CHARACTERIZING BALLAST WATER AS A VECTOR FOR NONINDIGENOUS ZOOPLANKTON TRANSPORT

by

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B.Sc., Memorial University, 2004

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

in

The Faculty of Graduate Studies

(Oceanography)

THE UNIVERSITY OF BRITISH COLUMBIA

(Vancouver)

September 2008

ABSTRACT

The global movement of aquatic non-indigenous species can have severe ecological, environmental and economic impacts emphasizing the need to identify potential invaders and transport pathways. Initial transport is arguably the most important stage of the invasion process owing to its role in selectively determining potential invasion candidates. This study characterizes a well defined human-mediated dispersal mechanism, ballast water transport, as a vector for the introduction of non-indigenous zooplankton. Ballast water exchange in the open ocean is the most widely adopted practice for reducing the threat of aquatic invasions and is mandatory for most foreign vessels intending to release ballast in Canadian waters. Ships entering Canadian ports are categorized into the following three shipping classes based on current regulations: overseas vessels carrying exchanged ballast water, intra-coastal vessels carrying exchanged ballast water or intra-coastal vessels carrying un-exchanged ballast water. This study characterizes zooplankton communities associated with each of these shipping classes sampled from ports on Canada's Pacific coast, Atlantic coast and the Great Lakes Basin. Ballast water samples were collected and analyzed from 77 vessels between 2006 - 2007. The ballast water environment was found to be diverse, with over 193 zooplankton taxa, 71 of which were non-indigenous to their receiving environments. Intracoastal vessels containing un-exchanged coastal water transported the greatest density of non-indigenous zooplankton into Canadian ports. Total zooplankton density was found to be negatively correlated with ballast water age The absence of mandatory ballast water exchange and the younger ballast water age of coastal un-exchanged vessels is likely responsible for the higher density of non-indigenous zooplankton in intracoastal un-exchanged vessels. Propagule pressure, invasion history and environmental suitability are all useful in evaluating invasion potential and all suggest that intracoastal un-

exchanged vessels pose the greatest invasion threat to Canadian aquatic ecosystems. In conclusion, although the risk of primary introductions from overseas ports may have been reduced through open-ocean exchange of ballast water, secondary introductions from previously invaded ports in North America may be the primary threat to Canadian aquatic ecosystems via this transport vector.

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ANOVA	Analysis of Variance
AU	Australian
BC	British Columbia
BOB	Ballast On Board
CA	California
CAISN	Canadian Aquatic Invasive Species Network
CDN	Canadian
EEZ	Exclusive Economic Zone
GLB	Great Lakes Basin
ICE	Intracoastal Exchanged
ICU	Intracoastal Un-exchanged
IMO	International Maritime Organization
MA	Massachusetts
MOE	Mid-ocean Exchange
NEP	Northeast Pacific
NIS	Non-indigenous Species
NIZ	Non-indigenous Zooplankton
NRC	National Research Council
NoBOB	No Ballast on Board
OR	Oregon
OTA	Office of Technology Assessment
RO/RO	Roll On/Roll Off
SD	Standard Deviation
SE	Standard Error
TC	Transport Canada
TOE	Transoceanic Exchanged
UBC	University of British Columbia
US	United States
YSI	Yellow Springs Instruments

ACKNOWLEDGEMENTS

I am extremely grateful for the generous support provided by my supervisor Claudio DiBacco. I am also thankful for the comments and suggestions provided by my committee members Colin Levings, Evgeny Pakhomov, Jeff Cordell and Thomas Therriault. Special thanks to ballast water sampling team members: Beth Piercey, Petra Logic, Francis Choi, Gavin Leung, Remi Daigle, Andrea Weise, Nathalie Simard, Chris McKindsey, Oscar Casas-Monroy, Matthieu Huot, Jean-Yves Couture, Colin van Overdijk, and Sarah Bailey. Taxonomic expertise was provided by Boris Borisov, Olga Kalata, Jeff Cordell, Nissa Ferm, Moira Galbraith (Pacific samples); and Karine Nantel (Atlantic & Great Lakes samples). Transport Canada vessel traffic data was provided by Veronica Lo. This research study was conducted as part of government funded Canadian Aquatic Invasive Species Network (theme I-III) whose mandate is to explore vectors and pathways of aquatic invasive species into aquatic ecosystems in Canada, characterize factors that influence establishment success and construct risk assessment models to direct future management policies. Funding for this research was provided by the Natural Science and Engineering Research Council of Canada, Fisheries and Ocean Canada and the Ontario Ministry of Environment.

INTRODUCTION

Invasion ecology

Biological introductions occur when organisms are transported to habitats outside of those in which they naturally evolved. Transportation can occur naturally through currents, tides, storms or climatic changes, or through human-mediated dispersal. Many of these "non-indigenous" or "alien" species fail to establish themselves in new habitats, owing to competition or predation by native biota (Case, 1990), or to a lack of favourable environmental conditions (Moyle & Light, 1996a). Other species may integrate themselves unnoticeably into their new habitat, causing little if any disturbance (Simberloff, 1981). A small proportion of these species will proliferate, spread and persist, leading to potentially significant negative effects for the environment, economy and human health. These species, termed "invasive" are so profound in their effects that they have led to the development of an entire discipline devoted to their understanding – invasion ecology.

Consequences

Biological invasions have been recognized as being a significant consequence of global environmental change resulting in potentially serious ecological, environmental and economic losses worldwide (Vitousek et al. 1997; Sala et al., 2000). As a leading cause of biodiversity loss, biological invasions have lead to the extinction of numerous species, often leading to the homogenization of biota within an environment (McKinney & Lockwood, 1999; Rahel, 2002). The most notorious documented case of mass extinction attributed to a biological invasion is that of the Nile Perch (*Lates niloticus*), which has lead to the extinction of over 200 endemic fishes since its introduction into Lake Victoria in the 1950's (Reinthal & Kling, 1997). While not as severe, similar cases of biodiversity loss has been documented throughout aquatic ecosystems in

every continent; North America has lost over 123 freshwater fauna (Ricciardi & Rasmussen, 1999).

From an environmental standpoint, biological invasions have been responsible for altering benthic habitats of freshwater and marine ecosystems. An epiphytic bryozoan, *Membranipora membranacea*, that infests kelp beds causing fragmentation and loss of fronds, has recently invaded the Atlantic coast of Canada and United States (Berman et al., 1992). Along with destructive grazing by sea urchins, this invasive species has helped facilitate the establishment of an invasive algal species, *Codium fragile* ssp. *tomentosoides*, which opportunistically exploits disturbed substrate and inhibits re-colonization by kelps and native seaweeds (Scheibling & Gagnon, 2006).

Biological invasions can alter fundamental ecological properties in an ecosystem, such as the flow of resources including nutrients, biomass or energy (Tansley, 1935; Odum, 1972). These ecological properties can include trophic level interactions, the dominant species in a community, an ecosystems physical features, nutrient cycling and plant productivity (Bertness, 1984; Vitousek, 1990). For instance, the invasion of the Black and Caspian Seas by the ctenophore *Mnemiposis leidyi*, has led to a cascade of effects which stemmed from its consumption of native non-gelatinous zooplankton. This in turn contributed to several ecosystem-wide effects which include: a near collapse of the anchovy industry (Kideys, 1994), sharp increases in phytoplankton production and biomass (Kideys & Romanova 2001), and subsequent changes to the nutrient levels in the Black Sea (Kideys, 1994).

Biological invasions can contribute to economic losses in several ways. The first includes losses associated with reduced output or yield of natural resources such as aquaculture and

commercial fisheries. For instance, the introduction of the club tunicate (*Styela clava*) into the Canadian Maritimes has reduced the output of mussels by 50% due to aquaculture fouling and competition (Colautti et al., 2006). A second potential economic loss includes the cost associated with combating invasions, either through quarantine, eradication or control. In the Great Lakes Basin, the cost for controlling the fouling of water intake pipes by zebra mussel (*Dreisenna polymorpha*) infestations over a ten year period was estimated to be US$3.1 billion dollars (OTA, 1993). Thirdly, invasions can negatively affect tourism by threatening recreational fisheries or diminishing the aesthetic value of marine habitats. A study by Colautti et al. (2006) estimated economic losses associated with 16 non-indigenous species (NIS) in Canada to range between CDN$13.3 to 34.5 billion per year. This is dwarfed by economic losses attributed to invasive species in the United States (US), which is estimated at US$137 billion annually (Pimentel et al., 2000).

The term "ecological roulette" has been used to describe the uncertainty associated with the consequences of introduced species (Carlton & Geller, 1993). Some species appear to integrate themselves into a new habitat and benignly co-exist with native species; however others completely alter the natural ecosystem in which they invade. It is clear that a greater understanding of the processes which govern species invasion success and outcome is necessary in order to limit the potential risks they pose to the environment and economy.

Invasions in aquatic ecosystems

Freshwater, estuarine and marine ecosystems are considered to be among the most severely invaded regions worldwide (Moyle, 1999). In North America alone, over 298 NIS of algae and invertebrates have been reported in marine habitats (Ruiz et al., 2000a) and an additional 170 NIS in the Great Lakes Basin (Ricciardi, 2001; Holeck et al., 2004). In some

aquatic ecosystems, biological invasions have completely altered natural food web dynamics. For example, the Great Lakes Basin food web is now dominated by the interaction and activities of non-indigenous species, such as *Dreissena polymorpha*, *Dreissena bugensis*, *Cercopagis pengoi*, and *Neogobius melanostomus* (Ricciardi & Rasmussen, 1998).

Zooplankton are an important component of pelagic food webs because of their intermediate role in transferring organic energy produced by photosynthetic organisms to higher trophic levels such as pelagic fish stocks that are exploitable by humans. Apart from predation, zooplankton are considered to be the most important factor controlling the year class strength of a large number of fish stocks known to be subject to strong fluctuations (Lenz, 2000). The productivity of a fish stock in a given year will largely depend on the availability of zooplankton at the right time and place during the first feeding period of fish larvae (Cushing, 1990). Zooplankton invasions pose a threat to commercial fish stocks by threatening native zooplankton species which serve as an important food source. Even if an introduced species does replace a native species in abundance, it may not provide a similar quality of food or reproduce and bloom at the safe time of the year, thereby limiting food available for fish larvae.

The invasion pathway is a multi-step process in which an organism must pass through a series of phases in order to establish themselves in a new environment (Figure 1; Williamson, 1996; Kolar & Lodge, 2001; Leung et al., 2002). The first phase and focus of this study, *initial dispersal*, requires that an organism utilizes some form of natural (i.e. currents, winds and animals) or human-mediated (i.e. shipping and aquaculture) transfer mechanism to move to a habitat outside its native range. If an organism is able to survive the transport vector filter then it moves onto the second phase of the pathway, *establishment*. The second phase requires that an organism establishes itself in its new environment and is able to persist through local

reproduction and recruitment (Moyle & Light, 1996b). This may be accompanied by a spread of the population throughout the recipient environment. Whether an organism is able to establish itself will depend on what Elton (1958) refers to as the ecological resistance of the new environment. This includes (i) environmental suitability such as temperature or salinity, (ii) biotic resistance such as prey availability, predation, competition, disease and parasites, and (iii) demographic resistance such as numbers or organisms introduced and reproduction. It may be difficult to infer whether a species has become established in a region. Some species may be so frequently released into an environment that they appear to maintain locally established regions when in fact they are continuously being stocked through dispersal from elsewhere (Vermeij, 1996). The third and final phase of the invasion pathway, *integration*, requires that the newly introduced species is able to move on its own, or utilize transport vectors to spread within its new habitat. During the invasion process, each of these phases selectively reduces the potential pool of aquatic invaders by exposing them to a variety of adverse physical, chemical and biological conditions. Non-indigenous zooplankton populations can be positively or negatively affected depending on their tolerance to environmental conditions and interaction with other biota. Only a small fraction of the initial species pool will survive and persist through all phases of the invasion process. The 'tens rule' (Williamson & Fitter, 1996a) suggests that of the initial pool of species transported to a new environment, only 10% of these species become introduced, only 10% of those introduced become established and only 10% of those established become invasive. These generalizations have been shown to be useful for predicting the fate of introduced birds, terrestrial plants and insects. Its application to aquatic species has received little if any attention.

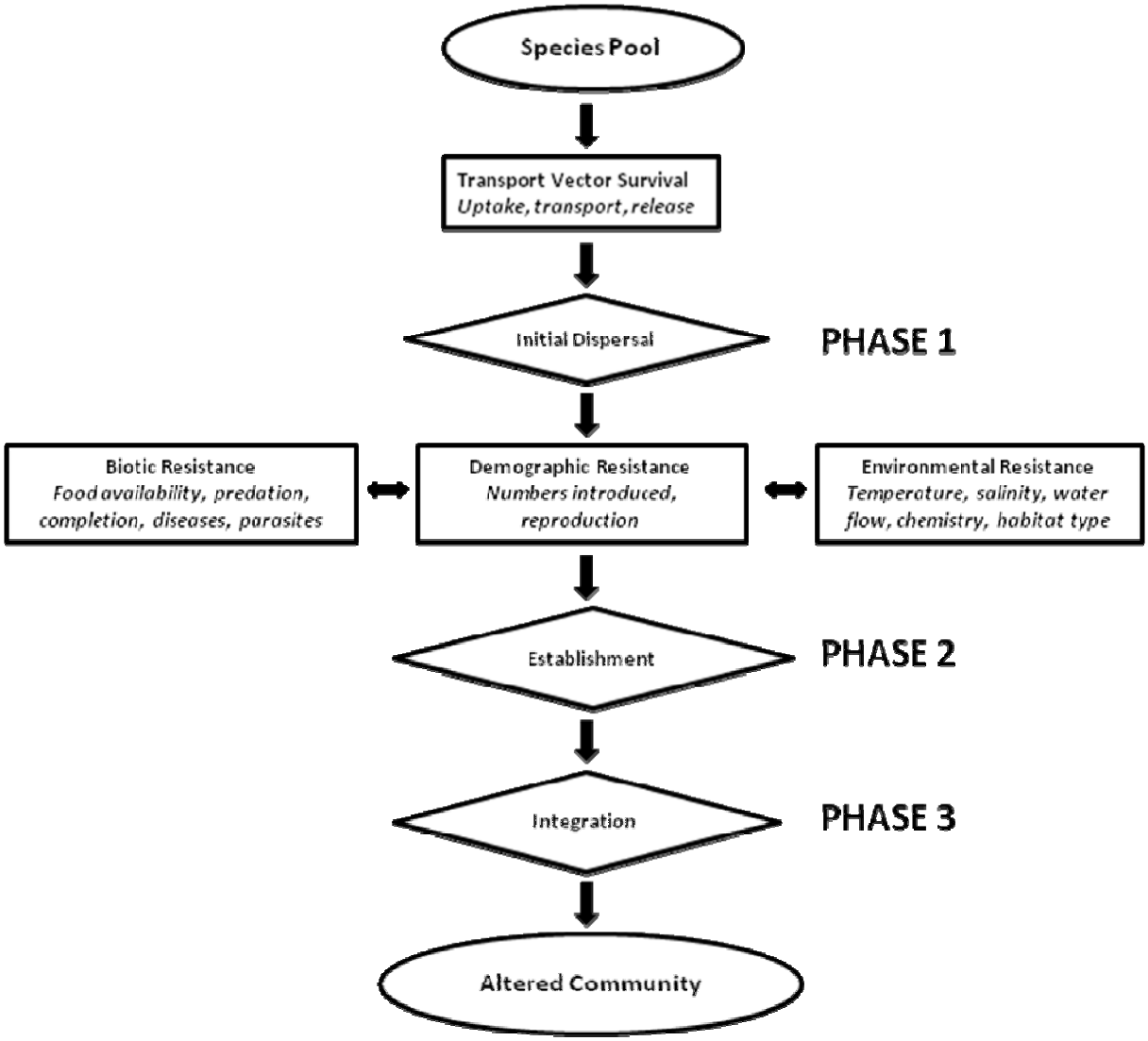

Figure 1. A conceptual model of the invasion pathway (adapted from Moyle & Light, 1996).

Initial dispersal (i.e. transport) is arguably the most important stage of the invasion pathway since it ultimately determines which species are introduced into a new environment and the introduction effort or 'propagule pressure' (discussed later) of species present. Interestingly, very few studies have concentrated their effort on this particular phase. A review of science and ecology papers by Puth & Post (2005) indicates that the majority of empirical research conducted to date (94.8%) has focused on the latter stages of the invasion process, particularly

establishment and spread in the recipient environment. It is evident that research on the transfer phase is lacking and more focus should be directed during this phase in order to understand the primary filter for preventing NIS introductions.

Vectors of aquatic invasions

The intentional and unintentional movement of aquatic species between water bodies worldwide has been largely mediated by human activity, although natural vectors do exist (e.g. tectonic movement, storms, and El Niño events). Increased globalization, trade and travel have all contributed to the worldwide spread of aquatic species between water bodies. International shipping transports 80% of the world's commodities and is fundamental to world trade (NRC, 1998), therefore it is not surprising that ship-mediated vectors such as hull fouling, ballast water and sediments are regarded as principal vectors for introductions of aquatic organisms (Grigorovich et al., 2003; Carlton, 1985). Estimates suggest that these vectors account for about 50% of species introduced into North American coastal ecosystems (Ruiz et al., 2000a) and 67% of those introduced into the Great Lakes Basin (GLB) (Grigorovich et al., 2003). The origins of introduced species vary, but largely reflect international shipping patterns. For example, Wonham and Carlton (2005) analyzed biogeographic literature pertaining to introduced species in the Northeast Pacific (NEP) Ocean and found that the vast majority of introduced species (82.9%) originated in the northern hemisphere, reflecting the dominance of European, Asian and eastern North American trade in the region.

Ballast is taken on by a ship at a source port to ensure stability and discharged en route or at its destination port to adjust for changes in cargo weight. Numerous species have been identified to live in, on or within close association with ships' ballast and are consequently introduced when ballast is discharged into a receiving environment. Changes in the identities of

introduced NIS have occurred concomitantly with changes in the nature of ship ballast. Initial introductions of NIS into North America consisted of terrestrial plants and insects owing to the terrestrial origins of the ballast used at this time (e.g. stones, bricks or debris). This pattern shifted in the early 20th century to aquatic organisms such as algae and invertebrates because of the replacement of solid ballast with water (Ricciardi and MacIsaac, 2000). It is estimated that approximately 10 billion tonnes of ballast water is transported globally each year (Rigby et al., 1995) containing densities of invertebrates in the tens of thousands per cubic meter (Piercey et al., 2000). The transport of NIS by ballast water was first suggested by Ostenfeld (1908) in an attempt to explain the discovery of the Asian diatom *Biddulphia sinensis* in the North Sea in 1903. Since this time, numerous other aquatic invasions have been attributed to ballast water including the notorious zebra mussel (*Driessena polymorpha*) invasion of the GLB (Hebert et al., 1991) and the western Atlantic ctenophore (*Mnemiopsis leidyi*) invasion of the Black and Caspian Seas (Ivanov et al., 2000).

Management and control

The management of aquatic invasions can be divided into three stages: (i) prevention (ii) eradication and (iii) control (Mack et al., 2000). Prevention is recognized by the scientific community as the primary and most effective method of aquatic invasive species management, largely because of the limited success and economic costs associate with eradication and control (Mack et al. 2000). The interminable environmental and biological nature of species introductions has been noted by Marsden (1993) who states "introductions, like extinctions, are forever". Indeed, a similar pessimistic attitude towards eradication of aquatic invasions has been echoed by scientists worldwide. Very few cases of effective eradication efforts exist, especially in aquatic ecosystems, but eradication of an aquatic invader is possible if it is detected early and

resources can be applied quickly (Simberloff, 1997). Bax et al. (2002) documented the eradication of the black-striped mussel (*Mytilopsis sp.*) from Darwin Harbour Australia shortly after its initial appearance in 1999. It is important to note that the eradication project was costly (Au$2.2 million) and took 2 years to complete (Bax et al., 2002).

The significance of ballast water as the primary vector for the transfer of aquatic NIS worldwide has received official recognition through the International Maritime Organization (IMO). In light of increasing human population growth and international trade, the IMO has formulated regulations for preventing the spread of harmful aquatic species and pathogens between aquatic ecosystems, the most widely adopted of which is mid-ocean exchange (MOE). It is during this process that vessels replace their original ballast water taken on board while the vessel is at port or near to the coast, with open ocean water. Mid-ocean exchange theoretically reduces the threat of NIS introductions by (i) discharging a high percentage of the coastal species into unfavourable environmental conditions in the open ocean, and in some cases (ii) increasing the salinity level within the ballast tank to a level not normally tolerated by freshwater or brackish species associated with coastal and inland ports (Taylor et al., 2002).

Coastal ballast water is replaced with open ocean water during MOE by one of two methods: (i) empty-refill or (ii) flow-through exchange. The empty-refill method involves emptying and subsequently refilling ballast tanks with ambient ocean water, which theoretically replaces 95% of the original water. The flow through method flushes tanks by taking up open ocean water and allowing the tank to overflow until three tank volumes (300%) of water have been exchanged. Exchanging three tank volumes was found to be necessary in order to replace approximately 95% of the original water since mixing of coastal and open ocean water occurs with this method (Rigby & Hallegraeff, 1994). Which exchange method is used is often

determined by the design of the ship. Ships that are capable of either method typically prefer flow-through exchange since the complete removal of ballast water during empty-refill exchange poses a greater risk to ship stability and safety (Murphy et al., 2004).

The theoretical premise behind the use of MOE to reduce invasion risk is based on two assumptions, namely (i) that the open ocean contains a lower density and diversity of species than coastal environments and (ii) open ocean species are less likely to survive the low salinity waters typical of many ports. Several studies have rejected the first assumption, arguing that MOE can actually contribute to a higher zooplankton density and diversity than what was taken up with source port waters. For example, MacDonald and Davidson (1998) reported a post-exchange increase in the diversity of diatoms and dinoflagellates of 16% and a 54% increase in their abundance. It is not surprising that the diversity increases following MOE considering that mid-ocean environments typically have a greater diversity than coastal habitats and some residual coastal species are usually still present in the tanks following exchange. Post-exchange densities have been observed to increase and remain higher than the initial density up until the final day of some transoceanic voyages (Wonham et al. 2001). The assumption that open ocean species are less likely to survive in low salinity coastal ports may be true, but only for freshwater ports or those that receive freshwater input. Indeed, some coastal ports such as Los Angeles, CA have salinities slightly below, equivalent to or even higher than the open ocean. The same will be true for similar ports which have high rates of evaporation and receive little precipitation and freshwater input from lacustrine and/or riverine systems.

The efficiency of ballast water exchange has been estimated to replace approximately 95% - 100% of the original water (Rigby 2001). Studies which have examined ballast water exchange efficiency have typically done so on the basis of salinity (Ruiz & Hines, 1997) or with

the use of dyes (Rigby & Hallegraeff, 1994). The efficiency of removing organisms can be viewed as distinct from water replacement and has been shown to vary greatly depending on the particular ship, voyage, exchange method and organisms present. Studies that have measured the exchange efficiency of plankton organisms have found effectiveness to vary depending on the taxa being considered. For example, Locke et al. (1993) found MOE to be 67% effective for zooplankton whereas Zhang and Dickman (1999) reported 87% effectiveness for diatoms and dinoflagellates. Exchange method also appears to be an important determinant of exchange effectiveness, since Cordell et al. (in press) reported lower densities of coastal taxa in vessels conducting empty-refill exchange as opposed to flow-through exchange. It is expected that the exchange efficiencies reported above may actually be underestimated since most of these studies were unable to distinguish entirely between coastal and open ocean species.

Since ballast water exchanged was initially recommended by the IMO in 1991, it has been adopted as either a voluntary or mandatory measure for ballast water management by 67 countries around the globe (IMO, 2004). Despite this, numerous aquatic invasions are still being documented in aquatic systems with ballast water as a likely vector. Coupled with accelerating changes in climate conditions and the modification of aquatic habitats worldwide, this vector presents a viable avenue for the translocation of NIS. Careful examination of current ballast water management practices should be performed to determine whether additional or alternative strategies should be implemented.

Canadian ballast water regulations

On 8 June 2006, Transport Canada (TC) initiated mandatory mid-ocean exchange (MOE) regulations for ocean-going vessels entering all Canadian ports (TC, 2006). These management procedures were implemented to minimize the transfer of harmful aquatic organisms and

pathogens in ships' ballast water, as recommended under IMO guidelines (IMO, 1997). Until this time, very few Canadian ports had implemented such procedures, with most ports adopting the voluntary guidelines introduced by TC in 1989. The port of Vancouver was one of the first Canadian ports to adopt mandatory MOE, with the port authority issuing standing orders as of 1997 (VPA, 2002). Based on current federal regulations, vessels are categorized into one of the following 3 classes (Figure 2): (i) transoceanic vessels carrying exchanged ballast water (TOE); (ii) intra-coastal vessels carrying exchanged ballast water (ICE); and (iii) intra-coastal vessels carrying un-exchanged ballast water (ICU). The distinction between each shipping category is based on the geographic origin of the ballast water intended to be discharged in Canadian waters. Current federal regulations require that trans-oceanic vessels intending to discharge ballast water from overseas within Canadian waters are to perform MOE outside the 200 mile exclusive economic zone (EEZ) in waters deeper than 2 km or in designated exchange areas (TC, 2006). However, vessels planning to discharge water from intra-coastal ports may not be required to exchange ballast water depending on its origin. Vessels originating from eastern Pacific ports south of Cape Blanco, OR (i.e., ICE) are required to comply with MOE regulations, but ships originating north of this location (i.e., ICU) are not required to perform MOE. The reason for this distinction is because the coastal environment north of Cape Blanco is thought to be contiguous with coastal BC due to northward flowing currents (Pickard and Emery 1996), and therefore, similar in native community composition. The same exemption is true for ships entering eastern

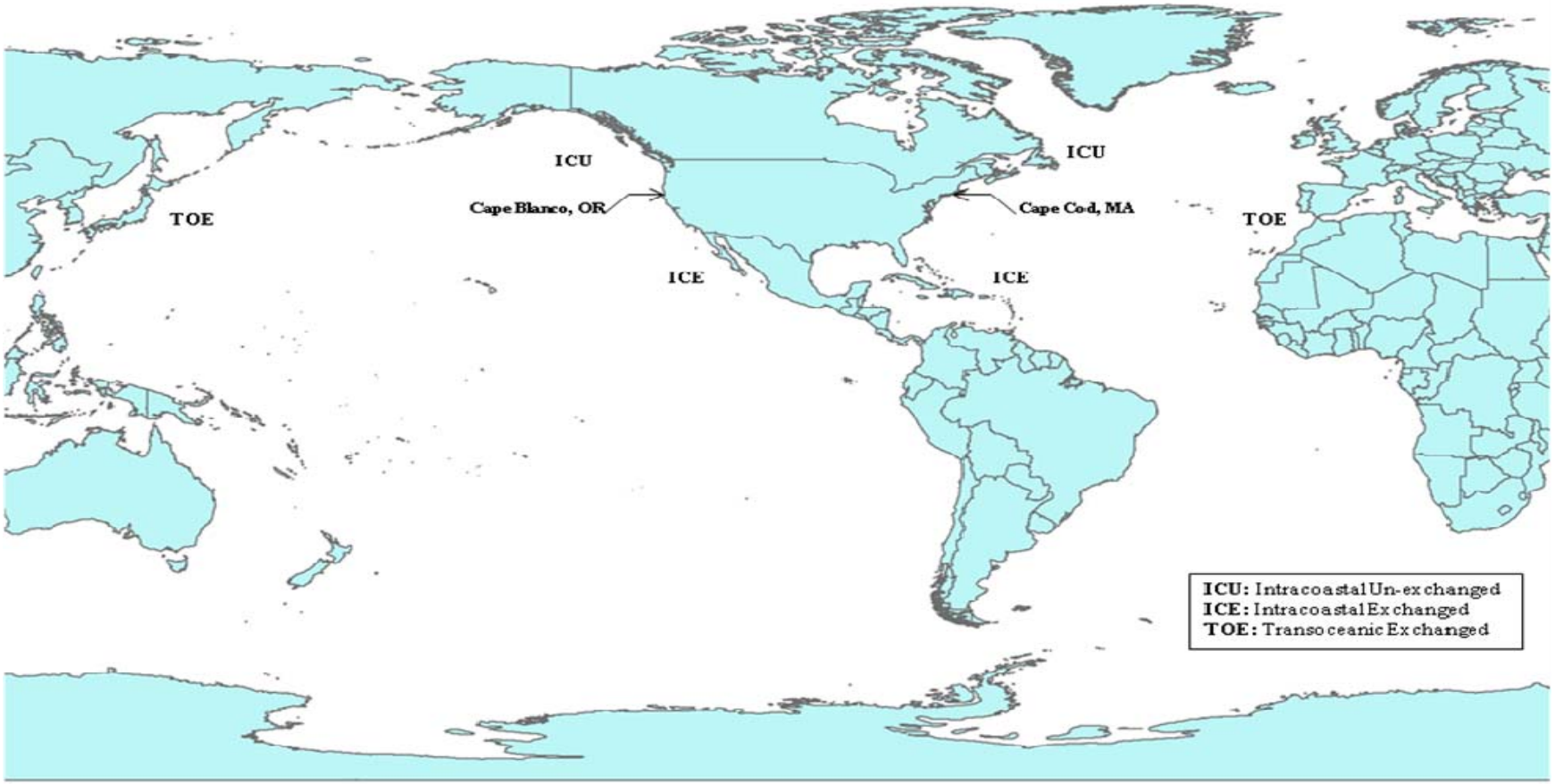

Figure 2. Map indicating the geographical extent or ports within shipping classes sampled in the present study and identified by current ballast water regulations in Canada.

Canadian ports whose last port of call was north of Cape Cod, MA. For those intra-coastal vessels that do have to perform MOE, they must exchange their ballast water at least 50 nautical miles from shore in waters deeper than 500m. Mandatory MOE regulations for the GLB were enforced well before they were for coastal ports largely because of the watershed's economic importance to both Canada and the US, and because of the high frequency of historical invasions (Riciarrdi 2001; Holeck et al., 2004). In 1993, mandatory MOE regulations were introduced for all vessels carrying 'ballast-on-board' (BOB) into the GLB and were made to follow protocols as outlined above for inter-coastal vessels (US Coast Guard, 1993). Exempt from these regulations are a category of vessels known as 'no-ballast-on-board' (NOBOB), which do not carry ballast water into the GLB, and comprise approximately 90% of the shipping traffic (Collauti et al., 2003). Despite the NOBOB classification, these vessels are believed to present a potentially serious invasion threat to the GLB because of residual volumes of un-pumpable ballast water and sediment the tanks often retain (Niimi & Reid, 2003). Residual ballast water sediment has been found to be diverse and abundant with resting stages of non-indigenous invertebrate species which may hatch in tanks or once released (Duggan et al., 2005; Bailey et al., 2005). As vessels take on ballast water while unloading cargo, the incoming water may re-suspend the residual ballast water and sediment which in turn may be discharged at the same or subsequent ports when loading cargo.

Although it might be expected that trans-oceanic vessels pose the greatest risk for non-indigenous zooplankton (NIZ) introductions due to source region differences in species composition, a recent study suggests otherwise. Cordell et al. (in press) characterized the zooplankton communities of vessels entering Puget Sound from intra-coastal and transoceanic vessels, and found significantly greater densities of high risk taxa in coastal vessels. Other

studies have also emphasized the importance of intra-coastal shipping in the regional and secondary spread of NIZ (Wasson et al., 2001; Lavoie et al., 1999). Wasson et al. (2001) emphasized the importance of intra-coastal transport when they documented the occurrence of 56 non-indigenous invertebrates in an estuary without international shipping. They argue that many of these species were transported through intra-coastal transport since 51 of these 56 species had been documented in neighbouring San Francisco Bay (SFB), and therefore represent secondary transfer. Secondary invasions refer to regional spread of an introduced species to additional habitats as the results of a previous primary invasion. As one of the most heavily invaded estuaries in the world, SFB is inhabited by approximately 150 non-indigenous invertebrates (Cohen & Carlton, 1998), many of which have spread to other regions in North America (Grosholz & Ruiz, 1995). In the case of this study, the exemption of ICU vessels may facilitate secondary invasions from previously invaded ports in North America. It is possible that intra-coastal shipping could play a bigger role in NIZ introductions since organism survivorship is highest in voyages of short duration (Williams et al., 1988), intra-coastal transport often represents a greater proportion of total ship traffic than inter-coastal visits (Verling et al., 2005) and because some intra-coastal ships are exempt from MOE regulations thereby increasing the likelihood of secondary invasions (e.g. Levings et al., 2004; Cohen et al., 1995).

Determining Invasion Risk

Despite evidence that aquatic invasions have been rising steadily during the past century (Levings et al., 2002; Mills et al., 1993a; Ricciardi 2001) limited progress has been made towards preventing future invasions due in part to our inability to identify likely invaders and implement effective management strategies (Endresen et al., 2004; Drake & Lodge, 2004). Considerable debate exists about which parameters are useful proxies for identifying potential

invaders and determining invasion risk. However, three parameters that are consistently used for evaluating invasion risk include: (i) propagule pressure, (ii) invasion history and (iii) host and recipient environmental similarity (Williamson, 1996; Colautti et al., 2006; Williamson & Fitter*b*, 1996; Riciarrdi & Rasmussen, 1998; Lodge, 1993). Each will be discussed in more detail below.

(i) Propagule pressure

Propagule pressure, also termed 'introduction effort', has been shown to hold predictive explanatory power in terms of establishment success or failure of introduced species (Williamson & Fitter, 1996*a*). That is, as the number of release events and/or number of individuals per given volume increase, so does establishment likelihood. In terms of ballast water introductions, this would translate into the number of vessels de-ballasting into a recipient environment, the average number of zooplankton individuals present in each release event and the average volume of ballast water released per vessel.

Determining invasion risk based on propagule pressure alone would suggest that the same propagule pressure in space and time would predict approximately the same number of invasions (Ruiz et al., 2000a). However, as studies have shown in natural ecosystems, the invasion process is a stochastic event, with propagule pressure and success varying on spatial and temporal scales. Numerous studies have quantified zooplankton density in ships ballast water, but the concentrations have been shown to vary (Harvey et al., 1999; Levings et al., 1998; Chu et al., 1997). The variability is explained by the fact that plankton densities in ballast water is an 'event-level' characteristic, differing between source regions, voyage lengths and seasons. Zhang & Dickman (1999) examined seasonal effects on phytoplankton densities in the ballast water of ships entering Hong Kong, China from Oakland, CA. They found that the density of harmful

phytoplankton varied considerably throughout the year, with the highest abundances occurring in April and February when temperatures were low in both ports. Additionally, they found that the period of greatest species richness of NIS did not correspond to the period of highest NIS abundance. This is not surprising since the dominance of a single species (i.e., bloom) is often associated with a lower diversity of other taxa due to competition for limiting resources (Margalef, 1967).

These examples of temporal and spatial variability emphasize the need to characterize variability in zooplankton density and species richness in vessels entering Canadian ports from different source regions and over multiple seasons. An understanding of how these variables vary in space and time will enhance our ability to determine the identity of likely invaders and high risk source regions (Williamson 1996, Drake & Lodge 2004, Endresen et al. 2004; Ruiz et al. 2000a).

(ii) Invasion history

Identifying species with a history of successful invasions has been suggested to be a useful tool for identifying likely invaders (Kolar and Lodge, 2001; Moyle & Marchetti, 2006; Kolar and Lodge, 2002). This method assumes that a species will continue to invade habitats outside its native range if favourable conditions and transport vectors exist. Ricciardi (1998) suggests that our ability to identify likely invaders can be enhanced by focusing on those species which are capable of utilizing human-mediated transport mechanisms, specifically ballast water transport. In order to achieve this species specific, profile-based approach to ballast water management, it is necessary to identify and quantify NIZ that are capable of capitalizing from ballast water transport.

A successful primary invasion can be considered one in which a species initially develops an established, self-sustaining population outside of its native range. The European Green Crab (*Carcinus maenus*) for example, has established spatially distinct populations in Australia, the northeast Pacific, northwest Atlantic and South Africa from source populations in the northeast Atlantic (Cohen et al. 1995). Similarly, the calanoid copepod *Pseudodiaptomus marinus*, has invaded the Hawaiian archipelago, northeast Pacific and the Indian Ocean with source populations from the northwest Pacific (Carlton, 1985; Carlton & Geller 1993; Grosholz & Ruiz, 1995). Based on invasion history, it can be expected that these species and others with successful invasion histories will continue to invade other coastal regions if favourable opportunities exist. From a management perspective, invasion history can be useful in identifying which non-indigenous species pose a threat to native environments and guide policy makers in adopting practices to limit exposure to these species.

Once a foreign species has established itself in a new environment it can continue to spread and invade neighbouring habitats, a process defined as secondary invasions. Some species benefit greatly from localized human-mediated dispersal mechanisms (i.e. hulls of recreational boats) while others are able to migrate using natural dispersal, such as planktonic larvae that disperse via currents (Zevina and Kuznetsova, 1965; Geller 1994). The secondary or intra-regional spread of individuals and their offspring can be an important component of a species invasion history. Zebra mussels (*Dreissena polymorpha*) for example, first became established in Lake St. Clair (GLB watershed) in 1988 (Hebert et al., 1991) through ballast water transport and have continued to expand from their initial location through natural dispersion and recreational boat traffic. Since their initial establishment, *D. polymorpha* can now be found in over 50% of the waterways in the US (Edwards, 1994).

(iii) Host and recipient environmental similarity

A critical factor influencing the survival of any biological invader is its compatibility with the biotic and abiotic environmental conditions in the invaded habitat (Lodge, 1993; Moyle & Light, 1996a; Mack 1996; Simberloff, 1986). Biological interactions between native and non-native communities are an important component in determining the survival of an introduced species (e.g. Grosholz, 2005; Stachowicz et al., 2002). Other studies however, have argued that equally if not more important in determining invasion risk is the abiotic suitability of the recipient habitat (Moyle & Light, 1996b; Baltz & Moyle, 1993). Moyle and Light (1996b) found that abiotic factors characterizing California streams, such as the timing and intensity of the spring flow rate, were most important in determining the success of invading fishes, regardless of whether native biota were already present. Further evidence to support the importance of environmental suitability is provided by the application of risk assessment models, which predict invasibility based on host-recipient environmental similarity (Herborg et al., 2007a). These models have successfully determined the range expansion of invasive species and invasibility of particular regions based primarily on environmental similarity to other inhabited environments. Herborg et al. (2007b) tested the use of ecological niche modelling to predict the range expansion of the invasive Chinese mitten crab (*Eriocheir sinensis*) in Europe and found invaded regions to have higher environmental similarity than un-invaded regions. Therefore, comparisons bewteen host (i.e. ballast water) and recipient environments can be a useful parameter for evaluating invasion risk.

Thesis Objectives

The purpose of this study was to investigate the role of ballast water as a vector for the transport of NIZ into Canadian waters. This study characterized ballast water zooplankton communities across three shipping classes (TOE, ICE and ICU) and regions (Pacific, Atlantic and Great Lakes). More specifically this study:

1. Characterized and described the species richness and density of total zooplankton and NIZ being transported into Canadian ports by the following 3 shipping classes: (i) TOE, (ii) ICE, and (iii) ICU. This determined which shipping class has the potential to introduce the highest density and diversity of zooplankton and NIZ into Canadian waters.

2. Compared the species richness and density of total zooplankton and NIZ in the ballast water of vessels from the following 3 regions: (i) Pacific Ocean, (ii) Atlantic Ocean, and (iii) Great Lakes Basin, for each of the shipping classes identified above. This determined whether regional differences exist in ballast water zooplankton communities and whether particular regions of Canada receive greater densities of NIZ where comparisons were possible.

3. Characterized the seasonal variability in total zooplankton and NIZ density and species richness for all vessels sampled in the Pacific region. This determined if seasonal difference exist in zooplankton ballast water community characteristics and whether greater densities of zooplankton and NIZ are transported at a particular time of the year.

4. Determined which shipping class identified above possesses the greatest invasion risk to Vancouver Harbour based on (i) an extrapolated estimate of NIZ released over the course of a year (i.e., propagule pressure), (ii) identifying the relative abundance of species with

a history of invasion success elsewhere, and (iii) comparisons of abiotic conditions between the ballast water and recipient environment. This determined which shipping class and species pose the greatest invasion threat and where management efforts should be concentrated.

5. Determined whether relationships exist between total zooplankton density and ballast water conditions, such as volume, age, temperature and salinity. This helped explain regional and shipping class differences in ballast water zooplankton densities.

Overall, this research study is intended to make a significant contribution to aquatic invasion science by increasing our knowledge of ballast water introductions by (1) identifying zooplankton species transported by ballast water transport (2) identify which non-indigenous zooplankton species pose a risk to Canadian aquatic environments, and (3) evaluating current ballast water regulations and their effectiveness in preventing zooplankton introductions. In the long term, this study is intended to make a significant contribution to the literature on ballast water invasions in order to facilitate future risk assessment and management decisions for controlling the global movement of organisms via ballast water.

METHODOLOGY

Study regions

Between September 2006 and November 2007, foreign ballast water was sampled and shipping crew surveyed aboard vessels arriving at various ports on Canada's Pacific and Atlantic coasts, as well as in the Great Lakes. These 3 geographically distinct regions represent the largest marine and freshwater habitats within Canadian boundaries, with the exception of the Arctic Ocean, and receive the majority of commercial shipping traffic in the country. The high occurrence of shipping along with a history of successful invasions in these regions suggests that these areas are likely to be prone to ballast-mediated invasions and should therefore be examined carefully.

Ballast water sampling protocol

Preliminary ballast water zooplankton sampling trials were conducted on 3 vessels during August and September 2006 to test a variety of sampling equipment and techniques. Attempts were made to sample ballast water through sounding pipes using a 12V well pump, as well as using plankton nets with various mesh sizes through open manholes. The sounding pipes are access points on the deck of the ship which allow the ship's crew to determine the water height within each ballast tank. These access points were chosen since they are always open and therefore do not require any assistance from shipping personnel. While it was possible to obtain samples using the sounding pipes, this sampling methodology was abandoned because: (i) the flow rate of the pump was inefficient for sampling the volume of water required (ii) the pump appeared to damage some soft-bodied zooplankton and (iii) the sounding pipes extended to the bottom of the ballast tanks thereby preventing pumps from sampling the entire depth range of the tank. Several studies have suggested that sounding pipes are not the best access point because they do not provide a good representation of the zooplankton community composition due to the

heterogeneous distribution of zooplankton within the ballast tanks (Murphy et al. 2002; IMO 2005). Instead, zooplankton sampling in ballast water tanks was achieved via vertical tows using a standard plankton net. Only ships that allowed access to ballast tanks through open manholes located on the deck of the ship were sampled in this study.

Ballast water samples were collected from vessels on an opportunistic basis. Sampling efforts were focused on bulk carriers and general cargo carriers since they are responsible for discharging the greatest percentage of ballast water internationally (Endresen et al., 2004); roll-on/roll-offs and oil tankers were sampled on occasion. Zooplankton samples were collected aboard each vessel in accordance with International Maritime Organization (IMO) guidelines for ballast water sampling (IMO, 2005). Approximately 1000L of ballast water was filtered through a 30-cm diameter, 125-µm mesh plankton net by conducting several vertical net tows through an open manhole (Figure 3). Typically nets were lowered to a depth of 2 to 13m depending on the depth of water in the tank and whether any obstructions were present. International Maritime Organization guidelines specify the use of a 50-µm plankton net for zooplankton sampling; however several studies have suggested that clogging can result from using mesh sizes smaller than 100µm (Tranter & Huron, 1967; Smith et al., 1968). This can lead to unequal filtration in various parts of the water column, differential pressures across apertures remaining open leading to the extrusion of organisms, and increased backflow at the mouth of the net (akin to bow wave) triggering an avoidance response by larger, faster swimming organisms (Likens & Gilbert, 1970; Harris et al., 2000). A 125-µm net was chosen in addition to a 50-µm net because it: (i) reduces the number of tows necessary to filter 1000 litres of water by half when compared to a 50-µm net (ii) reduces the probability of clogging and (iii) increases the likelihood of capturing

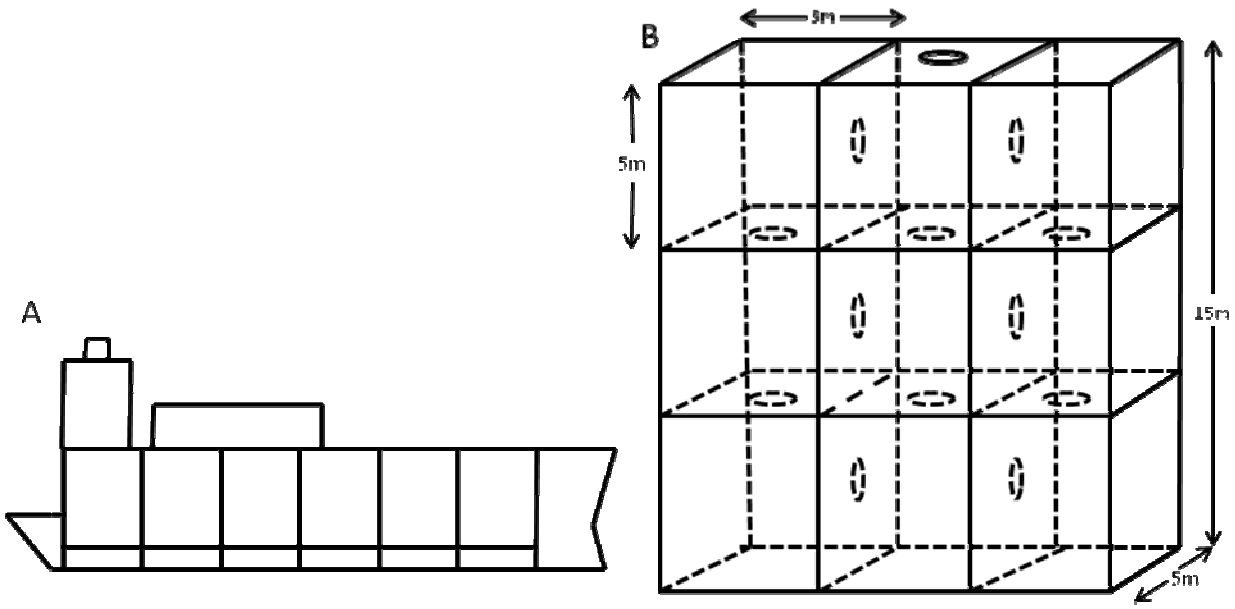

Figure 3. Illustration of (A) typical vessel sampled during study and (B) design of the ballast tanks. Note that ballast tanks are often divided into several compartments preventing sampling of entire water depth.

faster moving species. To ensure that smaller zooplankton species were not being overlooked by this alternative sampling method, one additional net tow was conducted using a 50-µm plankton net. Only one net tow was necessary to characterize the 50- µm to125-µm size class because smaller organisms (i.e. copepod nauplii, bivalve veliger larvae, rotifers) are usually more abundant thereby requiring less water to be filtered and because the majority of these organisms were unidentifiable to species level. The total volume of water filtered was determined using a mechanical flowmeter (General Oceanics Inc. Model 2030R) lashed to the inside of each plankton net. All samples were concentrated and preserved in 5% buffered formalin, then stored at room temperature until being enumerated and identified. Surface temperature and salinity measurements were taken for each tank sampled using a handheld YSI 30® instrument. International Maritime Organization ballast water reporting forms (Appendix A) detailing the ship's ballast water history (port of ballast water origin and uptake, volume and date of ballast water uptake, age of ballast water, as well as location, date and method of any MOE) were collected from all sampled ships. The sampling procedures described above were chosen to suit

both the scientific needs of this study and to minimize interference with shipping operations and personnel.

Ballast water sampling was initiated on the Pacific coast in September 2006. A co-ordinated nationwide sampling effort began in April 2006 and ended in October 2007 because. zooplankton productivity is generally highest in the Northern Hemisphere during spring and summer (Colebrook, 1979), thereby maximizing chances to detect zooplankton taxa taken up during ballasting operations and transported at this time of year.

Biological analysis

Zooplankton samples were sorted and individuals identified to lowest possible taxon, ontogenetic stage (e.g., nauplii) and sex when possible using stereomicroscopy. Enumeration and identification of small taxa (e.g., copepod nauplii and rotifers) was conducted by randomly sub-sampling from the entire sample using 1-ml and 3-ml Hensen-Stemple pipettes. Taxa that were present in high densities were quantified in 1/100 – 1/300 of the sample volume, whereas those that were less abundant were quantified in 1/10 of the sample volume. For larger taxonomic groups (e.g., decapod zoea and euphausiids), the entire sample was sorted for identification and enumeration of select taxa. For each taxon, up to 25 randomly selected individuals were measured in length and averaged to aid in species, ontogenetic and sex classification.

Zooplankton taxa were classified as being indigenous or non-indigenous to the regional aquatic habitats in which they were to be discharged using taxonomic and biogeographic literature (Gardner & Szabo, 1982; Brunel et al., 1998; Davis, 1949; Balcer et al., 1984; Shih et al., 1971; Razouls et al., 2008). In the Pacific region, this included nearshore, coastal, estuarine and freshwater habitats of BC. For the Atlantic region, this included similar habitats for the Gulf of St. Lawrence, and for the Great Lakes region this included all freshwater habitats of the

Laurentian Great Lakes watershed. Taxa that were not identified to species level were classified as 'unknown' if observed aboard vessels in both the Pacific and Atlantic regions, whereas any taxonomic group not native to freshwater environments were classified as 'non-indigenous' if observed aboard vessels in the Great Lakes region. Taxa were also classified as being freshwater, cryptogenic (i.e. true origins unknown) and/or already introduced and established in North American aquatic environments.

Data analysis

Zooplankton densities (individuals · m^{-3}) were calculated by dividing the total abundance of a given taxonomic group by the total volume of water filtered by the plankton net per vessel. Similarly, species richness (taxa · m^{-3}) was calculated based on the total number of taxa observed in a given volume of water (m^3). For the majority of samples, the total volume (m^3) of water filtered, V (Eq. 1) was calculated based on the tow distance (m) of the flowmeter through the ballast water, d (Eq. 2). Both equations were provided in the flowmeter manufacturer's manual:

$$V = \frac{\pi \cdot \delta^2 \cdot d}{4} \quad \textbf{(1)}$$

$$d = \frac{\tau \cdot \varphi}{999999} \quad \textbf{(2)}$$

where π = pi (3.14), δ = the plankton net diameter (0.30 m), d = the distance over which the plankton net is pulled through the ballast water (m), τ = the number of flowmeter revolutions which is based on the difference in the start and end count indicated on the flowmeter, φ = the rotor constant for this particular flowmeter model (26,873). In several cases, the volume of water inside the ballast tank was not sufficient to turn the flowmeter propeller and tow volumes were calculated based on an equivalent but different equation (Eq. 3):

$$V = d \cdot \pi \cdot \left(\frac{\delta}{2}\right)^2 \cdot \mu \quad \textbf{(3)}$$

where $\delta/2$ = the radius of the plankton net (0.15 m), and μ = the number of tows conducted per tank.

Propagule pressure (individuals deballasted · year^{-1}) was calculated based on data collected for this study (NIZ density and mean discharge volumes) and from Vancouver Harbour shipping traffic data (total number of ships · year^{-1}) provided by Lo et al. (unpublished data). Propagule pressure estimates were calculated for Vancouver Harbour because it was the only port in which all shipping classes were sampled and shipping traffic data was available. For each shipping class, propagule pressure of NIZ was calculated using the following equation (Eq. 4):

$$PP = \sigma \cdot \beta \cdot \theta \quad \textbf{(4)}$$

where σ = mean NIZ density per shipping class (ind · m^{-3}), β = mean discharge volume per shipping class (m^3), θ = the total number of release events in Vancouver Harbour from September 2006 to September 2007 (ships · year^{-1}). It is important to note that estimates are based on the assumption that the density of zooplankton in the single sampled ballast tank is representative of all tanks being deballasted.

Ballast water history, ballast tank and vessel characteristics were collected and analyzed based on the data provided on the IMO reporting forms. In few cases, missing data was collected through verbal communication with the vessel's Chief Officer or from the ballast water reporting log. Ballast water discharge volumes per vessel were calculated based on only those tanks which were listed to be discharged in the port of sampling. While the data provided on the reporting form is believed to be accurate, there is no reliable method to test this because forms are completed manually prior to arriving at ports of call.

Seasonal temperature and salinity data was obtained for three locations in the Strait of Georgia, BC; an inland sea connected to Vancouver Harbour. Complete seasonal data was not available for Vancouver Harbour. Data for the three regions was analyzed, including: the Main Strait of Georgia, off Point Grey, and near the mouth of the Fraser River. Data for the Strait of Georgia, BC was provided courtesy of R. Pawlowicz (UBC); for more information including sampling locations see Pawlowicz et al. (2007).

Shipping traffic data for Vancouver Harbour was analyzed for an entire year, spanning the time during which this study took place (September 2006 – September 2007). Vessels were categorized into each of their respective shipping classes (ICU, ICE and TOE) based on source ports origin of their ballast water to be discharged. Vessels which did not provide information regarding ballast water history were omitted. Data was provided courtesy of V. Lo (UBC).

Statistical analysis

All parametric analyses were performed using SYSTAT® Version 11 statistical software (SYSTAT Software Inc., 2004). Where appropriate, group variances were tested to assure homogeneity (Bartlett's test) and the residuals were examined for normality (Sokal and Rohlf, 1981). Zooplankton densities were positively skewed in that most densities were low with relatively few extremely large outliers. Furthermore, zooplankton density group variances were heterogeneous across factor levels. In an effort to normalize the date, equalize the variances and enhance the power of the parametric statistical tests, all zooplankton densities (total and non-indigenous) were log (x + 1) transformed prior to statistical analysis. In all cases this reduced the skewness of the data, but did not always satisfactorily homogenize the variances. Transformations were not necessary for species richness or ballast water environmental data.

The main factors of interest in this study (shipping class, region and season) were sampled unequally due to constraints in sampling personnel, shipping routes, and access to vessels. Thus sample sizes for comparing these factors were often unbalanced and vessels were not sampled across all shipping classes and seasons within each region. This limited the use of 2-way and nested design ANOVA's, which require data for all factors being compared. For instance, a lack of ICU vessels sampled in the Atlantic and Great Lakes regions prevented the use of a 2-way ANOVA to assess whether there was any interactive relationship between shipping classes and regions. The same analysis was used to look at ICE and TOE vessels only, but no interaction between factors was found justifying the use of 1-way ANOVA's.

One-way ANOVA's were used to test for differences in zooplankton densities, species richness, ballast water age, ballast tank volume, ballast water temperature and salinity between shipping classes (i.e. ICU, ICE, and TOE) and regions (Pacific, Atlantic and Great Lakes). In the Pacific region, ANOVA's were also used to test for seasonal differences in zooplankton densities and species richness, in addition to shipping class differences in the volume of ballast-on-board vessels and propagule pressure. It was assumed that an effect of any test was significant using an *a priori* α level of 0.05. If ANOVA models proved to be significant, unplanned multiple comparisons were used to distinguish group differences (Tukey; Zar, 1984).

Testing for relationships between ballast tank environmental conditions and total zooplankton density was done using stepwise multiple regression analysis (Zar, 1984). This determined which of the ballast tank environmental conditions measured explained most of the observed variability in zooplankton density.

Primer® Version 6 was used to produce a species accumulation plots (i.e., rarefaction curves) of the number of taxa observed in relation to the number of samples collected and

analyzed from each region in this study. Samples were randomly permuted 999 times and the resulting curves averaged to produce a smooth plot.

RESULTS:

Seventy-nine ballast water samples were obtained from 77 foreign vessels frequenting ports of the Pacific coast, Atlantic coast and Great Lakes Basin. Seventy-two samples were collected from ballast tanks while 7 samples were collected from cargo holds of vessels which can also serve as ballast water reservoirs. Seventy-four samples were used in regional and shipping class comparisons. The additional 5 samples were excluded from such comparisons because ballast water did not originate from any shipping class, but from the open ocean or source ports within the Atlantic Ocean (Pacific region only). All 79 samples were pooled in analyses not involving shipping class comparisons, such as ship type comparisons and regressions between zooplankton density and ballast tank environmental conditions.

Vessel and ballast water history

Pacific Coast

In the Pacific region, 43 ballast water samples were collected from vessels making berth at ports within the Strait of Georgia; including Vancouver (n= 42) and New Westminster (n= 1). Sampled vessels originated from 7 different countries and 24 foreign ports (Figure 4A). The majority of the ballast water samples collected were from TOE vessels (37%), followed by ICE (26%) and ICU (30%) vessels. A small fraction of the ballast water samples (7%) did not fall into any of these shipping classes. Differences in the number of ships sampled reflect relative number of ships available in each shipping class. Source ports for ballast water collected from TOE vessels were more diverse geographically, with samples originating from 14 different ports. Ballast water collected from ICE and ICU vessels originated from 7 and 3 ports respectively. Ballast water samples were collected over the course of four seasons; fall (01 September –

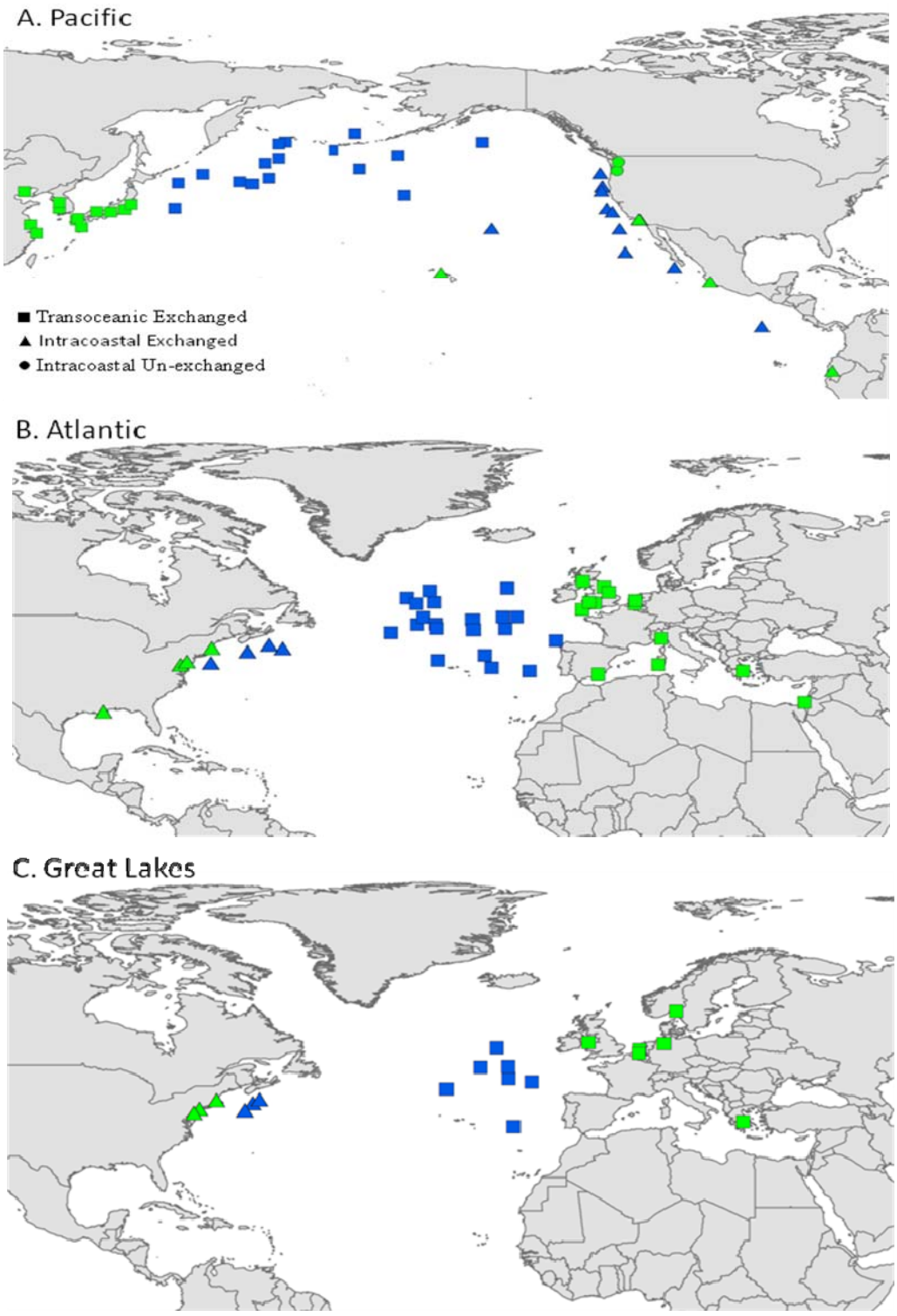

Figure 4. Map of ballast water source locations (green shapes) and mid-ocean exchange locations (blue shapes) for all vessels sampled in the (A) Pacific, (B) Atlantic and (C) Great Lakes regions between September 2006 and November 2007.

31 November; n=8), winter (01 December – 28 February; n=4), spring (01 March – 31 May; n=15) and summer (01 June – 31 August; n=16). The dominance of ships sampled during the spring and summer months is not a reflection of shipping traffic but a higher degree of sampling effort.

Atlantic Coast

In the Atlantic region, 24 ballast water samples were collected from vessels making berth at ports within the Gulf of St. Lawrence; including Sept-Îles (n=16), Baie-Comeau (n=3), and Port-Cartier (n=5). Sampled vessels originated from 8 different countries and 18 foreign ports (Figure 4B). The majority of the ballast water samples were collected aboard TOE vessels (83%), while 7% of samples were collected aboard ICE vessels and 10% of samples were collected aboard vessels that could not be classified into a shipping class. No ballast water samples were obtained from ICU vessels due to the lack of shipping traffic between the port of Sept-Îles and intra-coastal ports north of Cape Cod, MA. The dominance of ballast water samples from TOE vessels is a reflection of the trade between Western European ports and the ports sampled for this study. Source ports for ballast water collected from TOE vessels were more diverse than ICE vessels, with samples originating from 14 different European ports as opposed to 4 different American ports for ICE vessels. Ballast water samples were collected during the spring (54%) and summer 46%).

Great Lakes Basin

In the Great Lakes region, 12 ballast water samples were collected from vessels making berth at ports within Canada and the United States; including Milwaukee (n=1) located on Lake Michigan, Toledo (n=8) located on Lake Erie and Sarnia (n=3) located on Lake Huron. Samples were collected aboard vessels in American ports because of the difficulty in finding vessels with

enough ballast water to sample in Canadian ports. Sampled vessels originated from 7 different countries and 9 foreign ports (Figure 4C). The majority of the ballast water samples (58%) were collected from TOE vessels, while 25% of samples were collected from ICE vessels. The remaining ballast water samples (17%) collected did not originate from either shipping category. No ballast water samples were obtained from ICU vessels because current Canadian and American regulations require all foreign vessels originating from outside the Great Lakes Basin to exchange their ballast in the open ocean or declare NoBOB prior to entering the watershed. Source port ballast water for TOE vessels was collected from 6 different European ports, whereas source port water for ICE vessels originated in 3 different American ports. Ballast water samples were collected during the summer and fall, with the majority (64%) of collections occurring during the fall.

Ballasting operations

Vessels arrived in port carrying on average 70 ± 3% (± 1SE) of their total ballast water capacity (compare Figure 5A to Figure 5B). Bulk carriers represented the most commonly sampled ship type (n = 45) and were capable of carrying nearly twice the average volume of ballast water as any other vessel type (Figure 5A). Significant differences existed in the ballast water capacity of vessels (ANOVA, $F_{calc} = 4.3$; df = 4, 74; p = 0.004) with bulk carriers being capable of carrying a significantly higher volume of water than general cargo carriers (Figure 5A; Tukey's, p = 0.003). Vessels showed significant differences in the volume of ballast on board (BOB) (Figure 5B; ANOVA, $F_{calc} = 3.2$; df = 4, 73; p = 0.018). Bulk carriers carried a significantly higher volume of water compared to general cargo carriers (Tukey's, p = 0.011). This variability among vessels can be attributed to three factors. First, the volume of ballast on board a vessel was positively correlated with its total ballast water capacity (Figure 6; regression

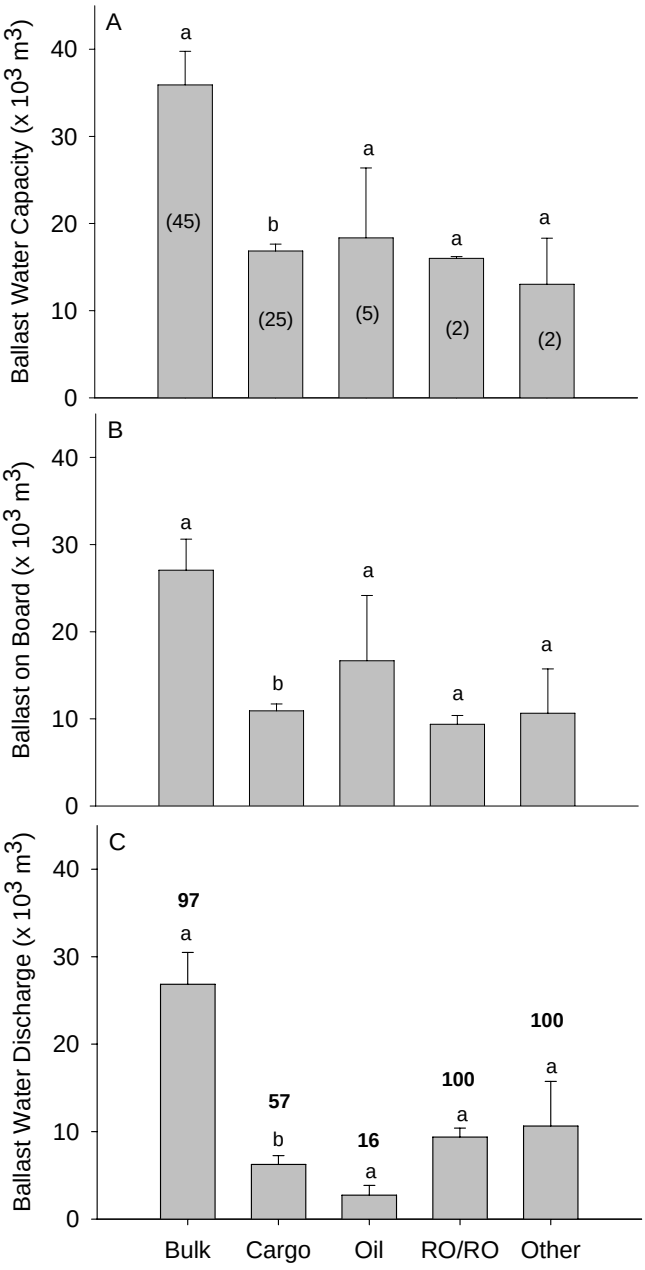

Figure 5. (A) Mean ballast water capacity, (B) ballast-on-board and (C) discharge volumes of vessels sampled between September 2006 and November 2007. Vessels defined as: bulk - bulk carrier; cargo – general cargo carrier; oil – oil tanker; RO/RO – roll-on/roll-off carrier; and other – other vessel types. Different letters above the bars indicate significant differences (Tukey's, $p < 0.05$) among means of vessel types. N-values superimposed on bars in (A) are for all sections. Values above error bars represent mean percentage (%) of BOB discharged at port of arrival. Error bars represent ± 1SE.

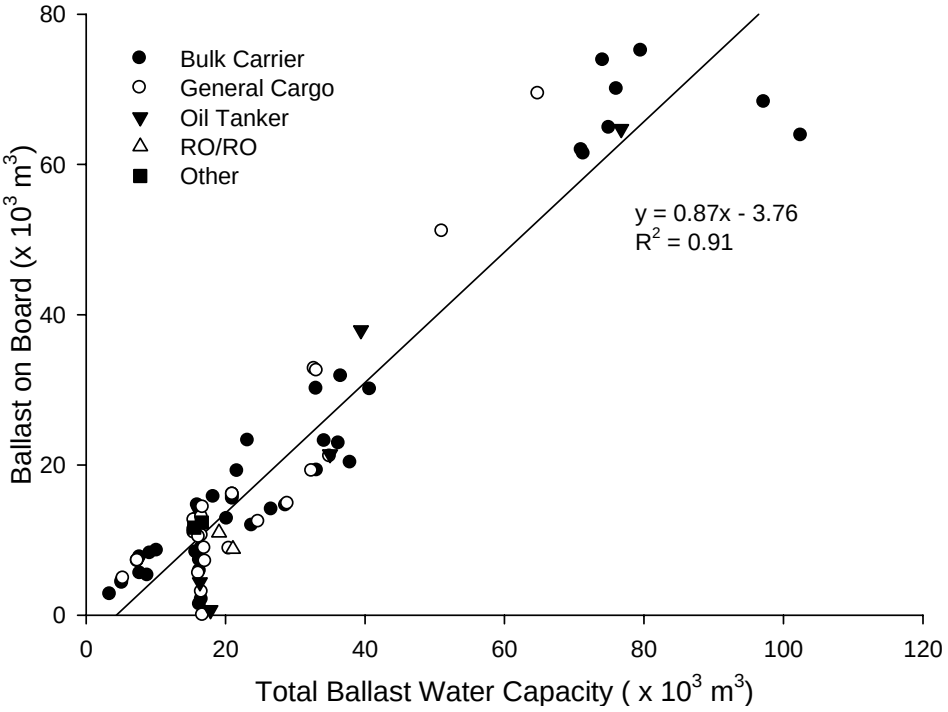

Figure 6. Relationship between total ballast water capacity and the volume of ballast water carried on board vessels at the time of sampling. Regression analysis ($R^2 = 0.91$; $p < 0.0001$).

analysis, $R^2 = 0.91$; $p < 0.0001$), which is largely dependent on the type and/or size of vessel (Figure 5A). Second, the volume of BOB a vessel varied depending on the port specific ballasting regulations and requirements. Vessels entering the Great Lakes region carried an average of 5190 ± 665 m^3 of ballast water compared to 13,915 ± 1,481 m^3 and 39,842 ± 4,983 m^3 for vessels entering Pacific and Atlantic ports, respectively. Thirdly, the variability in the volume of ballast water carried on board each vessel was dependent on the volume of cargo aboard the vessel. It would be expected that vessel with higher volumes of cargo would carry less ballast water; however, this relationship could not be tested due to the absence of cargo volume data on IMO reporting forms.

Wing tanks, located on the sides of vessels, were sampled for ballast water on most vessels (91%) because of ease of access to these types of ballast tanks. Cargo holds were sampled in all other cases (9%). Cargo holds were always sampled aboard bulk carriers, which

are capable of using their holds to carry ballast when necessary. Cargo holds carried a significantly greater (two sample t-test, $T_{calc} = 6.3$, df = 76, $p < 0.001$) volume of water (18,422 ± 1,859 m^3) than wing tanks (2,019 ± 250 m^3), and typically comprised 33 ± 2% of the total ballast water carried on vessels, as opposed to 14 ± 0.2% for wing tanks.

Ninety percent of vessels sampled across all shipping classes and regions reported de-ballasting some or all of their BOB, but the discharge volumes varied substantially (range = 0 - 75,110 m^3; mean ± 1SE = 17,830 ± 2,388 m^3) among vessels. This variability was partially attributed to the (1) volume of BOB, (2) ship type, and (3) volume of cargo loaded and un-loaded at ports of arrival. Discharge volumes varied significantly between vessel types ANOVA, F_{calc} = 5.8; df = 4, 73; $p < 0.001$). Bulk carriers discharged the greatest mean volume of ballast water (26,845 ± 3,642 m^3) representing on average 97% of the BOB (Figure 5C), and discharge volumes were significantly higher than general cargo carriers (Tukey's, $p < 0.001$). Nearly all the ballast water discharged by bulk carriers originated from 1 to 3 source ports (mean ± 1SE = 1.4 ± 0.9 ports). The 'other' vessel type discharged the second highest mean volume of ballast water (10,640 ± 5,091 m^3), representing 100% of the BOB and 1 to 2 source ports (mean ± 1SE = 1.5 ± 0.1 ports). This was followed by RO/RO's (9,376 ± 1,012 m^3; 100% of BOB; 3.5 ± 2.1 ports), general cargo carriers (6,233 ± 1,017 m^3; 57% of BOB; 2.7 ± 1.6 ports) and oil tankers (2,725 ± 1,117 m^3; 16% of BOB; 1.2 ± 0.4 ports).

Seventy-eight percent of all vessels sampled in this study reported exchanging their source port ballast water in the open-ocean. This value is largely reflective of the sampling design of the study and is not reflective of the whole shipping industry. The remaining 22% of vessels did not perform exchange because they were ICU vessels or contained water that originated in the open ocean, thereby not requiring MOE. Of the vessels that did perform MOE

(n = 62), 52% adopted to use the empty-refill exchange method, 45% used flow-through exchange, and 3% used an alternate exchange method. Nearly all the of vessels that performed flow-through exchange were bulk carriers (93%), whereas the vessels that performed empty-refill exchange were distributed relatively evenly amongst the most abundant vessel types.

Biological characteristics of ballast water

Absolute & Relative abundance

Zooplankton densities showed high temporal and spatial variability among vessels, ranging from 3.2 ind·m^{-3} to 63,562 ind·m^{-3}. The average zooplankton density across all sampled vessels (n = 79) was 8432 ± 1338 ind·m^{-3}(±1 SE). Zooplankton samples were taxonomically and ontogenetically diverse and well represented by holoplankton and meroplankton. A total of 193 distinct taxa were identified, of which at least 71 taxa were non-indigenous to the ballast water receiving environment (Appendix B). Quantitative analysis of all ballast water samples revealed that copepods numerically dominated ballast water zooplankton communities (Figure 7A). Copepods represented 81% of the entire zooplankton community, with densities as high as 44,497 ind·m^{-3}. The omnipresence of copepods was also reflected by the dominance of this taxa in ballast water zooplankton sampled, representing 71% of the taxa observed (Figure 7B). The relative abundance of other major taxonomic groups was much less, varying between density and species richness based abundances. Based on taxonomic group densities, rotifers were the second most abundant taxa (8%), followed by barnacle larvae (5%), cladocerans (2%) and bivalve larvae (2%). In terms of species richness based abundances, gastropods were the second most abundant taxa (4%), followed by barnacle larvae (2%), cladocerans (2%) and bivalve larvae (2%). A large

A B

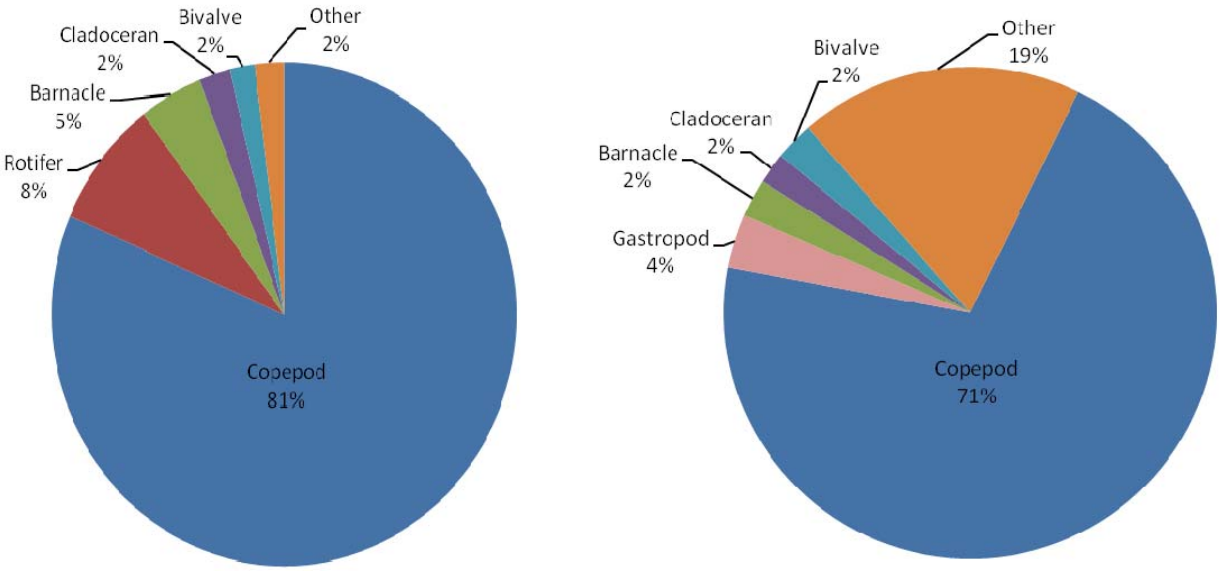

Figure 7. Relative abundance of the five most numerically dominant taxa based on (A) density of individuals, and (B) species richness.

discrepancy existed in the relative abundance of 'other' taxonomic groups when comparing density and species richness abundances. These taxa were numerically rare, representing only 2% of the total zooplankton density observed. However, they were diverse taxonomically representing 19% of the observed taxa.

Frequency of Occurrence

Copepods were the most common taxonomic group present in the ballast water of vessels sampled across all shipping classes and regions (Table 1). In the Pacific region, all but one sampled vessel contained copepods. The rank order of taxonomic groups among shipping classes that performed open-ocean exchange (i.e., ICE and TOE) in the Pacific region were similar, with barnacles being the second most frequented taxa, followed by polycheates, bivalves and chaetognaths. The rank order of taxonomic groups among the un-exchanged shipping class was different, with rotifers being the second most frequented taxa, followed by barnacles, cladocerans and gastropods.

Table 1. Frequency of occurrence for top five taxa present in vessels sampled between October 2006 and November 2007. Values expressed as a percentage of the number of ships sampled in each shipping class per region. For common frequencies between taxa in a column, rank order was based on highest mean density of taxa in the ballast water of vessels in corresponding shipping class and region.

Rank	Pacific			Atlantic		Great Lakes	
	ICU (n = 13)	ICE (n = 11)	TOE (n = 11)	ICE (n = 4)	TOE (n = 20)	ICE (n = 3)	TOE (n = 7)
1	Copepoda 100.0	Copepoda 90.9	Copepoda 100.0	Copepoda 100.0	Copepoda 100.0	Copepoda 100.0	Copepoda 100.0
2	Rotifera 76.9	Cirripedia 72.7	Cirripedia 37.5	Cnidaria 25.0	Gastropoda 50.0	Gastropoda 100.0	Gastropoda 52.7
3	Cirripedia 69.2	Polychaeta 54.5	Polychaeta 37.5	Amphipoda 25.0	Euphausiida 30.0	Bivalvia 100.0	Bivalvia 28.6
4	Cladocera 61.5	Bivalvia 54.5	Bivalvia 31.3	Gastropoda 25.0	Ostracoda 30.0	Bryozoa 33.3	Bryozoa 14.3
5	Gastropoda 53.8	Chaetognatha 45.5	Chaetognatha 31.3	Bivalvia 25.0	Amphipoda 20.0	Isopoda 33.3	Isopoda 14.3

In the Atlantic region, copepods were the most frequently encountered taxonomic group for both ICE and TOE, being present in 100% of the vessels sampled. The rank order of other taxonomic groups varied between shipping classes even though both shipping classes performed open-ocean exchange in the Northwest Atlantic (Figure 4B). In ICE vessels, cnidarians were the second most frequented taxonomic group, followed by amphipods, gastropods and bivalves. In TOE vessels, gastropods were the second most frequented taxonomic group, followed by euphasiids, ostracods and amphipods.

In the Great Lakes region, copepods were present in 100% of the sampled vessels and the rank order of the most frequented taxonomic groups was the same between both shipping classes, both of which performed open-ocean exchange.

Shipping class comparisons

Differences in total zooplankton density existed between shipping classes in the Pacific region (Figure 8A; ANOVA, $F_{calc} = 3.4$; df = 2, 37; p = 0.045). Significantly higher total zooplankton densities were found in the ballast water of ICU vessels than in TOE vessels

(Tukey's, p = 0.050). The same relationship was more pronounced for non-indigenous zooplankton densities in ballast water of ICU vessels (Figure 8B; ANOVA, F_{calc} = 4.8; df = 2, 37; p = 0.015; Tukey's, p = 0.01). There were no significant differences in total or non-indigenous zooplankton densities in ballast water shipping classes in the Atlantic region (Figure 8A and B). Significantly higher total zooplankton densities were observed in the ballast water of ICE than TOE vessels in the Great Lakes region (Figure 8A; student-t, t_{calc} = 3.2; df = 8; p = 0.01). Significantly higher and more pronounced differences in densities of non-indigenous zooplankton were also found in the ballast water of ICE versus TOE vessels in the Great Lakes region (Figure 8B; student-t, t_{calc} = 3.6, df = 8; p = 0.007).

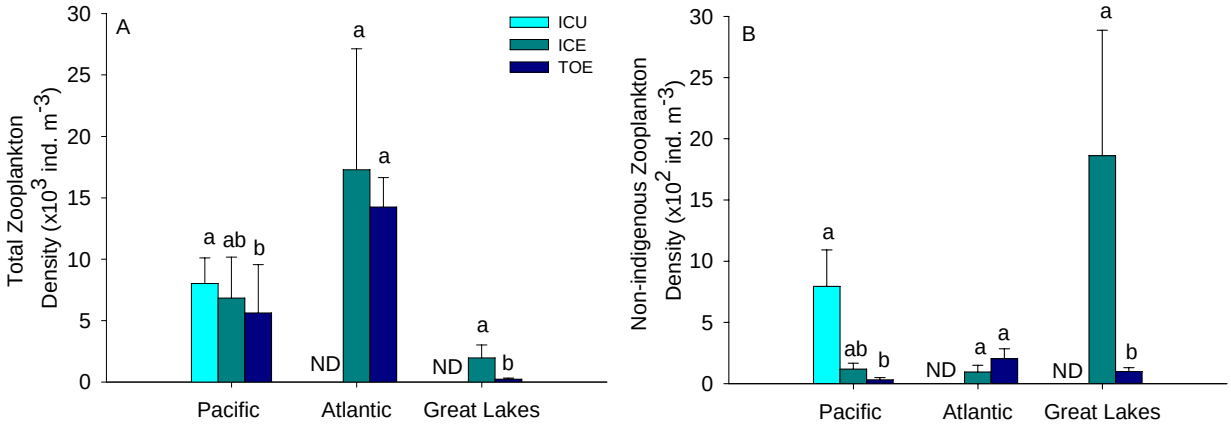

Figure 8. Summary of mean (±1SE) (A) total zooplankton density and (B) non-indigenous zooplankton density observed in the ballast water of vessels from each shipping class sampled in the Pacific, Atlantic and Great Lakes regions. Different letters above the bars indicate significant differences (Tukey's, p < 0.05) among means of shipping classes within a region. The absence of vessels for a particular shipping class is indicated with ND (no data).

There were no significant differences in total or non-indigenous zooplankton species richness between shipping classes in both the Pacific and Atlantic regions (Figure 9A and 9B). In the Great Lakes region, significantly higher total zooplankton species richness was found in the ballast water of ICE vessels than TOE vessels (Figure 9A; t-test, t_{calc} = 2.5; df = 8; p = 0.039). No significant differences in non-indigenous zooplankton species richness were observed between shipping classes in the Great Lakes region (Figure 9B).

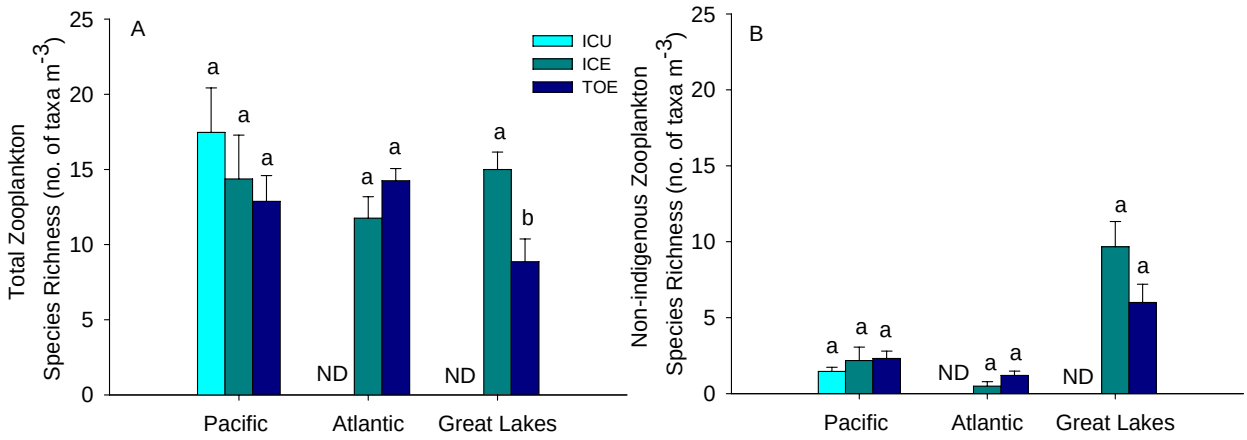

Figure 9. Summary of mean (±1SE) (A) total species richness and (B) non-indigenous species richness observed in the ballast water of vessels from each shipping class sampled in the Pacific, Atlantic and Great Lakes regions. Different letters above the bars indicate significant differences (Tukey's, $p < 0.05$) among means of shipping classes within a region (a, b). The absence of vessels for a particular shipping class is indicated with ND (no data).

Regional comparisons

Total and non-indigenous zooplankton densities differed among the 3 regions (Pacific, Atlantic and Great Lakes) within a particular shipping class (Figure 10). The total zooplankton density in the ballast water of TOE vessels was significantly different between shipping classes (Figure 10A; ANOVA, $F_{calc} = 16.1$; df = 2, 40, $p < 0.001$). Total zooplankton density was significantly higher in the Atlantic region than the Pacific (Tukey's, $p < 0.001$) and Great Lakes regions (Tukey's, $p < 0.001$). No significant differences in total zooplankton density existed among regions for ICE vessels. There was a significant difference in non-indigenous zooplankton density between regions for ICE vessels (Figure 10B; ANOVA, $F_{calc} = 3.6$; df = 2, 15; $p = 0.044$). The ballast water of ICE vessels in the Great Lakes region contained a significantly higher density of non-indigenous zooplankton than ICE vessels in the Pacific region (Tukey's, $p = 0.043$). There was no significant difference in non-indigenous zooplankton density among regions for TOE vessels. A comparison of ICU vessels across all regions was not possible due to a lack of samples from this shipping class in the Atlantic and Great Lakes regions.

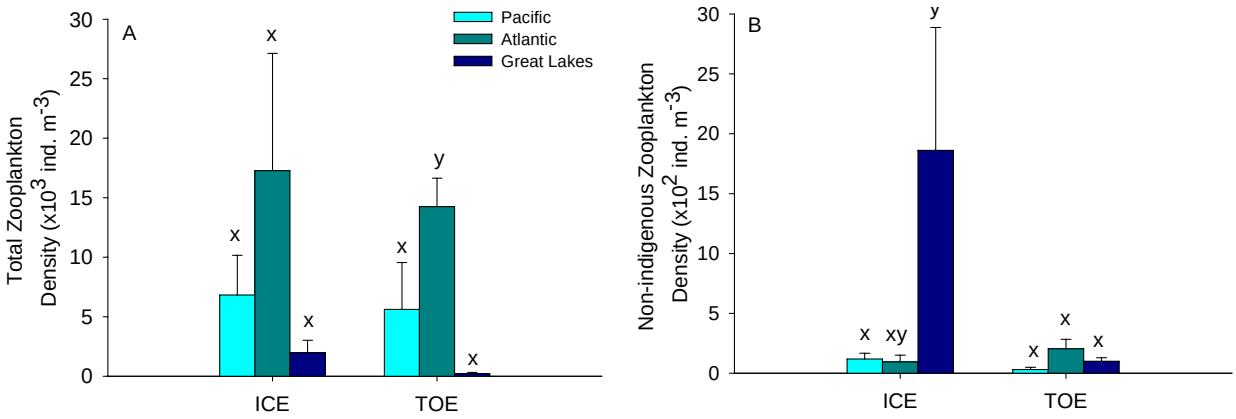

Figure 10. Summary of mean (±1SE) (A) total zooplankton density and (B) non-indigenous zooplankton density observed in the ballast water of vessels from each shipping class sampled in the Pacific, Atlantic and Great Lakes regions. Different letters above the bars indicate significant differences (Tukey's, $p < 0.05$) between regions within a shipping class.

Total zooplankton species richness was not significantly different between regions for both ICE and TOE vessels (Figure 11A). Significant differences in non-indigenous zooplankton species richness existed among the 3 regions, for both ICE (Figure 11B; ANOVA, $F_{calc} = 12.3$; df = 2, 15; $p = 0.001$) and TOE vessels (ANOVA, $F_{calc} = 16.3$; df = 2, 40; $p < 0.001$). Non-indigenous zooplankton species richness was significantly higher for ICE vessels in the Great Lakes regions, than in the Pacific (Tukey's, $p = 0.005$) and Atlantic regions (Tukey's, $p = 0.002$). Similarly, non-indigenous zooplankton species richness was also significantly higher for TOE vessels in the Great Lakes regions, than in the Pacific (Tukey's, $p < 0.001$) and Atlantic regions (Tukey's, $p < 0.001$).

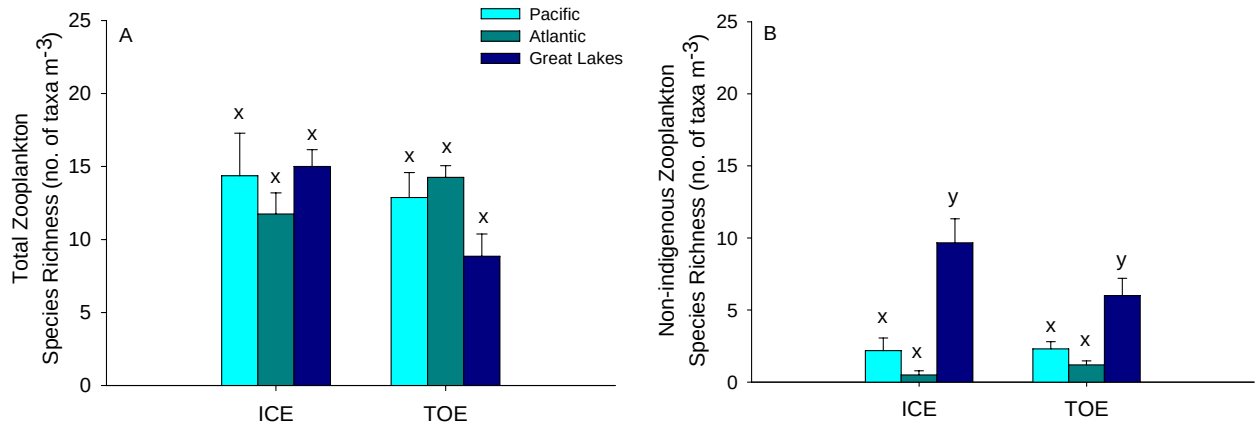

Figure 11. Summary of mean (±1SE) (A) total species richness and (B) non-indigenous species richness observed in the ballast water of vessels from each shipping class sampled in the Pacific, Atlantic and Great Lakes regions. Different letters above the bars indicate significant differences (Tukey's, $p < 0.05$) between regions within a shipping class.

Seasonal comparisons

There were no significant seasonal differences in the densities of total or non-indigenous zooplankton in the ballast water of vessels in the Pacific region (Figure 12). Similarly, no significant seasonal differences were found in total or non-indigenous zooplankton species richness in the ballast water of vessels in the Pacific region (Figure 13). Note that analysis of seasonal zooplankton densities was only possible for Pacific region data due a lack of seasonal samples in the Atlantic and Great Lakes regions.

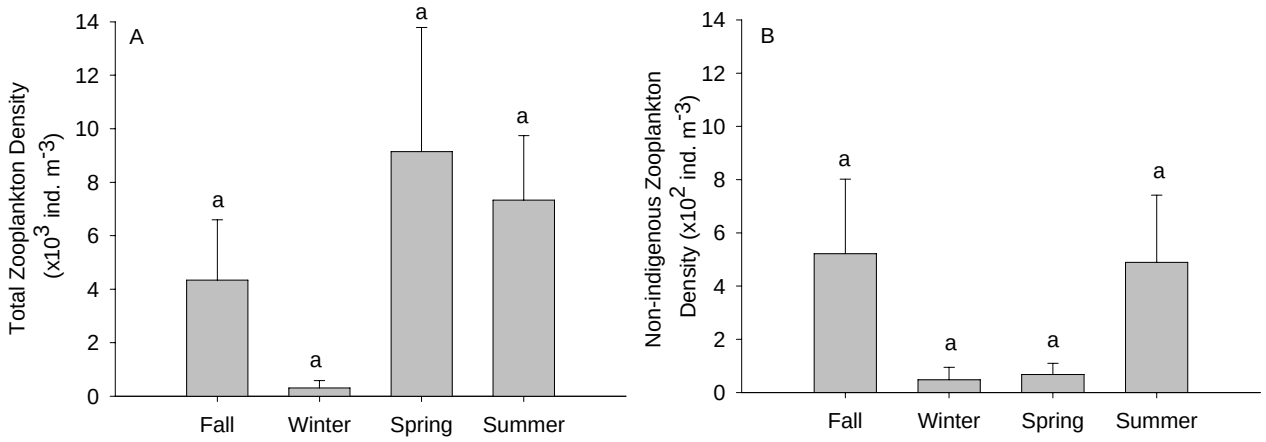

Figure 12. Summary of mean (±1SE) (A) total zooplankton density and (B) non-indigenous zooplankton density observed seasonally in the ballast water of vessels in the Pacific region. Different letters above the bars indicate significant differences (Tukey's, $p < 0.05$) among seasonal means.

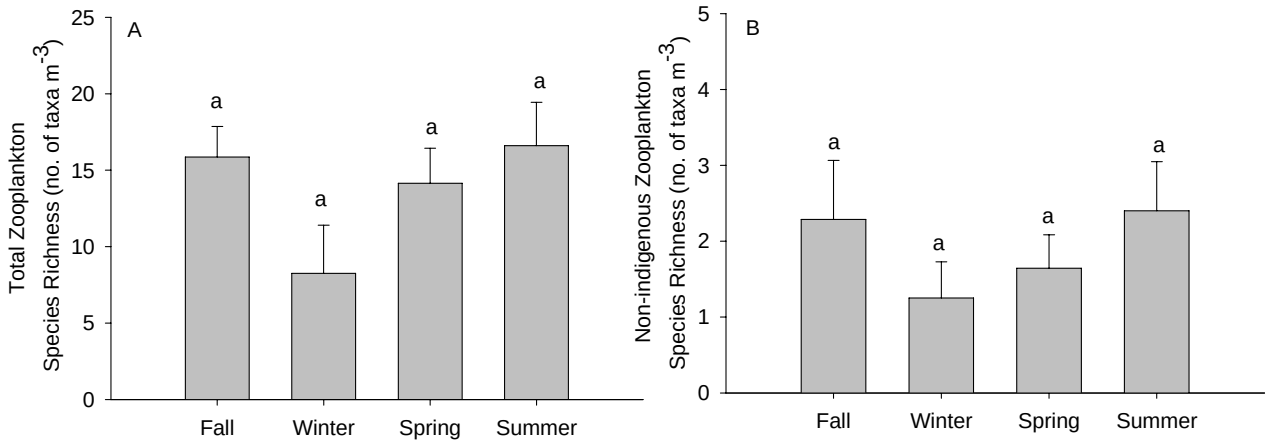

Figure 13. Summary of mean (±1SE) (A) total species richness and (B) non-indigenous species richness observed seasonally in the ballast water of vessels in the Pacific region. Different letters above the bars indicate significant differences (Tukey's, $p < 0.05$) among seasonal means.

Biological and environmental correlates

Ballast water data from all shipping classes and regions were combined to test for relationships between ballast water environmental conditions and total zooplankton densities. Pooling all samples allowed for better coverage of the ranges observed for the environmental variables since regional and shipping class differences were observed for these factors. Zooplankton densities were inversely correlated with ballast water age (stepwise multiple regression, $r^2 = 0.241$, $p < 0.001$; Figure 14A) and positively correlated with tank volume (stepwise multiple regression, $r^2 = 0.121$, $p = 0.002$; Figure 14B). There was no significant relationship between zooplankton density and ballast water temperature (Figure 14C; $p > 0.05$) or salinity (Figure 14D; $p > 0.05$).

Physical characteristics of ballast water

Shipping class comparisons

Ballast water environmental conditions varied across shipping classes in each of the three sampling regions (Figure 15). Ballast water age showed significant differences across shipping classes in the Pacific region (Figure 15A; ANOVA, $F_{calc} = 26.6$; df = 2, 37; $p < 0.001$). Mean

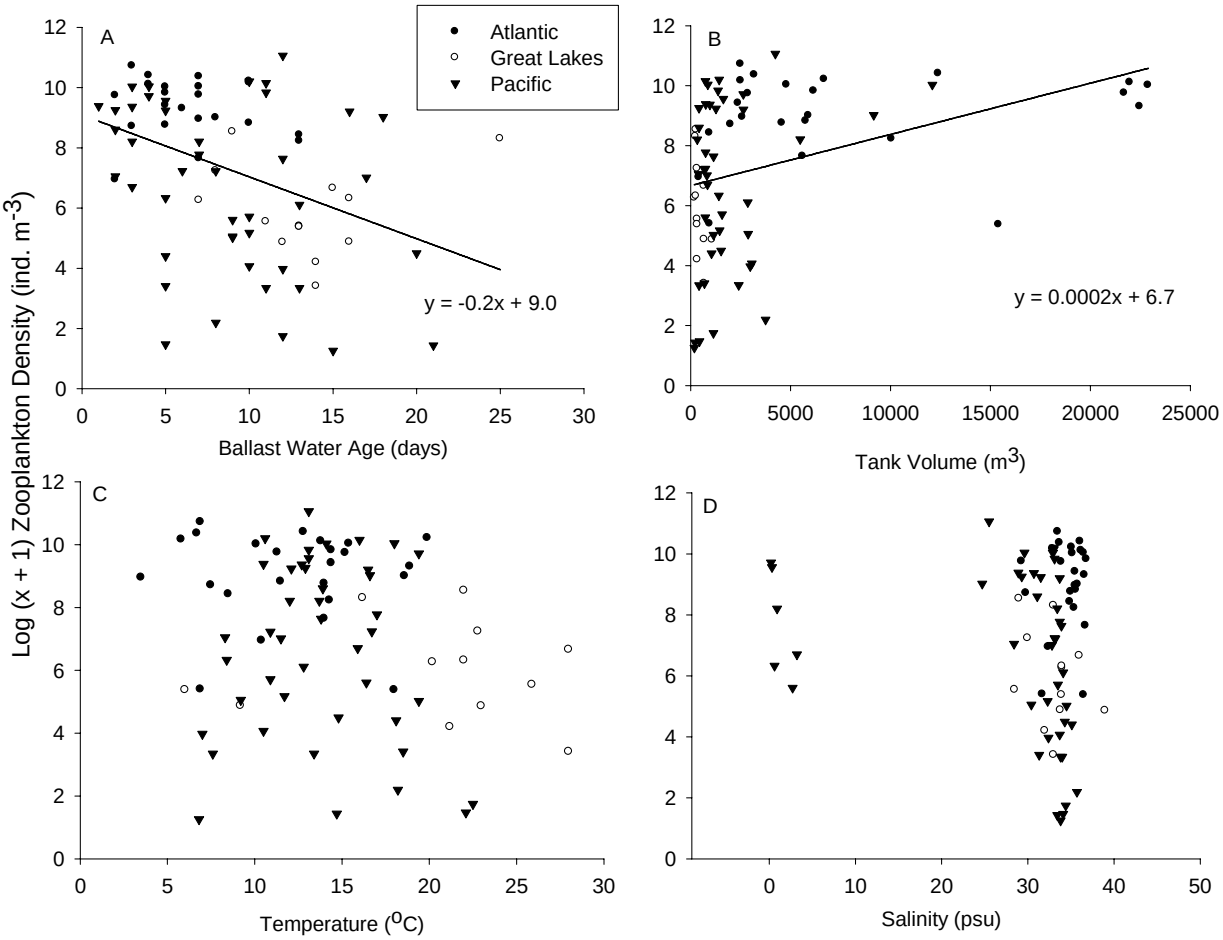

Figure 14. Total (log (x + 1)) zooplankton density (ind m^{-3}) as a function of (A) ballast water age, (B) tank volume, (C) temperature, and (D) salinity in vessels from all 3 shipping class frequenting all 3 regions. Significant relationships were found between zooplankton density and ballast water age (stepwise multiple regression, $r^2 = 0.46$; $p = 0.002$) and tank volume (stepwise multiple regression, $r^2 = 0.121$, $p = 0.002$). Neither temperature nor salinity was important in determining zooplankton density (Tukey's, $p > 0.05$).

(± 1 SE) ballast water age was significantly lower for ICU vessels (4.2 ± 0.7 d) than for ICE (8.1 ± 0.8 d; Tukey's, $p = 0.017$) and TOE vessels (13 ± 1.0 d; Tukey's, $p = 0.001$). Ballast water age was significantly higher for TOE vessels than ICE vessels (Tukey's, $p < 0.001$). In the Atlantic region, ballast water age was significantly younger (t-test, $t_{calc} = -3.4$; df = 22, 22; $p = 0.003$) for ICE vessels (2.5 ± 0.3 d) than TOE vessels (8.0 ± 0.7 d). No significant difference existed in ballast water age of shipping classes in the Great Lakes region. Note that ICU data was not included because estimates are not available for the Atlantic and Great Lakes regions.

Tank volume showed no significant differences between shipping classes in any of the 3 regions (Figure 15B), however TOE vessels had consistently higher mean tank volumes.

Ballast water temperature was found to be significantly different between shipping classes in the Pacific region (Figure 15C; ANOVA, $F_{calc} = 8.8$; df = 2, 37; $p = 0.001$). The mean (± 1 SE) ballast water temperature in ICE vessels was significantly higher (17.5 ± 1.1 °C) than that of ICU (13.0 ± 1.0 °C; Tukey's, $p = 0.007$) and TOE (12.2 ± 0.8 °C; Tukey's, $p = 0.001$) vessels. No significant difference in ballast water temperature was found between shipping classes in the Atlantic and Great Lakes regions.

Ballast water salinity was found to be significantly different between shipping classes in the Pacific region (Figure 15D; ANOVA, $F_{calc} = 15.6$, df = 2, 37, $p < 0.001$). The mean (± 1 SE) ballast water salinity in ICU vessels was significantly lower (16.7 ± 4.1 psu) than ICE (33.7 ± 0.4 psu; Tukey's, $p < 0.001$) and TOE (32.5 ± 0.7 psu; Tukey's, $p < 0.001$) vessels. In the Atlantic region, ICE vessels had a significantly lower (t-test, $t_{calc} = -2.6$, df = 22; $p = 0.017$) mean salinity (32.4 ± 0.9 psu) when compared to TOE (35.1 ± 0.4 psu) vessels. No significant difference in salinity was found between shipping classes in the Great Lakes region.

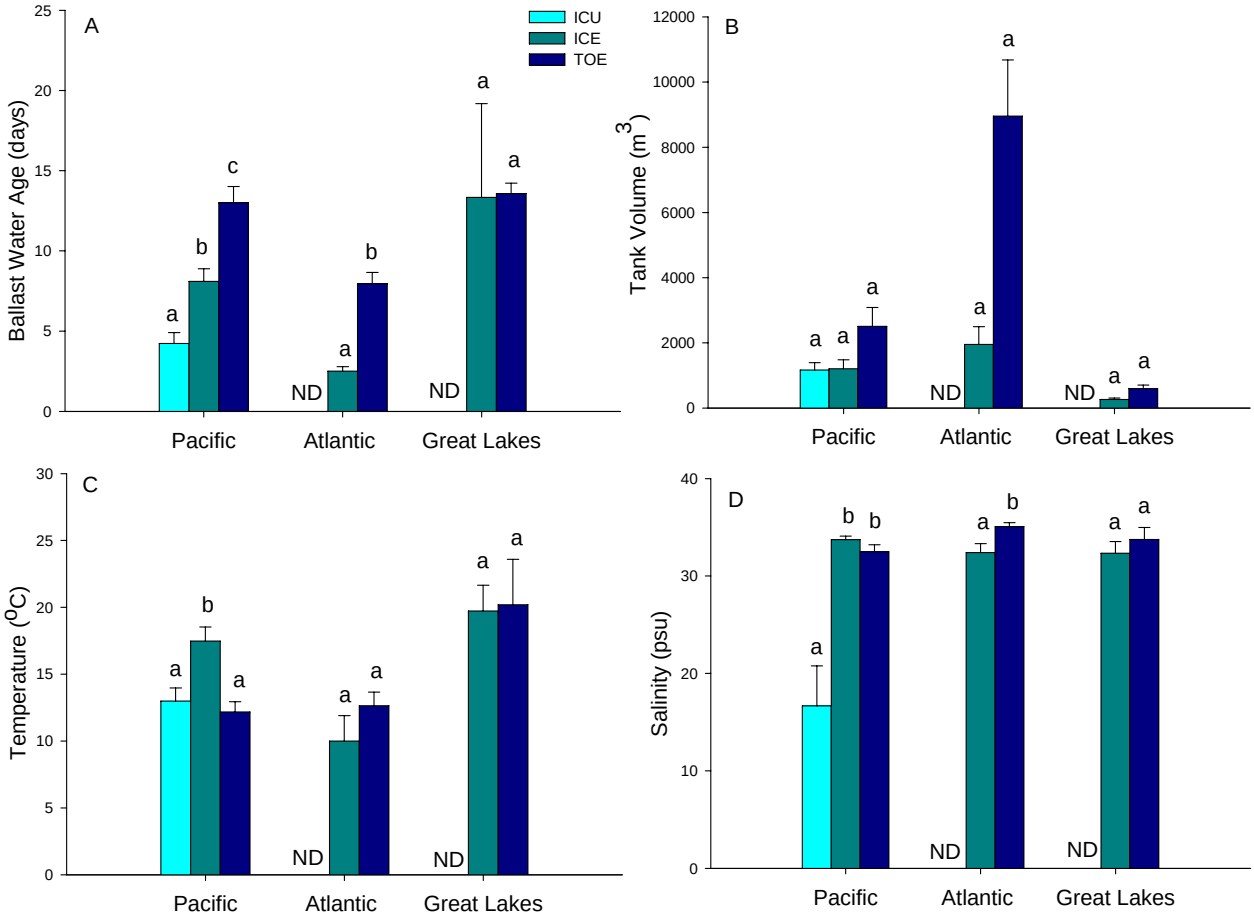

Figure 15. Summary of mean (± 1SE) ballast water (A) age, (B) tank volume, (C) temperature and (D) salinity for vessels in ICU, ICE and TOE vessels in the Pacific, Atlantic and Great Lakes Region. Different letters above the bars indicate significant differences (Tukey's, $p < 0.05$) among means of shipping classes within a region. The absence of vessels for a particular shipping class is indicated with ND (no data).

Regional comparisons

Ballast water environmental conditions showed significant differences among regions within each of their respective shipping classes (Figure 16). Ballast water age of ICE vessels was significantly different between regions (Figure 16A; ANOVA, $F_{calc} = 5.6$; df = 2, 15; $p = 0.016$). Mean ballast water age (± 1SE) of ICE vessels was significantly lower in the Atlantic region (2.5 ± 0.3 d;) than in the Great Lakes region (Figure 16A; 13.3 ± 5.8 d; Tukey's, $p = 0.013$). Significant regional differences in ballast water age also existed for TOE vessels (ANOVA, $F_{ratio} = 13.0$; df = 2, 40; $p < 0.001$). Ballast water age was significantly lower in the Atlantic region

(8.0 ± 0.7 d) than the Pacific (13.0 ± 1.0 d; Tukey's, $p < 0.001$) or Great Lakes regions (13.6 ± 0.7 d; Tukey's, $p = 0.001$).

Significant regional differences in tank volume existed for TOE vessels (ANOVA, $F_{calc} = 9.0$; $df = 2, 40$; $p = 0.001$) but not for ICE vessels (Figure 16B). Mean tank volume (± 1SE) was significantly higher for TOE vessels in the Atlantic region (8952.3 ± 1724.5 m^3) than the Pacific (2505.6 ± 577.5 m^3; Tukey's, $p = 0.003$) or Great Lakes regions (599.3 ± 104.6 m^3; Tukey's, $p = 0.004$).

Mean ballast water temperature was significantly different between regions for ICE vessels (Figure 16C; ANOVA, $F_{calc} = 8.4$; $df = 2, 15$; $p = 0.004$). Mean ballast water temperature (± 1SE) was significantly higher for ICE vessels in the Great Lakes (19.7 ± 1.9 °C; Tukey's, $p = 0.007$) and Pacific regions (17.4 ± 1.1 °C; Tukey's, $p = 0.007$) than in the Atlantic region (10.0 ± 1.9 °C). Significant regional differences in ballast water temperature were also found for TOE vessels (ANOVA, $F_{calc} = 6.9$; $df = 2, 40$; $p = 0.003$). Mean ballast water temperature was significantly higher for TOE vessels in the Great Lakes region (20.2 ± 3.4 °C) than in the Pacific (12.2 ± 0.8 °C; Tukey's, $p = 0.003$) and Atlantic (12.6 ± 1.0 °C; Tukey's, $p = 0.004$) regions.

Significant regional differences in mean ballast water salinity existed for TOE vessels (ANOVA, $F_{calc} = 4.5$; $df = 2, 40$; $p = 0.017$), but not for ICE vessels (Figure 16D). Mean ballast water salinity (± 1SE) was significantly higher for TOE vessels in the Atlantic region (35.1 ± 0.4 psu) than in the Pacific (32.5 ± 0.7 psu; Tukey's, $p = 0.012$) region.

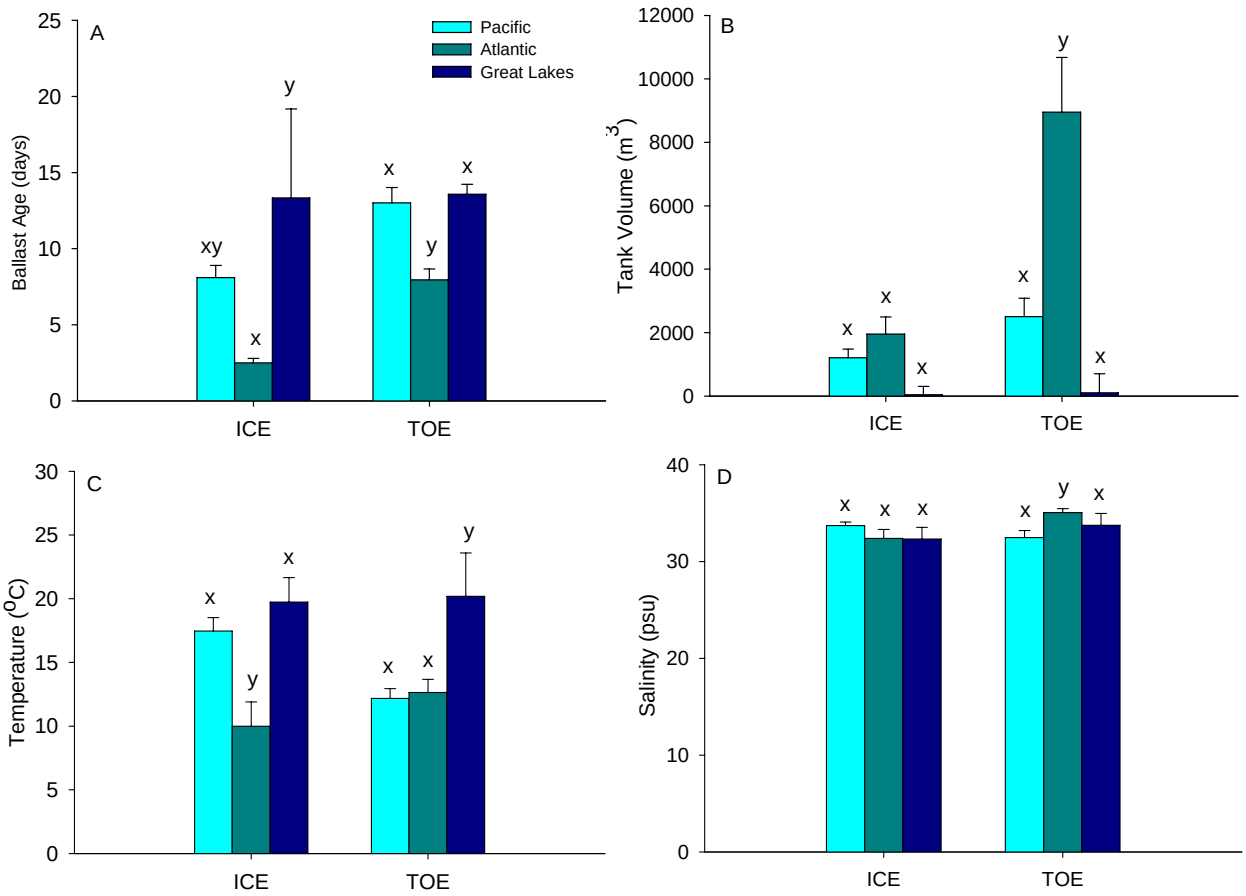

Figure 16. Summary of mean (± 1SE) ballast water (A) age, (B) tank volume, (C) temperature and (D) salinity for vessels in ICU, ICE and TOE vessels in the Pacific, Atlantic and Great Lakes regions. Different letters above the bars indicate significant differences (Tukey's, $p < 0.05$) between regions within a shipping class. The absence of vessels for a particular shipping class is indicated with ND (no data).

Determining invasion risk for Vancouver Harbour

Receiving environment & ballast water similarity

Mean surface temperatures (1-10m) for the Strait of Georgia (Figure 17) were highest in the summer (maximum = 17.5ºC) and lowest in the winter (minimum = 6.55ºC). The greatest temperature range was experienced during the summer (12.3-17.5ºC). Similarity in mean surface temperature conditions of the Strait of Georgia and ballast water aboard sampled vessels varied seasonally and across shipping classes (Figure 17). Ballast water temperature aboard TOE vessels was most similar to conditions in the Strait of Georgia (Figure 17C). Mean (±1SD)

ballast water temperatures fell within the surface temperature ranges for the Strait of Georgia during the fall, winter and summer. Mean ballast water temperature for ICU vessels fell within surface temperature ranges for the Strait of Georgia during the fall and winter. While mean ballast water temperature of ICE vessels was higher than surface temperatures for the Strait of Georgia, error bars did overlap during the summer.

Maximum surface salinities for the Strait of Georgia (Figure 18) were relatively similar across seasons, while minimum surface salinities showed more variability. The smallest and largest salinity ranges were experienced during the fall (20.3-28.5psu) and summer (12.5-27.8psu) respectively. There was very little overlap between mean (±1SD) ballast water salinities and seasonal surface salinity ranges for the Strait of Georgia. Mean ballast water salinities aboard ICE (Figure 18B) and TOE (Figure 18C) vessels were consistently higher than surface salinities of the Strait of Georgia. Mean ballast water salinities of ICU vessels (Figure 18A) overlapped with Strait of Georgia surface salinities during every season, and showed the greatest similarity during the fall and summer.

Vessels discharging in Vancouver Harbour

Between September 2006 and September 2007 a total of 887 vessels reported discharging ballast water in Vancouver Harbour, releasing approximately 6,721,336 m^3 of ballast water (Lo et al. unpublished data). Analysis of the ballast water history for these vessels revealed that 167 vessels were ICU, 252 vessels were ICE and 468 vessels were TOE (Figure 19A). The remaining vessels carried ballast water from ports outside of these shipping classes or details of the ballast water origins were not provided. The mean (± 1SE) volume of ballast water discharged by all vessels entering Vancouver Harbour between September 2006 and September 2007 was

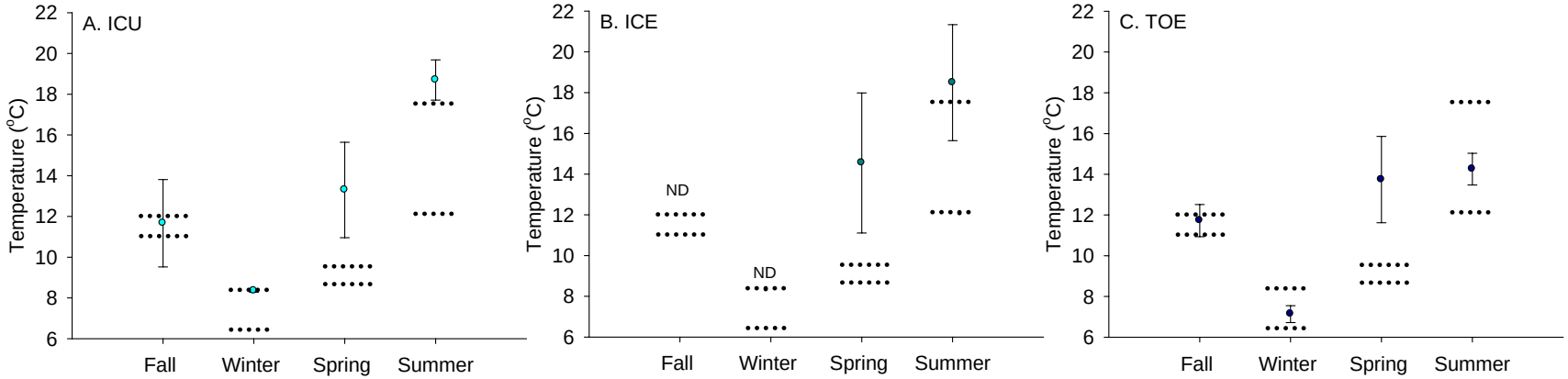

Figure 17. Summary of mean (±1SD) seasonal ballast water temperature for vessels in (A) ICU, (B) ICE, and (C) TOE vessels in the Pacific region. Dashed lines represent the minimum and maximum seasonal surface temperatures (1-10m) for several locations sampled within the Strait of Georgia, including near the Fraser River Mouth, the main Strait of Georgia, and off Point Grey. Strait of Georgia data were obtained from R. Pawlowicz (UBC).

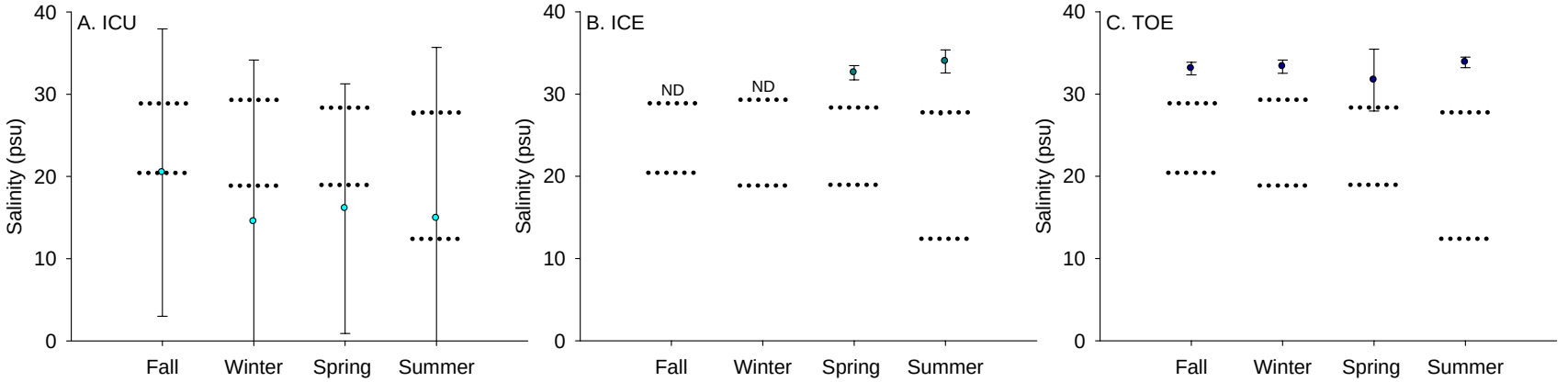

Figure 18. Summary of mean (±1SD) seasonal ballast water salinity for vessels in (A) ICU, (B) ICE, and (C) TOE vessels in the Pacific region. Dashed lines represent the minimum and maximum seasonal surface salinity (1-10m) for several locations sampled within the Strait of Georgia, including near the Fraser River Mouth, the main Strait of Georgia, and off Point Grey. Strait of Georgia data were obtained from R. Pawlowicz (UBC).

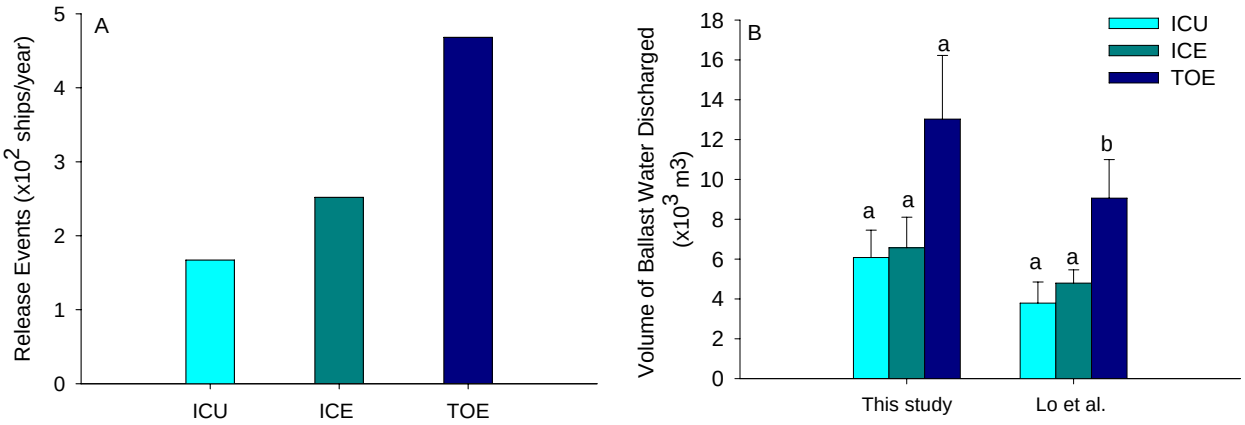

Figure 19. (A) Total number of release events and (B) mean volume of ballast water discharged by vessels entering the port of Vancouver between September 2006 and September 2007. Data are presented for vessels sampled in this study and all vessels entering the port of Vancouver during this time period (Lo et al., unpublished data). Different letters above the bars represent significant differences between shipping classes (Tukey's, $p < 0.05$).

significantly different (ANOVA, $F_{calc}= 5.8$, df = 3, 194, $p = 0.001$) between shipping classes (Figure 19B). TOE vessels discharged a significantly higher mean volume (9,057 ± 1,936 m^3) of ballast water than ICU (3,791 ± 1,065 m^3; Tukey's pairwise comparison test, $p = 0.050$) and ICE (4,797 ± 662 m^3; Tukey's pairwise comparison test, $p = 0.027$) vessels. In comparison, ballast water discharge volumes for vessels sampled in this study were not significantly different across shipping classes, yet the mean ballast water discharge volumes were highest for TOE vessels (13,027 ± 3,194 m^3), followed by ICE (6,573 ± 1,530 m^3) and ICU (6,072 ± 1,384m^3) vessels. Mean ballast water discharge volumes for vessels sampled in this study were slightly higher than all vessels discharging in Vancouver Harbour. This is likely because large vessels were targeted in this study (e.g. bulk carriers), whereas Lo et al. (unpublished data) report all discharged vessels, despite their size. To ensure this observation is not due to reporting or data entry errors, a comparison of discharge volumes reported on IMO forms collected from ships sampled both during this study as well as Lo's study were compared. There were no significant differences between the discharge volumes of common vessels reported in this study and V. Lo's study (Figure 20).

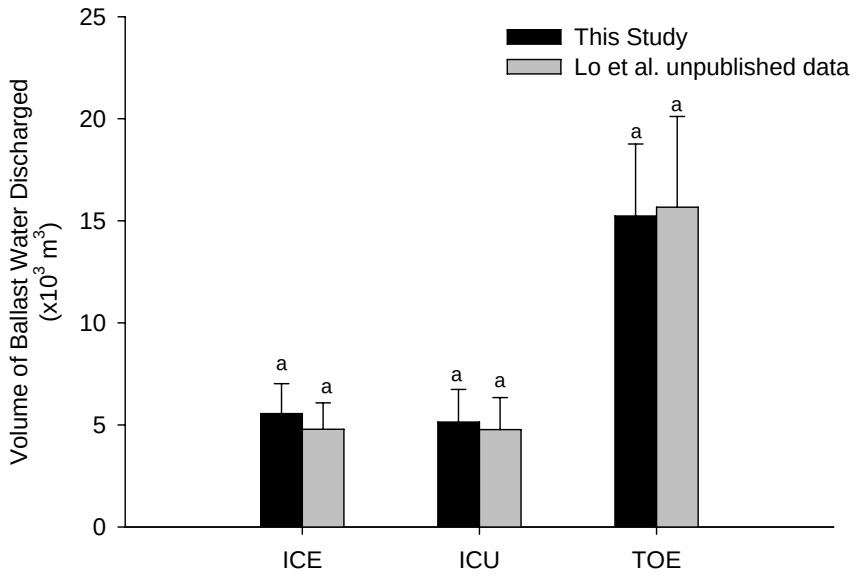

Figure 20. Comparison of mean volume of ballast water discharged by vessels sampled in this study based on IMO ballast water reporting forms collected during this study (black bars) and on data provided by V. Lo (grey bars). Only vessels reported in both studies were compared to determine if discrepancies exist between data sets. Common letters above the bars within respective shipping classes represent no significant differences between the 2 studies compared ($p>0.05$).

Propagule pressure estimate

Estimates of total zooplankton propagule pressure for vessels entering the port of Vancouver were variable between shipping classes (Figure 21). Total propagule pressure was highest in ICU vessels (3.37×10^8 ind · year^{-1}), followed by ICE (1.36×10^8 ind · year^{-1}) and TOE (1.22×10^8 ind · year^{-1}) vessels based on total ballast water discharge estimates for vessels entering Vancouver Harbour between September 2006 and September 2007. Estimates of total propagule pressure were highest in ICU vessels (3.07×10^9 ind · year^{-1}), followed by ICE (1.44×10^9 ind · year^{-1}) and TOE (1.08×10^9 ind · year^{-1}) vessels based on discharge volumes from vessels sampled in this study. No significant differences in propagule pressure were found between shipping classes. Estimates of propagule pressure for Atlantic and Great Lakes ports were not possible due to insufficient ship traffic data for these regions.

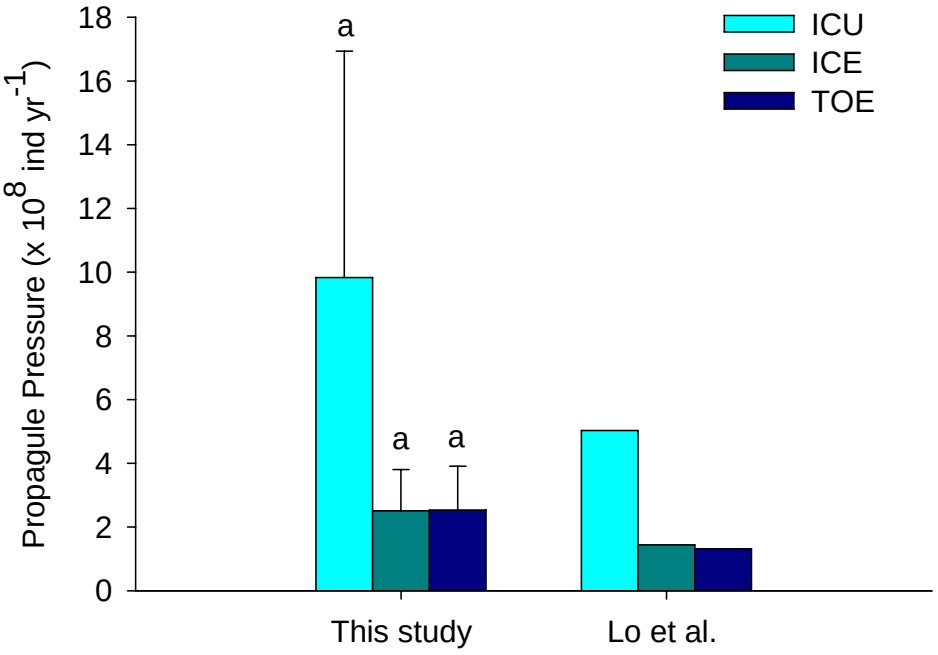

Figure 21. Estimate of total propagule pressure for non-indigenous zooplankton in the ballast water of vessels entering the port of Vancouver. Data are presented for vessels sampled in this study and all vessels entering the port of Vancouver during this time period (Lo et al., unpublished data). Different letters above the bars represent significant differences between shipping classes (Tukey's, $p < 0.05$). Propagule pressure estimates for Lo et al. data do not have error bars because zooplankton density data used in propagule pressure calculation were not available for all vessels.

DISCUSSION

Biological diversity of ballast water

The ballast water environment of vessels contained a diverse assemblage of aquatic organisms emphasizing the importance of this vector in transporting organisms between and within continents. All major holoplanktonic and meroplanktonic taxonomic groups were represented along with their developmental and ontogenetic stages. The present study identified 193 zooplankton taxa, belonging to 18 different phyla (Appendix B). Of those taxa identified to species level, 71 taxa were considered non-indigenous to the receiving environments in which they were to be discharged (Appendix B). Given that many 63 zooplankton taxa could not be identified to the species and that the true origins of some species were unclear (i.e., cryptogenic species), these are considered conservative estimates of the true diversity of NIZ in ballast water samples. Ballast water organisms originated from a variety of aquatic habitats, including freshwater, brackish water, coastal high-salinity, and open-ocean environments. A high diversity of NIZ from a variety of aquatic habitats clearly emphasizes that no aquatic environment is completely immune to aquatic invasive species introductions and associated invasion potential.

The total number of zooplankton taxa documented in this study was similar and sometimes higher in magnitude to that reported in previous ballast water studies conducted in the same regions. For example, Piercey et al. (2000) collected 67 ballast water samples from vessels entering Vancouver Harbour (24 more than this study), yet reported 92 zooplankton taxa compared to 148 taxa in this study (Figure 22A). Harvey et al. (1999) collected 94 samples in the Gulf of St. Lawrence, and reported 97 zooplankton taxa compared to 58 in this study. Duggan et al. (2005) collected 64 residual ballast water samples in vessels entering the GLB and reported 65 zooplankton taxa compared to 42 in this study. It is evident in from a species accumulation

plot (Figure 22B) that zooplankton taxa observations increase with sample size; however this study observed a higher number of zooplankton in the Pacific region despite collecting fewer samples than a previous report. This is likely due varying levels of taxonomic expertise and because the present study sampled 1000L of ballast water through open manholes using a 125-µm plankton net, whereas Piercey et al. (2000) sampled 500L of ballast water through sounding pipes with the use of a pump and 44-µm net. Previous studies have recommended using vertical net tows through open manholes to samples ballast water, since sounding pipes restrict sampling to the bottom of ballast tanks (Gollasch, et al., 2003; Smith et al., 1999). Sampling a greater vertical extent of ballast water allowed this study to report a higher number of zooplankton taxa which have been shown to distribute themselves unevenly throughout ballast tanks (Murphy et al., 2002). These results suggest that sampling design presents biases in terms of documenting zooplankton densities and taxa transported via ballast water.

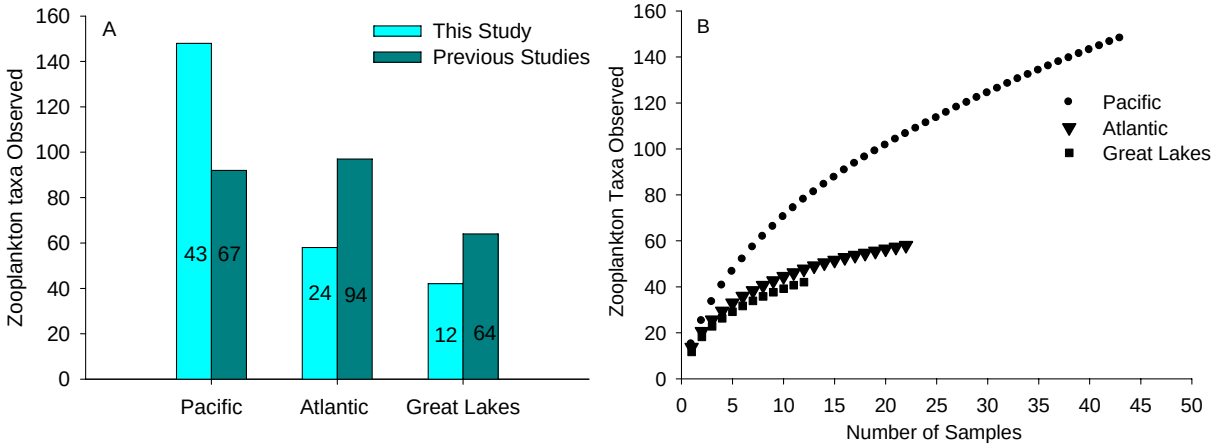

Figure 22. (A) Comparison of total number of zooplankton taxa observed in this study and previous studies from the same regions. Sample size for each study is superimposed on bars. (B) Species accumulation plot for the total number of zooplankton taxa observed in relation to the number of samples collected and analyzed in this study for the Pacific, Atlantic and Great Lakes regions. Samples were randomly permuted 999 times and the resulting curves averaged to produce a smooth plot.

Copepods dominated the ballast water zooplankton community composition by density and species richness (Figure 7). However, the rank order of other dominant taxa varied substantially between shipping classes and regions (Table 1). In the Pacific region, the rank order of the five most frequently sampled taxa was different between ballast water of un-exchanged (ICU) vs. exchanged shipping classes (ICE and TOE), which had the same rank order of dominant taxa despite exchanging in very different locations. As Figure 4A illustrates, the majority of ICE vessels exchanged their ballast water close to the continental margins of North America (<200 miles) because government regulations require them to be a minimum of 50 nautical miles from the coastline. The majority of TOE vessels exchanged their ballast water far from the North American coastline (>1000 miles) despite the fact that regulations require that this exchange is performed a minimum of 200 nautical miles from shore. Ballast water samples of both shipping classes frequently contained larval stages of taxa that are considered to be coastal in origin, such as barnacles (cirripedia) and bivalves (bivalvia). It is likely that both ICE and TOE vessels exchanged close enough to shore to collect these taxa. Even though TOE vessels did exchange their ballast tanks far from the North America coastline as required by law, many vessels were found to exchange their tanks immediately after leaving port and in close proximity to the Asian coastline (<500 miles). Additionally, many vessels were found to exchange their ballast water within close proximity to the Aleutian Islands. While exchanging near the Asian or Alaskan coast does not conflict with Canadian government regulations, it may still pose a potential invasion threat given that coastal taxa are frequently being taken up by ballast water exchange.

The rank order of most frequently sampled taxa in the Great Lakes region was different than the Pacific region, however much like the Pacific region both ICE and TOE shipping classes

presented similar results (Table 1). Although the rank order of dominant taxa for shipping classes in the Great Lakes region was similar, the frequency of occurrence for zooplankton taxa in TOE vessels was generally less. For instance, bivalves were present in 100% of ICE vessels but only 28.6% of TOE vessels. The lower frequency of this coastal taxonomic group in TOE vessels may be partially attributed to exchange occurring further from shore than ICE vessels, from higher mortality associated with longer voyages, or a combination of both. The Atlantic region showed more variability between shipping classes when compared to other regions. Unlike other regions, cnidarians, ostracods and amphipods were among the top 5 most frequently sampled taxa in the Atlantic. These regional differences may be associated with seasonal differences in sampling effort and hence community composition, differences in dominant zooplankton taxa between the Pacific and Atlantic Oceans, or a combination of both.

Patterns of zooplankton abundance in ballast water

Zooplankton densities were highly variable between vessels ranging from 3 to 63,562 individuals per m^3. On average, 8432 zooplankton individuals were observed per m^3 of ballast water (Figure 8A), most of which appeared to be viable based on observations during collection and tissue structure during identification. Thus, if average zooplankton density estimates are extrapolated to the average volume of water carried on board a vessel (i.e., mean BOB; Figure 5B), it is expected that a single de-ballasting vessel is capable of releasing up to 97 million individuals.

This study documented a high degree of variability in zooplankton density between shipping classes (Figure 8A) and regions (Figure 10A) which may be attributed to voyage specific ballast water environmental conditions. For example, total zooplankton density was negatively correlated with ballast water age (Figures 14A) as is shown in other studies (Cordell

et al., in press; Gollasch et al., 2000; Olenin et al., 2000; Wonham et al., 2001). Gollasch et al. (2000) reported a 90% reduction in total zooplankton density within the first 4 days of a 23-day voyage between Singapore and Germany. A similar study reported a 99% reduction in plankton density over the course of a 16-day voyage between Israel and the United States (Wonham et al., 2001). Therefore, we can expect to see lower densities of zooplankton associated with longer voyages. Across shipping classes in the Pacific region, ICU vessels contained significantly younger ballast water and contained higher total zooplankton densities in their ballast water than ICE and TOE vessels (Figure 8A). Across regions within Canada, ICE and TOE vessels from the Atlantic region contained the highest density of zooplankton (Figure 10A) along with the youngest ballast water age (Figure 16A). This data suggests that ballast water age has a negative effect on zooplankton densities and contributes to distinct regional differences in the density of zooplankton aboard vessels due to differences in voyage duration.

A significant positive relationship was also found between tank volume and zooplankton density (Figure 14B) suggesting that vessels carrying large volumes of water may pose a greater invasion risk. Bulk carriers transported the greatest mean volume of water (Figure 5A) and often utilized their cargo holds to carry additional ballast. Cargo holds carry a much greater volume of water than deck tanks and larger volumes of water may provide more stable environmental conditions (e.g., temperature) during transit and reduce associated mortality. Wonham et al. (2001) found that higher zooplankton mortality rates were associated with the deck tanks versus cargo holds of vessels. Regional differences in zooplankton densities observed in this study may be partially explained by differences in tank volumes of sampled vessels. Zooplankton densities of ICE and TOE ballast water were highest in the Atlantic region, which had higher tank volumes and younger ballast water age than the Pacific and Atlantic regions. While these

observations may be attributed to younger ballast water age, they may also be attributed to the higher tank volumes of vessels sampled in Atlantic region.

Studies have suggested that zooplankton densities are dependent on vessel design (Carlton 1985), tank type (Wonham et al., 2001), voyage conditions (Carlton et al., 1982; Gollasch et al., 2000), temperature (Gollasch, 1996; Gollasch et al., 2000), salinity (Gollasch, 1996), oxygen concentrations (Olenin et al., 2000; Gollasch et al., 2000; Wonham et al., 2001), and food availability (Carlton 1985). This study shows that ballast water age and tank volume affect zooplankton densities. However, it is difficult to conclude whether these factors are solely responsible for regional and shipping class differences or whether differences in initial zooplankton densities may have confounded the results of these relationships. For instance, ballast water in vessels sampled from the Great Lakes region were characterized by lower zooplankton densities, older water and lower tank volumes than the Pacific and Atlantic regions. Whether Great Lakes samples were lower in zooplankton abundance because of tank volume and ballast water age or because of unrelated regional differences in initial zooplankton abundance was not determined.

Determining invasion risk of shipping classes

Propagule pressure

Characterizing the supply of NIZ propagules to a region is essential in understanding potential invasion risk and developing effective management strategies. In terms of ballast-mediated introductions, propagule pressure is characterized by the density of individuals, volume

of ballast water discharged and frequency of release events during a specific period of time (e.g., 1-year). Since the rate of biological invasions in aquatic ecosystems is highly dependent on the volume of ship traffic to a region (Ricciardi 2001), ports that receive a disproportionate amount of global commerce are likely to be more vulnerable to biological invasions. The San Francisco Estuary for example, is one of the most frequented ports in the United States, and accordingly has been an epicentre of aquatic invasions for the last century. At least 200 non-indigenous species have been reported in the estuary (Cohen & Carlton, 1998), of which 9 are zooplankton (Ferrari and Orsi, 1984; Orsi and Ohtsuka, 1999; Bollens et al., 2002). In Canada, Vancouver Harbour is the most frequented port in the country receiving approximately 6.7 x 10^6 m^3 of ballast water from 1142 vessels between September 2006 and September 2007 (Lo et al. unpublished data). Levings et al. (2002) reported that the number of foreign vessels visiting Vancouver Harbour has increased steadily over the past century, which suggests that the volume of ballast water discharged and number of propagules released (i.e. propagule pressure) has also increased.

Previous studies have estimated propagule pressure based on ballast water discharge volumes or the frequency of release events alone. Verling et al. (2005) has cautioned against this approach given that variability in propagule abundance and species composition can be associated with the geographical source of inoculants (Grevstad 1999; Lonsdale 1999; Ruiz et al. 2000a). This study has documented considerable variability in propagule pressure between shipping classes (Figure 21), which reflects observed differences in the parameters used to estimate propagule pressure, including NIZ density (Figures 8B), number of release events (Figure 19A), and ballast water discharge volume (Figure 19B). Within the Pacific region, comparisons across shipping classes revealed that ICU exerted the greatest propagule pressure on

Vancouver Harbour. Despite being the least common shipping class and releasing on average the lowest volume of ballast water, ICU vessels exerted the greatest propagule pressure because of the significantly higher density of NIZ found in the ballast water of ICU vessels (Figure 7B).

Invasion history

Estuaries and coastal embayments of BC have been the site of numerous aquatic invasions during the past century. Levings et al. (2002) identified at least 66 non-indigenous and primarily benthic invertebrate taxa established within the Strait of Georgia, the majority of which contain a planktonic life stage capable of being transported via ballast water. Therefore one would expect to observe these species in the ballast water of vessels entering the region. Only 3 of 36 NIZ species observed in the ballast water of vessels from the Pacific region had a documented history of invasion success in North America (Appendix B), including calanoid copepods *Pseudodiaptomus forbesi*, *P. inopinus* and *P. marinus*. All three of these species originate from Asia, and despite being well documented in estuaries throughout the Northwest Pacific (Cordell et al., 2008; Meng & Orsi, 1991; Bollens et al., 2002), have yet to be reported in Canadian waters (Cordell & Morrison, 1996). An additional cryptogenic species, *Eurytemora affinis*, was present in the ballast water of a single TOE vessel sampled in the Atlantic region, but is not considered an invasion threat given its cosmopolitan distribution.

All three *Pseudodiaptomus spp*. were observed in the ballast water of vessels whose ballast water originated from North America (i.e., ICE and ICU vessels). The most frequently observed and most abundant of these species was *P. forbesi*, which was found in 23% of ICU vessels and at mean (±1SE) densities of 453 ± 317 ind · m^{-3} (Appendix B). This species represented 57% of the total zooplankton abundance observed in ICU vessels. Since its initial introduction via ballast water into the San Francisco Bay – San Joaquin Delta area, *P. forbesi* has

spread and established itself in several estuaries throughout the Northwest Pacific. In the Columbia River, where it is most abundant, *P. forbesi* has replaced *P. inopinus,* a previous invader, as one of the most dominant holoplanktonic organisms (Cordell et al., 2008). Only one of six ICU vessels originating from the Columbia River and sampled in this study contained *P. inopinus* and at very low abundance (< 1 individual · m^{-3}). *Pseudodiaptomus marinus* is the most notorious invader of the three copepod species, having established itself throughout much of the Northwest Pacific and the Hawaiian archipelago (Jones, 1966). In the present study, it was observed in low density (ca. 5 individuals · m^{-3}) aboard one sampled ICE vessel from Los Angeles, CA where it is known to be abundant.

While documented cases of copepod invasions are rare, at least 9 species of Asian copepods have been introduced via ballast water and successfully established themselves in the Northwest Pacific region (Cordell et al. 2008). Interestingly, none of the Asian copepod species documented in this study were observed aboard vessels whose ballast water originated in Asia (i.e., TOE vessels). Their absence from what would have historically been their primary transport vector into North America seems to suggest that open-ocean exchange of ballast water is effective in eliminating these species from the original coastal zooplankton community. For *P. inopinus* and *P. forbesi*, ballast water exchange may work particularly well, since these species are stenohaline, existing only in upper reaches of estuaries where salinities range from 0 to 10 psu (Cordell et al., 2007; Suh et al., 1991; Bollens et al., 2002; Orsi and Walter, 1991). *P. marinus* however, is known to exist in coastal ports with very high salinities (e.g., Los Angeles) and would be less likely to suffer osmotic shock from open ocean exchange. This is further evident from the presence of *P. marinus* in the post-exchange ballast water of vessels from other studies (e.g., Levings et al., 2004; Choi et al., 2005; Cordell et al., in press). Results from this

study suggest that although the primary transport vector for Asian NIZ may be eliminated or substantially reduced due to open-ocean exchange, secondary transport from neighbouring embayments with established NIZ is still a legitimate dispersal mechanism. The high occurrence of NIZ aboard ICU vessels, in addition to the short duration of voyages and absence of MOE exchange procedures may pose a serious invasion threat to coastal, estuarine and freshwater habitats in British Columbia.

Environmental suitability

In developing models to determine invasion risk, several studies have emphasized the need to understand the compatibility between the invader and the abiotic conditions of the receiving environment (Lodge, 1993; Simberloff, 1986). With respect to ballast water mediated introductions, the invasion risk posed by intracoastal or transoceanic vessels cannot be solely determined from the density and diversity of organisms released (i.e., propagule pressure) or the invasion history of species. Understanding the abiotic suitability of the receiving environment is important in determining survival outcome, which requires comparisons between donor and receiving environment to be made. Previous studies have demonstrated that temperature and salinity mismatch can lead to drastic reductions in the survival of zooplankton species (Wonham et al., 2001; Smith et al., 1999). In this study, ballast water temperature of TOE and ICU vessels was most similar to Strait of Georgia surface temperature conditions (Figure 17A). However, only ICU vessels contained water that was comparable to the Strait of Georgia with respect to salinity. All other shipping classes contained ballast water that was higher in salinity. This is understandable given that these vessels carry water from the open ocean and the Strait of Georgia receives considerable freshwater input from several major rivers (e.g., Fraser River; Pawlowicz et al., 2007). The environmental similarity between ICU ballast water and the Strait

of Georgia suggests that zooplankton mortality is likely to be lower than other shipping classes upon release. If this is true, zooplankton transported in the ballast water of ICU vessels may have a greater survival advantage and pose a greater invasion risk than zooplankton aboard ICE and TOE vessels.

This study documented the occurrence of at least 36 NIZ taxa in the ballast water of vessels entering aquatic environments of BC (Appendix B), including Vancouver Harbour and the Fraser River estuary. Despite both high densities and frequency of occurrence in ballast water for some species (e.g., *P. forbesi*), none of these species have been detected in BC waters. A study of 18 estuaries in the Northwest Pacific by Cordell & Morrison (1996) revealed that Asian copepod *P. inopinus* was present and often the dominant holoplanktonic species in 7 estuaries in Washington and Oregon, but did not occur in any of the 6 BC estuaries surveyed. In its native range, *P. inopinus* occurs in mainly fluvial oligohaline reaches of estuaries at salinities of 0–5psu (Oka et al., 1999; Suh et al., 1991). While Strait of Georgia surface salinities are much higher than this range (i.e.,. 12-18psu) salinities throughout the Fraser River estuary, a port laden environment, are comparable. Other estuaries throughout BC are also comparable in terms of salinity. Given the substantial invasion pathway, short voyage duration, high density of propagules and suitable salinity for *Pseudodiaptomus spp*., it is interesting that these species have not been documented in BC estuaries and harbours. Consequently, other factors must be influencing their establishment success. Cordell & Morrison (1996) suggest that temperature and the extent of salinity intrusion may be important in the establishment success of *P. inopinus*. Additionally, abiotic and biotic environmental factors such as substratum type, water quality (Moyle and Light, 1996), disturbance frequency and magnitude (Nichols et al. 1990; van den Brink et al. 1993), niche availability, and community composition and resistance (Case 1990;

Baltz and Moyle 1993), may also affect the establishment success of introduced species. Barry and Levings (2002) used a metapopulation model to suggest that *P. marinus* was capable of establishing itself in Vancouver Harbour provided that densities and release frequencies were high enough. They suggested that physiological and ecological tolerance of the recipient environment was necessary in evaluating invasion outcome.

Despite the fact that ballast water has been transported between and within continents for over a century, new invasions attributed to this transport vector still arise (Coles et al., 1999). The continual appearance of new invasions long after a transfer vector has become established may be attributed to changes in the host or recipient environment. Carlton (1996) suggests that abiotic changes such as temperature, water flow and salinity of the donor region could increase the abundance of a resident species, indigenous or non-indigenous, or create opportunities for regional species to expand into previously uninhabitable areas. As physical properties of the recipient environment change, new windows of opportunities could open up for species which were otherwise intolerant to previous conditions. For example, *P. inopinus* was first reported in the Columbia River estuary in 1992, nearly a century after ballast water had begun to be discharged in the river. Cordell et al. (1992) have suggested that the establishment of *P. inopinus* may have been encouraged by a synergistic effect of increased ballast water discharge, decrease freshwater discharge from the river and lower water temperatures over the past decade, although this remains to be tested. Therefore, while propagule pressure, invasion history and host-recipient environmental similarity may be useful in determining potential invasion risk, they do not unequivocally suggest anything about the invasion outcome.

Conclusion

Ballast mediated transport continues to serve as an important vector for the unintentional spread of aquatic organisms. This study characterized the ballast water zooplankton community as taxonomically diverse and abundant. Despite recent provisions to national ballast water management regulations, potentially harmful NIZ continue to enter Canadian aquatic environments in high abundance. This study suggests that the exclusion of some intracoastal vessels from mandatory mid-ocean exchange procedures is not justified from an ecological point of view and based on false assumptions. It was initially conceived that vessels excluded from performing MOE transported water from ports with a similar complement of species to Canadian aquatic habitats. However, as this study has shown, ICU vessels transported the greatest density of NIZ into ports of Canada's Pacific coast. This is largely attributed to a high invasion success rate of aquatic habitats within this exclusion zone, including several major source ports (e.g., Seattle and Portland) for vessels entering Canadian waters. Additionally, several parameters for determining invasion risk, including propagule pressure, invasion history and environmental suitability, all suggest that ICU vessels pose the greatest invasion threat to British Columbia. This suggests that the coastal movement of non-indigenous zooplankton, referred to as secondary invasions, via ballast water may by the primary threat to Canadian aquatic ecosystems through secondary transfer mechanisms.

Future Research

The invasion pathway is a multistep process involving several stages and filters that selectively determine the outcome of a species introduction (Fig. 2???). In order to properly evaluate risk and predict future invasions, it is important to have a better understanding of each these components of the invasion pathway and which ecological processes contribute to invasion success. This study focused on characterizing zooplankton communities in ballast water, to

census species entering Canadian ports with the potential to become established. In order to understand the dynamics of the invasion process, future research should be focused towards understanding ballast water community changes during uptake, transport and release. A greater knowledge of zooplankton community changes and associated physicochemical changes during the ballast water transfer process will allow ecosystem managers to development and implement new technologies and strategies for preventing future introductions. Additionally, more attention should be focused on interactions between potential invaders and the target community. This includes determining whether a species is suitable to abiotic and biotic conditions of the receiving environment, including species interactions, food and niche availability, and dispersal mechanisms. Although tedious and time consuming, evaluating invasion risk on a species-by-species basis will provide a greater understanding of the life history and species specific characteristics that favour invasion success.

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APPENDICES

Appendix A. Sample IMO ballast water reporting form collected aboard each ship sampled.

BALLAST WATER REPORTING FORM

1. VESSEL INFORMATION | **2. BALLAST WATER**

Vessel Name:	Type:	IMO Number:	Specify units: m^3, MT, LT, ST
Owner:	GT:	Call Sign:	Total Ballast Water on Board:
Flag:	Arrival Date:	Agent:	
Last Port and Country:		Arrival Port:	Total Ballast Water Capacity:
Next Port and Country:			

3. BALLAST WATER TANKS BALLAST WATER MANAGEMENT PLAN ON BOARDS YES ____ NO ____ MANAGEMENT PLAN IMPLEMENTED? YES ____ NO ____

TOTAL NO. OF TANKS ON BOARD _____ NO. OF TANKS IN BALLAST _____ **IF NONE IN BALLAST, GO TO NO. 5.**

NO. OF TANKS EXCHANGED _____ NO. OF TANKS NOT EXCHANGED _____

4. BALLAST WATER HISTORY: RECORD ALL TANKS THAT WILL BE DEBALLASTED IN PORT STATE OF ARRIVAL; IF NONE GO TO NO. 5.

Tanks/Holds (List multiple sources/tank separately)	BW SOURCE				BW EXCHANGE circle one: Empty/Refill or Flow Through					BW DISCHARGE			
	DATE DDMMYY	PORT or LAT. LONG.	VOLUME (units)	TEMP (units)	DATE DDMMYY	ENDPOINT LAT. LONG.	VOLUME (units)	% Exch.	SEA Hgt. (m)	DATE DDMMYY	PORT or LAT. LONG.	VOLUME (units)	SALINITY (units)

Ballast Water Tank Codes: Forepeak = FP, Aftpeak =AP, Double Bottom= DB, Wing= WT, Topside =TS, Cargo Hold = CH, O= Other

IF EXCHANGES WERE NOT CONDUCTED, STATE OTHER CONTROL ACTION(S) TAKEN: _____

IF NONE, STATE REASON WHY NOT: _____

5. IMO BALLAST WATER GUIDELINES ON BOARD (IMO RES. A.868(20))? YES ____ NO ____

RESPONSIBLE OFFICER'S NAME AND TITLE (PRINTED) AND SIGNATURE: _____

Appendix A. Identity, status of origin, frequency of occurrence and mean density (± 1SE) of zooplankton taxa observed in the ballast water of ICE, ICU and TOE vessels frequenting ports of the Pacific, Atlantic and Great Lakes region between September 2006 and November 2008.

Pacific Region

Taxon	Status[a]	Frequency of Occurrence (%)[b]			Mean Density (ind·m^{-3})		
		ICE	ICU[c]	TOE	ICE	ICU[c]	TOE
Crustacea							
Copepoda							
Copepod nauplii	UK	63.6	100	87.5	1494.2 ± 757.8	1648.3 ± 866.7	297.1 ± 109.2
Calanoida							
Acartia (Acartiura) longiremis (Lilljeborg, 1853)	IN	27.3	30.8	43.8	14.7 ± 12.0	8.4 ± 5.0	225.5 ± 208.2
Acartia clausi (Giesbrecht, 1889)	NI	0.0	7.7	6.3	0.0	2.3 ± 2.3	0.2 ± 0.2
Acartia danae (Geisbrecht, 1889)	IN	9.1	0.0	0.0	0.1 ± 0.1	0.0 ± 0.0	0.0
Acartia hudsonica (Pinhey, 1926)	IN	18.2	15.4	37.5	1.0 ± 0.7	3.6 ± 3.4	83.5 ± 41.1
Acartia negligens	NI	9.1	0.0	0.0	12.3 ± 12.3	0.0	0.0
Aetideus divergens (Bradford, 1971)	IN	0.0	23.1	0.0	0.0	0.4 ± 0.2	0.0
Aetideus sp.	UK	0.0	7.7	0.0	0.0	0.2 ± 0.2	0.0
Calanus glacialis (Jaschnov, 1955)	NI	0.0	0.0	31.3	0.0	0.0	4.9 ± 3.3
Calanus marshallae (Frost, 1974)	IN	0.0	7.7	0.0	0.0	6.0 ± 6.0	0.0
Calanus pacificus (Brodsky, 1948)	IN	45.5	15.4	18.8	3.0 ± 1.3	12.8 ± 10.4	3.8 ± 3.3
Candacia sp.	UK	0.0	0.0	6.3	0.0	0.0	0.0
Canthocalanus pauper (Giesbrecht, 1888)	NI	0.0	0.0	0.0	0.0	0.0	0.0
Centropages abdominalis (Sato, 1913)	IN	27.3	23.1	25.0	1.8 ± 1.2	0.5 ± 0.3	18.8 ± 17.4
Centropages furcatus (Dana, 1852)*	NI	0.0	0.0	0.0	0.0	0.0	0.0
Centropages sp.	UK	0.0	7.7	0.0	0.0	0.2 ± 0.2	0.0
Centropages tenuiremis (Thompson & Scott, 1903)	NI	0.0	7.7	0.0	0.0	0.5 ± 0.5	0.0
Clausocalanus arcuicornis (Dana, 1849)	IN	9.1	0.0	0.0	0.2 ± 0.2	0.0	0.0
Clausocalanus sp.	UK	18.2	15.4	18.8	22.5 ± 15.1	1.0 ± 0.9	0.3 ± 0.2
Ctenocalanus vanus (Giesbrecht, 1888)	IN	9.1	0.0	0.0	4.7 ± 4.7	0.0	0.0
Delibus nudus (Sewell, 1929)	NI	0.0	0.0	6.3	0.0	0.0	0.3 ± 0.3
Epischura sp.	UK	9.1	30.8	0.0	0.3 ± 0.3	24.2 ± 21.5	0.0
Eucalanus bungii (Giesbrecht, 1892)	IN	0.0	7.7	0.0	0.0	0.1 ± 0.1	0.0
Eucalanus sp.	UK	0.0	0.0	6.3	0.0	0.1 ± 0.1	0.0
Eucalanus subcrassus (Giesbrecht, 1888)	NI	9.1	0.0	0.0	0.1 ± 0.1	0.0	0.0
Euchaeta flava (Giesbrecht, 1888)	NI	9.1	0.0	0.0	0.0 ± 0.0	0.0	0.0
Euchaeta indica (Wolfenden, 1905)*	NI	0.0	0.0	0.0	0.0 ± 0.0	0.0	0.0
Euchaeta sp.	UK	0.0	0.0	6.3	0.0	0.0	0.2 ± 0.2
Euchirella amoena (Giesbrecht, 1888)	NI	9.1	0.0	0.0	1.1 ± 1.1	0.0	0.0

Eurytemora americana (Williams, 1906)	IN	0.0	7.7	6.3	0.0	0.7 ± 0.7	0.1 ± 0.1
Eurytemora pacifica (Sato, 1913)	IN	9.1	0.0	6.3	2.1 ± 2.1	0.0	0.6 ± 0.6
Family Diaptomidae†	UK	0.0	30.8	0.0	0.0	5.8 ± 3.7	0.0 ± 0.0
Labidocera kroyeri (Brady, 1883)	NI	0.0	0.0	6.3	0.0	0.0	0.5 ± 0.4
Mesocalanus tenuicornis (Dana, 1849)*	IN	0.0	0.0	0.0	0.0	0.0	0.0
Metridia pacifica (Brodsky, 1950)	IN	18.2	30.8	25.0	1.1	11.0 ± 10.4	2.8 ± 2.4
Microcalanus pusillus (Sars, 1903)	IN	0.0	7.7	6.3	0.0	0.1 ± 0.1	0.8 ± 0.8
Microcalanus pygmaeus (Sars, 1900)	IN	9.1	15.4	12.5	2.5	0.1 ± 0.1	0.3 ± 0.2
Neocalanus cristatus (Kröyer, 1848)	IN	0.0	7.7	6.3	0.0	0.1 ± 0.1	0.1 ± 0.1
Neocalanus plumchrus (Marukawa, 1921)	IN	9.1	7.7	31.3	1.2	10.4 ± 10.4	15.3 ± 12.4
Neocalanus sp.	UK	0.0	0.0	0.0	0.0	0.0	0.0
Order Calanoida	UK	9.1	0.0	0.0	0.1	0.0	0.0
Parvocalanus crassirostris (F. Dahl, 1894)	NI	9.1	0.0	0.0	0.2	0.0	0.0
Paracalanus denudatus (Sewell, 1929)	NI	18.2	0.0	0.0	1.2	0.0	0.0
Paracalanus parvus (Claus, 1863)	IN	54.5	53.8	62.5	217.6 ± 136.7	639.9 ± 320.2	158.4 ± 113.7
Paracalanus sp.	UK	9.1	0.0	0.0	1.6 ± 1.6	0.0	0.0
Pseudocalanus minutus (Kröyer, 1845)	IN	9.1	15.4	12.5	1447.0 ± 1447.0	1.2 ± 0.8	5.2 ± 4.9
Pseudocalanus newmani (Frost, 1989)	IN	36.4	38.5	56.3	129.0 ± 112.7	18.6 ± 14.9	91.9 ± 57.2
Pseudocalanus sp.	UK	0.0	7.7	6.3	0.0	0.3 ± 0.3	0.0
Pseudodiaptomus forbesi (Poppe & Richard, 1890)‡	NI	0.0	23.1	0.0	0.0	453.4 ± 317.0	0.0
Pseudodiaptomus inopinus (Burckhardt, 1913)‡	NI	0.0	7.7	0.0	0.0	0.1 ± 0.1	0.0
Pseudodiaptomus marinus (Sato, 1913)‡	NI	9.1	0.0	0.0	0.5 ± 0.5	0.0	0.0
Pseudodiaptomus sp.	UK	9.1	0.0	0.0	3.1 ± 3.1	0.0	0.0
Scolecithricella minor (Brady, 1883)	IN	0.0	0.0	6.3	0.0	0.0	0.0
*Scolecithricella sp.**	UK	0.0	0.0	0.0	0.0	0.0	0.0
Temora discaudata (Giesbrecht, 1889)*	NI	0.0	0.0	0.0	0.0	0.0	0.0
Tortanus (Boreotortanus) discaudatus (Thompson & Scott, 1897)	IN	36.4	0.0	6.3	3.4 ± 1.9	0.0	0.2 ± 0.2
Cosmocalanus darwinii (Lubbock, 1860)	NI	0.0	0.0	6.3	0.0	0.0	0.0
Cyclopoida							
Acanthocyclops robustus (Sars, 1863)†	IN	0.0	7.7	0.0	0.0	4.1 ± 4.1	0.0
Corycaeus (Ditrichocorycaeus) anglicus (Lubbock, 1857)	IN	45.5	38.5	37.5	14.6 ± 8.4	182.6 ± 85.4	8.5 ± 5.5
Corycaeus sp.	UK	0.0	7.7	6.3	0.0	0.5 ± 0.5	0.1 ± 0.1
Oithona nana (Giesbrecht, 1892)	NI	18.2	0.0	12.5	3.1	0.0	1.4 ± 1.3
Diothona oculata (Farran, 1913)	NI	54.5	0.0	12.5	93.7 ± 41.4	0.0	0.7 ± 0.6
Oithona plumifera (Baird, 1843)	IN	9.1	7.7	6.3	0.1 ± 0.1	0.1 ± 0.1	0.0
Oithona similis (Claus, 1866)	IN	63.6	53.8	100.0	3198.0 ± 1860.8	145.2 ± 74.5	1007.0 ± 527.4
Oncaea borealis (Sars, 1918)*	IN	0.0	0.0	0.0	0.0	0.0	0.0
Oncaea sp.	UK	27.3	0.0	0.0	0.6	0.0	0.0

Oncaea clevei (Früchtl, 1923)	NI	9.1	0.0	12.5	0.1 ± 0.1	0.0	4.5 ± 4.3
Hemicyclops japonicus (Itoh & Nishida, 1993)	NI	0.0	0.0	18.8	0.0	0.0	0.4 ± 0.3
Oncaea dentipes (Giesbrecht, 1891)	NI	0.0	0.0	6.3	0.0	0.0	7.1 ± 6.9
Oncaea media (Giesbrecht, 1891)	NI	9.1	0.0	0.0	0.2 ± 0.2	0.0	0.0 ± 0.0
Oncaea scottodicarloi (Heron & Bradford-Grieve, 1995)	NI	18.2	53.8	43.8	1.9 ± 1.5	336.0 ± 118.5	3.5 ± 2.4
Oncaea venusta (Phillipi, 1843)	NI	9.1	0.0	6.3	2.0 ± 2.0	0.0	4.4 ± 4.3
Oncaea zernovi (Shmeleva, 1966)	NI	0.0	0.0	18.8	0.0	0.0	2.4 ± 2.0
Sapphirina sp.	UK	0.0	7.7	0.0	0.0	0.1	0.0
Order Cyclopoida	UK	9.1	15.4	0.0	0.0	1.7 ± 1.2	0.0
Harpacticoida							
Macrosetella gracilis (Dana, 1848)	NI	9.1	0.0	0.0	0.5	0.0	0.0
Family Tachidiidae	UK	0.0	7.7	0.0	0.0	0.7 ± 0.7	0.0
Microsetella norvegica (Boeck, 1864)	IN	36.4	30.8	18.8	7.1 ± 3.4	2.4 ± 1.6	7.9 ± 6.0
Microsetella rosea (Dana, 1848)*	IN	0.0	0.0	0.0	0.0	0.0	0.0
Microsetella sp.	UK	9.1	0.0	0.0	0.9	0.0	0.0
Order Harpacticoida	UK	72.7	84.6	43.8	10.9 ± 3.8	172.6 ± 81.0	1.6 ± 0.7
Tigriopus sp.	NI	0.0	0.0	6.3	0.0	0.0	0.1 ± 0.1
Tigriopus japonicus (Mori, 1932)	NI	0.0	7.7	18.8	0.0	1.0	0.2 ± 0.1
Amphipoda							
Corophium crassicorne	NI	0.0	23.1	6.3	0.0	0.4 ± 0.2	0.1 ± 0.1
Suborder Hyperiidea	UK	0.0	7.7	0.0	0.0	0.3 ± 0.3	0.0
Hyperia sp.	UK	0.0	15.4	0.0	0.0	0.1 ± 0.1	0.0
Metopa sp.	UK	0.0	0.0	6.3	0.0	0.0 ± 0.0	0.1 ± 0.1
Themisto pacifica (Stebbing, 1888)	IN	9.1	0.0	12.5	0.5 ± 0.5	0.0 ± 0.0	0.1 ± 0.1
Cumacea							
Cumella vulgaris	IN	0.0	7.7	0.0	0.0	0.1 ± 0.1	0.0
Decapoda							
Gaetice depressus (de Hann, 1835)	NI	9.1	0.0	0.0	0.4 ± 0.4	0.0	0.0
Hyas coarctatus (Leach, 1815)	NI	0.0	0.0	6.3	0.0	0.0	0.1 ± 0.1
Pinnixa rathbuni (Sekiguchi, 1983)	NI	9.1	15.4	6.3	1.0 ± 1.0	0.3 ± 0.2	0.1 ± 0.0
Family Hippolytidae	UK	0.0	15.4	0.0	0.0	0.3 ± 0.2	0.0
Chionoecetes sp.	UK	9.1	7.7	0.0	0.1 ± 0.1	0.7 ± 0.7	0.0
Infraorder Brachyura	UK	9.1	0.0	0.0	0.1 ± 0.1	0.0	0.0
Family Lithodidae	UK	0.0	0.0	6.3	0.0	0.0	0.1 ± 0.0
Family Oregoniidae	UK	0.0	7.7	6.3	0.0	0.1 ± 0.1	0.1 ± 0.1
Hemigrapsus. sp.	UK	0.0	7.7	0.0	0.0	0.2 ± 0.2	0.0
Family Pandalidae	UK	0.0	7.7	0.0	0.0	0.1 ± 0.1	0.0

Cladocera							
Bosmina longirostris (O.F. Müller, 1776)†	IN	0.0	7.7	0.0	0.0	56.5 ± 56.5	0.0
Bosmina longispina (Leydig, 1860)†	IN	9.1	38.5	6.3	2.2 ± 2.2	1010.2 ± 804.5	0.1 ± 0.1
Daphnia sp.†	UK	9.1	23.1	0.0	0.1 ± 0.1	9.5 ± 6.3	0.0
Pseudevadne tergestina (Claus, 1877)	IN	0.0	15.4	6.3	0.0	0.2 ± 0.2	0.2 ± 0.2
Suborder Cladocera	UK	9.1	0.0	0.0	0.1 ± 0.1	0.0	0.0
Ceriodaphnia sp.†	UK	0.0	7.7	0.0	0.0	0.1 ± 0.1	0.0
Pleopsis polyphaemoides (Leukart, 1859)	IN	0.0	15.4	6.3	0.0	11.3 ± 7.8	17.6 ± 17.1
Podon sp.	UK	9.1	0.0	0.0	0.1 ± 0.1	0.0	0.0
Euphausiacea							
Nematoscelis difficilis (Hansen, 1911)*	IN	0.0	0.0	0.0	0.0	0.0	0.0
Thysanoessa raschii (M. Sars, 1864)	IN	9.1	23.1	0.0	0.7 ± 0.7	17.3 ± 16.6	0.0
Ostracoda							
Class Ostracoda	UK	9.1	7.7	0.0	1.5 ± 1.5	0.4 ± 0.4	0.0
Euconchoecia bifurcata (Chen & Lin, 1984)	NI	0.0	0.0	0.0	0.0	0.0	0.0
Isopoda							
Order Isopoda	UK	9.1	23.1	25.0	0.1 ± 0.1	0.5 ± 0.3	0.4 ± 0.2
Cirripedia							
Infraclass Cirripedia	UK	27.3	7.7	0.0	1.3 ± 0.7	938.1 ± 931.1	0.0
Balanus sp.	UK	45.5	61.5	37.5	79.1 ± 76.0	900.0 ± 605.9	276.3 ± 215.0
Chaetognatha							
Sagittoidea							
Sagitta elegans	IN	36.4	23.1	31.3	2.8 ± 1.4	2.1 ± 1.7	0.8 ± 0.4
Sagitta sp.	UK	9.1	7.7	0.0	3.1 ± 3.1	0.1 ± 0.1	0.0
Phylum Chaetognatha	UK	0.0	7.7	0.0	0.0	1.1 ± 1.1	0.0
Chordata							
Tunicata							
Oikopleura dioica (Fol, 1872)	IN	9.1	7.7	12.5	2.3 ± 2.3	4.6 ± 4.6	11.8 ± 102
Class Appendicularia	UK	0.0	7.7	0.0	0.0	0.1 ± 0.1	0.0
Ascidia sp.	UK	0.0	7.7	0.0	0.0	0.2 ± 0.2	0.0
Pisces							
Class Pisces	UK	0.0	7.7	0.0	0.0	0.1 ± 0.1	0.0
Ciliophora							
Tintinnida							
Family Tintinnidae	UK	0.0	7.7	6.3	0.0	43.6 ± 43.6	0.0
Cnidaria							
Hydrozoa							
Obelia sp.	UK	9.1	30.8	0.0	8.1 ± 8.1	30.7 ± 27.8	0.0
Suborder Calycophorae	UK	9.1	30.8	0.0	0.1 ± 0.1	0.7 ± 0.4	0.0
Order Leptothecatae	UK	18.2	7.7	6.3	0.4 ± 0.3	6.3 ± 6.3	0.0

Anthozoa							
Order Actiniaria	UK	0.0	7.7	0.0	0.0	1.1 ± 1.1	0.0
Ctenophora							
Phylum Ctenophora	UK	0.0	0.0	6.3	0.0	0.0	0.2 ± 0.2
Pleurobrachia pileus (Fabricius, 1780)	IN	9.4	0.0	0.0	0.2 ± 0.2	0.0	0.0
Dinoflagellata							
Noctiluca scintillans (McCartney, 1810)	IN	18.2	15.4	25.0	1.1 ± 0.8	47.0 ± 43.5	3.3 ± 2.6
Echinodermata							
Echinoidea							
Class Echinoidea	UK	0.0	15.4	6.3	0.0	9.2 ± 7.7	0.0
Asteroida							
Order Asteroida	UK	0.0	23.1	6.3	0.0	9.3 ± 6.9	0.0
Ophiuroidea							
Class Ophiuroidea	UK	0.0	7.7	6.3	0.0	4.3 ± 4.3	0.3 ± 0.2
Holothuroidea							
Class Holothuroidea	UK	0.0	7.7	0.0	0.0	0.6 ± 0.6	0.0
Bryozoa							
Phylum Bryozoa	UK	9.1	15.4	0.0	0.2 ± 0.2	7.7 ± 6.3	0.0
Foraminifera							
Phylum Foraminifera	UK	9.1	0.0	6.3	0.1	0.0	0.0
Mollusca							
Gastropoda							
Clione limacina (Phipps, 1774)	IN	0.0	0.0	6.3	0.0	0.0	0.1 ± 0.1
Limacina helicina (Phipps, 1774)	IN	36.4	46.2	18.8	1.3 ± 0.7	30.6 ± 11.6	0.9 ± 0.6
Limacina sp.	UK	0.0	7.7	0.0	0.0	85.5 ± 85.5	0.0
Class Gastropoda	UK	0.0	7.7	0.0	0.0 ± 0.0	1.1 ± 1.1	0.0
Bivalvia							
Class Bivalvia	UK	54.5	46.2	31.1	15.0 ± 5.9	802.0 ± 464.0	6.5
Polyplacophora							
Class Polyplacophora	UK	0.0	7.7	0.0	0.0	0.1 ± 0.1	0.0
Nematoda							
Phylum Nematoda	UK	0.0	7.7	0.0	0.0	1.4 ± 1.4	0.0
Phoronida							
Phylum Phoronida	UK	18.2	0.0	0.0	0.8 ± 0.6	0.0	0.0
Platyhelminthes							
Phylum Platyhelminthes	UK	27.3	23.1	0.0	0.3 ± 0.2	0.5 ± 0.4	0.0
Polychaeta							
Phylum Polychaeta	UK	54.5	46.2	37.5	9.7 ± 5.3	183.1 ± 88.3	1.2 ± 0.6
Rotifera							
Keratella sp.†	UK	0.0	0.0	0.0	0.0	24.5 ± 24.5	0.0
Asplancha sp.†	UK	0.0	0.0	0.0	0.0	2.0 ± 2.0	0.0
Phylum Rotifera	UK	18.2	76.9	31.3	2.9 ± 2.4	89.6 ± 42.1	3443.3 ± 3226.2

Atlantic Region

Taxon	Status[a]	Frequency of Occurrence (%)[b]			Mean Density (ind·m^{-3})		
		ICE	ICU[c]	TOE	ICE	ICU[c]	TOE
Crustacea							
Copepoda			-	65.0			
Copepoda nauplii	UK	75.0			185.8 ± 147.2	-	84.8 ± 29.0
Calanoida			-	45.0			
Acartia clausi (Giesbrecht, 1889)	IN	50.0	-	10.0	425.3 ± 424.4	-	118.3 ± 51.8
Acartia longiremis (Lilljeborg, 1853)	IN	0.0	-	15.0	0.0	-	1.8 ± 1.4
Acartia sp.	UK	25.0	-	5.0	2.9 ± 2.9	-	7.4 ± 4.2
Aetideus armatus (Boeck, 1872)	IN	0.0	-	90.0	0.0	-	0.1 ± 0.1
Calanus finmarchicus (Gunnerus, 1770)	IN	100.0	-	5.0	3558.0 ± 2929.4	-	1236.0 ± 680.1
Calocalanus styliremis (Giesbrecht, 1888)	IN	0.0	-	10.0	0.0	-	4.7 ± 4.7
Candacia armata (Boeck, 1872)	IN	0.0	-	30.0	0.0	-	4.5 ± 4.0
Centropages hamatus (Lilljeborg, 1853)	IN	25.0	-	15.0	88.5 ± 88.5	-	50.6 ± 35.4
Centropages sp.	UK	25.0	-	30.0	2.9 ± 2.9	-	38.2 ± 34.4
Centropages typicus (Kröyer, 1849)	IN	75.0	-	25.0	55.4 ± 29.5	-	20.0 ± 12.0
Clausocalanus arcuicornis (Dana, 1849)	NI	0.0	-	5.0	0.0	-	13.2 ± 6.5
Clausocalanus sp.	UK	0.0	-	0.0	0.0	-	3.1 ± 3.1
Euchaeta norvegica (Boeck, 1872)	IN	25.0	-	5.0	30.3 ± 30.3	-	0.0
Eurytemora affinis (Poppe, 1880)!!	IN	0.0	-	10.0	0.0	-	1.0 ± 1.0
Eurytemora herdmani (Thompson & Scott, 1897)	IN	25.0	-	5.0	2648.9 ± 2648.9	-	22.0 ± 21.6
Eurytemora sp.	UK	0.0	-	10.0	0.0	-	0.9 ± 0.9
Metridia longa (Lubbock, 1884)	IN	0.0	-	10.0	0.0	-	0.7 ± 0.6
Metridia lucens (Boeck, 1864)	IN	25.0	-	10.0	0.7	-	3.8 ± 2.6
Metridia sp.	UK	0.0	-	35.0	0.0	-	10.5 ± 8.2
Microcalanus pygmaeus (Sars, 1900)	IN	0.0	-	100.0	0.0	-	79.5 ± 61.0
Order Calanoida nauplii	UK	100.0	-	75.0	6766.1 ± 5708.9	-	2300.5 ± 791.4
Paracalanus parvus (Claus, 1863)	IN	50.0	-	15.0	22.8 ± 21.9	-	2086.3 ± 792.7
Pseudocalanus elongatus (Boeck, 1865)	NI	25.0	-	90.0	44.2 ± 44.2	-	6.5 ± 3.9
Pseudocalanus sp.	UK	100.0	-	50.0	1029.4 ± 660.1	-	612.6 ± 307.7
Temora longicornis (Müller, 1972)	IN	50.0			45.7 ± 26.4	-	892.2 ± 449.4

Cyclopoida							
Corycaeus (Ditrichocorycaeus) anglicus (Lubbock, 1857)	IN	100.0	-	10.0	0.0	-	17.9 ± 13.4
Oithona similis (Claus, 1866)	IN	0.0	-	95.0	1940.7 ± 960.8	-	5347.4 ± 1430.0
Oithona spinirostris (Claus, 1863)	IN	0.0	-	20.0	0.0	-	3.1 ± 1.8
Oncaea media (Giesbrecht, 1891)	IN	0.0	-	5.0	0.0	-	1.8 ± 1.8
Oncaea mediterranea (Claus, 1863)	NI	0.0	-	10.0	0.0	-	59.0 ± 58.4
Oncaea sp.	UK	0.0	-	20.0	0.0	-	22.5 ± 14.8
Oncaea subtilis (Giesbrecht, 1892)	NI	0.0	-	40.0	0.0	-	86.5 ± 43.6
Order Cyclopoida	UK	0.0	-	10.0	0.0	-	1.2 ± 0.9
Harpacticoida							
Sapphirina sp.	UK	25.0	-	5.0	40.4 ± 40.4	-	1.6 ± 1.6
Clytemnestra scutellata	NI	0.0	-	5.0	0.0	-	0.0
Euterpina acutifrons (Brian, 1921)	NI	0.0	-	20.0	0.0	-	38.5 ± 33.9
Microsetella norvegica (Boeck, 1864)	IN	75.0	-	90.0	25.1 ± 18.7	-	675.1 ± 282.2
Order Harpacticoida	UK	25.0	-	25.0	50.6 ± 50.6	-	16.4 ± 8.1
Order Harpacticoida nauplii	UK	75.0	-	10.0	0.0	-	3.5 ± 3.1
Amphipoda							
Scina borealis (Sars, 1883)	NI	0.0	-	5.0	0.0	-	0.6 ± 0.6
Themisto abyssorum (Boeck, 1870)	IN	25.0	-	15.0	10.1 ± 10.1	-	20.9 ± 20.2
Cumacea							
Order Cumacea	UK	0.0	-	5.0	0.0	-	1.0 ± 1.0
Decapoda							
Pagurus sp.	UK	0.0	-	5.0	0.0	-	0.7 ± 0.7
Order Decapoda	UK	25.0	-	0.0	30.3	-	0.0
Cladocera							
Evadne sp.	UK	0.0	-	5.0	0.0	-	0.8 ± 0.8
Euphausiacea							
Class Euphausiacea	UK	0.0	-	30.0	0.0	-	6.0 ± 2.7
Cirripedia							
Infraclass Cirripedia	UK	0.0	-	30.0	0.0	-	46.1 ± 34.8
Mysidacea							
Order Mysida	UK	0.0	-	10.0	0.0	-	3.1 ± 2.3
Ostracoda							
Class Ostracoda	UK	0.0	-	30.0	0.0	-	3.7 ± 1.8
Cnidaria							
Hydrozoa							
Aglantha digitale (Müller, 1776)	IN	0.0	-	5.0	0.0	-	0.4 ± 0.4
Obelia sp.	UK	0.0	-	5.0	0.0	-	1.5 ± 1.5
Sarsia princeps (Haeckel, 1879)	NI	25.0	-	0.0	50.6 ± 50.6	-	0.0

Chordata							
Tunicata							
Oikopleura sp.	UK	0.0	-	10.0	0.0	-	68..6 ± 47.4
Polychaeta							
Tomopteris helgolandica (Greef, 1879)	IN	0.0	-	5.0	0.0	-	1.0 ± 1.0
Class Polychaeta	UK	25.0	-	45.0	212.3 ± 212.3	-	36.7 ± 17.5
Bryozoa							
Phylum Bryozoa	UK	0.0	-	5.0	0.0	-	0.6 ± 0.6
Chaetognatha							
Parasagitta elegans (Verrill, 1873)	IN	0.0	-	10.0	0.0	-	8.6 ± 7.9
Echinodermata							
Phylum Echinodermata	UK	25.0	-	5.0	2.9 ± 2.9	-	4.4 ± 4.4
Mollusca							
Bivalvia							
Class Bivalvia	UK	25.0	-	15.0	2.9 ± 2.9	-	15.6 ± 12.0
Gastropoda							
Limacina sp.	UK	25.0	-	50.0	3.3 ± 3.3	-	156.7 ± 70.3
Chelicerata							
Arachnida							
Class Arachniidea	UK	0	-	5.0	0.0	-	0.7 ± 0.7

Great Lakes Region

Taxon	Status[a]	Frequency of Occurrence (%)[b]			Mean Density (ind·m^{-3})		
		ICE	ICU[c]	TOE	ICE	ICU[c]	TOE
Crustacea							
Copepoda							
Class Copepoda	UK	33.3	-	28.6	1.1 ± 1.1	-	0.9 ± 0.6
Class Copepoda eggs	UK	0.0	-	14.3	0.0	-	0.2 ± 0.2
Calanoida							
Acartia clausi (Giesbrecht, 1889)	NI	100.0	-	57.1	359.3 ± 354.7	-	6.2 ± 3.2
Acartia sp.	NI	0.0	-	14.3	0.0	-	1.2 ± 1.2
Acartia tonsa (Dana, 1849)	NI	33.3	-	0.0	2.1 ± 2.1	-	0.0
Centropages hamatus (Lilljeborg, 1853)*	NI	0.0	-	0.0	0.0	-	0.0
Centropages typicus (Kröyer, 1849)	NI	33.3	-	0.0	10.7 ±10.7	-	0.0
Centropages sp.	NI	0.0	-	14.3	0.0	-	0.5 ± 0.5
Eurytemora herdmani (Thompson & Scott, 1897)	NI	33.3	-	0.0	2.1 ± 2.1	-	0.0
Metridia lucens (Boeck, 1864)	NI	33.3	-	14.3	4.3 ± 4.3	-	0.1 ± 0.1
Microcalanus pygmaeus (Sars, 1900)	NI	33.3	-	14.3	10.7 ± 10.7	-	0.5 ± 0.5
Paracalanus parvus (Claus, 1863)	NI	33.3	-	42.9	201.6 ± 201.6	-	4.3 ± 3.0
Parvocalanus crassirostris (F. Dahl, 1894)	NI	33.3	-	0.0	3.8 ± 3.8	-	0.0
Pseudocalanus elongatus (Boeck, 1865)	NI	0.0	-	14.3	0.0	-	0.3 ± 0.3
Pseudocalanus sp.	NI	33.3	-	42.9	17.2 ± 17.2	-	1.9 ± 0.9
Pseudodiaptomus coronatus (Williams, 1906)	NI	33.3	-	0.0	15.2 ± 15.2	-	0.0
Temora longicornis (Müller, 1972)	NI	33.3	-	0.0	0.9 ± 0.9	-	0.0
Tortanus discaudatus (Thompson & Scott, 1897)*	NI	0.0	-	0.0	0.0	-	0.0
Order Calanoida nauplii	UK	100.0	-	42.9	67.9 ± 30.6	-	12.1 ± 10.3
Cyclopoida							
Oithona nana (Giesbrecht, 1892)	NI	33.3	-	14.3	18.9 ± 18.9	-	0.2 ± 0.2
Oithona similis (Claus, 1866)	NI	100.0	-	71.4	149.2 ± 113.2	-	38.0 ± 32.7
Oithona sp.	NI	100.0	-	57.1	1045.6 ± 822.7	-	0.9 ± 0.9
Oncaea media (Giesbrecht, 1891)	NI	33.3	-	57.1	2.1 ± 2.1	-	0.1 ± 0.1
Oncaea subtilis (Giesbrecht, 1892)	NI	0.0	-	28.6	0.0	-	3.8 ± 3.6
Oncaea sp.	NI	0.0	-	57.1	0.0	-	0.9 ± 0.9
Oncaea venusta (Phillipi, 1843)	NI	0.0	-	14.3	0.0	-	0.1 ± 0.1

Harpacticoida							
Euterpina acutifrons (Brian, 1921)	NI	0.0	-	28.6	0.0	-	0.4 ± 0.3
Order Harpacticoida	UK	100.0	-	57.1	7.6 ± 5.9	-	111.0 ± 107.0
Macrosetella gracilis (Dana, 1848)	NI	33.3	-	0.0	0.4 ± 0.4	-	0.0
Microsetella norvegica (Boeck, 1864)	NI	100.0	-	42.9	10.4 ± 7.7	-	1.1 ± 0.7
Euphausiacea							
Class Euphasiacea	NI	33.3	-	0.0	0.2 ± 0.2	-	0.0
Mysidacea							
Order Mysida*	UK	0.0	-	0.0	0.0	-	0.0
Ostracoda							
Class Ostracoda	UK	0.0	-	14.3	0.0	-	0.2 ± 0.2
Cirripedia							
Infraclass Cirripedia	UK	33.3	-	0.0	0.2 ± 0.2	-	0.0 ± 0.0
Isopoda							
Order Isopoda	UK	33.3	-	14.3	0.9 ± 0.9	-	0.4 ± 0.4
Polychaeta							
Phylum Polycheata	UK	100.0	-	42.9	17.2 ± 2.2	-	0.8 ± 0.4
Mollusca							
Gastropoda							
Phylum Gastropoda	UK	0.0	-	0.0	0.0	-	0.0
Limacina sp.	NI	100.0	-	57.1	5.9 ± 3.5	-	8.3 ± 6.7
Bivalvia							
Bryozoa							
Phylum Bryozoa	UK	33.3	-	14.3	1.8 ± 1.8	-	0.4 ± 0.4
Foraminifera							
Phylum Foraminifera	UK	0.0	-	14.3	0.0	-	0.4 ± 0.4
Chelicerata							
Arachnida							
Class Arachnida	UK	0.0	-	14.3	0.0	-	0.2 ± 0.2

[a] Determination of native status based on taxonomic and biogeographic literature. Taxa are defined as indigenous (IN) if native to (1) coastal, estuarine or freshwater environments of British Columbia for the Pacific Region, (2) coastal, estuarine or freshwater habitats of the Gulf of St. Lawrence for the Atlantic Region, and (3) the Great Lakes Basin for the Great Lakes Region. Those taxa not native to each region are defined as non-indigenous (NI) or unknown (UK) if taxa could not be identified to species level. Taxa not identified to species level and are not native to freshwater habitats were defined as non-indigenous if found in ballast water of ships entering Great Lakes Region.

[b] Frequency of occurrence based on absence/presence data for each vessel per category.

[c] No ICU vessels were sampled in Atlantic and Great Lakes regions.

* Taxa which were observed in the ballast water of vessels that did not fit into either shipping class.

† Freshwater taxa only.

‼ Cryptogenic species

‡ Species with a history of invasion success in North America.