ZOOPLANKTON COMMUNITIES IN CANADIAN PORTS: COMPOSITION, RELATIVE ABUNDANCE AND OCCURRENCE OF INVASIVE SPECIES

BY

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A Thesis

Submitted to the Graduate Faculty

In Partial Fulfilment of the Requirements

For the Degree of

MASTER OF SCIENCE

Department of Health Management

Faculty of Veterinary Medicine

University of Prince Edward Island

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Thesis/dissertation Title: ZOOPLANKTON COMMUNITIES IN CANADIAN PORTS: COMPOSITION, RELATIVE ABUNDANCE AND OCCURRENCE OF INVASIVE SPECIES	
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Abstract

Aquatic invasive species (AIS) cause significant damage to their host ecosystems, altering habitats and native species dynamics. Initiatives to improve our understanding and management of leading stressors of ecosystems like AIS are being developed through collaborative research within the Canadian Aquatic Invasive Species Network (CAISN). This thesis was developed in the context of preventing or reducing dispersal of AIS, facilitating early detection tools to rapidly and effectively identify AIS. In fact, one of the priorities of CAISN aimed at comparing results of several different approaches using traditional and molecular taxonomy to support the development of a national surveillance program.

The overarching objective of this thesis was to identify zooplankton species from 16 "high risk ports" based on shipping traffic and ballast water discharges across Canada, four in each of the four coastal regions (Atlantic, Pacific, Arctic, Great Lakes). Zooplankton collections were conducted by four different CAISN teams, one for each region. 250μm mesh plankton net was used by means of oblique tows during summer periods of 2011 and 2012. The zooplankton was immediately preserved in 95% ethanol and fractioned for parallel projects including traditional and molecular taxonomy. A target taxonomic group (crustaceans) with a global invasion history was studied using a dissecting microscopic. As a result, approximately 14,400 specimens and 86 different taxa, 81 of which were crustaceans, have been identified to their lowest taxonomic level and reference list are provided. Among those, 13 NIS were identified, including *Carcinus* *maenas*, the green crab and *Cercopagis pengoi*, the fishhook waterflea. The number of NIS identified was fairly conservative considering the number of known NIS in each coastal region of Canada. Ordination tests were used to assess the similarities and dissimilarities among ports and regions, and regression analyses were used to assess the relationship between propagule pressure and NIS occurrence. Considerable variation was found among and within regions and significant relationships (albeit modest) between propagule pressure (measured as ship traffic) and the number of NIS were found. Overall, the Artic and Atlantic Oceans were the most different and the Pacific and Atlantic Oceans were the most similar in terms of zooplankton composition. No significant relationships existed when the relative abundance of zooplankton were analysed but significant relationships existed when the number of NIS were analyzed. A separate analysis focused on the comparison among samples and ports of the Atlantic region. A first inventory list of plankton communities associated to ports in this region was developed and ordination analyses were used to assess their level of similarity. Overall, Port Hawkesbury and Halifax Harbour were the most similar and Bayside and Sept-Iles were the most different in terms of zooplankton composition. The data from the 16 ports included in this thesis will be deposited in a National AIS database providing baseline information to support future policy development, and studies on vectors and environmental assessments.

Acknowledgements

Many people have assisted and contributed to the successful completion of this project. First and foremost, I would like to thank my co-supervisor, Dr. Pedro Quijon; I do not know what I would have done without him. I would also like to thank my co-supervisor, Dr. Jeff Davidson for his support throughout this grueling experience. I would like to acknowledge Dr. Mary McNiven for her time and patience. I also want to thank Thomas Landry for his critics which pushed me to an unknown confidence level. I would also like to thank the four sampling teams from the Arctic, Pacific, Atlantic and the Great Lakes regions, my laboratory colleagues, Renée Bernier for her unforgettable help with zooplankton identifications and verifications and Erica Watson for her undivided attention for my needs. I would like to give thanks to Moira Galbraith from IOS who helped with zooplankton verification for the Pacific samples, and Rob Young, PhD candidate from University of Guelph that helped with general information. I would also like to thank the financial support from CAISN/NSERC that made this project possible and DFO, Moncton that always found money to hire and re-hire me and of course UPEI/AVC. Last but not least, I would like to give many thanks to my family that continuously supported me through this experience; I would have never made it through without them. I would like to dedicate this work to them as they never stopped encouraging me even when I thought it was impossible to keep going.

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1. General introduction

1.1. Biological Invasions

The term "biological invasion" dates back to Charles Darwin during his HMS Beagle voyage (1833) where he described the displacement of some native species by introduced species (Hatcher and Battey, 2011). However, the term was only globally recognized after Charles Elton's (1958) book "The Ecology of Invasions by Animals and Plants" was published (Hatcher and Battey, 2011). Biological invasions involve the colonization of new locations by non-native species (Bright, 1999; Vitousek *et al*., 1996) and they represent a significant ecological and economic threat to biodiversity, fisheries, aquaculture, and tourism activities, among others (CCFAM, 2004; Hanfling *et al*., 2011). They occur over time and may involve complex ecological interactions (Perrings *et al*., 2002) that have the potential to modify ecosystems (Hoagland, 2006) and the dynamics that occur between different trophic levels. Given the lack of knowledge about most invaders, their introductory pathways, and the consequences of their potential colonization success, policy makers, industry, government and researchers are challenged to make accurate management decisions (Hoagland, 2006).

The "introduced" status of a species is recognized by a combination of evidence from genetics, ecology, biogeography, systematics, history, palaeontology and archaeology. As a result, marine communities are composed of three categories of organisms: native species, non-indigenous species (NIS) and cryptogenic species. Native species are defined as organisms that are present in a region as a result of natural processes and with no known human-mediated intervention. NIS are exotic, alien or introduced species living outside their original distributional range, because they have been displaced deliberately or accidentally by anthropogenic activities. These species are not necessarily invasive to their new environment but do have the potential to become a nuisance or a pest. Cryptogenic species have an unknown origin; they cannot be verified as either native or introduced (Carlton, 1996). Another term often used to describe or replace NIS is “aquatic invasive species” (hereafter AIS). For the purpose of this thesis, AIS will be considered as a subset of NIS and will be further defined as species that can cause damage to their host ecosystem, alter habitats and be detrimental to native species dynamics.

The introduction of an organism may result in its establishment and subsequent range expansion either deliberately or accidentally (Carlton, 1987). By definition, introductions involve human-mediated dispersal of NIS across natural barriers and into new geographical regions. Similarly, range expansions (in the context of NIS) relate to the spread of these species from an area of introduction (Carlton, 1987). Marine coastal organisms have been dispersed by anthropogenic activities since at least the nineteenth century. Before that period, vessel traffic was much more limited and was restricted to whaling, exploration and fur trading and sailing between the Pacific and Atlantic coast of North America (Essig, 1934). Shipping across the Pacific, however, rapidly increased since gold was discovered in California in 1849, and the traffic has been increasing ever since, moving organisms that have altered many benthic and planktonic communities (Carlton, 1987). Most marine invasions have been reported in bays and estuaries (Ruiz *et al*., 1997) and have been extensively studied (Carlton, 1979; Cohen and Carlton, 1995;

Cohen *et al*., 1998; Hines and Ruiz, 2000; Ruiz *et al*., 2000). These environments are characterized by steep gradients in physical characteristics, the alteration of biological communities, human influence and pollution (Olenin and Leppakoski, 1999). Additional authors such as Galil (2008), Olenin and Leppakoski (1999) and Ranasinghe *et al*. (2005) have studied patterns of invasions in other marine coastal environments such as harbours, ports, bays and closed inlets.

Ecosystems, including coastal environments, are self-sustainable entities consisting of dynamic groups of organisms that interact with each other and with the environment (Geyer, 2011). Because the environment is constantly undergoing changes, communities and populations, including zooplankton, are also expected to change. Analyses of changes in marine ecosystems relative to long-term climate and environmental changes show that the species diversity of some marine communities has been decreasing in the last few decades (Geyer, 2011). Zooplankton species are highly sensitive to oceanic changes including those related to biological invasions. The target taxonomic group of this thesis, namely crustaceans (Engelkes and Mills, 2011) have a global invasion history (Devin *et al*., 2005; Karatayev *et al*., 2009; MacIsaac, 2011).

Within North America, a very well documented example of an altered ecosystem is the Great Lakes: the Eurasian zebra mussel (*Dreissena polymorpha*) (Roberts, 1990) has increased water clarity by its filtering mechanism and, in addition, fouling structures causing major damage. Two significant cladoceran (crustaceans) invaders of the Great Lakes are the Spiny water flea (*Bythotrephes longimanus*) and the Fishhook water flea (*Cercopagis pengoi)*. They first appeared in Lake Ontario in 1998, possibly as a secondary introduction, which was the result of an intentional or unintentional introduction into a new area, and then their dispersal from that point of entry into areas by human mediated introduction (sensu Jansson 2000). They originally came from the Baltic Sea via ballast water (Cristescu *et al.* 2001) and they have since spread in Lake Michigan and five New York Finger Lakes (MacIsaac *et al.* 1999; Makarewicz *et al.* 2001). Examples of marine invasive species are also found in Atlantic Canada, for example, in Prince Edward Island (hereafter PEI). PEI waters harbour two invasive crustaceans, the European green crab (*Carcinus maenas*) (Klassen and Locke, 2007) and the Japanese skeleton shrimp (*Caprella mutica*) and four tunicate species, the Clubbed tunicate (*Styela clava),* (Bourque *et al*., 2007) the Golden Star tunicate (*Botryllus schlosseri),* the Violet tunicate (*Botrylloides violaceus)* and the Vase tunicate (*Ciona intestinalis*).

1.2. Aquatic Invasive Species

AIS are a growing problem in Canada due to the lack of knowledge available about these species (CCFAM, 2004).The risks that are involved in a new invasion cannot be properly known without an understanding of the spatial and temporal dispersal rate of the invader, its distribution and abundance (Stohlgren and Schnase, 2006). As summarized below, other implications include the potential interactions of AIS with native species and their habitats. The rate of biological invasions in the last 500 years has increased exponentially due to anthropogenic activities like transoceanic vessel traffic (Ricciardi, 2007). As a result, AIS are reducing the ecological and economic integrity of aquatic ecosystems worldwide (Lejeusne *et al*., 2010). AIS are not only a threat to our economy and to the ecological integrity of our aquatic ecosystems but also a global threat to aquatic biodiversity. Stressed environments are targets for non-indigenous species the same way that they are targeted by opportunist species. Invaders colonize these environments quickly by out-competing native species for food and habitat (Occhipinti-Ambrogi and Savini, 2003). Vacant niches are open for colonisation when native species and their environment are stressed, favoring the establishment of invaders (Occhipinti-Ambrogi and Savini, 2003). For example, climate change has caused native species to be displaced, leaving an open door for opportunistic species to invade (Giaccone and Di Martino, 1997). Several authors including Delgado *et al*. (1996), Gracia *et al*. (1996), Meinesz and Hesse (1991) and Meinesz *et al*. (1993) have suggested that the success of some invaders is due to their ecological fitness and some characteristics of the receiving environment. Native species can act as natural barriers (resistance) to invaders. However, if native species are in decline due to above mentioned stressors (whether environmental or human-related), invading species are more likely to be successful.

Stressors can include wastewater discharge, organic pollution from aquaculture sites, drastic salinity changes and climate change. In this context, it is crucial to understand the links between human and natural disturbances in order to help prevent and not facilitate future invasions (Occhipinti-Ambrogi and Savini, 2003). In order to control AIS, we first need to identify “hot-spots” of invasions based, for example, on global shipping traffic. Extremely high population densities attained by invasive species are what usually leads to ecologic change and economic loss (Nichols *et al*., 1990). Biological invasions are one of the factors contributing to the major changes that stressed marine ecosystems are facing (Occhipinti-Ambrogi and Savini, 2003). Aquatic ecosystems are already susceptible because in most cases AIS have no natural predators and many of them can easily out-compete native species for food and habitat (Baltz and Moyle, 1993; Case, 1990). This is also true for terrestrial invasive species but in return, the latter can potentially influence nearby water systems (US EPA, 2012). Due to their detrimental effects to the environment and the industries it sustains, the costs of AIS control measures globally reach approximately $1.4 trillion per year (MacIsaac, 2011).

Examples of AIS include the colonial ascidian (*Botrylloides violaceus).* This species was originally introduced from the Northwest Pacific Ocean, using ships' hull. The species has now spread along the Atlantic and Pacific coasts of North America and since its introduction, has caused negative effects on coastal ecosystems and aquaculture activities (e.g. Bock *et al*., 2010). The dispersal of colonial ascidians is mostly by sexually produced propagules as indicated by molecular data (Bock *et al*., 2010). However, asexual budding of individual zooids creates large mats, constantly breaking and forming new mats, increasing its local abundance and potential dispersal (Carver *et al*., 2006). Mitigation measures like high-pressure washing in aquaculture lines do not prevent the local spread of such species via asexual propagules (Bock *et al*., 2010) due to the fragmenting and regeneration of these so called mats. Other impacts and mitigation options have been identified for the vase tunicate (*Ciona intestinalis*) and the clubbed tunicate (*Styela clava*), species fouling aquaculture gear (Bourque *et al*., 2007).

Another example of AIS is the European green crab. Green crabs on a global scale have negatively affected native species and their host ecosystems (Cohen *et al*., 1995; Grosholz and Ruiz, 1995; Klassen and Locke, 2007). Interestingly, when a new green crab invasion occurs, their population size can either grow quickly potentially intensifying interactions with native species by quickly limiting available resources (Pintor *et al*., 2009; Simberloff and Gibbons 2004) or can undergo a lag time, where no clear impacts are evident for some time. Lafferty and Kuris (1996) investigated the economic impacts of the green crab on the west coast of the United States. They reviewed studies by Elner (1981), Glude (1955), MacPhail *et al*. (1955), Moulton and Gustafson (1956) and Ropes (1968) in which these authors concluded that the green crab was one of the contributing factors to the decline of the soft-shell clam fishery in northern New England and Nova Scotia. Economic losses of the landings of the Dungeness crab, mussels, rock crab, oyster and bait fisheries in northern and southern California and Puget Sound have also been attributed to the invasiveness (predation and competition) of the green crab (Hoagland, 2006).

1.3. Propagule pressure, vectors and pathways

Many definitions are used for propagule (individual organism in its larval form) pressure (hereafter PP) but the most common refers to the number of individual propagules released in a single event (Briski *et al*., 2011; Drake and Lodge, 2006; Elton, 1958; Lockwood *et al*., 2009; Simberloff, 2009). PP can also refer to the collective pressure over time instead of an individual event. Three phases can be identified: the introductory or survival phase, the establishment phase and the spread phase. The introductory or survival phase involves the initial transport of propagules over natural barriers and their introduction into a new environment either naturally (wind, current, etc.) or by human mediation (shipping, aquaculture, etc.) Then comes the establishment phase (Johnston *et* *al*., 2009; Minchin and Gollasch, 2003; Wonham *et al*., 2001) which requires the NIS to survive the resistance from the host environment or biota in order to settle and undergo successful reproduction. Resistance factors can be anthropogenic, physical (salinity and temperature) or biological (predation and competition). The last phase of a successful introduction (spread phase) involves the growth of the AIS population and its spread to a wide geographical range.

PP can be used as a predictor of the establishment success of AIS. Related terms include propagule size, which is an estimation of the absolute number of individuals released in one event and propagule number, which is the number of discrete release events. As the number of releases increases, the propagule pressure also increases (Lockwood, 2005). If there are frequent introductory events containing a large and consistent number of individuals, the species will have a higher probability of successfully invading a new area. One way to understand propagule pressure is to look at historical data, such as the number of individuals that have been introduced and whether or not the introduction was accidental or intentional and caused by anthropogenic activities (Colautti *et al*., 2006). There are many ways in which AIS can enter new coastal ecosystems; most often, they arrive as hull fouling organisms, within ballast water discharges (Gollasch, 2006; Ricciardi, 2006) or among loads of commercial shellfish (Carlton, 1987; Savini *et al*., 2010). Hull fouling organisms are those that attach to the ship's hull, "hitching a ride" to other ports and releasing eggs and larvae, and potentially spreading and establishing in a new environment. Ballast water discharge is one of the primary pathways for coastal marine and freshwater organisms to enter a new environment, and is responsible for one-third of AIS that successfully invade ecosystems worldwide (Hewitt and Campbell, 2010). Total ballast water tonnage in a vessel can range from a few hundred to over 100,000 tonnes (t). Any volume of water can carry viable organisms that are continuously and rapidly transported worldwide (Gauthier and Steel, 1996). A ballast water monitoring study from the South Pacific reported an array of live organisms within those waters, including polychaetes, copepods, amphipods, bivalve and crustacean larvae and many fish species (Medcof, 1975; Middleton, 1982; Paxton and Hoese, 1985; Springer and Gomon, 1975).

English coal shippers developed bulk carriers in 1850 where they used ballast water instead of rock and sand (Carlton, 1985). Similarly, in the late 1870's iron-hulled ships started to regularly use ballast water tanks for balance instead of "dry" ballast. As a consequence, vessels transported and released entire planktonic communities all over the world, overcoming large biogeographic barriers for a large variety of marine and freshwater organisms. Earlier, when ballast was made up of rock and sand, it was more common to see terrestrial insects and plants as invaders on the Atlantic Coast than it was to see AIS. When ballast changed from dry to wet, AIS numbers significantly increased, but yet, it was only in the last few decades that they gained more importance (Adams *et al*., 2012). It was not until 1990 that ballast water research began in Canada (Locke *et al*., 1993). The year before, Transport Canada had issued management guidelines to prevent the spread and introductions of NIS to the Great Lakes and to the St. Lawrence River above Montreal (Transport Canada, 2011). Since early 2000, all vessels in all waters are required to do open-ocean ballast water exchange to reduce the risk of possible NIS (Transport Canada, 2007). Hence, the occurrence of significant invasions prompted a management response. The United States had their own regulations drawn up in 1990, the

Non-indigenous Aquatic Nuisance Prevention and Control Act. Their goal was the same as the Canadian guidelines, to interrupt species transfers by directing ships to exchange their ballast water in the open-ocean before port arrival, resulting in significantly fewer viable coastal organisms (propagules) arriving at a receiving port (Davidson, 2012) . As a result, guidelines have been placed in different parts of the world (i.e. IMO, Australia and Port of Vancouver) with mandatory policies to reduce the transfer of non-native organisms (Davidson, 2011; Hewitt and Campbell 2009)

Since the introduction of modern ships, the rate of AIS dispersal has dramatically increased (Carlton, 1985; Carlton and Geller, 1993; Gollasch *et al*., 1999; Gollasch *et al*., 2000; Harvey *et al*., 1999; Ruiz *et al*., 2000; Williams *et al*., 1988). Factors that potentially contributed to increase this rate include shorter transit times, larger ships and new traffic patterns making connections between ports that were not connected before. As a consequence, harbours and ports have been increasingly exposed to transoceanic vessel traffic, and have dramatically increased the number of NIS present and detected (Chapman *et al*., 2013; Ruiz *et al*., 2000). Despite that, we have only been researching non-indigenous species in the last 40 years, even though invasions have been occurring much longer than that.

If shipping activities increase as a result of ongoing climate change and mining exploration, propagule pressure is also expected to increase, making the Arctic more vulnerable to future invasions (Chan *et al*. 2012). An example of a highly invaded ecosystem is the Great Lakes, the world’s largest freshwater system (US EPA 2006) and one of the most ecologically diverse ecosystems in North America (OMNR 2009). Between 160 and185 aquatic NIS have established in the St. Lawrence River system connecting the Great Lakes to the Atlantic Ocean, making the system one of the most highly invaded ecosystems worldwide (Holeck *et al*. 2004; Ricciardi 2006). Approximately 65-73% of these NIS were transported to the Great Lakes by ballast water since the opening of the St. Lawrence Seaway in 1959 (Kelly *et al.* 2009; Grigorovich *et al.* 2003; Ricciardi 2006). It is estimated that on a daily basis 10,000 taxa are transported globally by ballast water alone (Carlton, 1999), but only 5-20% of those species successfully establish in a new region (Lockwood *et al*., 2007). Once a population of an invasive species establishes itself in a new location, its growth rate may be rapid, often in response to reduced competition, parasitism affecting the native biota, and predation release (Behrens Yamada *et al*., 2005). The impacts of these invasions are detrimental to biodiversity and the fisheries; they also represent a concern for infrastructure (a billion dollar cost) and human health (Daskalov *et al*., 2007; Hallegraeff 1998; MacIsaac *et al*., 1992; Ruiz *et al*., 2000). AIS are also affecting local fisheries and aquaculture (Hanfling *et al*., 2011). For example, mussel socks in Prince Edward Island (PEI) have been overwhelmed by the invasive tunicate *Styela clava* among three other species in some bays and estuaries (Bourque *et al*., 2007).

Canadian coastal ecosystems are being challenged with the dispersal of non-native species, altering species composition of native marine communities (Carlton, 1987). The Arctic is one of such ecosystems and constitutes nearly 75% of Canada's coastline (Standing Senate Committee on National Security and Defence 2011). Shipping plays an important role in Arctic waters, supporting communities and providing resources for domestic and international trade (McCalla, 1994). Future development in mining extraction will most likely increase shipping activities to the Arctic ports (DFO 2012;

Stewart and Howland, 2009) which will also increase invasion risk. The opening of new waterways and shipping channels through the Arctic Ocean also will entail similar challenges (ACIA 2004; Chan *et al*. 2012, 2013; Niimi 2004). Even though no ship-mediated NIS have been identified yet in the Arctic, these activities will increase over time increasing also propagule pressure, making ports in the Arctic more susceptible to NIS (Chan *et al*. 2012).

Other two large Canadian ecosystems include the Atlantic and Pacific coastal areas. Eighty two established NIS are known to the Atlantic coast (Locke, pers.comm.), including some of the fouling organisms that cause serious economic losses to the shellfish industry (Drouin and McKindsey 2007; Scheibling and Gagnon 2006) and have increased operation and production costs for aquaculture industries (Howes *et al*. 2007; Locke *et al.* 2009). Meanwhile, the Pacific coast supports a large shipping industry and serves as the Canadian gateway to the Pacific (Transport Canada 2007). At least 112 NIS have established in the marine waters of Canada's Pacific coast, (Daniel and Therriault 2007; Gillespie 2007; Levings *et al*. 2002) 98 of which were recorded in the Strait of Georgia (Therriault, pers. comm.).

1.4. Biodiversity, biogeography, diversity patterns and measures

The UN Conference on Environment and Development in Rio (1992) defined biodiversity as "the variability among living organisms from all sources including, *inter alia*, terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are a part; this includes diversity within species and of ecosystems" (Gray, 1997). There are different levels of diversity including species diversity and genetic diversity, which is the variability within a species and among its populations (Gray, 1997). Different levels of diversity are a result of evolutionary history, genetic mutations, speciation and ecological interactions. Currently, global biodiversity is threatened, among several other factors, by an increase of anthropogenic activities, the introduction of NIS and climate change (ACIA 2004; Chan *et al*. 2012, 2013; Galil 2008; Niimi 2004). Biodiversity is extremely important because it influences the ecosystems' productivity and arguably all species play an important role in the ecosystems' ecological integrity. Biodiversity is linked to species richness and healthy ecosystems, so the greater the diversity and the healthier the ecosystem, the less vulnerable they become. There is evidence suggesting that healthy ecosystems maintain nutrient recycling, stabilize environmental physical and biological properties and have the ability to recover after natural disasters (Beaumont *et al*., 2007).

Biogeography is the study of the distribution of species and their ecosystems in geographic space and in geological time (Golikov *et al*., 1990). Biogeographic regions are defined by factors like bathymetry, hydrography, productivity and trophic linkages (DFO, 2009). When considering biogeographic regions, it is important to consider the spatial scale because higher levels in the hierarchy (larger spatial scales) are influenced by geomorphic and oceanographic processes, whereas lower levels of hierarchy (smaller spatial scales) are more influenced by biological processes such as species interactions (DFO, 1999). For example, taxonomic similarities among species within communities, depth ranges, water masses, and sedimentation characteristics can all play a role in defining a particular biogeographic region (Golikov *et al*., 1990).

The biodiversity and distribution of the zooplankton, the group on which this thesis is focused, is affected by a series of biotic and abiotic factors. Among these factors, salinity and temperature are particularly important and are influenced by freshwater input and upwelling (Orsi, 1986). Human mediated activities can also affect the zooplankton small and large scale distribution. These activities include transoceanic vessel traffic, recreational boating, fishing and industry. Seasonal patterns could, however, influence the diversity of zooplankton communities due to reproductive cycles (Plourde and Runge, 1993). Spatial and temporal variability are important factors to consider when measuring zooplankton abundance and diversity. Spatial scale can be related to the distance between habitats or, in this study, the distance among sites relative to the dispersal of plankton (Fahrig, 1989). Replicate samples should be collected at a same time of the year and preferably over time for accurate description of spatial variability. Seasonal dynamics can also have an influence on species distribution and fluctuations in plankton abundance. For example, in the Gulf of St. Lawrence, productivity is not critically limited by light or by winter months as the warmer water cools and mixes with the deeper water, promoting nutrient recycling and increasing productivity (Archambault *et al*., 2010).

1.5. Zooplankton main group from a NIS perspective: Crustaceans

Zooplanktons are free-living drifting animals occupying the pelagic zone (Sieburth *et al.*, 1978) and include all major taxonomic groups (Lebrasseur and Fulton, 1967). They can be categorized as holoplankton, or animals that remain planktonic throughout their life cycle, such as some families of copepods, pteropods mollusk and some amphipods and as meroplankton, or animals that have a planktonic larval stage and also a benthic adult stage, such as barnacles, bivalves and crabs (Mackas *et al.*, 2007). A single species can occupy several trophic levels depending on its ontogenetic stage. Other classifications of zooplankton include those based on diet, motility, size and morphology; they can be grouped, among others, as microzooplankton, mesozooplankton, or macrozooplankton (Mackas *et al*., 2007). They are among the most important primary and secondary consumers in the lower trophic levels and they play a vital role in the food web dynamics of both marine and freshwater ecosystems. These organisms also play a role in biogeochemical cycles such as carbon and nutrients (Keister *et al.*, 2012). Zooplankton are the single most important link in the trophic web to nearly all marine animals including fish, seabirds, and top predators (Keister *et al.*, 2012).

The contribution of crustaceans to NIS detected in North American and Canadian waters is substantial. In a study done by Ruiz *et al*. (2000), out of 298 NIS in North America 28% were crustaceans. According to Razouls *et al.* (2005-2014), 261 native copepod species exist in the North West Atlantic Ocean, 204 native copepod species exist in the Strait of Davis, Labrador Sea and 294 native copepod species exist in the North East Pacific Ocean. Copepods often represent the numeric majority in the zooplankton (at least in terms of what we call “net plankton”. Other common forms of plankton caught in samples include rotifers, barnacle larvae, crab zoeae and gastropod veligers (Johnson and Allen, 2005). In the Atlantic coast, the upper Bay of Fundy (Atlantic Ocean), for example, is comprised of mostly small estuarine copepod species such as *Eurytemora herdmani* and *Acartia tonsa* (Daborn, 1976). Similarly, a study conducted by Johnson *et al*. (2011) in the Southern Gulf of St. Lawrence included prominently species associated with nearshore environments such as bays and estuaries: *Acartia* species,*Tortanus discaudatus* and *Eurytemora* spp.

The zooplankton associated with the Arctic Ocean is made up of medusas, arrow worms, copepods, amphipods, euphausiids, and sea butterflies. These zooplankton communities are influenced by hydrodynamic characteristics that create changes in surface water temperature, salinity, stratification and mixing conditions (Harvey *et al*. 2001). Hudson Bay area including the Hudson Strait is typically made up of Arctic species, such as the pteropod *Clione limacina*, amphipod *Themisto libellula* and copepods like *Calanus hyperboreus*, *C. glacialis*, and *C. finmarchicus* and of euphausiids (Harvey *et al*. 2001; Roff and Legendre 1986). James Bay and southeastern Hudson Bay is highly influenced by the seasonal freshwater inputs and estuarine characteristics (Grainger and McSween 1976). Two euryhaline copepod species, *Acartia longiremis* and *Centropages hamatus* are particularly important in these regions (Harvey *et al*., 2001; Roff and Legendre 1986).

In the pacific coast, the zooplankton described from the Strait of Georgia is comprised of mostly euryhaline species as well and occasional freshwater species associated with the freshwater runoff from the Fraser River. Oceanic zooplankton do get carried into the basin when deep water is formed in the San Juan de Fuca channels, this mainly happens during summer and early fall (Fulton, 1968). Similarly, in the limnetic waters of the Great Lakes, usually just one or two calanoids and one cyclopoid species dominate at one time. In the littoral areas more copepods are present because of the diverse habitats and the shallow benthic zones which are usually dominated by harpactacoids. The most common copepods found in the Great Lakes are those from the families Pseudocalanidae, Centropagidae, Temoridae, Diaptomidae and Cyclopidae. Cladocereans are also a dominant crustacean in the Great Lakes, including major AIS like *Cercopagis pengoi* and *Bythotrephes longimanus*. These communities are usually dominated by zooplankters of the Bosminidae and Daphnidae families (Balcer *et al.*, 1984).

1.6. Thesis Objectives

The main goal of this thesis was creating a comprehensive zooplankton inventory of native and NIS species in four Canadian coastal ecosystems. Comparing zooplankton communities among regions will provide insight in the development of effective tools for the early detection and rapid response to invaders in their planktonic life stage. This information will also help to properly prepare for commercial shipping through Arctic waters by providing appropriate information on species composition while facilitating the identification of new NIS. Furthering invasion research to develop early detection strategies for the identification and surveillance of aquatic invasive species is important. Given the major threats listed previously, it is important to know the zooplankton species diversity (and their potential differences) in Canada's coastal ecosystems. In coastal areas, zooplankton communities are expected to be higher in diversity and generally are affected by more anthropogenic stressors than in offshore oceanic areas (Angel, 1993). Therefore, research efforts should begin in these areas, particularly in those that are more at risk of stressors like invasions.

The 16 “high risk ports” in which the first part of this thesis focused were based on vessel traffic and ballast water volumes (as surrogates of discharge volumes) (MacIsaac, 2012, pers. Comm.). The target taxonomic group (crustaceans) was chosen based on existing knowledge of its previous invasion history in Canadian waters and elsewhere. Relationships between indicators of propagule pressure and the occurrence of NIS are also known to exist (Locke *et al*, 2007) and are therefore assessed here using available data from those 16 ports.

The specific objectives of this thesis are the following:

1) To identify and to develop a first inventory list of native and NIS zooplankton species (focused on crustaceans) in four Canadian coastal regions (Atlantic, Pacific, Arctic and the Great Lakes) and to assess the relationships between NIS occurrence and PP.

2) To compare zooplankton composition patterns among ports (and among samples within ports) in one of these regions (Atlantic) for which replicated samples were made available.

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Chapter 2: Occurrence of non-indigenous species in plankton communities from 16 high risk Canadian ports and their relationship to two indirect measures of propagule pressure

2.1. Abstract

Having the largest coastline in the world, Canada is exposed to the introduction and potential impact of a growing number of marine and freshwater non-indigenous species (NIS). The limited knowledge available on these NIS has prompted basic studies on their occurrence, distribution and abundance. This study analyzed zooplankton samples collected by the Canadian Aquatic Invasive Species Network (CAISN) from 16 high-risk ports in four major coastal regions: the Great Lakes, the Pacific, the Arctic and the Atlantic. The two main objectives of this study were, first, to provide an inventory of plankton communities, specifically crustaceans associated with each port in order to document the occurrence of NIS, and second, to assess the relationship between propagule pressure indices (shipping traffic and ballast water) and the number and relative abundance of NIS. Based on samples collected in late summer/early fall, this study found considerable differences among ports located in the three marine coastal regions and as expected, stronger differences between these and the ports located in the Great Lakes. The Atlantic and Pacific Oceans were the most similar and the Atlantic and the Arctic Oceans were the most different in terms of zooplankton composition. NIS numbers per port ranged between one and four and were positively and significantly (albeit modestly) related to shipping traffic and in one case to ballast water volume but

showed no significant relationships when assessed against the relative abundance of NIS. Although preliminary, these results provide key baseline information and represent a first step towards future spatially designed studies per region or location.

2.2. Introduction

In the emerging science of invasion biology, studies have traditionally focused on the processes and patterns of invasions (Ruiz *et al*., 2000) and on their influence on human activities, biodiversity and ecosystem function (Daskalov *et al*., 2007; Hallegraeff, 1998; MacIsaac *et al*., 1992; Ruiz *et al*., 2000). During the past several decades, non-indigenous species (hereafter NIS) have gained increasing media attention as a result of the impacts of several high profile invaders (e.g. the zebra mussel, *Dreissena polymorpha*) (Roberts, 1990). However, in order to understand invasions, basic studies aiming to describe the occurrence of NIS and their contribution to native communities remain critical (Stohlgren and Schnase, 2006). Planktonic forms are essential for the spread and introduction of NIS into uninvaded waters, so their identification is important in order to understand when and how invasions occur. The vast diversity of plankton forms frequently forces researchers to target particular taxonomic groups, based on information available on their previous invasion histories. According to the literature, crustaceans have a large number of species with well-known invasion histories (Hanfling *et al*., 2011; Molnar *et al*., 2008). For the purpose of this study, they were also chosen because of their feasibility of preserving with ethanol (this is important due to parallel molecular studies in CAISN) and also because representatives of these taxa are known to have invaded at least one of the regions under this study. Studies of plankton communities with a focus on the occurrence of NIS are especially important in areas of "high risk" of invasion, such as ports (Ruiz *et al*., 2000). These areas become the entry point of many NIS and the starting point for subsequent expansions in their new region (Ruiz *et al*., 2000).

In North America, the number of invasive species has increased exponentially during the past two centuries, likely due to the increase in shipping vessels, trade, and fishery activities (Ruiz *et al*., 2000). In fact, since the introduction of modern day ships and ballast water practices in the mid-1850's (Carlton, 1985) the rate of dispersal of NIS has dramatically increased (Carlton, 1985; Carlton and Geller, 1993; Gollasch *et al*., 1999; Gollasch *et al*., 2000; Harvey *et al*., 1999; Ruiz *et al*., 2000b; Williams *et al*., 1988) although this is also due to an increase in awareness. Shipping activities involve the risk of hull fouling, ballast water transport and discharge, and the trade and transport of various commercial shellfish are among the various modes of transportation (Carlton, 1987). All these potential "vectors" contribute to the number of NIS propagules being released at receiving ports, through a process termed "propagule pressure" or the density and frequency of organisms being introduced into a new environment (Smith *et al*., 1999). There are many cases of failed introductions (Carlton & Geller 1993) but there is little doubt that increased propagule pressure improves the probability of successful invasions (Duggan *et al*., 2005; Colautti *et al*., 2008). Furthermore, several studies have related the increase in propagule pressure to the increase in ship numbers, size, speed and scale of the global shipping traffic (Carlton, 1996; Ruiz *et al*., 1997; Gollasch, 1998; Lavoie *et al*., 1999).

Directly related to shipping traffic are ballast water practices, which are responsible for one-third of the documented marine biological invasions worldwide (Hewitt and Campbell, 2010). Ballast water practices originated in the 1850's when English coal shippers developed bulk carriers using water rather than rock and sand ballast (Carlton, 1985) as a mechanism for the ship's balance. Ballast water tanks became common with iron-hulled ships, and had the ability to transport and release entire planktonic communities into new environments worldwide (Davidson and Simkanin, 2012). Some of the best known cases include the late 1980's Eurasian zebra mussel (*Dreissena polymorpha*) introduction to the Laurentian Great Lakes (Roberts, 1990), toxic dinoflagellates from Australia introduced in Japan (Hallegraeff and Bolch, 1991), epidemic cholera (*Vibrio cholera* 01) moving from South America to the United States (McCarthy and Khambaty, 1994) and the American ctenophore (*Mnemiopsis leidyi*) from the Americas to the Black and Azov Seas (Shiganova, 1998). All of these invasions had serious ecological and economic consequences leading to the development of ballast water management practices. In Canada, these began in 1989 with the aim to prevent the dispersal of non-indigenous species to the Great Lakes and the Lawrence Seaway (Transport Canada, 2011; IMO, 2004).

Regardless of the vector and mechanism used by NIS to reach destination ports, their occurrence and potential impacts cannot be properly understood without the existence of inventories of plankton communities. This study addressed this problem by describing the composition and relative abundance of zooplankton communities in 16 Canadian ports located in the Great Lakes, Pacific, Arctic, and Atlantic coastal regions. These ports were considered "high risk" because they had relatively high levels of shipping traffic for the region. Based on samples collected in late summer/early fall by four separate sampling teams, this study had two objectives: a) to provide an inventory of zooplankton species composition (focusing on crustaceans) and similarity within and among regions documenting the occurrence of NIS in each region; and b) to study the relationship of international shipping traffic and ballast water volumes, as measures of propagule pressure, and to the number of NIS collected in each port.

2.3 Materials and methods

2.3.1. Study area

A total of 16 Canadian ports, four in each of the main coastal regions (Great Lakes, Pacific, Arctic and Atlantic) were chosen for sampling based on information available on vessel traffic and ballast water discharge (MacIsaac, 2011) (Figure 2.1).

The Great Lakes. This region includes a collection of freshwater lakes (Superior, Ontario, Huron, Erie and Michigan) that connect to the Atlantic Ocean via the St. Lawrence Seaway and the Great Lakes Waterway. The four ports sampled in this region were Hamilton Harbour (Lake Ontario), Port of Montreal (St. Lawrence River), Nanticoke Port (Lake Erie) and Port of Thunder Bay (Lake Superior).

Figure 2.1. Map of the 16 Canadian ports, four in each of the main coastal regions (Great Lakes, Pacific, Arctic and Atlantic).

The Pacific. Three of the four ports are located in the Strait of Georgia which extends from 48°50'N to 50°00'N and covers a surface area of 6,800 km². Port of Victoria is located in the Strait of Juan de Fuca. In the summer, the freshwater inflow from the Fraser River (80% of the total freshwater input) mixes with the uppermost saline waters of the Strait of Georgia, to create an upper layer of brackish water, which adopts a counter-clockwise circulation pattern (Gustafson *et al.*, 2000). In this region, Port Metro Vancouver and Roberts Bank Superport are both located on the mainland of British

Columbia whereas Nanaimo Harbour and Port of Victoria are located on Vancouver Island.

The Arctic. This region is divided in five biogeographic regions: the Arctic Basin, the Arctic Archipelago, the Western Arctic, and the Eastern Arctic which includes Lancaster Sound and the Baffin Bay-Davis Strait, and the Hudson Bay Complex (DFO, 2009). These biogeographic units are defined by the influence of freshwater inputs, bathymetry and the distribution of ice coverage. The Hudson Bay is in fact a sea and is the largest one of its kind, covering an area of 822,324 km^2. Its waters flow into the Arctic Ocean due to the cyclonic circulation in its center (Harvey *et al*., 2001). Samples were collected from Churchill Port (Hudson Bay – Hudson Bay Complex) and Iqaluit Port (Davis Strait – Eastern Arctic) in August 2011 and from Deception Bay (Hudson Strait – Hudson Bay Complex) and Steensby Inlet (Foxe Basin – Eastern Arctic) in August 2012 (samples from the three other regions were all collected in 2011).

The Atlantic. The Scotian Shelf, (including Bay of Fundy), the Newfoundland-Labrador Shelves and the Gulf of St. Lawrence constitute this region. The ports sampled included Port de Sept-Iles (Gulf of St. Lawrence), and Bayside Port, Halifax Harbour and Port Hawkesbury, all located in the Southern Scotian Shelf (DFO, 2009). However, Bayside Port is likely influenced by the Gulf of Maine and the Bay of Fundy (a subunit of the Scotian Shelf) system (Spalding *et al*., 2007) whereas Port Hawkesbury is more likely influenced by a combination of the Gulf of St. Lawrence and the Scotian Shelf systems (DFO, 2009).

2.3.2. Collection of zooplankton samples

Four CAISN field teams sampled separately the four coastal regions during ice-free periods in late summer/early fall of 2011 and 2012. Six sites per port were randomly chosen to collect samples with geo-referenced 250 µm oblique plankton net hauls (50 cm diameter opening and 250 cm long). Depth at the sampling sites ranged from 15 to 49 m and information such as date, location, tow and site # were recorded; all samples collected were immediately preserved in 95% ethanol. The samples collected were subdivided so fractions were made available for parallel traditional taxonomic analysis (this study) and molecular analysis (DNA barcoding at University of Guelph and pyrosequencing at McGill University). Unfortunately, sample replication within ports was subsequently lost due to time, logistical, and data issues: individual samples from the six sites per port were combined into a single sample per port, except for the Atlantic samples which were not pooled. Therefore, the samples received and used in this study were restricted to four samples per region (replicated samples from the Atlantic region are analyzed in detail in Chapter 3).

2.3.3. Sample laboratory processing

Each sample was sieved through a 63 µm dip net and thoroughly washed with tap water, and then diluted into a 600 ml beaker with a 400 ml volume of solution in total. The sample was then placed on a stirrer (200 mini-stirrer with 1.27cm magnetic spinbar) until a homogenous sample was obtained. Sub-sampling was performed using 1ml, 2ml, 5ml or 10ml Hensen Stempel pipettes and placed into a Ward counting wheel PVC (1810-E90) and counted under a dissecting microscope until approximately 300 individuals per sample were counted. This subsampling technique was previously validated by Horwood and Driver (1976) taking multiple aliquots and recording total number of individuals subsampled and their variation among subsamples. Estimates indicated a coefficient of variation between 10 and 15% which is considered acceptable for reliable estimations of zooplankton densities (Van Guelpen *et al*. 1982). Sub-samples were initially sorted into four groups: crustaceans, molluscans, ascidians and "others". The identification focused then on the main group (crustaceans) which were identified to the lowest possible taxonomic level using reference manuals and taxonomic keys available. Specimens were counted and sorted under a dissecting microscope (Wild M420, Eyepiece 16X/14B, Leica Wild Makrozoom microscope 2.0X objective magnifier) and a compound microscope (Leica DMLS). Identifications were then validated by a taxonomist familiar with the biota of each region (Renée Bernier – Atlantic and Arctic regions, Moira Galbraith – Pacific region and no one was available for the freshwater zooplankton of the Great Lakes region). The validity of taxonomic names was verified using the Integrated Taxonomic Information System (http://www.itis.gov). After photography, preserved specimens of each taxon were sent to the University of Guelph for barcoding and subsequently to the University of McGill for pyrosequencing analysis.

2.3.4. Community and statistical analysis

Preliminary analysis of field records made evident that some of CAISN`s field teams neglected to record proper flow meter data resulting in data for which abundance of zooplankton per unit water volume could not be determined. Zooplankton community composition was therefore compared using relative values per species (densities per sample expressed as percentages) rather than absolute densities estimated from water volumes which is normally the base of zooplankton studies. The Bray and Curtis index (Magurran, 1988) was used to assess similarity among zooplankton communities among regions. These comparisons were conducted using clustering and multivariate routines in the software package PRIMER v6. From Bray-Curtis similarity matrices, non-metric multidimensional scaling (nMDS) ordinations were performed to visually examine the clustering of samples. Analysis of similarity (ANOSIM) was then used to test the significance of the differences detected among regions. SIMPER analyses were also performed to detect what species drove similarity and dissimilarity between regions. These analyses were based on 4 samples per region, one sample per port.

2.3.5. Shipping traffic and ballast water

International vessel traffic and ballast water discharge data collected in 2006 (Statistics Canada, 2006) was obtained for 13 of the 16 ports of this study. That year was chosen because it had the best records for the ports under consideration. No data was available for Steensby Inlet (Arctic region) as this port was inactive in 2006, or from Iqaluit (Arctic) and Robert Banks (Pacific) (Adams *et al*., 2012; Statistics Canada, 2006). Vessel traffic (# international arrivals/year) and ballast water discharge (tonnes/year) were used as independent variables to conduct linear regressions with two dependent variables: the number of NIS and the relative abundance of NIS (%) recorded from the plankton samples (Table 2.1). Linear regressions were conducted using data available from all the ports (n=13) and then using data from the subset of marine ports only (Pacific, Arctic and Atlantic; n=9), due to their differences with plankton communities from the Great Lakes. Differences in the number of ports per region (see Table 2.1) were not taken into consideration for the regression analyses: Data from each individual port was used as a separate (distinct) value for the purposes of the regressions. Multiple regressions were not considered in these analyses considering that only two independent variables were available for the analysis (shipping and ballast water). In addition, in preliminary runs, multiple regressions did not provide an outcome that was better (i.e. higher R^2 values) than the ones obtained with linear regressions.

Table 2.1. The # of international vessels in 2006 (shipping) and ballast water discharge per vessel (tonnes '000t) were used as separate independent variables to conduct linear regressions with two dependent variables: the number of NIS and the relative abundance of NIS (%) recorded from the zooplankton samples.

Region	Port	Independent variables		Dependent variables	
		Shipping	Ballast water	# NIS	% NIS abundance
Lakes	Montreal	1598	24856.6	2	83
	Hamilton	417	4610.8	3	33
	Thunder Bay	203	3302.1	1	22
	Nanticoke	350	8465.8	3	68
Arctic	Deception Bay	1	6	1	7
	Churchill	13	337.8	1	37
Pacific	Vancouver	3691	87764	3	4
	Victoria	4766	38322.8	3	2
	Nanaimo	649	7907.8	2	17
Atlantic	Halifax	771	30173.3	1	1
	Port Hawkesbury	395	20229.1	1	1
	Bayside	55	1086.9	1	1
	Sept-Iles	280	11505.4	1	1

2.4. RESULTS

2.4.1. Zooplankton species composition

As expected, preliminary similarity analyses among the four regions showed that the zooplankton species composition of the Great Lakes was significantly different from the three marine regions (Global R=0.964, ANOSIM p= 0.001). Hence, Great Lakes zooplankton communities were described separately from those in marine regions.

2.4.1.1. The Great Lakes

Table 2.2 summarizes species composition in the four ports of this region. In total, the Great Lakes zooplankton consisted of 7 calanoid copepod taxa, 5 cyclopoid copepod taxa, and 8 cladoceran taxa. With regards to similarity among ports, there was considerable overlap on zooplankton taxonomic composition. The zooplankton from Hamilton was dominated by taxa like the cladocerans *Bosmina* sp., *Daphnia* sp., and the cyclopoid copepod *Eucyclops agilis*. Meanwhile, the zooplankton in Nanticoke was composed primarily of *Cercopagis pengoi*, *Bosmina* sp. and *Daphnia* sp. cladocerans. Species composition in Thunder Bay and Montreal was dominated by the cladocerans *Bosmina* sp. and *Daphnia* sp., and secondarily by the calanoids *Leptodiaptomus silicis* and *Eurytemora affinis*, respectively (Table 2.2).

Table 2.2. Zooplankton species composition and their occurrence in four ports of the Great Lakes region. Port names are as follows: HA: Hamilton, NA: Nanticoke, TB: Thunder Bay, MO: Montreal. NIS are identified with bold and double asterisks.

	Great Lakes ports			
Taxa	HA	NA	TH	MO
Acanthocyclops vernalis	*	*		
Bosmina sp.	*	*	*	*
Cercopagis pengoi		**		
Ceriodaphnia sp.	*			*
***Cyclops* sp.(*)**	**			
***Daphnia* sp.(*)**	**	**	**	**
Diacyclops thomasi	*	*	*	
Diaphanosoma sp.	*	*		*
Epischura lacustris		*	*	
Eucyclops agilis	*	*	*	
Eurytemora affinis	**	**		**
Heterocope septentrionalis			*	
Holopedium gibberum		*		
Leptodiaptomus sicilis			*	
Leptodiaptomus silicoides	*	*	*	
Leptodora kindti		*	*	*
Leydigia quandrangularis	*			
Mesocyclops edax	*	*		*
Skistodiaptomus oregonensis	*	*	*	*
Skistodiaptomus reighardi		*		

(*) Note: The genus *Cyclops* and *Daphnia* possibly belong to NIS. There are some species of these two genuses' that are known NIS to the Great Lakes region; however, due to taxonomic difficulties, they were left at the genus level. Therefore, it is unknown if the specimen from these samples are in fact NIS or not.

2.4.1.2. The marine regions

In total, 68 zooplankton taxa were identified from the 12 ports sampled in the marine regions (Table 2.3).

Table 2.3. Zooplankton species composition and their occurrence in the three marine regions: Pacific, Arctic and Atlantic. Port names are as follows: VA: Vancouver; VI: Victoria; NA: Nanaimo; RB: Robert Banks; IQ: Iqualut; CH: Churchill; SI: Steenby Inlet; DB: Deception Bay; HL: Halifax; BA: Bayside; SI: Sept-Iles; PH: Port Hawkesbury. Names in bold are NIS in one or more regions and identified with double asterisks on those ports in which they are NIS.

Taxa	Pacific				Arctic				Atlantic			
	VA	VI	NA	RB	IQ	CH	SI	DB	HL	BA	SI	PH
Acartia sp.									*	*	*	*
Acartia hudsonica	*	*	*	*		**		**	*	*	*	*
Acartia longiremis	*	*	*	*	*	*	*	*	*		*	*
Acartia tonsa									*		*	*
Balanus sp.	*	*	*	*	*	*	*	*				
Bivalvia veliger											*	*
Brachyura megalopa								*				
Calanoida	*	*	*	*					*	*	*	*
Calanus sp.			*					*				*
Calanus finmarchicus												
Calanus marshallae	*											
Cancer sp.									*			
Cancridae		*	*	*								
Carcinus maenas									**	**		**
Centropages sp.												*
Centropages abdominalis	**	**	**									
Centropages hamatus					*	*			*	*	*	*
Centropages typicus						*			*			
Corycaeus anglicus	**		**	**								
Crangonidae			*									
Dajidae			*									

Table 2.3. Continued from previous page

Taxa	Pacific				Arctic				Atlantic			
	VA	VI	NA	RB	IQ	CH	SI	DB	HL	BA	SI	PH
Eurytemora sp.				*					*	*		*
Eurytemora affinis									*	*		*
Eurytemora Americana				*					*	*		
Eurytemora herdmani						*			*	*		*
Evadne sp.	*	*	*	*					*		*	*
Evadne nordmanni									*			*
Evadne tergestina									*			*
Gastropoda veliger	*							*	*	*	*	*
Grapidae	*		*									
Halicyclops magniceps								*				
Harpacticoida					*			*			*	
Hippolytidae		*	*	*								
Ischyrocerus anguipes					*		*	*				
Labidocera aestival							*					
Limacina sp.								*				
Majidae			*									
Metridia pacifica	*											
Microsetella norvegica								*			*	
Monstrilloida									*			
Mysis stenolepis								*				
Neotrypaea californiensis	*		*	*								
Oithona sp.											*	
Oithona atlantica			*								*	*

Table 2.3. Continued from previous page

Taxa	Pacific				Arctic				Atlantic			
	VA	VI	NA	RB	IQ	CH	SI	DB	HL	BA	SI	PH
Oithona similis	*	*	*	*	*			*	*		*	*
Ostracoda								*				
Pagurus acadianus												*
Paracalanus sp.			*	*								
Paracalanus indicus			*									
Paracalanus parvus	**										*	
Pleopis polyphemoides	*								*	*	*	*
Podonidae	*	*	*	*					*		*	*
Pseudocalanus mimus	*	*	*	*								
Pseudocalanus minutus	*	*	*	*								
Pseudocalanus moultini	*		*	*					*		*	*
Pseudocalanus newmani	*	*	*	*					*	*	*	*
Pseudocalanus sp.	*		*	*	*	*	*	*	*	*	*	*
Solidobalanus hesperius				*								
Tachidiidae					*							
Talitroidea								*				
Temora longicornis									*	*	*	*
Themisto abyssorum								*				
Themisto pacific			*									
Tisbidae												*
Tortanus discaudatus									*	*		*
Triconia borealis					*							

The Pacific region was numerically dominated by 16 calanoid copepods, 3 cyclopoid copepods, 1 harpactacoid copepod, 3 cladocerans, 2 barnacles, 3 crabs, 1callianassids, 2 caridean shrimp, 1 gastropod, 1 amphipod and 2 isopod taxa, including 1 parasitic one. Among these taxa, a number of NIS were present: Corycaeus anglicus (cyclopoid) was present in all four ports, Centropages abdominalis (calanoid) was collected in Vancouver, Nanaimo and Victoria; Microsetella norvegica (harpactacoid) was only found in Victoria and Paracalanus parvus (calanoid) was only found in Vancouver. The main taxa driving similarity within the Pacific region were the cladocerans Podonidae and Evadne sp. and the calanoid Acartia hudsonica (Table 2.3).

The Arctic region was numerically dominated by 9 calanoid copepods, 2 cyclopoid copepods, 4 harpactacoid copepods, 3 amphipod, 1 crab, 1 barnacle, 2 gastropod, 1 ostracod and 1 mysid taxa. Among these taxa, only one NIS was present: the calanoid copepod *Acartia hudsonica*. This species was present in Churchill and Deception Bay. The main taxa driving similarity within the Arctic region were a calanoid *Pseudocalanus* sp. and a barnacle *Balanus* sp. (Table 2.3).

The Atlantic region was numerically dominated by 15 calanoid copepods, 2 cyclopoid copepods, 3 harpactacoid copepods, 4 cladocerans, 3 crabs, 1 barnacle, 1 gastropod and 1 monstrilloid copepod. Among these taxa, only one known AIS was present: a brachyuran *Carcinus maenas*, the European green crab which was present in Halifax, Bayside and Port Hawkesbury. The main taxa driving similarity within the Atlantic region were two calanoid copepods, *A. hudsonica* and *T. longicornis* (Table 2.3).

Figure 2.2 and Table 2.4 summarize the levels of dissimilarity among the marine regions and the three main species contributing to this dissimilarity in each comparison.

Strong dissimilarity between Pacific and Arctic ports (76.51%) was related to the differences in abundance of Podonidae cladocerans, *Pseudocalanus* sp. (calanoid) and the cladoceran *Evadne* sp. The first taxon (Podonidae) was also the main taxon driving dissimilarity between Pacific and Atlantic regions (67.75%). The highest dissimilarity was found between Arctic and Atlantic communities (80.69%) and was related to the abundance of calanoids *T. longicornis*, *Pseudocalanus* sp. and *A. hudsonica* (Figure 2.2, Table 2.4).

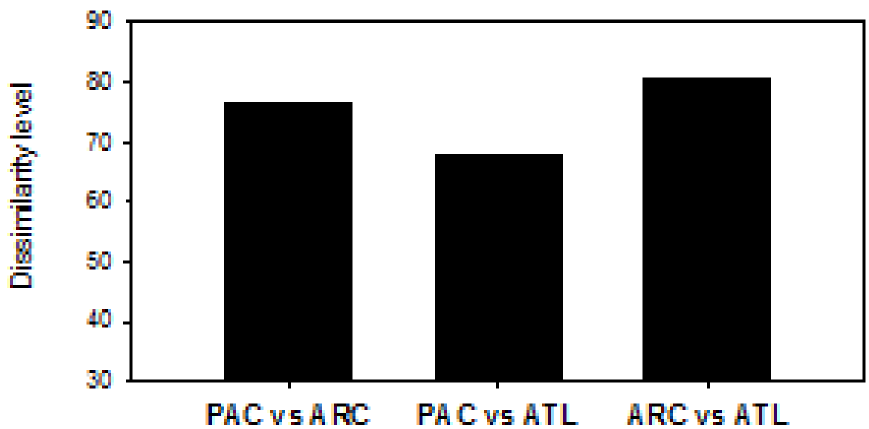

Figure 2.2. Levels of Bray-Curtis dissimilarity (expressed in percentage) between regions. PAC: Pacific region, ARC: Arctic region, ATL: Atlantic region.

High dissimilarity levels among regions were also reflected in the MDS and cluster plots (Figs. 2.3 and 2.4), in which the ports of each region were clearly segregated from each other. At a 40% Bray Curtis similarity (continuous lines surrounding groups of ports in Fig. 2.3), the zooplankton communities of each region were clearly segregated. At this level, the plankton community from Churchill was also separated from the rest of the Arctic ports (Figs. 2.3 and 2.4). ANOSIM confirmed that differences among regions were all significant (Global R=0.905, ANOSIM p=0.02).

Table 2.4. Summary of pairwise dissimilarity levels between marine regions based on SIMPER analyses. The cumulative contribution to dissimilarity of the three species is also presented.

Region	Average dissimilarity (%)	Species cumulative contribution to dissimilarity (top three species and their %)		
Pacific vs Arctic:	76.51	Podonidae (13.42%)	*Pseudocalanus* sp. (21.12%)	*Evadne* sp. (28.46%)
Pacific vs Atlantic:	67.75	Podonidae (10.58%)	*Temora longicornis* (18.33%)	*Eurytemora herdmani* (23.47%)
Arctic vs Atlantic:	80.69	*Temora longicornis* (7.65%)	*Pseudocalanus* sp. (15.24%)	*Acartia hudsonica* (22.72%)

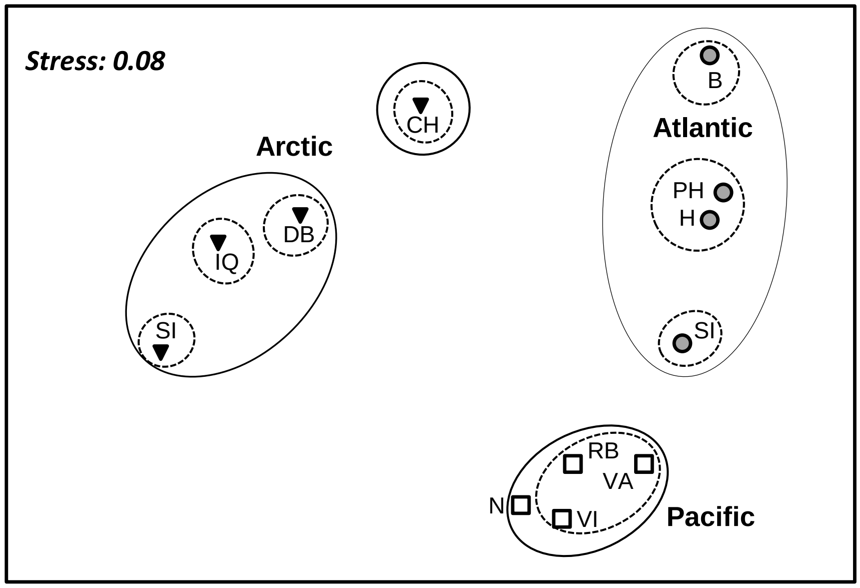

Figure 2.3. MDS plot based on zooplankton community composition from 12 ports in three marine regions: Pacific (NA: Nanaimo, VI: Victoria, VA: Vancouver, RB: Roberts Bank), Arctic (SI: Steensby Inlet, IQ: Iqualuit, DB: Deception Bay, CH: Churchill), and Atlantic (B: Bayside, PH: Port Hawkesbury, H: Halifax, SI: Sept-Iles). Based on Bray-Curtis similarity and square root transformed % of abundance data. Continuous and dashed lines surround ports at 40 and 60% similarity, respectively.

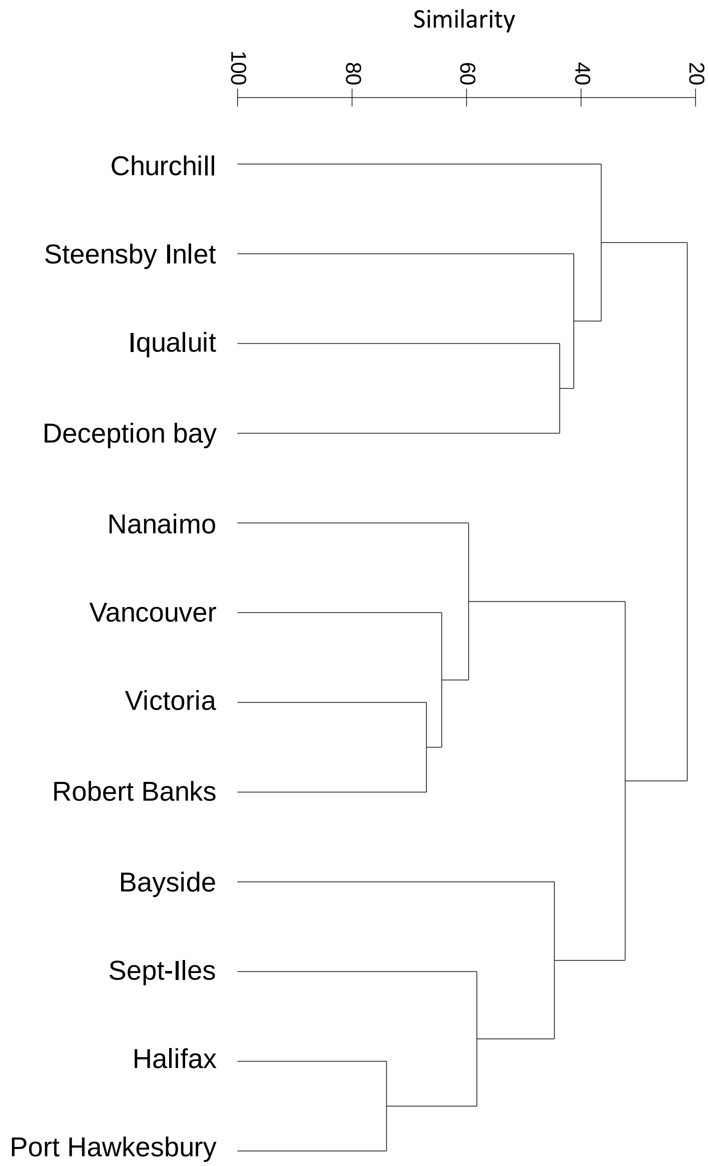

Figure 2.4. Cluster of zooplankton community composition from samples collected from the same 12 ports and three marine regions as depicted in Fig. 2.3. Based on Bray-Curtis Similarity and square-root transformed data as in Fig. 2.3.

Table 2.5 summarizes the results of linear regressions conducted between indirect measures of propagule pressure (shipping traffic and ballast water) and the number and relative abundance of NIS. Some of these regressions are also plotted in Figure 2.5. When data from all (13) ports were used, only one regression analysis identified a significant relationship: the number of NIS was positively associated with shipping levels (R^2= 0.34; p=0.021; Table 2.5). When data from marine ports only were used (n=9), the number of NIS was significantly and positively associated with both shipping traffic (R^2= 0.87; p<0.001) and ballast water (R^2= 0.57; p= 0.018; Table 2.5). Neither of the independent variables explained significantly the variation in relative abundance of NIS (Table 2.5).

Table 2.5. Results of linear regressions using shipping levels and ballast water volume as independent variables and number of NIS and their relative abundance as dependent variables. Regressions were run with data available from all the ports, and then with data from marine ports only (excluding the Great Lakes). These are % of all zooplankton in all ports.

Data	Independent variable	Dependent variable	
		Number of NIS	Relative abundance of NIS
All ports (n=13)	Shipping level	Y=1.374+(0.00039*X); **R^2=0.34; P=0.021**	Y=23.892-(0.00255*X); R^2=0.02; P=0.647
	Ballast water volume	Y=1.436+(0.00002*X); R^2=0.23; P=0.102	Y=25.437-(0.00022*X); R^2=0.04; P=0.513
Marine ports (n=9)	Shipping level	Y=1.006+(0.00047*X); **R^2=0.87; P<0.001**	Y=10.077-(0.00185*X); R^2=0.07; P=0.480
	Ballast water volume	Y=1.037+(0.00002*X); **R^2=0.57; P=0.018**	Y=11.057-(0.00015*X); R^2=0.11; P=0.375

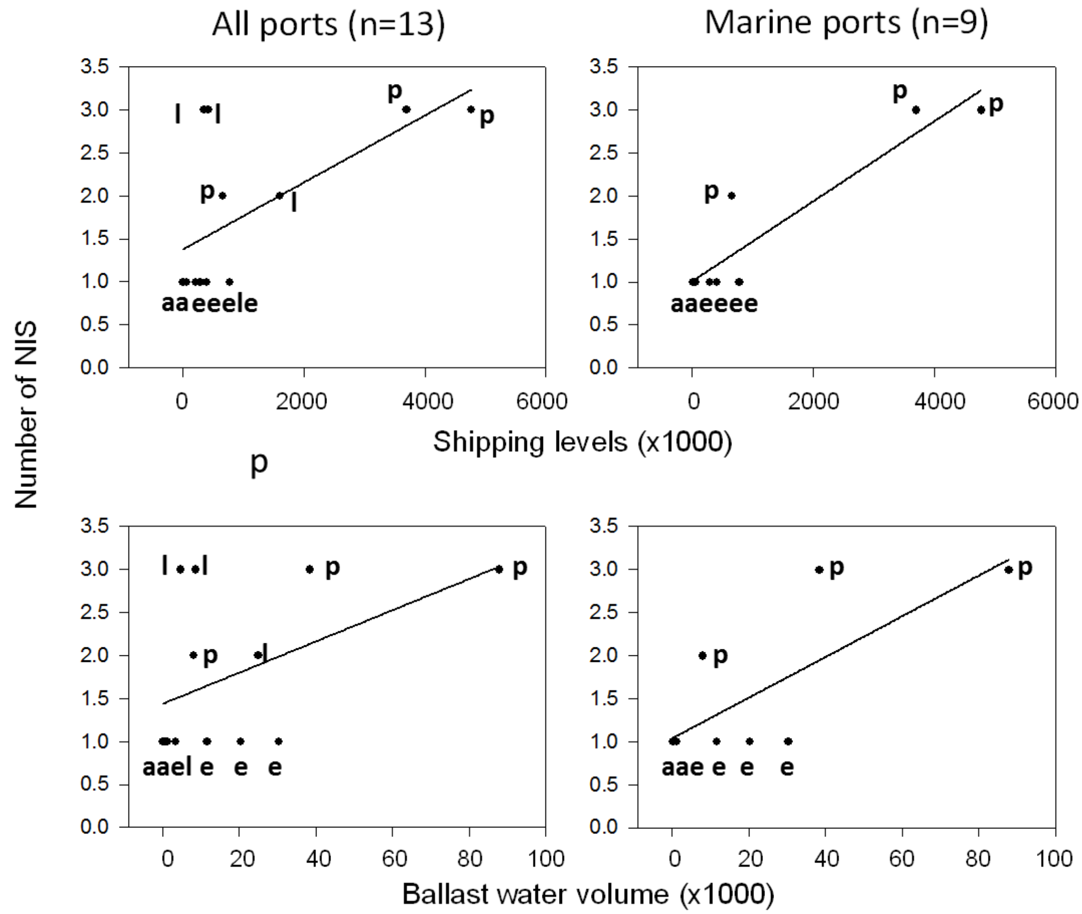

Figure 2.5. Summary of linear regressions between shipping levels (# of international vessel arrivals/year) and ballast water volumes (tonnes/year) (independent variables) and number of NIS in the samples collected from all the ports (n=13) and from the marine ports only (n=9). Letters near symbols stand for regions: l: Great Lakes; p: Pacific; a: Arctic; e: Eastern or Atlantic ports.

2.5. Discussion

Zooplankton composition and distribution are determined by large-scale factors like latitude and major ocean currents (DFO 2009) and a variety of local-scale factors (Johnson and Allen, 2005). Among the later, temperature and salinity vary spatially (Solomon, 2006) and vertically in the water column (Nybakken, 2003) alongside with other factors like water turbidity and nutrient levels (Breitburg, 1997; Boesch, 2001; Epifanio & Garvine, 2001; Kimmel *et al*., 2006). Latitudinal and cross-shelf gradients are stronger in coastal areas than in pelagic systems, and therefore have a stronger influence on zooplankton community structure (Archambault *et al*., 2010). In Canadian coastal systems, for example, it has been suggested that the diversity of groups like crustaceans are more diverse in the Arctic than in the Pacific and the Atlantic coast, but that the western coast is more species rich than the eastern coast (Archambault *et al*., 2010). However, the lack of systematic surveys in the Arctic has not allowed this hypothesis to be exhaustively studied and there is no doubt that many more species are yet to be discovered. Community similarities within and among regions and the contribution of NIS are assessed here using zooplankton communities associated with ports. Then, the relationship between propagule pressure and the numbers of NIS found is discussed and the overall limitations of this study identified.

2.5.1 Zooplankton composition and similarity within regions

2.5.1.1 The Great Lakes

As expected, the Great Lakes zooplankton composition was very different from the ones described for the marine regions, so there was no need to further assess these differences. Four NIS were identified in the Great Lakes, of which *Cercopagis pengoi*, a cladoceran species native to the Caspian-Ponto Sea, was the dominant NIS in my samples in this region. This species was first observed fouling fishing lines in the summer of 1998 in Lake Ontario (MacIsaac *et al*., 1999) and has since spread to Lake Erie and the Finger lakes in New York State (MacIsaac *et al.* 1999b, Makarewicz *et al.* 2001). When it was first discovered, four of the eight sampling sites in Lake Ontario were heavily invaded by *C. pengoi* (US EPA, 2012). However, not one specimen of this species was collected there during this study, this could be partly due to time of year and depth of sampling. In Nanticoke, Lake Erie, nearly 75% of the individual zooplankton collected were *C. pengoi*, but none of these organisms were collected in the other three ports of the region.

A second important NIS, *Eurytemora affinis*, a calanoid, accounted for nearly 68% of the zooplankton abundance in the samples collected from Montreal, and this species was also present but in lower relative densities in Nanticoke and Hamilton. Interestingly, *E. affinis* is native to the North Atlantic marine environment, and there are no known physiological mechanisms to explain how it was capable of colonizing these freshwater systems (Lee, 1999). *Daphnia* sp. (cladoceran) and *Cyclops* sp. (cycloipod) include well known NIS (*D. galeata galeata, D. lumholtzi, C. strenuous* and *Megacyclops viridis*), but neither taxon could be identified to the species level; therefore, it has not been determined if the individuals sampled in this study were NIS or not. Although NIS may have been present in these two groups, the lack of species-level identification limited the ability of this study to assess their contribution to the zooplankton of this region. Larvae of Zebra mussel (*Dreissena polymorpha*), one of the most notorious NIS in the Great Lakes region and a strong driver of water clarity and plankton primary production (Lebrasseur, 1954) were surprisingly not found in this study. This is due to the time of sampling; oogenesis occurs in the autumn and fertilization occurs in the spring and once the larvae are released, usually three to five days after fertilization, they move within the water column for just one month before settling onto the substratum (Banson *et al*., 2014), and both set of samples in this study were collected in July and September.

2.5.1.2 The marine regions

The Pacific ports were the most similar in terms of zooplankton composition. This is consistent with their close geographic distribution and some of the physical properties of their waters. With the exception of Victoria, the Pacific ports were all found in the same eco-region: the Strait of Georgia. This eco-region has a narrow range of annual temperatures (~7 °C) which is in strong contrast with other regions (e.g. the Atlantic) where annual variations reach ~20 °C. The Strait of Georgia is considered a mid-latitude transitional area between the polar seas of the Arctic and the warmer waters located at lower latitudes (Davenne and Masson, 2001). The California Current affects the waters west of Vancouver Island and, coincidentally, two of the four NIS detected in this study in this region (*Corycaeus anglicus,* a cyclopoid and *Paracalanus parvus,* a calanoid*)* are suspected to have travelled up with this current system rather than ship-based transport (Galbraith, pers.comm.). Two other NIS, *Centropages abdominalis* (calanoid) and *Microsetella norvegica* (harpactacoid) (the latter has been found in this region but not during the present study) are believed to have been introduced via ballast water (Razouls *et al*., 2005-2014). The most heavily invaded ports in the Pacific were Vancouver (three NIS in addition to *M. norvegica*) and Nanaimo (in which NIS accounted for 16.5% of the plankton abundance). In comparison, only one NIS was collected in Roberts Bank (Deltaport) despite its closeness to Vancouver (only 40 km away). The port of Vancouver services over 3500 ships annually and since August 2013 Roberts Bank has been receiving the world's largest container ship coming from Asia (Waltz, 2014). Given the high level of traffic in Vancouver and this new development in Robert Banks, NIS numbers are expected to increase in the region. A likely arrival is the larvae of the European green crab (*Carcinus maenas*) already reported along the outer coast of Vancouver Island (Gillespie *et al*., 2007).

Arctic ports had the lowest levels of similarity among ports (39.25%) and, as suggested by Archambault *et al*. (2010), shared the lowest levels of plankton diversity. The low among-port similarity level was likely due to the large distance among them and the fact that each port is located in a different eco-region: Hudson Bay, Hudson Strait, Frobisher Bay and Foxe Basin (Archambault *et al.*, 2010). The port of Churchill is exposed to freshwater run-off from Churchill and Seal Rivers and Nymaykoos Lake. Steensby Inlet has freshwater inputs from the Foxe Basin region where three lakes drain into the inlet. Even though both of these ports have freshwater influences, they differ in zooplankton composition. Churchill was dominated by two calanoid copepods, *Acartia hudsonica* (NIS) and *A. longiremis*, and Steensby Inlet was dominated by one calanoid copepod, *Pseudocalanus* sp. and one barnacle, *Balanus* sp. Finally Iqaluit and Deception Bay are more influenced by saltwater conditions (Archambault *et al*., 2010). The Labrador Sea current flows into Frobisher Bay and into the Port of Iqaluit, whereas the Hudson Strait is influenced by currents coming from the Arctic and the Atlantic oceans both flowing into Deception Bay (Archambault *et al*., 2010). They were both primarily dominated by a calanoid copepod, *Pseudocalanus* sp., whereas, Iqaluit was also dominated by a harpactacoid from the family Tachidiidae and Deception Bay also comprised of many unidentified calanoid copepod nauplii. The Arctic region was dominated by euryhaline copepods like *Acartia longiremis*, *Centropages hamatus*, and *Pseudocalanus* sp. in addition to *Balanus* sp. larvae. Although similarity among Arctic ports was not driven by the only NIS collected in the region (*Acartia hudsonica*) this species was well represented in two of its ports (Deception Bay and Churchill).

In comparison to Pacific and Arctic ports, those in the Atlantic region had an intermediate level of similarity. According to DFO (2009) Atlantic ports belong to two eco-regions: the Scotian Shelf and the Gulf of St. Lawrence. However, a third eco-region (the Bay of Fundy) has been proposed by some authors based on the unique tidal influence affecting this area (up to 15 m at the head of the bay; Kelley and Kelley, 1995). Bayside (located in the Bay of Fundy) is in the St. Croix Estuary, where the tidal influence is still 7m. Halifax and Port Hawkesbury are solely influenced by marine waters are the most similar in the Atlantic region. The distinct tidal influence and water properties are likely to explain the low level of similarity between these ports. Bayside also had the highest density of *Carcinus maenas* larvae, the only NIS identified from the samples of this region; this is probably due to the fact that it has been present in the Bayside area for approximately 50 years. Similarity among ports was driven primarily by the native calanoid copepods *Acartia hudsonica* and *Temora longicornis*. Daborn (1976) found that the zooplankton community of the inner coastal waters of Bayside was dominated by small estuarine species such as *Eurytemora herdmani* and *Acartia tonsa*. The later species was not collected during this study but was present in the other three Atlantic ports. Port Hawkesbury is the second largest Canadian port with respect to annual tonnage, due to large volumes of crushed rock, gravel shipments and oil trans-shipments. Despite the ship traffic involved, surprisingly no additional NIS were detected in this port, although this could depend on sampling efforts. The fourth Atlantic port, Sept-Iles, is found on the northern shore of the Gulf of St. Lawrence, at the mouth of the St. Lawrence River, which discharges 420 km^3 of freshwater annually (Fennel *et al*., 2009). Low similarity levels with other Atlantic ports are likely due to this large freshwater input and its unusual exposure to the hypoxic waters of the St. Lawrence Estuary (Gilbert *et al*., 2005 and 2007).

2.5.2. Zooplankton dissimilarity among marine regions

The main species driving dissimilarities between the Arctic and the Pacific regions were *Pseudocalanus* sp. (calanoid) and two cladocerans, *Evadne* sp. and a species from the family Podonidae. *Pseudocalanus* sp. was present in all four Arctic ports and represented ~31% of the plankton relative abundance, but was only a minor component (7%) in the samples from the Pacific. In counterpart, the two cladocerans were not found in the Arctic but comprised 57% and 11% (respectively) of the relative abundance in the Pacific samples. This is consistent with a previous study by Shaffer *et al*. (1995) who found that these two cladocerans dominated the zooplankton in estuaries in and around the San Juan Archipelago (near Victoria).

Dissimilarity between Arctic and Atlantic regions was driven by *Pseudocalanus* sp., which again was numerically better represented in the Arctic waters. In contrast, *Acartia hudsonica* (calanoid) was well represented in the Atlantic but not in the Arctic, and *Temora longicornis* (calanoid) was only found in the samples of the Atlantic region. Among these species, *A. hudsonica* is new to the Arctic region (Churchill and Deception Bay). A study done by Bailey and Hachey (1950) suggests that there is an increasing influence of the Atlantic into the Arctic waters in terms of temperature and salinity. Therefore, the presence of *A. hudsonica* in the Arctic might be due to a range expansion associated with that increasing influence or to a recent arrival in ballast water.

Differences in community structure between the Pacific and the Atlantic were due primarily to two calanoids, *Temora longicornis* and *Eurytemora herdmani*, both absent in the Pacific but relatively well represented in the Atlantic. A third taxon driving dissimilarity between these regions was Podonidae, a family well represented in the Pacific samples but comprising less than 1% of the zooplankters of the Atlantic ports sampled. However, past researchers have identified *Podon* sp. all over the Southern Gulf of St. Lawrence (Locke, pers. comm.), therefore, the lack of Podonidae in this study may be due to the spatial limitation. *T. longicornis* was present in all four Atlantic ports but while it was a numerically dominant species in Halifax, Sept-Iles and Port Hawkesbury, it was only a minor component of the zooplankton of Bayside. These differences may be related to salinity, which is high in the three first ports (near 30 ppt; Dadswell, 1979) and much lower in Bayside, a freshwater influenced port where salinities reach 14.6 ppt (Martin *et al*., 1999).

2.5.3. Propagule pressure and NIS

This study found positive relationships between the two indices of propagule pressure (ship traffic and ballast water volume) and the number of NIS detected in the zooplankton samples. Among those, significant relationships were detected between ship vessel traffic and number of NIS from marine ports. These results agree with preliminary studies conducted during CAISN's first phase of projects (Lo *et al*., 2012) where researchers found that the number of aquatic invasive species in both the Pacific and the Atlantic regions was related to the number of vessels arriving at each port, where most of them are international vessels (an indicator of ship traffic). These results suggest that ship vessel traffic is a reasonably good indicator of propagule pressure and only a fairly reliable predictor of the number of NIS to be found in port waters. However, it must be mentioned that at the scope of this project, there was evidence of spatial aggregation of ports that explain to some extent the significant relationships found. For instance, ports located in the Arctic and Atlantic regions (labelled "a" and "e" in Fig. 2.5) were located at the lower left end of the relationships, whereas those from the pacific region ("p") were always located at the upper right side. Estimations of ballast waters were less reliable as predictors of number or relative abundance of NIS in plankton communities associated with port waters.

These results concur with several studies that have identified positive relationships between shipping traffic or other measures of propagule pressure, and the number of NIS in a given area. Colautti *et al*. (2006) stated that NIS success was often related to propagule pressure and Briski *et al*. (2012) found positive relationships between colonization pressure (estimated as the number of species released in a new area) and estimations of propagule pressure measured by ballast water of exchanged ships. Locke and Therriault (unpublished data) indicated that a total of 82 and 98 NIS have been identified in the Atlantic and Pacific regions, respectively. This is consistent with a recent study by DiBacco *et al*. (2012) which concluded that vessels entering the west coast of Canada entail a significantly higher NIS propagule pressure than those entering the east coast. Other authors such as Cordell *et al*. (2009), Lawrence and Cordell, (2010) and Wasson *et al*. (2001) have reported similar findings.

Up to 185 NIS have been identified in the Great Lakes over the last decade (Ricciardi, 2011). From these, an estimated 31% of NIS introductions have been suggested to be directly related to ballast water discharge and, indirectly, ship vessel traffic (Molnar *et al*., 2008). Meanwhile, the port of Vancouver was ranked second in vessel traffic in Canada and first in terms of ballast water volumes. This is consistent with the number of NIS detected in this study compared to the other marine ports and with the interpretation of the regression analyses. Interestingly, though, the port of Nanticoke has the highest recorded abundance of NIS, but international arrivals were 350 vessels in 2006, suggesting that vessel traffic and ballast water may not be necessarily the best nor the only estimators of propagule pressure (see study limitations).

The number of NIS in the Arctic was expected to be low (following Archambault *et al*., 2010 observations), although this vast region remains the least known: no comprehensive studies to date have collected and identified zooplankton communities. Regardless of the actual number of NIS currently living in the Arctic, this number is expected to increase due to the new Mary River Project (iron ore mine located on North Baffin Island in the Qikqtani Region of Nunavut, Arctic). This project will certainly increase ship vessel traffic, ballast water levels, and the potential for hull fouling (MacIsaac, 2011; Chan *et al*., 2012). In addition, the further opening of the northern passage as a result of ice melting is also expected to increase vessel traffic, and therefore, propagule pressure. In both scenarios, if the results of our regressions stand correct, the number of NIS is expected to increase. Oil exploration could also increase shipping pressures on the Scotian Shelf. For example, 85% of vessels that arrived in Come-by-Chance, NL, contained ballast water (Blakeslee *et al*., 2010).

2.5.3. Study limitations

Two study limitations have been already identified in the Methodology: lack of within-port replication and lack of absolute field density estimations. Although by using relative abundance this study was able to assess levels of similarity; however, caution must be exercised considering the potential bias of very different volumes of water being collected during the sampling of different ports. Both factors likely have an incidence on the likelihood of NIS to be successful in the new invaded habitat. The use of rough numbers of ship traffic per port is also considered to be a limitation; the origin of ships and their cargo, and possibly the seasonality of ships should also be considered. In addition, season of collection may also represent a limitation: Pacific ports were sampled in late July, Arctic ports in August, and Atlantic ports between late August to September. Even though the temporal scale is relatively narrow for a study of this geographic scale, an important seasonal transition may account for at least some of the community differences detected in this study and therefore cannot be ignored. For example, during the warmest summer months high salinities and nutrient concentrations combine with poor dissolved oxygen conditions in some of the areas studied here. These conditions, which change towards the fall, are likely to affect zooplankton community composition.

Considering the vast spatial scope of this study, the number of ports per region was fairly limited due to logistical considerations. In addition, the type of habitats surrounding these ports, whose assessment was beyond the scope of this study, may have a profound influence on the zooplankton composition. The lists of species composition presented in this study include several taxa that could not be identified at the species level. This is related to time restrictions and access to taxonomy expertise not readily available for each region. As a supplement to traditional taxonomy, genetic diversity patterns should be further researched as molecular tools are becoming more important in the study of the diversity of planktonic organisms.

Several regression analyses generated non-significant results. This could potentially suggest added complexity that could not be captured by linear regressions with relatively low number of samples (ports) or that no relationship in fact existed. It is well known that one determinant of invasion success is the relationship that exists between invaders and recipient communities and habitats (Ricciardi and Atkinson 2004; Strauss *et al*. 2006; Colautti *et al*. 2006). Thus, even when invasion success is influenced by dispersal opportunity and propagule pressure (Smith *et al*., 1999; Kolar and Lodge, 2001) these are clearly not the only factors driving the number and abundance of NIS in a given area.

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Chapter 3: Port zooplankton and the occurrence of non-indigenous species in Canada's Atlantic region: a first inventory of species composition and relative abundance

3.1. Abstract

Regional studies of plankton communities associated with ports are important in order to identify indigenous and non-indigenous species in areas considered to be of high risk for invasions. Canada's Atlantic region is particularly sensitive to those invasions, but no studies investigating the contribution of non-indigenous species to zooplankton communities have been conducted in ports of this region. The main objective of this study was to document the composition and relative abundance of indigenous and non-indigenous crustacean zooplankton species in four ports (Halifax, NS; Bayside, NB; Port Hawkesbury, NB; and Sept-Iles, QC). Replicated (six samples per port) zooplankton collections were made in August and September of 2011 using oblique or vertical plankton tows with a 250 µm mesh net. In this first inventory of port zooplankton communities, only two non-indigenous species were collected, larvae of European green crabs, *Carcinus maenas*, in all three ports except Sept-Iles and a calanoid copepod, *Acartia tonsa*, that was collected in Sept-Iles. Zooplankton communities were numerically dominated by copepods and showed clear differences in composition and relative abundance among samples and ports. Halifax Harbour and Port Hawkesbury, both subject to Scotian Shelf influences, exhibited the highest levels of zooplankton

community similarity. Bayside, in the Bay of Fundy, had the most distinctive zooplankton community.

3.2. Introduction

Biological invasions involve the colonization of new locations by non-indigenous species (hereafter NIS) (Bright, 1999; Vitousek *et al*., 1996). These invasions typically involve human-mediated dispersal (commercial fishing, aquaculture, recreational boating etc.) of NIS across natural barriers into new geographical regions. These invasions are followed by range expansions or the dispersal of these species (currents, population growth) from an area of first introduction into additional geographical locations where the species did not formerly exist (Carlton, 1987). NIS have gained attention because many of them constitute a threat to global and regional biodiversity, ecosystem dynamics, fisheries and other economic activities (Stohlgren and Schnase, 2006). However, the risks associated with invasions cannot be properly assessed without basic studies of NIS distribution and their contribution to native communities (Stohlgren and Schnase, 2006).

Most marine invasions have been reported in bays and estuaries (Ruiz *et al*., 1997) (Carlton, 1979; Cohen and Carlton, 1995; Cohen *et al*., 1998; Hines and Ruiz, 2000; Ruiz *et al*., 2000) but researchers like Galil (2008), Olenin and Leppakoski (1999) and Ranasinghe *et al*. (2005) have also studied invasions in other marine coastal environments. There is little doubt that long-term climate and environmental changes continue to occur and some evidence already suggests that species diversity of some marine communities has been decreasing in the last few decades (Geyer, 2011). Among these communities, zooplankton assemblages are highly sensitive to oceanic changes including those related to biological invasions (IUCN, 2000). This relates to the fact that a majority of marine NIS possesses a planktonic larval stage during their life cycle (Ruiz *et al*., 2000) which is essential for their dispersal and expansion into new environments.

The high diversity of zooplankton organisms entails a level of taxonomic difficulty that imposes a limit to the taxonomic scope of community studies. This study was conducted under the umbrella of the Canadian Aquatic Invasive Species network (CAISN), which chose groups that were common to both fresh and salt water in addition to having a history of invasion. Zooplanktonic crustaceans were chosen as the target group (Hanfling *et al*., 2011; Molnar *et al*., 2008). In fact, crustaceans are the most successful taxonomic group in terms of diversity of aquatic invasive species (Devin *et al*., 2005; Engelkes and Mills, 2011; Karatayev *et al*., 2009). For example, Karatayev *et al*., (2009) reported that 53% of the aquatic invaders in European freshwater ecosystems were crustaceans, whereas Olenin and Leppakoski (1999) and Ranasinghe *et al*., (2005) reported similar numbers in brackish waters, and Galil (2008) in marine environments. The most common invasive crustaceans are decapods, copepods, cladocerans and mysids (Ricciardi, 2011).

Studies on zooplankton communities with a focus on the potential occurrence of NIS are critical, particularly in areas likely to be invaded. Along shorelines and coastal areas, ports have been traditionally considered sites at high risk of invasions. Vessel traffic and ballast water discharge, both of which are related to the location of ports, have been also linked to the introduction of NIS (Ruiz *et al*., 2000; see also Chapter 2). The regional study of port zooplankton biota has been identified as a practical way to assess the occurrence of NIS and constitute the first step to analyse their distribution and potential spread. This study focuses on zooplankton communities associated with four "high risk" ports located in the Atlantic region. Based on samples collected during August and September 2011, this study describes species composition and similarity within and among ports with a focus on the presence and contribution of NIS. The two specific objectives of this study were a) to develop a first inventory of native and non-native zooplankton species (crustaceans and mollusks) in selected ports of the Atlantic region, and b) to assess the level of plankton community similarity among ports.

3.3. Methods

3.3.1. Study Area

The Scotian Shelf, the Newfoundland-Labrador Shelves and the Gulf of St. Lawrence constitute the main geographical components of the Atlantic region. The ports sampled in this study include Port de Sept-Iles (Gulf of St. Lawrence), Bayside Port, Halifax Harbour and Port Hawkesbury (Fig. 3.1). Although all of these ports, except Sept-Iles, are located in the Southern Scotian Shelf (DFO, 2009), Bayside Port is likely influenced by the Gulf of Maine/Bay of Fundy system (Spalding *et al.*, 2007) whereas Port Hawkesbury is more likely influenced by the Gulf of St. Lawrence and Scotian Shelf systems (DFO, 2009).

Bayside Port is an ice-free port located near the mouth of the St. Croix River in Passamaquoddy Bay (Bay of Fundy). Vessel traffic in this port is related to gypsum and potatoes and the new development of a food storage facility that has increased the total volume of vessel traffic, making this the fastest growing port in the Canadian Maritimes. The St. Croix River has a length of 144 km and a total drainage area of 3900 km^2.

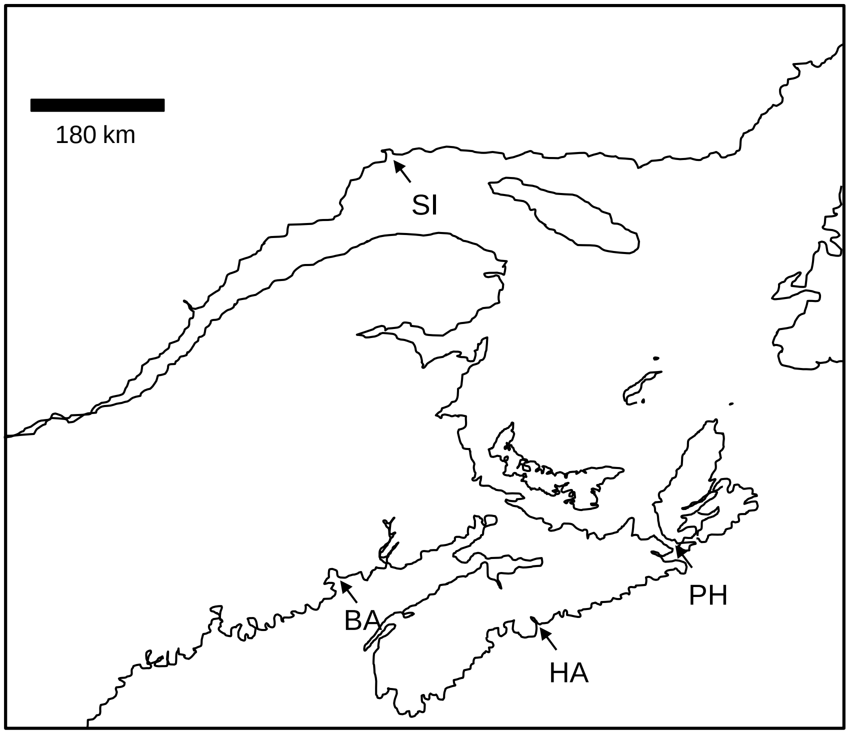

Fig. 3.1. Map outline of Atlantic Canada with the approximate location of the ports where zooplankton samples were collected. HA: Halifax harbour, BA: Bayside port, SI: Sept-Iles, PH: Port Hawkesbury.

Port de Sept-Iles, located in the lower estuary of the St. Lawrence River, connects to the Atlantic Ocean through the Belle-Isle and Cabot Straits (Moritz *et al*., 2012). Seaward surface waters and landward deeper waters control the estuarine circulation (Saucier *et al*., 2003). Three channels characterize the topography of the northern part of the Gulf of St. Lawrence: the Laurentian, the Esquiman and the Anticosti (Moritz *et al*., 2012); the port is located in the Laurentian channel.

Halifax Harbour is the third largest container port in Canada (after Vancouver and Montreal). It handles a range of bulk products including wheat and gypsum. Traffic through this port has increased steadily over the past decade (Pinfold, 2010).

Port Hawkesbury is associated with the Strait of Canso port complex, located between mainland Nova Scotia and Cape Breton Island. The Canso Causeway, a permanent link between these two areas built in the mid 1950's, forms the northern boundary of the port and ensures that the port remains ice-free year round. The Strait of Canso is 20 km long, up to 1.5 km wide, has a limiting depth of 27 m and can handle vessels of up to 500,000 deadweight tonnes (dwt) (Pinfold, 2010).

3.3.2. Collection of zooplankton samples

A field team from the Canadian Aquatic Invasive Species Network (CAISN) collected samples from the four ports indicated above during late summer/early fall 2011. Six samples per port (24 samples in total) were randomly chosen to collect samples with geo-referenced 250 µm oblique or vertical plankton net hauls (50 cm diameter opening and 250cm long) (Zhan, A. pers. comm, 2011). Samples were preserved in 95% Ethanol and information such as date, location, tow and site number# were recorded. Depth at the sampling sites ranged from 15 to 49 m. All the samples were subdivided so fractions were made available for traditional taxonomic analysis (this study) and parallel molecular analyses (DNA barcoding, University of Windsor).

3.3.3. Sample Laboratory Processing

Each sample was poured into a 63 µm dip net and thoroughly washed with tap water. Each sample was placed in a 600 ml beaker with water added to bring the volume of the diluted sample to 400 ml in total. The sample was then placed on a stirrer (200 mini-stirrer with 1cm magnetic spinbar) until a uniform sample was obtained. Subsampling was performed using 1ml, 2ml, 5ml or 10ml Hensen Stempel pipettes and subsamples were placed into a Ward counting wheel PVC (1810-E90) until a minimum of 300 individuals per sample were counted. This subsampling technique was validated by taking multiple aliquots and recording total number of individuals subsampled and their variation among subsamples (e.g. Horwood and Driver, 1976). Estimates indicated a coefficient of variation between 10 and 15% which is considered acceptable for reliable estimations of zooplankton densities (Van Guelpen *et al*. 1982).

Sub-samples were initially sorted into three groups: crustaceans, molluscans and "others". Crustaceans, the most prominent and the target group of this study, were then identified to the lowest possible taxonomic level using reference manuals and taxonomic keys available. Specimens were counted and sorted under a dissecting microscope (Wild M420, Eyepiece 16X/14B, Leica Wild Macrozoom microscope 2.0X) and identified using dissecting and compound microscopes (Leica DMLS). Identifications were then validated by a regional taxonomist. The validity of taxonomic names was verified using the Integrated Taxonomic Information System (http://www.itis.gov). A representative of each taxon for each region was also photographed and sent to the University of Guelph for barcoding as part of a major CAISN project.

3.3.4. Community and statistical analysis

Absolute density estimations (number of zooplankton organisms per volume of water sampled) were not consistent across ports due to inconsistent use of flowmeters to determine volume sampled during the field collection. Consequently, zooplankton community composition was compared using composition of species and their representation (percentage) in the samples rather than using absolute densities. The Bray-Curtis index (Magurran, 1988) was used to assess similarity among zooplankton communities, specifically, among samples and among ports within the region. These comparisons were conducted using clustering and multivariate routines in the software package PRIMER v6. From Bray-Curtis similarity matrices, non-metric multidimensional scaling (nMDS) ordination were performed to visually examine the clustering of samples. Analysis of similarity (ANOSIM) was then used to test whether the differences detected among ports were or not significant at a 5% probability level (Clarke and Warwick, 2001)

3.4. RESULTS

3.4.1. Zooplankton species composition

Crustacean zooplankton communities in the region were numerically dominated by 15 calanoid copepods, 2 cyclopoid copepods, 3 harpacticoid copepods, 4 cladocerans, and larvae of 3 crab species (Table 3.1). Among these taxa, only two NIS were present in the samples: *Carcinus maenas* (European green crab) which was present in Halifax, Bayside and Port Hawkesbury, and *Acartia tonsa* (Copepoda: Calanoida) which was collected in Sept-Iles and one sample of Halifax. Two additional NIS were detected in Sept-Iles samples collected in a separate survey conducted in December: the calanoid harpactacoids *Clymenestella scutellata* and *Tisbe graciloides*), but these are not listed in Table 1as they are not part of the present study due to lack of seasonal data.

Table 3.1. Species composition and occurrence (+ = presence) in the samples collected from each Atlantic Canada port. Names in bold are NIS in one or more port and identified with double asterisks on those ports in which they are NIS. Boldface means NIS.

	Halifax Harbour						Bayside Port					
Atlantic	H1	H2	H3	H4	H5	H6	B1	B2	B3	B4	B5	B6
Acartia hudsonica	+	+	+	+	+	+	+	+	+	+	+	+
Acartia longiremis	+	+		+	+	+						
Acartia spp.	+	+	+	+	+	+		+	+			
Acartia tonsa				+								
Bivalvia veliger												
Calanoida	+	+	+	+	+	+		+	+			
Calanus spp.												
Cancer spp.			+									
Carcinus maenas					++				++	++		
Centropages hamatus	+	+	+	+	+	+		+	+			+
Centropages typicus	+											
Centropages spp.												
Copepodites			+	+	+	+				+		
Eurytemora affinis	+											
Eurytemora americana	+					+	+	+	+			
Eurytemora herdmani	+	+	+	+	+	+	+	+	+	+	+	+
Eurytemora spp.	+	+		+		+	+	+	+	+	+	+
Evadne nordmanni	+	+	+	+	+							
Evadne spp.	+		+	+	+	+						
Evadne tergestina	+	+	+	+	+	+						
Gastropoda veliger						+						+
Harpactacoid												
Microsetella norvegica												
Monstrelloida	+											
Oithona atlantica												
Oithona similis				+								
Oithona spp.												
Pagurus acadianus												
Paracalanus parvus												
Pleopis polyphemoides	+	+	+	+	+	+		+			+	
Podon spp.				+								
Pseudocalanus moultini			+	+								
Pseudocalanus newmani	+	+	+	+	+	+	+	+	+	+		+
Pseudocalanus spp.	+	+	+	+	+	+					+	
Temora longicornis	+	+	+	+	+	+		+		+		+
Tisbidae												
Tortanus discaudatus		+					+	+	+	+	+	+

Table 3.1. Continued from previous page.

	Sept-Isle						Port Hawkesbury					
	S1	S2	S3	S4	S5	S6	P1	P2	P3	P4	P5	P6
Acartia hudsonica	+	+	+	+	+	+	+	+	+	+	+	+
Acartia longiremis			+	+	+	+	+	+	+	+	+	+
Acartia spp.			+		+			+		+		+
Acartia tonsa			++					+	+			+
Bivalvia veliger	+		+	+	+	+					+	+
Calanoida	+	+	+	+	+	+	+	+	+	+	+	+
Calanus spp.											+	
Cancer spp.												
Carcinus maenas									+	+	+	+
Centropages hamatus			+		+	+	+	+	+	+	+	+
Centropages typicus												
Centropages spp.								+			+	
Copepodite	+	+	+	+	+			+	+	+		
Eurytemora affinis											+	
Eurytemora americana												
Eurytemora herdmani								+	+	+	+	+
Eurytemora spp.							+				+	
Evadne nordmanni										+		
Evadne spp.	+	+	+	+	+	+			+			+
Evadne tergestina								+				
Gastropoda veliger						+	+	+	+	+	+	
Harpactacoid		+										
Microsetella norvegica	+		+	+								
Monstrelloida												
Oithona atlantica	+				+		+	+	+	+	+	+
Oithona similis	+	+	+	+	+	+		+	+			+
Oithona spp.	+	+			+							
Pagurus acadianus									+			
Paracalanus parvus			+		+							
Pleopis polyphemoides	+	+						+	+		+	
Podon spp.			+	+	+	+	+					+
Pseudocalanus moultini	+			+	+	+						+
Pseudocalanus newmani	+		+	+	+	+	+	+	+	+	+	+
Pseudocalanus spp.	+		+	+	+	+						+
Temora longicornis	+	+	+	+	+	+	+	+	+	+	+	+
Tisbidae										+		
Tortanus discaudatus							+	+	+		+	+

Table 3.2 summarizes the levels of dissimilarity among the four ports of the region and identifies the three main species contributing to each dissimilarity estimation. The highest dissimilarity was found between Bayside and Sept-Iles (85.37%; see also Fig. 3.2) and was related to three calanoida copepods; *A. hudsonica*, *T. longicornis* and *E. herdmani*. The dissimilarity between Bayside and Port Hawkesbury (64.73%) and between Halifax and Bayside (56.30%) was also driven bycalanoid copepods (*A. hudsonica* and *T. longicornis*) in addition to *Pseudocalanus newmani* and *Temora discaudatus*. Sept-Iles and Port Hawkesbury's (59.76%) dissimilarities were driven by the calanoid copepods *P. newmani* and *Centropages hamatus* and one cladoceran: *Evadne* spp. The lowest levels of dissimilarity were found between Halifax and Sept-Iles (54.75%) and between Halifax and Port Hawkesbury (44.98%) (Fig. 3.2). The main species driving these dissimilarities were the calanoid *E. herdmani* followed by the cyclopoid copepod, *Oithona similis*, and three other calanoid copepods; *A. hudsonica* and *P*. *newmani*, *C. hamatus*.

Dissimilarities among samples were also reflected in the MDS and cluster plots (Figs. 3.3 and 3.4). At a 60% level of similarity, the plots distinguished three individual ports (groups separated as Halifax, Bayside and Port Hawkesbury), and scattered samples from Sept-Iles linked in three different groups (Figs. 3.3 and 3.4). ANOSIM confirmed that differences among ports were all significant (Global R=0.849, ANOSIM p=0.01).

Table 3.2. Summary of pairwise dissimilarity (percentage) levels between ports in the Atlantic region based on SIMPER analyses. The cumulative contribution to dissimilarity of the top three species is also presented.

Region	Average dissimilarity	Species cumulative contribution to dissimilarity (three top species and their %)		
H vs B:	56.30	*A. hudsonica* (15.89)	*T. longicornis* (30.99)	*T. discaudatus* (37.14)
H vs SI:	54.75	*E. herdmani* (13.86)	*O. similis* (21.00)	*A. hudsonica* (28.04)
H vs PH	44.98	E. *herdmani* (11.45)	*P. newmani* (18.81)	*C. hamatus* (24.67)
B vs SI:	85.37	*A. hudsonica* (17.32)	*T. longicornis* (27.69)	*E. herdmani* (36.09)
B vs PH:	64.73	*A. hudsonica* (14.46)	*T. longicornis* (25.74)	*P. newmani* (35.31)
SI vs PH	59.76	*P. newmani* (7.83)	*Evadne* spp. (14.99)	*C. hamatus* (21.55)

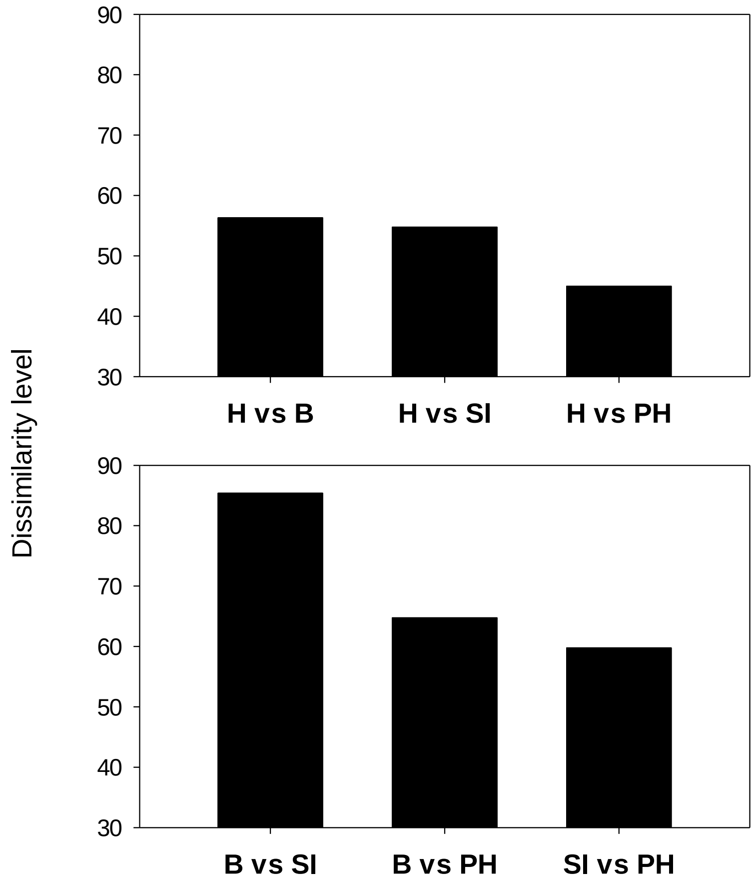

Fig.3. 2. Levels of Bray-Curtis similarity among samples collected within a same port (top), and levels of dissimilarity (percentage) among communities associated to different ports (bottom panels) of the Atlantic Region. H= Halifax, B= Bayside, SI= Sept-Iles, PH= Port Hawkesbury

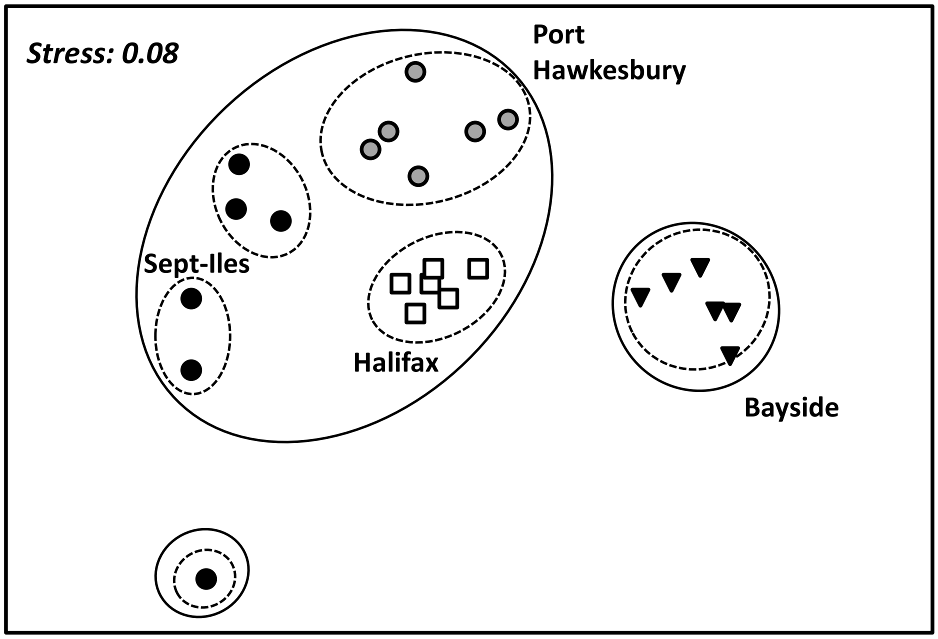

Fig. 3.3. MDS plot based on zooplankton community composition from 24 samples collected from four Atlantic ports: Bayside, Port Hawkesbury, Halifax, and Sept-Iles. Based on Bray-Curtis similarity and square-root transformed data. Continued and dotted lines represent 40% and 60% similarity levels, respectively.

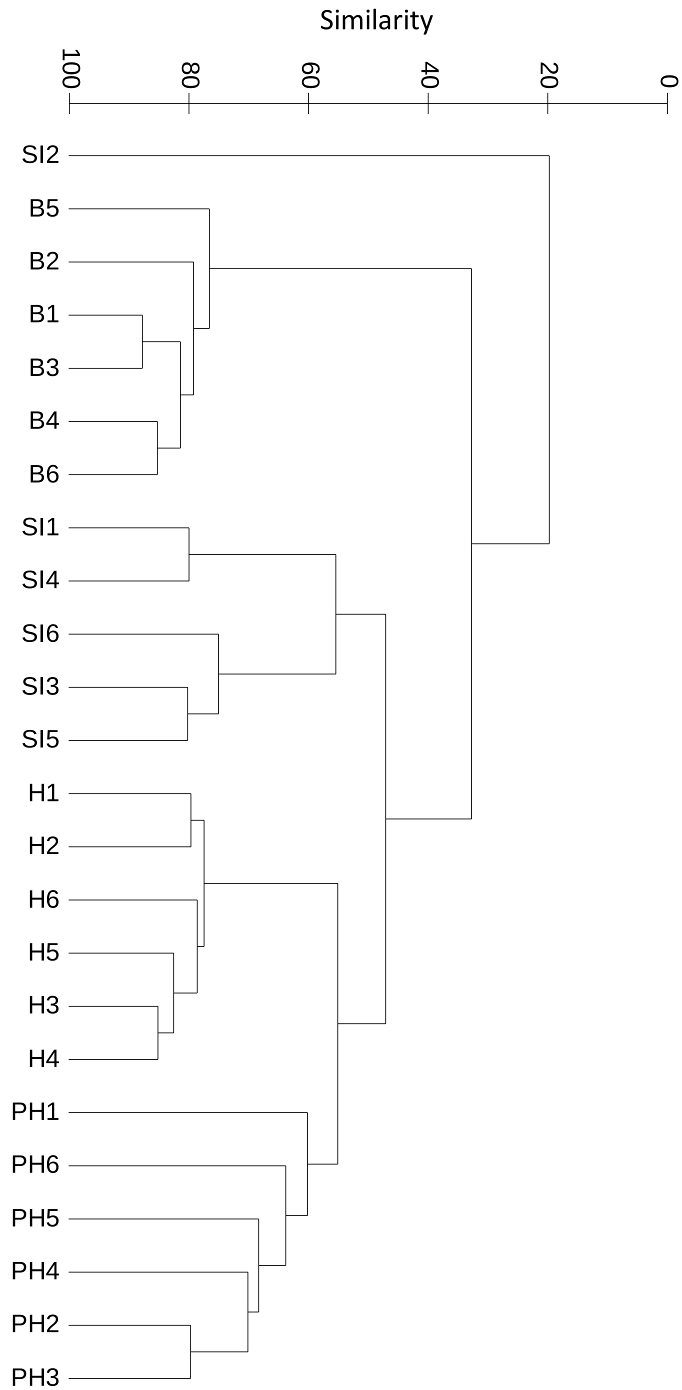

Fig. 3.4. Cluster of zooplankton community composition from 24 samples collected in four Atlantic ports: B: Bayside, SI: Sept-Isle, H: Halifax, and PH: Port Hawkesbury. Based on Bray-Curtis Similarity and square-root transformed data.

3.5. Discussion

A minimum of 22 NIS from a variety of taxonomic groups have been recorded in the Scotian Shelf, and at least seven of these NIS have been shown to generate ecological and economic issues (MacLean *et al*., 2013). The number of NIS collected in this study can be considered very conservative, as many more were expected to be present in the plankton samples. Climate change, increasing shipping traffic, aquaculture activities, recreational boating and various forms of habitat disturbance are all pressing alterations (MacLean *et al*., 2013) that the Scotian Shelf is facing. This is likely leading towards the facilitation of introduced species that continue to inoculate, establish and spread into new environments (MacLean, 2013). In the span of 70 years, the volume and frequency of international vessels has sharply increased and has been correlated with the worldwide growth in the number of NIS (Carlton, 1996). That increase in NIS worldwide was expected to be reflected in the zooplankton communities associated to ports of Atlantic Canada. In addition, given the level of connectivity (water masses structure) and environmental similarity (e.g., one large biogeographic zone) among these ports, their zooplankton communities were expected to be relatively similar. Surprisingly though, the number of NIS collected was relatively low (two species, and two other NIS confirmed from other samples) and communities were distinctively different among ports.

The similarity observed among port communities can be attributed to the contribution of species like the calanoid copepods *Acartia hudsonica* and *Temora longicornis*. This is consistent with a study conducted by Johnson *et al*. (2011) in the Southern Gulf of St. Lawrence, where these authors collected these species in a variety of nearshore environments including bays and estuaries. Copepods often represent the majority of a typical zooplankton sample, including between 50 and 90% of the densities of the organisms collected (Ruiz *et al*., 2000). They are also the dominant taxonomic group in samples collected from ballast waters, which are believed to be prime vectors for the transport of NIS (Carlton, 1996). For instance, DiBacco *et al*. (2011) collected 63 ballast water samples from ships arriving in Atlantic Canada between 2007 and 2009. In these samples, 96 zooplankton taxa were identified and copepods accounted for 89% of the total zooplankton density.

The strong dissimilarity detected among port communities may be explained by several other calanoid copepods, such as *Centropages hamatus*, *Eurytemora herdmani* and *Pseudocalanus newmani*. These biological differences may be dictated by the geographical location of the ports (distance) and therefore to their connectivity and environmental similarities. The ports sampled in this region belong to two distinctive eco-regions: the Nova Scotian Shelf and the Gulf of St. Lawrence (DFO 2009). However, some authors have identified the Bay of Fundy, where the port of Bayside is located, as possibly a third distinctive eco-region characterized by its unusual tidal influence (Kelley and Kelley, 1995). Samples from Bayside were among the most distinctive zooplankton associations detected in this study, clearly separated from all the other zooplankton communities.

Halifax Harbour and Port Hawkesbury, both located on the Atlantic shore of the Scotian Shelf, are the most directly influenced by marine waters and, as expected, were the least dissimilar in terms of zooplankton community composition in the region. These ports receive approximately 1000 and 2000 international ships, respectively, on an annual basis (Kelly, 2004). Most of these ships arrive from the eastern shores of the United States and Western Europe (MacLean *et al*., 2013), which was expected to be reflected in an influx of planktonic NIS from those regions. Port Hawkesbury is the second largest Canadian port with respect to annual tonnage (after Vancouver), due to large volumes of crushed rock, gravel shipments and oil trans-shipments. The Port handled 31.6 million metric tonnes of goods in 2006, and 21.6 million tonnes of crude petroleum (Statistics Canada, 2006). Despite the potential for high NIS propagule pressure in Halifax and Port Hawkesbury, surprisingly only one NIS (*Carcinus maenas*) was detected in these ports. Zooplankton samples from Sept-Iles, from where one NIS was collected (*Acartia tonsa*) and two other have been reported, were particularly intriguing. This port is located on the northern shore of the Gulf of St. Lawrence, at the mouth of the St. Lawrence River (Fennel *et al*., 2009). The deep-waters of the St. Lawrence Estuary have been characterized as hypoxic (Gilbert *et al*., 2005 and 2007) and, perhaps, it represents a transition habitat for plankton communities. When the deep-waters circulate landwards, it is possible that it is reducing the oxygen levels in the upper layers of the water column (Gilbert *et al*., 2005). Although some of the samples from Sept-Iles were relatively similar to the zooplankton collected in Halifax and Port Hawkesbury, other samples were quite different. Again, this could be due to oxygen and salinity levels in the Port of Sept-Iles. Further studies on these communities are clearly required in order to understand the differences detected here.

Bayside had the highest relative density of *Carcinus maenas* larvae in the Atlantic samples, a NIS that was also the most widespread in the samples of this region. This species has successfully colonized many coastal ecosystems across Canada (except the

Arctic) and continues to spread in this and other regions (Klassen & Locke, 2007). The green crab was introduced in 1870 in Long Island (Klassen & Locke, 2007) and the introduction was probably due to the transport of adult crabs in dry ballast and ship hulls. This species underwent successive invasions that resulted in its arrival in the Halifax harbour area by the 1970's (Carlton and Cohen 2003). It was then collected around the coasts of the Gulf of St. Lawrence (northwest NS, eastern NB, PEI, Magdalen Islands) in the 1990's (Blakeslee *et al*. 2010; Klassen and Locke 2007) and in Newfoundland in 2007 (Blakeslee *et al*. 2010). The relatively high abundance of *Carcinus maenas* in Bayside may relate to the fact that this port is affected by relatively low salinities (~15 ppt) due to the tidal influence of and the strong the Bay of Fundy. The species was also in this area since 1951, so has had more time to establish and build up large populations of females able to hatch larvae. An alternative explanation is the pattern of circulation of the area which may facilitate the retention of larvae in the area.

With regards to other relevant zooplankton species, the estuarine copepod *Eurytemora herdmani*, a dominant species in the zooplankton samples of Bayside, prefers turbid and shallow areas. Its maximum abundances in the Bay of Fundy have been found nearby, in Passamaquoddy Bay (Daborn, 1976). Hildebrand (1981) described two distinct zooplankton communities in these waters: an estuarine one consisting mainly of *Acartia hudsonica*, *E. herdmani*, *Centropages hamatus* and meroplanktonic larvae, which was associated to low tide conditions, and a second marine community dominated by *Pseudocalanus* spp. and *Sagitta* spp. associated to high tide conditions. Even though tide conditions could not be considered in this study, these two copepod communities are in general consistent with the composition found in the samples collected in this study.

Temora discaudatus, another calanoid copepod present in the Bayside samples, is usually found in higher salinity waters than *A. hudsonica* and *E. herdmani*, (Johnson *et al*., 2011) but *T. discaudatus* was also equally present in terms of abundance in Halifax and Port Hawkesbury.

This study likely underestimated the number of NIS present in the region: very few were identified in the samples and it is known that many exist in adult forms all along the coastal areas for the Atlantic region. Potential limitations that may explain this issue are similar to some of the ones identified in the previous chapter: First, the inability to conduct simultaneous collection of samples, despite the fact that there is an important seasonal transition between August and September that may account for some of the community differences detected. Second, this study is limited in terms of number of ports, which despite being among the most important in the region, not necessarily encompass the large diversity of zooplankton port communities to be found in Atlantic Canada (samples from ports in Newfoundland and Labrador, for example, have not been considered at all). Third, limited availability of taxonomy expertise remains a challenge for the proper identification of species in this and other regions. Despite these limitations, this study provides the first systematic inventory of zooplankton organisms from port samples in the region, and a preliminary assessment of the contribution of NIS to those communities. Both types of information will be important for future studies addressing the region's zooplankton communities in ports and other high risk areas. Indeed the number of NIS is expected to grow as an increasing number of NIS is expected to arrive and establish in the next decade. For example, the Chinese mitten crab is a recently introduced species that remains for the moment restricted to the waters of the St.

Lawrence River (Veilleux and de Lafontaine, 2007). Using a mathematical model focusing on the habitat requirements where this species thrives, Herborg *et al*. (2007) predicted that the entire Atlantic region is a suitable habitat for its establishment. This species has been named one of the worst invaders due to its ability to rapidly grow and cause negative impacts on new host environments (Veilleux and de Lafontaine, 2007). Its larvae are likely going to be identified as part of the zooplankton communities surrounding ports and coastal areas of the region in the near future.

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Chapter 4: Summary of results and conclusions

The two main objectives of this thesis were to provide an inventory of the zooplankton (crustaceans) composition associated with ports, and to document the presence of NIS and their potential contribution to these communities. The study of communities associated with ports was deemed important because these areas are considered zones of high risk of invasion (Ruiz *et al*. 2000) and relatively high numbers of NIS were expected to be collected. Ports have been recognized as points of entrance for new invasions, from where subsequent range expansions towards other coastal areas can take place or further new invasions can be initiated. During the first phase of CAISN (2006-2011), this research network conducted an assessment of NIS diversity in ports from different Canadian coasts. Their preliminary results suggested that NIS diversity was likely related to the number of ships that visited a port but not necessarily to the volume of water de-ballasted. Those results are generally consistent with the ones presented in Chapter 2, even though the highest diversity of NIS was found in the areas of Vancouver and Yarmouth, two port areas that were not considered in this study.

Chapter 2 encompassed zooplankton communities associated with 16 ports, four from each Canadian coastal region: Pacific, Atlantic, and Arctic Oceans and the Great Lakes. Spatially, the composition and relative abundance of taxa in these zooplankton communities were drastically different among regions. Zooplankton communities in the Great Lakes were significantly different from those of marine regions, which was expected, since there is limited overlap in species composition between marine and freshwater bodies. The Great Lakes communities were heavily dominated by cladocerans, particularly *Bosmina* sp. and *Daphnia* sp. The number of NIS detected in this study was relatively low considering the number of NIS known to exist in the geographic locations in which the sampled ports are located. A total of 185 NIS have been documented in the Great Lakes (Ricciardi, 2011) but only two NIS and possibly two more were present in the plankton samples collected from those ports. Arctic ports, as expected, had a lower number of NIS and an overall lower number of zooplankton taxa than all other regions. The increasing opening of new transportation routes in the Arctic, as the result of ice melting and global warming conditions is expected to facilitate the arrival and establishment of an increasing number of NIS. Regardless of region, the number of NIS collected and identified in these chapters' under-represents the actual number of NIS. Other studies have accounted already for greater number of NIS (Ricciardi, 2011 and Locke and Therriault, unpublished data).

Although incomplete, some published evidence explains the routes and mechanisms used by some NIS to invade these regions. For instance, *Paracalanus parvus* and *Corycaeus anglicus*, two NIS with established populations in the Strait of Georgia are known to have travelled up with the California current from their original distribution ranges (M. Galbraith, pers. comm.). Similarly, *Microsetella norvegica* and *Centropages abdominalis*, two established NIS in the Pacific region are known to have been introduced via ballast water (M. Galbraith, pers. comm.). A similar mechanism (ballast water) was the most likely responsible for the original invasion of *Carcinus maenas* to the Atlantic region (Blakeslee *et al*., 2010), and the invasion of the Great Lakes by *Cercopagis pengoi* (MacIsaac *et al*., 1999). Interestingly, *Acartia hudsonica* was collected by this study for the first time in two of the four ports in the Arctic, Deception

Bay and Churchill. There is no clear evidence to explain how that species arrived to those waters but since *A. hudsonica* is holoplanktonic, ballast water is a possible mechanism.

Considering the wide geographic variation among ports and the availability of published records on shipping rates and ballast water volumes per port, Chapter 2 also assessed if these two variables (shipping and ballast water) were or not statistically related to the number and relative abundance of NIS. Shipping rates and ballast water volumes were used as surrogates of propagule pressure, known to influence the number of NIS present in a given area. The number of NIS was significantly associated with shipping rates and positively but non-significantly associated with ballast waters. Although these conclusions are supported by several significant regressions, the level of explanation of these analyses was in most cases only modest. This is probably due to the limited number of ports considered in the analysis, and the large variation observed in zooplankton communities. Future studies should consider a higher number of port zooplankton communities and possibly more recent records of shipping rates or ballast water per port. This will help to confirm the validity of these positive relationships because shipping rates have significantly increased in the last decade.

Chapter 3 focused on port zooplankton communities of a single region (Atlantic Canada) but provided results from replicated samples conducted at each individual port. As in Chapter 2, an inventory of zooplankton species associated with ports was documented, including the presence of confirmed NIS. Again, the number of NIS (two in addition to two other species collected separately) underrepresented the actual number of NIS known for this region (82, Locke and Therriault, unpublished data.). The results of Chapter 3 also suggest that there is a considerable level of dissimilarity among zooplankton communities associated with individual ports, particularly among those located in distinct ecoregions of the Atlantic. In fact, ports located directly adjacent to the Scotian Shelf (Halifax and Port Hawkesbury) were the most similar in terms of species composition and relative abundance. As expected, communities of ports in the Gulf of St. Lawrence (Sept-Iles) and the Bay of Fundy (Bayside) were considerably more dissimilar. This was likely due to differences in salinity or oceanographic features such as the large tidal amplitude in the Bay of Fundy.

These oceanographic differences are likely to play a role in determining the number NIS that became established in these areas. Wolff (1999) has shown that the percentage of NIS species in waters of low salinity is higher than that in water with salinities higher than 20 psu. This author suggests that this is likely due to the reduced level of native diversity present in these brackish waters, which may facilitate the establishment of NIS with opportunistic life history traits. Similarly, Moyle and Light (1996) have suggested that coastal ecosystems highly influenced by anthropogenic activities (for instance, heavy port development) do not resist invasions as well as areas of lower levels of disturbance.

Differences detected among zooplankton communities associated with ports of the Atlantic coast suggest that NIS management protocols may not necessarily work in the same way for an entire region (or for different regions). Further analyses and predictive models should be developed in order to understand the complexities associated with the invasion of multiple sites per region. For instance, it is well known that one determinant of invasion success is the receiving habitat and community (Ricciardi and Atkinson 2004; Strauss *et al*. 2006; Colautti *et al*. 2006). Thus, even when invasion success may be governed by dispersal opportunity and propagule pressure (Smith *et al.*, 1999; Kolar and Lodge, 2001), it is evident that these are not the only factors driving the number and abundance of NIS in a given area.

The results of this thesis are part of a major CAISN initiative to document and understand the diversity of port zooplankton communities. CAISN focuses on four main themes, all linking basic research with an applied aspect of NIS: early detection, rapid response, NIS as multiple stressors, and reduction of uncertainty in prediction and management. This thesis provides critical information that relates primarily to the first theme, and its results, obtained using traditional taxonomy, complement the efforts conducted by other research teams with the use of molecular identification techniques. More specifically, this thesis falls into the “Surveillance for AIS Throughout Canada's Coastal Waters” project, which aims to compare the reliability of molecular detection with traditional taxonomy. Once both types of studies are complete (traditional taxonomy and barcoding) CAISN aims to develop pyrosequencing techniques that would facilitate rapid detection of NIS and determine which locations and which technologies are best suited for future monitoring studies. One of CAISN’s goals is to achieve a complete AIS database which will provide references for future changes in policy, vector activities and how the environment should be assessed

Based on the methodological limitations identified in Chapter 2 and 3, future studies focusing on traditional plankton identification should consider at least two general recommendations. First, a higher number of ports per region and a higher level of replication should be considered strong priorities. Zooplankton communities are known to exhibit high degrees of patchiness, both spatially and temporally (Sorochan and Quijon

2014). Hence, increased replication and increased number of locations (ports) is necessary in order to make the inventories presented here more comprehensive. Second, a rigorous selection of the conditions in which samples are collected is essential in order to make inventories comparable and more informative. Short term variation associated with tidal cycles, for example, is likely to have a strong effect on planktonic composition and abundance. Future zooplankton studies should be fully standardized in order to reduce the subsequent variation in data. This will facilitate the comparison of communities and the measurement of the actual contribution of new (upcoming) NIS to be detected in the plankton.

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5. Glossary

Abiotic Factor	Non-living physical and chemical factors in the environment
Aquatic Invasive Species	Species living outside their native range that cause ecological and economical damage
Ballast Water	Water pumped into ballast tanks for the ships balance
Bathymetry	Measurement of depth of water
Biodiversity	Diversity of living things in a specific habitat or ecosystem
Biofouling	Fouling of pipes and underwater infrastructure by aquatic organisms
Biogeography	Geographical distribution of plants and animals
Biological Invasion	The colonization of new locations by non-indigenous species
Biotic Factor	Living things that shape an ecosystem
Cryptogenic Species	Species of unknown origin
Ecological Integrity	Overall health of an ecosystem
Evenness	Quantifies how equal a community is in an given area
Holoplankton	Organisms that are planktonic throughout their entire life cycle
Hydrography	The science of surveying and charting bodies of water
Meroplankton	Organisms that are planktonic for part of their life cycle
Native Species	Species native to their natural habitat
Neustonic	Living on surface or just below water level

Non-Indigenous Species	Species living outside their native range
Ontogeny	Origin and development of an individual organism
Propagule	Section of an organism that can fully reproduce asexually
Popagule Pressure	Number of individual species released into a new environment
Species Richness	Number of different species represented in a given area
Zooplankton	Microscopic organisms living throughout the water column

6. Taxonomy References

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