Ballast water transport of non-indigenous zooplankton to Canadian ports

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Ballast water is one of the primary transport vectors for the transfer and introduction of non-indigenous zooplankton (NIZ). Regulations require vessels from overseas to conduct mid-ocean exchange before discharging ballast in Canadian ports. Intracoastal vessels from nearby ports may be exempt from exchange, whereas intracoastal vessels from more distant ports are required to exchange. Zooplankton in the ballast water of transoceanic exchanged (TOE), intracoastal exchanged (ICE), and intracoastal unexchanged (ICU) vessels arriving at Canada's west (WC) and east (EC) coasts were examined. NIZ density, propagule pressure, taxon richness, and community composition were compared among the three shipping classes. The WC ports received greater densities of NIZ and had greater NIZ propagule pressure than EC ports. Within WC vessels, NIZ propagule pressure and density were significantly greater in ICU vessels. TOE vessels on the EC had the greatest NIZ propagule pressure and density. ICU vessels entering Vancouver ports represented the greatest invasion risk to Canadian waters. These vessels likely mediate secondary invasions by facilitating the transport of unexchanged ballast directly from ports previously invaded, whereas short ICU voyage duration enhances organism survivorship and vessels transport NIZ over natural dispersal barriers.

Keywords: aquatic invasive species, ballast water, intracoastal transport, mid-ocean exchange, non-indigenous species, secondary invasion.

Introduction

The introduction and spread of non-indigenous species (NIS) is recognized as a significant global threat, resulting in negative impacts on biodiversity (Pimentel et al., 2000; Sala et al., 2000), economic losses through money spent on control, and impacts on natural resource commercial ventures such as aquaculture (Pimentel et al., 2000). Mitigating such impacts relies on a better understanding of the invasion pathway. The first step in this pathway is initial introduction, the process by which organisms are transported from native to new habitats, outside their natural range (Wonham et al., 2001; Puth and Post, 2005). This results in primary invasions, which are followed by secondary invasions as the species disperses from its new habitat.

Ballast water is a main vector for initial transport of NIS (Carlton, 1985; Grigorovich et al., 2003; Simkanin et al., 2009; Lawrence and Cordell, 2010). Some have suggested that the cosmopolitan distribution of many coastal species may be partially attributed to ballast water transfer (Hallegraeff and Bolch, 1992). Ballast is taken up by vessels at the source port to ensure stability,

then discharged *en route* or at the destination port to adjust for changes in cargo weight. Ballast water is a phyletically non-selective transport vector (Carlton and Geller, 1993), and variability among vessel type, source region, transit route, and duration can influence the nature, density, and viability of organisms being transported (Verling et al., 2005).

The International Maritime Organization (IMO) has developed regulations to reduce the spread of NIS between ecosystems involving mid-ocean exchange (MOE) protocols that require vessels to replace ballast water collected at the port of origin with open-ocean water. MOE can reduce the threat of NIS by (i) discharging a high percentage of the coastal species into the open ocean, (ii) increasing ballast water salinity to a level not normally tolerated by brackish or freshwater species associated with coastal and inland source ports, and (iii) replacing coastal species in ballast tanks with open-ocean species that are less likely to survive in brackish/freshwater environments of destination ports (Taylor et al., 2002). Studies have shown that MOE is not entirely effective at purging all coastal species and that exchange efficacy varies with vessel type, voyage duration,

exchange method, and species composition (Locke et al., 1993; Wonham et al., 2001; Levings et al., 2004; Cordell et al., 2008, 2009).

Transport Canada (2006) initiated mandatory MOE for commercial vessels intending to discharge ballast water in coastal Canadian ports as well as all vessels intending to discharge within the Great Lakes Basin. This effectively resulted in three general shipping classes, including transoceanic vessels that originate overseas and conduct ballast water exchange *en route* to Canada (TOE, transoceanic exchanged). Regulations require all transoceanic vessels to perform MOE a minimum of 200 nautical miles from the coast in water deeper than 2 km or in designated exchange areas (Transport Canada, 2006). The other two shipping classes include intracoastal vessels that are either exchanged (ICE) or unexchanged (ICU), a distinction based on the source region of the ballast water. On the North American west coast (WC), exchange is not required for vessels travelling exclusively from ports north of Cape Blanco, Oregon, and British Columbia ports (ICU), whereas vessels from source ports located farther south are required to exchange (ICE). On the North American east coast, no exchange is required for vessels originating from north of Cape Cod, MA (ICU), but vessels originating from south of that location must exchange (ICE).

Transoceanic ships are often the vector for initial dispersal of NIS, bringing taxa from their native habitat to a new region (Carlton and Geller, 1993). This primary invasion is followed by secondary invasions via intraregional spread, which can be mediated by natural dispersal or anthropogenic vectors (Wasson et al., 2001). Intracoastal ballast water transport is gaining increasing attention as a significant vector for secondary transport of NIS (Wasson et al., 2001; Cordell et al., 2009; Simkanin et al., 2009; Lawrence and Cordell, 2010). Intracoastal vessels often have shorter voyages, and organism survivorship is greater on shorter voyages (Williams et al., 1988). Simkanin et al. (2009) examined intracoastal ballast water transfer along with NIS presence in receiving ports and suggested that intracoastal transport may be a significant vector for the secondary spread of NIS. Cordell et al. (2009) analysed zooplankton in ships arriving in Puget Sound and found that intracoastal transport poses a greater invasion risk, based on the presence of high-risk coastal taxa, than transoceanic transport, a result supported by propagule pressure estimates from Lawrence and Cordell (2010). Verling et al. (2005) examined intracoastal and transoceanic vessel traffic and zooplankton densities in several ports on the east and west coasts of the United States and found significant differences in vessel types and source regions among receiving ports, and differing zooplankton survival among voyage types. All these factors contribute to differences in propagule pressure and therefore the threat of invasion experienced by coastal Canadian ports. A similar regional comparison of Canadian ports has not been carried out.

This study aimed to build on the previous work done on ballast water as a vector for NIS, with special consideration of the ICU shipping class. We sampled zooplankton from the ballast water of vessels arriving in Canadian west and east coast (EC) ports, classifying them as indigenous or non-indigenous to these ports, to assess the invasion risk. All results were compared among the TOE, ICE, and ICU shipping classes to assess whether the current exemption of ICU vessels from MOE is justified based on zooplankton density, taxonomic richness, propagule pressure, and community composition. In doing so, we provide the first assessment of ballast-mediated invasion risk in multiple regions of Canada.
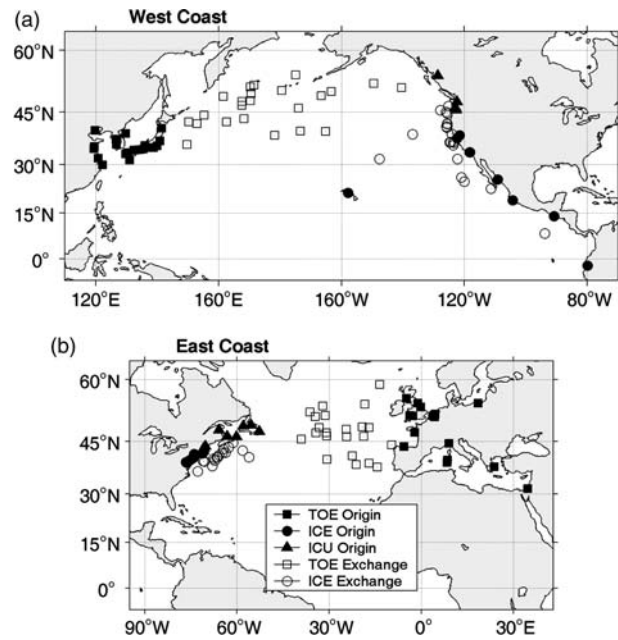

Figure 1. Location of source ports of ballast water samples and exchange locations for (a) west coast, and (b) east coast.

Material and methods

Samples of ballast water were collected from vessels arriving at ports on the EC (Atlantic) and WC (Pacific) of Canada (Figure 1). WC samples were collected from vessels arriving at terminals within the Port of Vancouver, and EC samples at ports in Quebec (Baie-Comeau, Port-Cartier, Sept-Îles), Nova Scotia (NS; Auld's Cove, Halifax, Dartmouth, Liverpool, Point Tupper, Sheet Harbour), and New Brunswick (Saint John).

On the WC, all three shipping classes were sampled during each of two sampling periods: October 2006 to September 2007 (hereafter referred to as 2007), and May–October 2008 (Table 1), which allowed for interannual comparisons among shipping classes. EC samples were collected from April 2007 to August 2008 (Table 1). Because of the limited availability of ICU vessels for sampling, we were unable to collect samples from all shipping classes in each sampling year, and interannual comparisons are restricted to WC data only.

Ballast water and zooplankton sampling

Ballast water sampling was limited to vessels on which the crew could gain easy access to ballast tanks via manhole covers on the vessel deck. Other methods of access to the ballast water, such as pumping water through sounding pipes, were eliminated during a preliminary study because pumps damaged soft-bodied zooplankton and sampling depth was restricted to the tank bottom. On each ship, one tank was sampled for zooplankton.

Zooplankton sampling was based on modified IMO guidelines for ballast water sampling (IMO, 2005). The results presented here are based on samples collected with a 125-μm Nitex plankton net 30 cm in diameter. We initially used both a 50- and 125-μm plankton net, but found that the 125-μm net more effectively sampled larger volumes of water and greater densities of larger (>125 μm) zooplankton than the 79-μm net recommended by IMO guidelines. The 50-μm plankton net tended to clog rapidly and consisted mostly of rotifers and unidentifiable copepod

Table 1. Sampling statistics for ballast water, including sampling region (WC, west coast; EC, east coast), shipping class (ICE, intracoastal exchanged; ICU, intracoastal unexchanged; TOE, transoceanic exchanged), sampling period, number of vessels sampled, number of ballast water source ports, and ballast water age (d; mean $\pm$ s.e.).

Region	Shipping class	Sampling period	Number of vessels sampled	Number of source ports	Ballast age
WC ($n = 70$)	ICE	4 April – 5 June 2007	11	9	7.8 ± 0.7
		11 May – 2 October 2008	10		
	ICU	24 October 2006 – 6 July 2007	13	6	4.4 ± 0.5
		13 May – 14 October 2008	10		
	TOE	26 October 2006 – 22 June 2007	16	21	12.2 ± 0.8
		7 May – 22 October 2008	10		
EC ($n = 63$)	ICE	5 May – 11 June 2007	3	15	1.8 ± 0.2
		6 June – 14 August 2008	14		
	ICU	13 June 2007	1	11	2.6 ± 0.6
		7 June – 5 August 2008	10		
		7 July – 3 August 2009	13		
	TOE	29 April – 13 August 2007	20	17	8.2 ± 0.7
		26 June – 8 August 2008	2		

naupliar stages. The vast majority of identifiable zooplankton species were sampled in the 125-µm net and consisted predominantly of copepodids (see Results below). There were no copepodid species sampled exclusively in the 50-µm net. This sampling strategy effectively reduced onboard sampling time and hence minimized delays that could impact commercial schedules. Standardized zooplankton densities reported, especially of dominant copepod species, are comparable with those reported in other studies, despite the differences in plankton-net mesh sizes.

The zooplankton net was lowered to the maximum accessible depth inside the ballast tank and retrieved by hand at a speed of $\sim$1 m s^{-1}; this was repeated until $\sim$1000 l of ballast water had been filtered. Tow depth depended on the size of the tank, the depth of ballast water, and the presence of tank obstructions, but nets were generally lowered to a mean depth ($\pm$ s.e.) of 5 ± 0.4 m. All samples were preserved in 5% buffered formalin and stored at room temperature until sorting.

Surface salinity was measured within the upper 1 m of the water column for each tank with a hand-held YSI (model 30) temperature–salinity probe. IMO reporting forms for ballast water were collected from all vessels sampled and provided vessel-specific data, including the volume and age of sampled ballast water, the source port of the ballast on board, and the date and location of MOE, if conducted. The forms also provided information on each vessel's total ballast capacity and the total volume of ballast on board.

Zooplankton samples were sorted with a stereomicroscope and individuals identified to the lowest feasible taxonomic level and ontogenetic stage (e.g. nauplii). Zooplankton were classified as indigenous, non-indigenous (NIZ), or cryptogenic, depending on their status in the port sampling region where they would be discharged. Generally, an individual could only be classified as indigenous or non-indigenous if identified to species level; most taxa not identified to species level were categorized as cryptogenic.

The classification of indigenous and non-indigenous species was based on local taxonomic and biogeographic literature pertaining to nearshore, coastal, estuarine, and freshwater habitats of the WC (Davis, 1949; Shih *et al.*, 1971; Gardner and Szabo, 1982; Razouls *et al.*, 2008) and EC regions, with the EC including the Gulf of St Lawrence (Shih *et al.*, 1971; Brunel *et al.*, 1998; Razouls *et al.*, 2008). The results here focus on NIZ, but results for indigenous zooplankton density, taxon richness, and propagule pressure are provided as Supplementary Figures S1–S3.

Zooplankton densities were standardized to ind. m^{-3} for each vessel by dividing the abundance of a taxonomic group by the volume of water filtered for that sample. Sample volume was estimated by multiplying sample depth, mouth area of the net, and the number of vertical tows conducted. Taxon richness (number of taxa m^{-3}) was determined in a similar manner, using the total number of distinct taxa in a sample divided by the volume of water filtered.

Propagule pressure was defined as the potential number of individuals released per sampling period. Sampling period is defined as the region-specific span of time covered during each round of sampling (Table 1). Propagule pressure ($= \sigma\beta\theta$) was estimated for each region and shipping class by calculating the product of mean NIZ density (σ), mean vessel discharge volume (β), and total number of shipping class-specific release events in a region (θ) within the specified sampling period. Zooplankton densities (σ) were calculated as the mean of densities in vessels sampled for which we had discharge information on the ballast water. Vessel discharge volumes (β) were obtained from IMO forms, and only ships sampled in this study were included in these calculations, resulting in conservative estimates of ballast water discharge. In cases where a vessel reported no discharge, or if that information was missing, the vessel was excluded from this analysis. The number of release events (θ) was based on vessel arrival data and was restricted to ships discharging water in the ports of interest during the sampling times for each region (Table 1). Vessel arrival data for ports sampled (as listed above) were obtained from Transport Canada's Canadian Port State Control System.

Statistical analysis

All statistical tests were conducted using SYSTAT (v. 13). Densities were log ($x + 1$)-transformed before analysis to normalize values and equalize variances. Non-transformed values are presented. Taxon richness and ballast water properties were not transformed. For the unbalanced ANOVAs, we used type III sum of squares, and when significant differences ($\alpha = 0.05$) were found, Tukey's *post hoc* tests were used to identify groupings within the factors.

To compare NIZ densities, taxon richness, propagule pressure, and ballast water properties among shipping classes, two-way ANOVA with the main factors of region (EC, WC) and shipping class (ICU, ICE, TOE) was used. Two-way ANOVA tests were also used to compare NIZ density, taxon richness, and propagule

Table 2. Relationship between levels of the main effects in all analyses of NIZ density, richness, and propagule pressure.

Parameter for regional comparison	Region	Shipping class	Interaction
Density	WC > EC, $F_{1,127} = 36.4$, $p < 0.001$	NS	Region × Class, $F_{2,127} = 15.8$, $p < 0.001$
Taxon richness	WC > EC, $F_{1,127} = 42.0$, $p < 0.001$	WC > EC (marginal), $F_{2,127} = 4.8$, $p = 0.06$	NS
Propagule pressure	WC > EC, $F_{1,101} = 85.7$, $p < 0.001$	ICU > ICE > TOE, $F_{2,101} = 19.7$, $p < 0.001$	Region × Class, $F_{2,101} = 31.0$, $p < 0.001$

Parameter for interannual comparison	Year	Shipping class	Interaction
Density	2008 > 2007, $F_{1,64} = 4.5$, $p = 0.04$	(ICU = ICE) > TOE, $F_{2,64} = 9.2$, $p < 0.001$	NS
Taxon richness	NS	NS	NS
Propagule pressure	2008 > 2007, $F_{1,54} = 24.5$, $p < 0.001$	ICU > ICE > TOE, $F_{2,54} = 21.0$, $p < 0.001$	Year × Class, $F_{2,54} = 7.1$, $p = 0.002$

NS, non-significant result. Comparisons are two-way ANOVA of region (east coast, west coast) or year (2007, 2008), and shipping class (ICE, ICU, TOE).
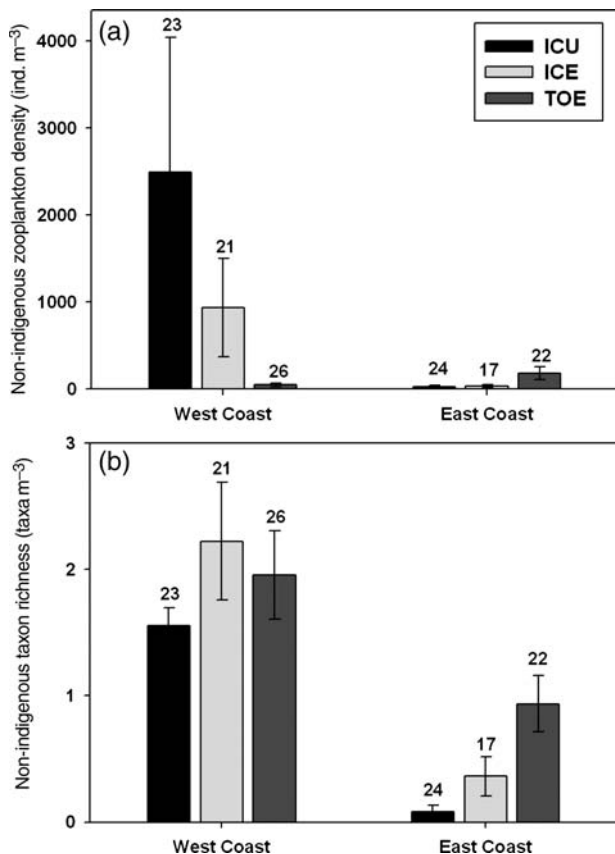
Figure 2. Mean (± s.e.) non-indigenous (a) zooplankton density, and (b) taxon richness for each shipping class in all regions. The numbers above the bars are sample sizes.

pressure between WC sampling periods (2007 and 2008) and shipping classes. WC samples were pooled across seasons, because we found no differences between spring (defined for our purposes as 1 March–31 May), summer (1 June–31 August), and autumn (1 September–30 November). There were insufficient winter samples (1 December–28 February; Table 1) to compare across shipping classes (ICU = 1, TOE = 4, ICE = 0), so winter was excluded from the analyses.

Primer® (version 6) was used to compare differences in zooplankton community composition among shipping classes and to identify the key species that distinguished these groups. Total zooplankton abundance data were fourth-root transformed before being used in the ordinations, to downweight highly abundant taxa and to allow rare or less abundant taxa to have more influence (Clarke and Warwick, 2001). Non-metric multidimensional scaling (NMDS) was used to compare patterns of community composition, because it can handle non-normal data and zero values (McCune and Grace, 2002). Ordinations were produced with 50 iterations, a minimum stress of 0.01, and were based on Bray–Curtis similarity matrices. Differences in composition between groups were tested using Primer's one-way analysis of similarity (ANOSIM) procedure, run with 999 permutations. A similarity percentage procedure was used to identify species contributing to observed differences between shipping classes within the different regions.

Results

Vessel and ballast water characteristics

Samples of ballast water were collected from 70 WC and 63 EC vessels over 3 years (Table 1). WC vessels consisted predominantly of bulk carriers (39.7%) and cargo vessels (45.6%), and EC vessels were mainly bulk carriers (58.6%) and tankers (31%). WC vessels had significantly older ballast water (Table 1; $F_{1,128} = 28.4$, $p < 0.001$). EC vessels had a significantly greater volume of ballast on board (27 477 ± 2779 vs. 12 492 ± 1007 m^3; $F_{1,125} = 30.5$, $p < 0.001$) and a higher salinity (31.7 ± 0.5 vs. 26.6 ± 1.4 psu; $F_{1,133} = 10.9$, $p = 0.001$). An interannual comparison of WC vessels found that ballast water age, ballast on board, ballast capacity, and salinity did not differ between 2007 and 2008.

Zooplankton density and taxon richness

NIZ were significantly more abundant in ships arriving on the WC, in terms of both density and taxon richness (Table 2; Figure 2). There were no differences in NIZ density between the three shipping classes, and just marginal differences in NIZ taxon richness (Table 2; Figure 2). Unlike NIZ, indigenous zooplankton density was greatest in EC vessels, whereas indigenous taxon richness was not different between regions (Supplementary Figure S1). The WC interannual comparison
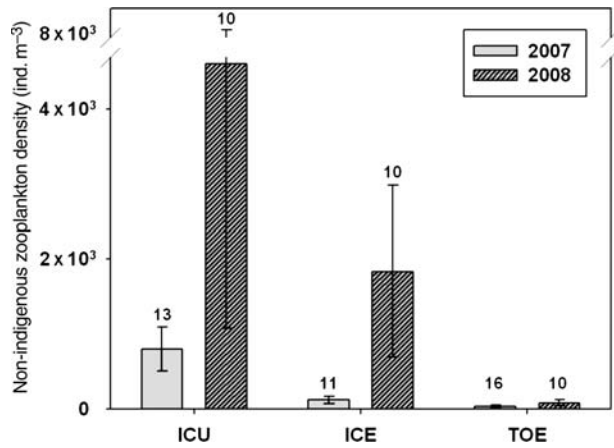
Figure 3. Mean (± s.e.) NIZ density for each sampling period on the west coast. The numbers above the bars are sample sizes.
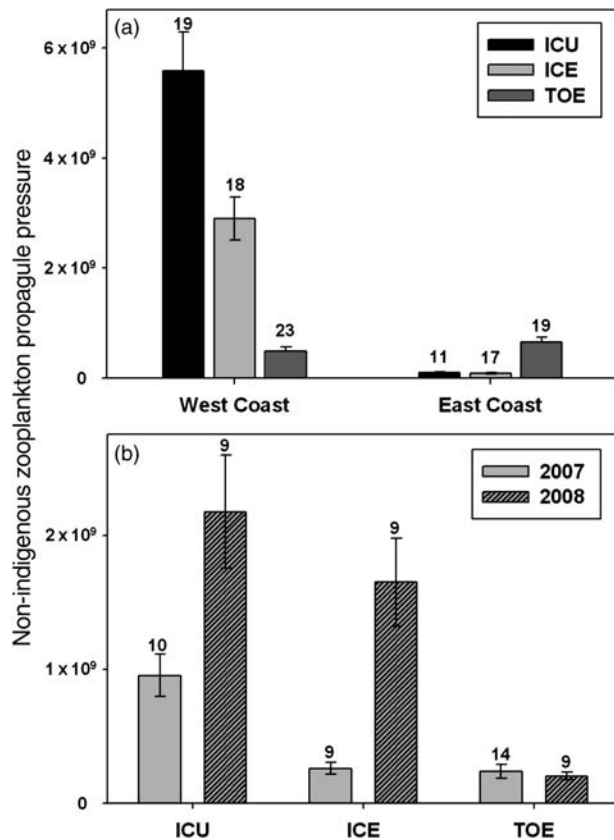

Figure 4. Mean (± s.e.) non-indigenous propagule pressure (individuals released per sampling period) for (a) each region in each shipping class, and (b) west coast samples only for the two sampling periods.

found NIZ density to be significantly greater in 2008 (Table 2; Figure 3), whereas taxon richness was not different (Table 2). NIZ density was greater in ICE and ICU vessels than TOE (Table 2; Figure 3). There was no effect of year or shipping class on indigenous zooplankton density or taxon richness (Supplementary Figure S2).
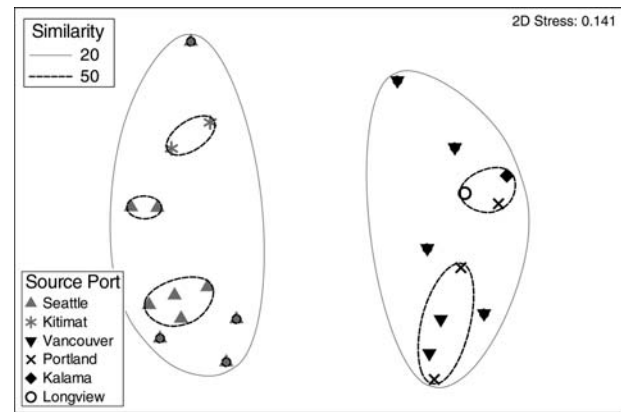

Figure 5. NMDS for the west coast, ICU samples only showing the location of ballast source. Vessels from Seattle and Kitimat are significantly different from vessels from Vancouver, Portland, Kalama, and Longview ($p = 0.001$).

Propagule pressure

Vessels from the WC had significantly greater NIZ propagule pressure than EC vessels, whereas WC ICU vessels had the greatest NIZ propagule pressure and TOE the lowest (Table 2; Figure 4). Indigenous zooplankton propagule pressure was greatest in EC vessels (Supplementary Figure S3). The interannual comparison of WC vessels revealed that NIZ propagule pressure was greater in 2008, and highest in ICU and lowest in TOE vessels (Table 2; Figure 5). Indigenous zooplankton propagule pressure, however, was greatest in TOE vessels (Supplementary Figure S3).

Zooplankton assemblages and community structure

Zooplankton samples were taxonomically diverse, with both holo-plankton and meroplankton. A complete list of the taxa found in each region is provided in Supplementary Table S1. There were 176 taxa in WC and 96 in EC vessels. On the WC, 29.4% of the zooplankton taxa sampled were NIZ, whereas just 10.4% were NIZ on the EC. Copepods dominated the samples, constituting 88.9 and 73.2% of total zooplankton density for the EC and the WC, respectively. Calanoid copepods were the most frequently observed copepod, at 62.7% of the EC and 47.3% of the WC copepod density.

West coast

The ordination for WC ballast water zooplankton communities identified differences among shipping classes (ANOSIM, global $r = 0.212$, $p < 0.001$), with all classes significantly different (ANOSIM, pairwise comparison, $r = 0.161–0.299$, $p < 0.001$). NIZ were an important driver in the differences seen between shipping classes and were often more abundant in ICU samples than in ICE or TOE. The non-indigenous copepod *Pseudodiaptomus forbesi* made up 22% of the density observed in WC ICU vessels, accounting for 4.5% of the dissimilarity between ICU and ICE vessels, and 5.2% of the dissimilarity between ICU and TOE vessels.

Within the WC ICU shipping class, vessels separated into two distinct groups (ANOSIM, global $r = 0.892$, $p = 0.001$; Figure 5): vessels from ports on the Columbia River (CR; Portland, OR, Vancouver, Kalama, and Longview, WA; $n = 11$), and vessels from Seattle, WA, and Kitimat, BC ($n = 12$). The difference between these two groups was characterized by

P. forbesi, which was absent in vessels from Seattle/Kitimat and present in 64% of vessels from the CR. The absence of *Paracalanus parvus*, an indigenous calanoid copepod in CR vessels and its presence in 83% of Seattle/Kitimat vessels also contributed to the differences observed. Within the WC ICE shipping class, only vessels from Mexico ($n = 3$) and Los Angeles ($n = 12$) had significantly different communities (ANOSIM, pairwise comparison, $r = 0.746$, $p = 0.007$). TOE vessels did not group by any discernible pattern and were not different based on port of origin.

East coast

The zooplankton community composition in EC vessels was different among all three shipping classes (ANOSIM, global $r = 0.537$, $p = 0.001$; pairwise comparison, $r = 0.326{-}0.77$, $p = 0.001$). No groups were distinguished by NIZ, and NIZ were not generally present at high density. There was no difference in community composition between source ports for either EC ICU or ICE when samples were grouped by country or state of origin, respectively. Within the EC TOE shipping class, vessels showed significant differences in community composition overall (ANOSIM, global $r = 0.338$, $p = 0.022$), but only one pairwise test between source ports in Italy ($n = 3$) and the UK ($n = 8$) was significant (ANOSIM, $r = 0.484$, $p = 0.036$).

Discussion

West coast

The shipping category that poses the greatest risk of introducing NIZ to the west coast of Canada was ICU vessels. On the WC, ICU vessels had the greatest NIZ density and NIZ propagule pressure of the three shipping classes. This pattern was consistent across years, although densities and propagule pressure were significantly greater in 2008. This finding supports a growing body of work highlighting the importance of intracoastal vessels in the transport of NIS (Wasson *et al.*, 2001; Cordell *et al.*, 2009; Simkanin *et al.*, 2009; Lawrence and Cordell, 2010). In contrast, on the EC, TOE vessels had the greatest NIZ density and NIZ propagule pressure.

Our findings of higher NIZ densities and propagule pressure in WC ICU vessels are consistent with the work of Cordell *et al.* (2009) and Lawrence and Cordell (2010). We used a larger mesh size (125 µm), but our results are comparable because the 125-µm mesh provided a better estimate of density than the 50-µm mesh, which caught mainly rotifers and would clog easily (see above). Our study also differs from the earlier ones with respect to the classification of taxa. We considered high-risk taxa to be any taxa non-indigenous to the region, whereas Cordell *et al.* (2009) consider high risk to be any coastal NIS, which includes holoplankton and meroplankton of shallow-water taxa and are characteristic of source ports and nearshore ballast water. They found greater high-risk zooplankton densities in intracoastal vessels relative to transpacific vessels entering Puget Sound (Cordell *et al.*, 2009), and we observed higher NIZ densities in ICU than in TOE vessels. Lawrence and Cordell (2010) reported that propagule pressure of high-risk taxa in Puget Sound was greatest in ICU ballast, intermediate in ICE, and lowest in TOE ballast. This pattern mirrors the pattern of NIZ propagule pressure observed in vessels entering the WC region.

Whereas transoceanic vessels are responsible for the initial dispersal of many NIS, intracoastal transport clearly plays a role in the secondary spread (Wasson *et al.*, 2001). In busy ports, introductions can be virtually constant (Wasson *et al.*, 2001), and over time many North American ports have become invasion hotspots (Drake and Lodge, 2004). Such invaded ports can serve as sources of secondary invasion for other ports, through such vectors as transport of unexchanged ballast (Simkanin *et al.*, 2009) and natural dispersal on currents (Wasson *et al.*, 2001). San Francisco Bay (SFB) represents one of the most heavily invaded port systems in North America and indeed the world (Cohen and Carlton, 1998). Simkanin *et al.* (2009) found that of four US Pacific coast port systems, NIS identified were most often recorded first in SFB. Wasson *et al.* (2001) suggested that SFB acted as a stepping stone for the secondary invasion of invertebrates into Elkhorn Slough, an estuary 150 km to the south of SFB. Elkhorn Slough does not receive international vessels, and those authors suggested a number of intraregional vectors, including natural dispersal and anthropogenic transport, that could have been responsible for its highly invaded status.

The heavily invaded SFB likely poses less of a threat for the introduction of NIS to west coast Canadian ports because vessels originating from there are required to conduct ballast water exchange *en route*. However, the CR has received unexchanged ballast water from SFB in the past (Simkanin *et al.*, 2009) and has likely received NIS directly or indirectly from SFB (Cordell *et al.*, 2008). Therefore, ICU vessels from the CR have the potential to introduce NIS into low salinity or mainly freshwater Canadian ports. In our analyses of zooplankton community composition, we found that ICE (e.g. from SFB) and ICU vessels (e.g. from the CR) differed in part because of the presence of the non-indigenous copepod *P. forbesi* in ICU vessels. *Pseudodiaptomus forbesi* is present in SFB and was first recorded in the CR in 2002, from where it has spread and become established upstream, sometimes dominating zooplankton assemblages (Cordell *et al.*, 2008). It is possible that the CR has become another stepping stone for secondary invasions of brackish and freshwater taxa in the NE Pacific (Cordell *et al.*, 2008), much like SFB. To date, *P. forbesi* has not been recorded in Canadian waters, despite being present in 30% of ICU vessels with an average ($\pm$ s.e.) density of 7264 ± 4830 ind. m^{-3}. However, we are not aware of any recent field surveys designed to seek this species in Canadian waters. The continued arrival of unexchanged ballast water from this system demonstrates a real threat to Canada's WC, because it may facilitate the spread of NIZ, like *P. forbesi*.

The intraregional spread of invasive species can be natural through the dispersal of species from the initial port of invasion (Wasson *et al.*, 2001). The real consequence of intraregional transport of invasive species in ballast water is that it can overcome natural barriers to dispersal. The European green crab (*Carcinus maenas*) is a successful worldwide invader and provides an excellent case study in the dispersal of an invasive species. A self-sustaining population of green crab was discovered in SFB in 1989 (Cohen *et al.*, 1995), with the initial invasion thought to have taken place via the transport of larvae in ballast water (Cohen and Carlton, 2003). Since that primary invasion, green crabs have spread, through natural dispersal on currents and via anthropogenic intraregional transport (Behrens Yamada *et al.*, 2005). Models have shown that it is possible that the spread north from SFB to BC could have taken place through natural dispersal (See and Feist, 2010). In a coastal survey in 2008, adult green crab populations were sampled as far north as Winter Harbour, BC, on the west coast of Vancouver Island (G. Gillespie, pers. comm.). Adult green crabs have yet to be reported in the Strait

of Georgia (SoG) despite there being ideal green-crab habitat there (Jamieson *et al.*, 1998; Gillespie *et al.*, 2007).

One possible explanation for the absence of established green crab populations in the SoG may have to do with the effect of the large freshwater input into the SoG and Puget Sound from spring and summer snowmelt, which establishes strong estuarine circulation with a net outflow in the upper 100 m of the water column (Thompson, 1981). This estuarine circulation likely serves as a natural barrier to the dispersal of the surface-dwelling larvae of green crab, their main development stage of dispersal (Behrens Yamada and Kosro, 2010), preventing them from entering and establishing in the SoG or Puget Sound. Recent zooplankton surveys conducted in Barkley Sound on Vancouver Island's west coast revealed that green crab zoeae aggregated near the depth of the pycnocline regardless of tidal or diel phase (unpublished data). This vertical distribution in conjunction with strong estuarine circulation characteristic of the SoG (LeBlond, 1983; Masson and Cummins, 2000) most likely prevents zoeae from dispersing from the nearshore coastal zone into Puget Sound or the SoG. This is corroborated by the absence of any established population of green crabs in the SoG (Gillespie *et al.*, 2007), despite them having been recorded on the coast of Vancouver Island since the early 1990s (Jamieson *et al.*, 2002).

The intraregional transport of green crab larvae via ballast water represents one way that green crabs might overcome the natural, hydrodynamic barrier. The intraregional transport of non-indigenous species past a natural barrier has been suggested on Canada's EC. Cohen and Carlton (2003) predicted that green crabs would not expand north of the lower Gulf of St Lawrence in Canada because of their temperature preferences. Since that prediction, however, green crabs have become established in southeastern Newfoundland (NL). Genetic analyses have confirmed that the recent population spread resulted from a secondary invasion from NS and not a new invasion from Europe (see summary by Blakeslee *et al.*, 2010). Intracoastal vessel transport between NS and NL is considered the most likely vector. Given the documented history of ecosystem (Grosholz *et al.*, 2000) and economic impacts caused by green crabs in other estuaries (Pimentel *et al.*, 2000), intraregional transport represents an area of concern for managers, so needs to be addressed.

Cordell *et al.* (2009) sampled 380 vessels on intracoastal and transoceanic voyages. We identified and classified three taxa as NIZ which Cordell *et al.* (2009) found only in transoceanic vessels, but which we found in ICU vessels discharging ballast in the Port of Vancouver. *Pseudodiaptomus inopinus* was present in 17% of ICU vessels and came from Portland, Seattle, and Kitimat. *Temora turbinata* and *Oncaea scottodicarloi* were present in 4.3 and 30% of ICU ships, respectively, and came from Seattle. The former species is already established in Oregon and Washington, but has not yet been reported in Canadian waters (Cordell and Morrison, 1996; Cordell *et al.*, 2010). Little information exists about *T. turbinata* and *O. scottodicarloi* as potential invaders, but both species were classified by Cordell *et al.* (2009) as oceanic, so may not survive in coastal waters.

East coast

The Atlantic coast of North America has a lower proportion of harmful alien species than the Pacific coast (Molnar *et al.*, 2008). Our data have shown that EC ICU vessels contain far fewer NIZ than WC ICU vessels. The risk of NIZ introductions posed by ICU ships on the WC, as demonstrated by high NIZ densities

and propagule pressure, was not reflected in EC ICU vessels. In contrast, EC ICU vessels were characterized by greater densities of indigenous zooplankton. Differences in NIZ densities between the two coastal regions may be attributable in part to significant salinity differences in ballast water. WC vessels had a much lower mean salinity than EC vessels, providing an environment more similar to brackish, coastal habitats, and selecting for NIZ adapted to similar environmental conditions. The high mean salinity water of EC vessels would select against non-native species most likely adapted to brackish coastal conditions. Our analysis of community composition suggests that NIZ do not play a significant role in the zooplankton community in EC ICU vessels.

Propagule pressure

Propagule pressure is often positively associated with the establishment and impact of invasive species (Colautti *et al.*, 2006), and it has been suggested as a predictor of invasion risk. Our estimates of propagule pressure are robust, taking into account observed estimates of mean zooplankton density, mean ballast water discharge, and shipping-class-specific vessel arrival data within each region. Previous studies have emphasized that ballast volume (Lawrence and Cordell, 2010) or the frequency of vessel arrivals (Verling *et al.*, 2005) alone can yield erroneous results. For example, EC vessels had the greatest quantity of ballast water on board and most water discharged in our study, but this did not translate to the highest estimates of propagule pressure, because the EC had the lowest NIZ propagule pressure. On the WC, vessel arrival data alone would have misidentified the TOE shipping class as the greatest threat for introducing NIZ, because it represented the most arrivals during our sampling periods, according to 952 vessel arrivals logged in the Canadian Port State Control System database. In fact, NIZ propagule pressure was higher for EC TOE than for WC TOE vessels, despite that coast receiving far fewer vessels (78). WC ICU vessels were also fewer than WC TOE, but had a greater NIZ propagule pressure because of the greater NIZ densities observed in ICU ships.

MOE and ICU management

MOE remains one of a limited number of defences against ballast water introductions of NIS. However, it is not a barrier to dispersal so much as a filter (Locke *et al.*, 1993). MOE can be an effective mechanism to replace coastal organisms with those from the open ocean (Locke *et al.*, 1993; Wonham *et al.*, 2001; Cordell *et al.*, 2009), but many factors affect the success of the exchange, including exchange method (Choi *et al.*, 2005) and location of the exchange (Endresen *et al.*, 2004). As MOE does not remove all NIS (Levings *et al.*, 2004), it is merely reducing propagule pressure along with the probability of introductions, rather than eliminating it. Ideally, the organisms being released will be open-ocean taxa, which are less tolerant or intolerant of coastal conditions and less likely to outcompete native species and survive in receiving ports (Wonham *et al.*, 2001). However, environmental conditions can vary considerably between ports. For example, the Metro Port of Vancouver consists of 28 marine terminals that service container, bulk, and automobile carriers. Depending on the season and terminal location within the Vancouver watershed, salinity will vary from marine to brackish to freshwater. Therefore, independent of ballast-water history, where and when the ballast water is exchanged will play a key role in determining the likelihood that species existing within ballast water will be able to survive and potentially establish *in situ*.

The similarity in environmental conditions, including salinity and temperature, between source and receiving ports is an important factor in determining the probability that a released organism will survive (Smith et al., 1999). In general, coastal port conditions, which tend to be influenced by freshwater run-off, differ from open-ocean conditions. The fact that ICU ships transfer species between coastal ports in relatively proximity, i.e. hundreds of kilometres. with similar conditions would be expected to facilitate invasion. Whereas restricting ICU vessel traffic is not economically feasible, other management strategies can be applied to ICU vessels. Many treatment protocols for ballast water are currently being developed and tested. For example, filtering and UV treatment removes zooplankton and bacteria from ballast water (Waite et al., 2003), and treatment with NaCl has resulted in high rates of zooplankton mortality (Bradie et al., 2010). To be effective, though, the use of such methods would need to be feasible on the short durations of intracoastal voyages, bringing the ballast water into compliance with local regulations and standards.

In addition to ballast treatment, changes to exchange procedures may also work to reduce invasion risks. Restrictions on exchange practices for ballast water could help regulate where unexchanged water can be released, based on salinity criteria. For example, the risk of introducing NIS might be reduced if ships carrying ballast of low salinity from the CR performed ballast exchange in a brackish receiving port or exchanged ballast water in a fully marine area, e.g. regions of the SoG, before entering freshwater ports, e.g. Fraser River. Such management changes would require that source/receiving port combinations be assessed separately, but may present an effective mean of reducing the propagule pressure observed in the WC region from ICU ships.

Conclusions

Smith and Kerr (1992) stated that NIS management of Canadian coastal regions needs to take into account differing invasion pressures of different regions. We have shown that the east coast and the west coast are under different invasion pressures. The results of this study indicate that current management practices for ballast water, and their compliance to them, appear to be effective for lowering NIS risk on the EC, but the exclusion of some intracoastal vessels from exchange is not justified for WC traffic. There is also the potential for ICU ships to pose a similar threat on the EC, as demonstrated by the secondary invasion of green crabs demonstrated by Blakeslee et al. (2010). Our findings strongly suggest that ICU vessels on the WC pose the greatest invasion threat to Canadian waters and that the WC has been and continues to be vulnerable to secondary invasions. The primary threats to Canada's coastal waters are different for each region, and regulations on ballast water need to meet unique regional requirements.

Supplementary material

Supplementary material is available at the ICESJMS online version of the manuscript. There is a table of all taxa observed, status of origin, frequency of occurrence, and mean density by region and ship class, and graphically (three figures) the results of shipping class and regional comparisons of indigenous zooplankton density, taxon richness, and propagule pressure, and interannual comparisons of indigenous zooplankton density, taxon richness, and propagule pressure on the west coast.

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