NIS in ballast tanks in consequence (Humphrey 2008). While prolonged exposure to the hostile environment of ballast tanks (e.g., no light, reduced dissolved oxygen; Reid et al. 2007) reduces survival of active invertebrates, this may not be the case for their dormant eggs.

In view of the varying results of ballast water regulations from previous studies (Gray et al. 2007; Humphrey 2008; Briski et al. 2010), the variation in density of active zooplankton transported to different regions (Humphrey 2008), and the high survivorship of active zooplankton during relatively short voyages (Humphrey 2008), we compared abundance and species richness of invertebrates and their dormant eggs in ballast sediment sampled from ships that used different pathways to the Pacific and Atlantic regions of Canada and to the Laurentian Great Lakes. Ship pathways considered include transoceanic voyages with MOE (''transoceanic exchanged'' [TOE]), coastal voyages with MOE (''coastal exchanged'' [CE]), and coastal voyages without MOE (''coastal not exchanged'' [CNE]). By sampling ships on particular pathways, we were able to test five hypotheses: (1) the invasion risk posed by invertebrates and their dormant eggs transported in ballast sediment does not vary among regions, (2) the invasion risk posed by ships on transoceanic and coastal voyages is equal, (3) the invasion risk posed by ships performing MOE and those exempt from MOE is equal, (4) the length of voyage does not effect either abundance or species richness inside tanks, and (5) the amount of sediment in tanks does not effect either abundance or species richness.

Methods

Ships' particulars and sediment estimation—Ships sampled in this study serviced both international and domestic Canadian routes (Fig. 1), although those arriving to the Atlantic and Great Lakes regions typically operated between Atlantic ports, whereas those arriving to Pacific Canadian ports traveled mainly among Pacific ports (Table 1). Ballast water reporting forms, which included total ballast capacity, previous dates and locations of ballast uptake and discharge, and last port of call, were used to discern pathways of operation. TOE ships were defined as ships arriving from any continent except North America. CE ships arrived from ports in the southern

Table 1. Summary statistics for ships sampled in three regions (Pacific, Great Lakes, and Atlantic) of North America by broad geographic route. Ship pathways are transoceanic with midocean exchange (TOE), coastal with midocean exchange (CE), and coastal exempt from midocean exchange (CNE). Individual ship transits have been grouped according to country or state of last port of call and region of arrival. The number of samples, mean voyage duration, and mean density of dormant eggs per 40-g subsample of sediment and per 10,000 m^3 of ballast capacity are indicated for each route with standard error (SE) when applicable. Note the scale (10^5) for dormant egg density per 10,000 m^3 of ballast capacity.

Region	Ship pathway	Last port of call	No. of samples	Mean (SE) voyage duration	Mean (SE) egg density per 40 g	Mean (SE) egg density per 10,000 m^3 ballast capacity^{-1} ($\times 10^5$)
Pacific	TOE	China	8	19.5 (1.5)	9.8 (3.9)	2.5 (1.4)
		Japan	9	18.6 (1.6)	2.2 (1.5)	0.06 (0.03)
		South Korea	1	30	7.5	0.7
		Taiwan	2	27.5 (6.5)	0 (0)	0 (0)
	CE	California	15	11.8 (1.4)	21.2 (8.3)	1.5 (0.7)
		Guatemala	2	14.5 (0.5)	0 (0)	0 (0)
		Mexico	2	12.5 (1.5)	5.5 (5.5)	1.5 (1.5)
	CNE	British Columbia, Canada	1	6	0	0
		Oregon	6	8.6 (1.5)	6.5 (4.5)	0.4 (0.4)
		Washington	14	7.3 (2.0)	3.9 (2.6)	1.2 (1.1)
Great Lakes	TOE	Belgium	2	27.5 (3.5)	17.1 (2.3)	0.2 (0.01)
		Brazil	1	36	15.25	1.2
		Germany	4	18.0 (1.1)	20.2 (8.7)	36.2 (34.1)
		Greece	1	37	2	0.05
		Italy	2	34.0 (17.0)	4.0 (3.0)	0.5 (0.3)
		Netherlands	1	43	1	0.01
		Spain	1	15	7.5	1.7
		United Kingdom	2	22.5 (8.5)	54.5 (48.0)	85.1 (84.9)
	CE	Massachusetts	1	9	41.5	47.7
		New Brunswick, Canada	2	15.0 (0.0)	0 (0)	0 (0)
		New Jersey	2	20.5 (11.5)	33.2 (20.5)	6.5 (6.2)
Atlantic	TOE	Belgium	3	11.0 (3.0)	96.8 (52.3)	130.0 (90.4)
		Brazil	1	15	0	0
		France	1	44	129.5	47.1
		Italy	1	15	2.75	0.06
		Israel	1	18	11.5	22.2
		Netherlands	2	12.5 (2.5)	162.5 (31.5)	68.5 (65.4)
		Poland	1	13	0	0
		Spain	1	12	13.75	34.9
		Turkey	2	19.5 (0.5)	5.8 (5.6)	16.4 (16.3)
		United Kingdom	9	11.12 (0.9)	155.3 (104.2)	160.0 (88.3)
	CE	Florida	1	2	1.5	0.08
		Louisiana	1	23	6	0.06
		Maryland	2	9.0 (2.0)	412.7 (338.7)	42.8 (36.2)
		Massachusetts	2	4.0 (0.0)	12.8 (11.9)	4.3 (2.1)
		New Hampshire	2	4.0 (0.0)	34.8 (4.1)	260.0 (70.5)
		New York	5	5.0 (0.0)	138.1 (126.6)	26.2 (10.3)
		Pennsylvania	4	6.2 (1.4)	34.1 (17.6)	250.0 (180.0)
		Virginia	3	10.0 (2.1)	16.6 (11.8)	11.7 (5.8)
	CNE	Maine	5	2.2 (0.4)	24.7 (17.5)	4.3 (2.8)
		Massachusetts	6	1.6 (0.2)	103.0 (63.7)	28.3 (23.7)
		New Brunswick, Canada	2	6.5 (3.5)	152.0 (15.5)	370.0 (340.0)
		Newfoundland, Canada	4	9.5 (2.2)	219.1 (119.6)	53.7 (43.1)
		Nova Scotia, Canada	3	5.3 (2.3)	0 (0)	0.0 (0.0)
		Prince Edward Island, Canada	1	6	50.25	3.1

Table 2. Sampling scheme for ships sampled in three regions (Pacific, Great Lakes, and Atlantic) of North America by ship pathway: transoceanic with midocean exchange (TOE), coastal with midocean exchange (CE), and coastal exempt from midocean exchange (CNE).

	Pacific region				Great Lakes region			Atlantic region			
	Total	TOE	CE	CNE	Total	TOE	CE	Total	TOE	CE	CNE
No. of ships sampled	60	20	19	21	17	13	4	58	22	20	16
No. of tanks sampled	60	20	19	21	19	14	5	63	22	20	21
No. of ships with two tanks sampled					2	1	1	5			5
No. of tanks with four spatial samples sampled					6	6					

United States, defined as south of Cape Blanco, Oregon on the Pacific coast, and south of Cape Cod, Massachusetts, on the Atlantic coast. CNE ships arrived from American and Canadian ports north of Cape Blanco on the Pacific coast and Cape Cod on the Atlantic coast and were exempt from MOE (Canadian Government 2006; Fig. 1). Ships operating in different regions tended to vary in size, with a mean ballast capacity of 21,835, 11,726, and 32,381 m^3 for Pacific, Great Lakes, and Atlantic regions, respectively. Voyage length also varied across pathways, with mean durations of 19.5, 10.2, and 6.0 days for TOE, CE, and CNE pathways, respectively (Table 1).

Sampling—One hundred and forty-two sediment samples from 135 ships were collected between May 2007 and August 2009, inclusive (Table 2). We sampled 20 TOE, 19 CE, and 21 CNE tanks in the Pacific region. Comparable numbers in the Atlantic region were 22 TOE, 20 CE, and 21 CNE tanks, while in the Great Lakes region we sampled 14 TOE and five CE tanks (Table 2). In seven cases, results from two tanks sampled from the same ship were averaged following Bailey et al. (2005). All sampled tanks were inspected for presence of macroinvertebrates such as Cnidaria, Decapoda, Gastropoda, and Bivalvia, which were collected when found. Sampling teams also inspected sediment depth and percent cover inside ballast tanks. Information on the amount of sediment per tank, combined with architectural diagrams of ships' tanks, was used to estimate the amount of sediment carried per ship; since ship size varied by region, we used a standardized 10,000-m^3 ballast capacity for shipwise comparisons. Whenever possible, 6 kg of sediment were collected per tank. In all cases, sediment was collected from different areas inside the ballast tank and homogenized. The only exception to this protocol occurred with six TOE tanks in the Great Lakes region for which four different areas inside the tanks were sampled and processed separately to determine if spatial differences in biological composition exist within tanks: Sorensen's coefficient of similarity was calculated for 16 pairs of samples randomly selected from within tanks and compared to those of 16 randomly drawn pairs of samples between tanks from different ships using a Mann–Whitney *U*-test (SPSS version 11.5.0). Furthermore, to determine if the density of dormant eggs within tanks varied, we compared egg density counts of four replicated samples taken from six TOE tanks in the Great Lakes region using one-way analysis of variance (ANOVA; SPSS version 11.5.0) following a logarithmic transformation to meet assumptions of parametric test. Since Sorensen's coefficients of similarity for 16 randomly drawn pairs of samples within tanks were significantly higher than those of 16 randomly drawn pairs of samples between tanks from different ships (means of 0.85 and 0.17, respectively; Mann–Whitney *U*-test, $Z = -4.423$, $p < 0.05$) and densities of eggs collected from different areas in tanks did not differ significantly from each other (ANOVA, $F_{3,20} = 0.042$, $p > 0.05$), data from replicated samples were averaged and processed as single samples.

Density counts, viability experiment, and identification—Macroinvertebrates were separated from sediment and stored in 95% ethanol until processed. Sediment was thoroughly homogenized in the laboratory and stored at 4°C. Four 40-g subsamples were taken from each sediment sample for egg density counts (Briski et al. 2010). Eggs were separated from sediment using colloidal silica Ludox® HS 40 (Briski et al. 2010). Dormant eggs were enumerated under a dissecting microscope and grouped by size and gross morphology, and a maximum of 20 dormant eggs per morphological group was taken for molecular identification (Briski et al. 2010). Identification of dormant eggs was conducted using molecular methods and traditional morphological taxonomy of hatched individuals (Briski et al. 2010).

The remaining sediment was stored for 4 weeks to break the diapause of dormant eggs before hatching experiments were conducted (Cáceres 1997; Briski et al. 2010). For hatching experiments, dormant eggs were isolated from 40-g sediment subsamples using a sugar flotation method (Briski et al. 2010). Extracted dormant eggs were placed in vials containing sterile synthetic pond water (salinity of zero; Briski et al. 2010) or a sterile seawater medium with salinity of 15‰ or 30‰ (Briski et al. 2010). Four replicates were placed in each of the 0‰, 15‰, and 30‰ treatments at 20°C. We used the number of hatched eggs as a proxy measure of egg viability, although we acknowledge that some eggs that did not hatch may have been viable but did not receive appropriate hatching cues (Cáceres 1997).

Statistical analysis—Variation in density and viability of dormant eggs in 40-g sediment subsamples between regions and between different ship pathways within regions were analyzed using nested ANOVA, where ship pathways were nested within regions. Additionally, post hoc Bonferroni tests were applied (SPSS version 11.5.0). Significance levels for statistical comparisons were adjusted for multiple pairwise comparisons by Bonferroni-type correction with a family-wise error rate of 0.05. After completing analyses

of 40-g sediment subsamples, additional tests were conducted on extrapolated egg abundance and number of viable eggs by multiplying the average of four 40-g subsamples from each ship by the amount of sediment carried per 10,000-m^3 ballast capacity for that ship. Sediment weight and extrapolated egg abundance and their viability were compared to find possible differences per region and per ship pathway using two-way multivariate analysis of variance (SPSS version 11.5.0).

Species richness of the larger ship population was estimated based on the number of rare species recorded from sampled vessels using a first-order jackknife method (Chao and Shen 2006). Separate richness estimates were generated for the three regions and three ship pathways, enabling examination of region or application of MOE as determining factors. Sample-based species rarefaction curves were generated and contrasted for all sampling regions and pathways. Confidence intervals (95%) were generated to test for significant differences between sampling regions and ship pathways (Chao and Shen 2006; Gotelli and Entsminger 2006). First-order jackknife estimates were calculated using species prediction and diversity estimation (SPADE) software (Chao and Shen 2006), while rarefaction curves were generated with 5000 random iterations using ecological simulation (EcoSim; Gotelli and Entsminger 2006).

We used parametric (Pearson's) correlation to explore the relationship between voyage length (calculated using ballast water reporting forms) and density and viability of dormant eggs in 40 g of sediment. We also used Pearson's correlation analysis to explore the relationship between sediment weight inside tanks with density and viability of dormant eggs in 40-g sediment subsamples and with density of dormant eggs of NIS in 40-g sediment subsamples. All data were log transformed to meet statistical assumptions. Macroinvertebrates were excluded from 40-g subsample analyses because they were quantified on a tankwise basis rather than per 40 g of sediment.

Results

Invasion risk by region—Our samples revealed significant regional variation in abundance and species richness of invertebrates and their dormant eggs, with ships arriving to the Atlantic region carrying a two- and one-order-of-magnitude higher abundance of invertebrate dormant eggs than those arriving to the Pacific and Great Lakes regions, respectively (Tables 3–5). Active macroinvertebrates were found only in ships arriving to the Atlantic region. Maximum density of macroinvertebrates was two individuals per tank (Table 6).

Seventeen, 24, and 29 distinct species of zooplankton and macroinvertebrates, representing 14 taxonomic groups, were identified from the Pacific, Great Lakes, and Atlantic region samples, respectively (Table 6). Corresponding richness estimates for the vessel populations were 18, 29, and 37 species, respectively (Fig. 2). Species richness estimates were significantly higher for the Great Lakes and Atlantic regions than for the Pacific region (Fig. 2). Copepods were the dominant taxonomic group found in ships, representing 66%, 35%, and 88% of total taxa arriving to the Pacific, Great Lakes, and Atlantic regions, respectively. Cladocerans represented 26%, 32%, and 7%, respectively, while rotifers represented 4%, 26%, and 3%, respectively. All other taxonomic groups represented less than 1% of taxa in all three regions.

Three of 17 species identified in the Pacific region were nonindigenous freshwater cladocerans (*Bosmina freyi*, *Ceriodaphnia dubia*, and *Daphnia retrocurva*) that are likely incapable of surviving in the primarily marine conditions in the region (Table 6). In the Great Lakes, five of 24 species identified were NIS (*Daphnia magna*, *Cergopagis pengoi*, *Pleopis polyphemoides*, *Podon intermedius*, and *Acartia tonsa*), including two taxa capable of survival in the Great Lakes (*D. magna* and *A. tonsa*) and one species already established there (*C. pengoi*; Table 6). In the Atlantic region, nine of 29 distinct species were NIS (*Hyalinella punctata*, *Plumatella emarginata*, *D. magna*, *Daphnia galeata*, *Daphnia cucullata*, *Calanus euxinus*, *Carcinus maenas*, *Littorina littorea*, and *Hyotissa numisma*), including two species able to tolerate salinity conditions typical in the Atlantic region (*C. euxinus* and *H. numisma*) and two already established there (*C. maenas* and *L. littorea*; Table 6).

Invasion risk by ship pathway and exchange status—Our results showed that there was no significant difference in taxonomic composition or abundance of invertebrates or their dormant eggs between transoceanic and coastal vessels or between vessels performing MOE and those exempt from MOE. Neither dormant egg densities nor viability were significantly different between pathways within any of the three regions (Tables 1, 4, and 5). Eight, eight, and nine distinct species were identified from the TOE, CE, and CNE samples in the Pacific region, respectively (Table 6). Thirty and nine distinct species were identified from the TOE and CE samples in the Great Lakes region, respectively, while 18, 19, and 19 species were identified from the TOE, CE, and CNE samples in the Atlantic region, respectively (Table 6). Corresponding species richness estimates were not significantly different for ship pathways within the Pacific and Atlantic regions; however, a significant difference was observed between pathways in the Great Lakes region (Fig. 2).

Effect of voyage length and amount of sediment—The length of voyage did not have any influence on the abundance or viability of invertebrate dormant eggs (Pearson's correlation, $r^2 = 0.017$, $p = 0.120$, and $r^2 = 0.005$, $p = 0.403$, respectively; Table 1), although short voyages were positively related to presence of active macroinvertebrates inside tanks. Active macroinvertebrates were found only in ships with voyage length of 5 d or less.

Sediment tended to accumulate peripherally in tanks, with only a thin layer spread across the tank bottom (mean depth 3 mm); maximum bottom area coverage was 100%, and maximum depth was 25 cm. Sediment weight (per 10,000-m^3 ballast capacity) of TOE and CE ships in the Atlantic region averaged 9800 and 14,300 kg, respectively (Table 3). CE ships from the Great Lakes region average

Table 3. Mean (standard error [SE]) and median dormant egg density and viability per 40-g subsample of sediment collected from ships arriving to three regions (Pacific, Great Lakes, and Atlantic) of North America by ship pathway: transoceanic with midocean exchange (TOE), coastal with midocean exchange (CE), and coastal exempt from midocean exchange (CNE). Amount of sediment and dormant egg density and viability per 10,000 m^3 of ballast capacity are also indicated to standardize for differences in ship size. Note the difference in scale (10^6 vs. 10^5) for dormant egg density and viability per 10,000 m^3 of ballast capacity, respectively.

Region	Ship pathway	Density per 40 g		Viability per 40 g		Sediment per 10,000 m^3 ballast capacity^{-1} (kg)		Density per 10,000 m^3 ballast capacity^{-1} ($\times 10^6$)		Viability per 10,000 m^3 ballast capacity^{-1} ($\times 10^5$)	
		Mean (SE)	Median	Mean (SE)	Median	Mean (SE)	Median	Mean (SE)	Median	Mean (SE)	Median
Pacific	TOE	5.2 (1.8)	1	0	0	2500 (1100)	500	0.3 (0.1)	0.02	0	0
	CE	17.3 (6.8)	4.2	0	0	1500 (700)	500	0.2 (0.08)	0	0	0
	CNE	4.5 (2.1)	0	0	0	3600 (1800)	200	0.1 (0.1)	0	0	0
Great Lakes	TOE	18.4 (7.1)	11.0	1.2 (0.5)	0.4	2500 (1300)	300	3.4 (2.2)	0.03	1.9 (1.3)	0.01
	CE	33.6 (7.5)	40.5	1 (0.6)	0.4	16,000 (12,400)	6000	8.6 (6.0)	1.9	0.7 (0.6)	0.03
Atlantic	TOE	129 (47.6)	52.1	4.5 (2.4)	0	9800 (2000)	7700	28.6 (14.2)	5.5	5.1 (3.2)	0
	CE	84 (43.2)	16.7	4.9 (4.2)	0	14,300 (3900)	5200	13.9 (6.0)	2.3	7.4 (4.1)	0
	CNE	100 (27.2)	48.5	29.8 (25.5)	0	2100 (1100)	900	7.8 (5.1)	0.5	19.5 (13.5)	0

Table 4. Results of nested analyses of variance addressing density and viability of dormant eggs in 40-g sediment subsamples collected from three different ship pathways (transoceanic with midocean exchange [TOE], coastal with midocean exchange [CE], and coastal exempt from midocean exchange [CNE]) in three regions (Pacific, Great Lakes, and Atlantic) of North America.

Variable	Density			Viability		
	df	F	p	df	F	p
Intercept	1	179.04	0.000	1	24.846	0.001
Region	2	19.123	0.002	2	10.437	0.005
Pathway (region)	5	1.672	0.146	5	0.738	0.596

16,000 kg of sediment. All other ships carried an average of $<$ 4000 kg of sediment (Table 3). Further, ships containing larger amounts of ballast sediment carried higher densities of dormant eggs (Pearson's correlation, $r^2 = 0.029$, $p = 0.022$). However, sediment volume in ballast tanks was not correlated with viability ($r^2 = 0.001$, $p = 0.339$) or density of NIS dormant eggs ($r^2 = 0.018$, $p = 0.056$).

Discussion

The risk of aquatic invasions from ships ballast sediment is not uniform across regions despite implementation of common, national management practices across Canada. We found that ships arriving to the Atlantic region not only carry more ballast sediment than do those arriving to the Pacific and Great Lakes regions but also transported more species at higher abundance within that sediment. The amount of sediment carried by ships may depend on the geography of ship activities. TOE and CE ships in the Atlantic region typically carried an order of magnitude more ballast sediment than those in the Pacific or Great Lakes regions. As ships arriving to the Atlantic region frequently visit ports that are shallow and sandy in the North and Baltic Seas, the high amount of accumulated sediment found in their ballast tanks is not surprising. In contrast, TOE and CE ships arriving to the Pacific region operate almost exclusively at Pacific ports, which are mainly rocky and deep. TOE ships servicing the Great Lakes region contained less sediment than those arriving to the Atlantic region even though they operate largely between the same ports. Approximately 90% of tanks on ships entering the Great Lakes are "empty" (MacIsaac et al. 2002) and managed with saltwater flushing rather than MOE, which may more effectively purge accumulated sediments (Briski et al. 2010); however, the varying degrees of enforcement of regulations in the two regions may confound our ability to assess effects of management practices directly.

While MOE effectively manages active invertebrates in ballast water (Gray et al. 2007; Humphrey 2008), we found that it had no discernible effect on dormant eggs in ballast sediment. This could be attributed to the unique ability of dormant eggs to withstand very harsh environments (Cáceres 1997), remaining viable inside ballast tanks from several months to longer than a year (Briski et al. 2011).

Table 5. Results of two-way multivariate analysis of variance addressing the effect of region and ship pathway on the amount of sediment, abundance of dormant eggs, and their viabilities.

Variable	df	*F*	*p*
Region			
Univariate *F*-tests			
Sediment	2	10.428	<0.001
Abundance of dormant eggs per ship	2	36.696	<0.001
Viability of dormant eggs per ship	2	17.099	<0.001
Multivariate test			
Wilks's lambda=0.576	6	13.950	<0.001
Ship pathway			
Univariate *F*-tests			
Sediment	2	5.273	0.006
Abundance of dormant eggs per ship	2	0.839	0.434
Viability of dormant eggs per ship	2	0.223	0.800
Multivariate test			
Wilks's lambda=0.919	6	1.889	0.083
Interaction			
Univariate *F*-tests			
Sediment	3	5.656	0.001
Abundance of dormant eggs per ship	3	0.953	0.417
Viability of dormant eggs per ship	3	0.145	0.932
Multivariate test			
Wilks's lambda=0.847	9	2.527	0.008

Furthermore, ballast tanks with greater sediment buildup contained higher densities of dormant eggs, implying that thicker sediment provides more protection for dormant eggs (Bailey et al. 2006; Briski et al. 2010).

As with MOE, the length of voyage did not influence density or viability of dormant eggs. However, this study indicates that active macroinvertebrates survived only in ships undertaking shorter voyages. Reid et al. (2007) reported a rapid decline of dissolved oxygen concentration inside ballast tanks, with 90% of initial oxygen content lost in 10 d at temperatures above 20°C. With shorter voyages, water inside tanks is renewed more frequently, which may enhance survival of active macroinvertebrates. As transatlantic voyages take approximately 15 d (Table 1; MacIsaac et al. 2002), there is low probability for transportation of active adult macroinvertebrates, such as crabs, bivalves, and gastropods, from Europe to North America. However, free-swimming larvae of these taxa have been found frequently in ballast waters of transoceanic ships even after MOE (Humphrey 2008), suggesting that species such as zebra mussel *Dreissena polymorpha*, quagga mussel *Dreissena rostriformis bugensis*, and the European green crab *Carcinus maenas* could have been transported to North America as free-swimming larvae in ships' ballast water.

Several species recorded during this study are NIS of global concern (Invasive Species Specialist Group [ISSG] 2011). For example, the fishhook waterflea *Cercopagis pengoi*, found in two Great Lakes ships, is already established in this system (ISSG 2011). *C. pengoi* is a predator and may suppress both invertebrate and vertebrate taxa (Leppäkoski et al. 2002; ISSG 2011). The European green crab *Carcinus maenas*, found in two ships servicing Atlantic Canadian ports, is a generalist predator that is attributed with the decline of other crab and bivalve species (ISSG 2011). Although these two NIS are already established in North America, a risk exists of increasing genetic variation with additional introductions of new individuals (Simberloff 2009*b*). Increased genetic variation from continued propagule pressure could lead to production of new genotypes that are better adapted to the local environment or adjacent areas, potentially enhancing further spread (Simberloff 2009*b*). Importantly, to our knowledge, this is the first record of these two species from ballast tanks, providing evidence that ballast water and sediment are active vectors of their human-mediated dispersal. Other problematic NIS (ISSG 2011) recorded in this study include the crab *Rhithropanopeus harrisii*, ascidian *Botryllus schlosseri*, and bivalve *Mytilus galloprovincialis*.

Numerous vectors operate in all biogeographic regions, with the relative importance of each vector changing through time (Ricciardi 2006). The cumulative propagule pressure has resulted in a diverse community of established NIS globally. Our study indicates that the relative importance of the ballast sediment vector also changes regionally within Canada, currently posing the greatest invasion risk for ports in the Atlantic region. With no active animals recorded and no viable dormant eggs, our study shows very low invasion risk for the Pacific region from this vector. Furthermore, considering that the Great Lakes receive visits by fewer and smaller ships than either Vancouver or Atlantic ports (S. Bailey unpubl.), we conclude that the relative risk of sediment-borne invasions is lower for this region. However, this conclusion should be taken with caution, as even small propagule number can

Table 6. List of invertebrate taxa identified in this study, arranged taxonomically. Egg taxa were identified directly from dormant eggs using mitochondrial markers cytochrome c oxidase subunit I (COI) and 16S rDNA (16S) following Briski et al. (2010) and morphologically after eggs hatched. Active animals were identified using mitochondrial markers COI and 16S and morphologically. Occurrence lists number of ships that the species was collected from, out of a possible 135. Abundance lists the range (median) number of eggs identified from 40-g sediment subsamples or range (median) number of active macroinvertebrates per tank (§). Nonindigenous species (NIS) are in bold. NIS capable of tolerating ambient salinity in the recipient port (II).

	Pacific region		Great Lakes		Atlantic region	
Taxon	Occurrence	Abundance	Occurrence	Abundance	Occurrence	Abundance
Porifera						
Ephydatia fluviatilis	1*	0.25				
Cnidaria						
Actinaria, unidentified					1‡	1§
Rotifera						
Asplanchna sp.	7‡	0.25–1 (0.25)			5*†	0.25–14.5 (1.75)
Brachionus calyciflorus	2*	0.25–3.25 (1.75)	8*†	0.25–43.25 (11.75)		
Brachionus plicatilis					18*†‡	0.25–25.5 (4.87)
Brachionus sp.			2*†	0.25–0.5 (0.37)		
Synchaeta sp.	6*†	0.25–7.25 (2.25)			13*†‡	0.25–6.75 (0.25)
Bryozoa						
Hyalinella punctata					**1†**	**4**
Plumatella casmiana			2*	0.25 (0.25)		
Plumatella emarginata			3*†	0.25–2.5 (0.25)	**28*†‡**	**0.25–12.25 (1.5)**
Plumatella reticulata			1*	0.25		
Pectinatella sp.	8*†‡	0.25–4 (0.25)				
Anomopoda						
Bosmina freyi	**3†**	**0.25–8.5 (6.25)**				
Bosmina sp.			1*	0.25	6†	0.25–8.5 (0.62)
Ceriodaphnia dubia	**3†**	**3–46.8 (4.255)**				
Daphnia magna			**2*†**	**0.25–51.5 (25.87)‖**	**11*†‡**	**0.25–21 (2.25)**
Daphnia retrocurva	**5***	**0.25–2.25 (0.25)**				
Daphnia mendotae			1*	1.75		
Daphnia parvula			2*	0.25–1.5 (0.87)		
Daphnia pulex			2*†	0.25–2.25 (1.25)		
Daphnia galeata			**1***	**2‖**	**2†**	**1.5–4 (2.8)**
Daphnia cucullata					**1***	**1.8**
Daphnia sp.	7*†	0.25–17.5 (5.75)	1*	0.25		
Ilyocryptus sp.					1†	3
Moina sp.			2*	0.5–1 (0.75)	1†	1
Anomopoda, unidentified			1*	70.25		
Ctenopoda						
Diaphanosoma brachyurum			2*	0.25–0.75 (0.5)		
Diaphanosoma sp.			1*	0.25	3*	1–2 (1.75)
Onychopoda						
Evadne nordmanni					19*‡	0.25–136.5 (9.75)
Cercopagis pengoi			**2***	**0.25–0.5 (0.37)‖**		
Pleopis polyphemoides	9‡	0.25–7 (0.75)	**1***	**0.25**	2†	2–9.75 (5.88)
Podon intermedius			**1†**	**0.25**		
Copepoda						
Acartia tonsa			**1***	**0.25‖**		
Acartia omorii	1‡	17.75				
Acartia pacifica	2†‡	6–116.75 (61.37)				
Calanus euxinus					**4***	**54–194 (157.88)‖**
Ctenocalanus vanus	1‡	26.75				
Diacyclops thomasi			1*	31.5		
Eurytemora affinis					8*†	1.75–652.5 (87.12)
Leptodiaptomus siciloides			1†	5		
Calanoida, unidentified	25*†‡	0.25–45 (4.25)	11*†	0.75–41.5 (7.5)	38*†‡	0.25–872.25 (20.87)
Harpacticoida, unidentified					9†‡	5.5–384 (14)

Table 6. Continued.

Taxon	Pacific region		Great Lakes		Atlantic region	
	Occurrence	Abundance	Occurrence	Abundance	Occurrence	Abundance
Amphipoda						
Americorophium sp.					1*	11
Eurythenes sp.	1†	5				
Parhyale sp.	1‡	1				
Decapoda						
Carcinus maenas					**2‡**	**2§‖**
Rhithropanopeus harrisii					1†	1§
Annelida						
Neanthes virens					1‡	1§
Platynereis sp.					2†	2–3 (2.5)§
Gastropoda						
Littorina Littorea					**1‡**	**2§‖**
Bivalvia						
Hyotissa numisma					**2†‡**	**1–2 (1.5)§‖**
Mya arenaria					4*†‡	1§
Mytilus galloprovincialis					1‡	1§
Scapharca sp.	1‡	1.25				
Ascidia						
Botryllus schlosseri			**1***	**0.25**		

* Transoceanic ship (TOE).
† Coastal ship performed midocean exchange (CE).
‡ Coastal ship exempt from midocean exchange (CNE).

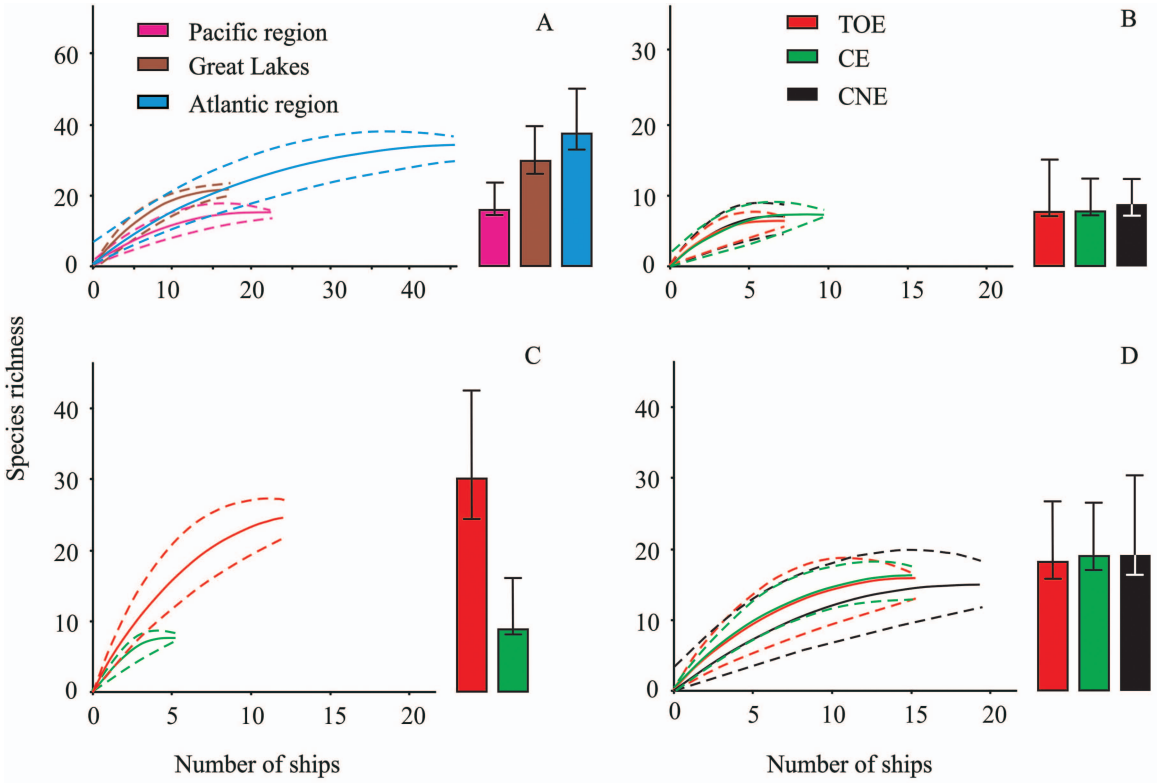

Fig. 2. Sample-based rarefaction curves (± 95% CI) for ships sampled (A) in all regions, (B) in the Pacific region, (C) in the Great Lakes region, and (D) in the Atlantic region. Also shown are species richness estimates for the vessel population (first-order jackknife ± 95% CI). Note the difference in scale for each x- and y-axis.

lead to successful establishment of NIS when environmental and biological factors match for arriving species (Simberloff 2009*b*).

Efficacy of ballast water management appears to vary by region. We have identified the amount of sediment as the single most important factor for management of invertebrates and their dormant eggs in ballast sediment. As more sediment in tanks portends transfer of higher abundances and higher species richness, ballast activities should be undertaken with the objective to limit or avoid entrainment of sediments by avoiding uptake in turbid or shallow water. This objective can be difficult to achieve, however, because of tight schedules and dock work rules as well as the fact that sediment uptake cannot be avoided in many ports (Reid et al. 2007). Nevertheless, frequent inspection and cleaning of ballast tanks could result in important reductions in abundance and species richness of invertebrates and their dormant eggs in ballast sediment. The reduction in sediment amount is also beneficial to the ship's owner, as less sediment carried in tanks increases the vessel's permissible cargo capacity.

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