THE ROLE OF RECREATIONAL BOATING IN THE INTRODUCTION AND SPREAD OF MARINE INVASIVE SPECIES

by

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Abstract

Introduction and spread of non-indigenous species is a significant threat to the preservation of global biodiversity. Human-mediated vectors are responsible for transporting potentially invasive species to new habitats throughout the world. This research investigates the role of recreational boating movements as a vector for introduction and spread of invasive species.

A baseline survey of subtidal fouling communities was conducted using artificial substrates in marinas of the southern Strait of Georgia, British Columbia. There was both a high presence of non-indigenous species and high non-indigenous species fouling cover in some marinas, indicating a likely negative impact on native communities. A dive survey which examined the species present on the underwater surfaces of recreational boats in marinas throughout British Columbia showed that more than two-thirds of boats examined had macrofouling present and one-quarter had one or more known non-indigenous species. In combination, a boater questionnaire was used to describe the movements and behaviours of the boaters themselves and behaviour patterns indicated a risk of non-indigenous species transport. The results of the dive survey and boater questionnaire were then used to develop a model that predicts the presence of fouling on boats based on three variables (age of antifouling paint, time in water and incidence of long trips).

The biomechanical properties of non-indigenous species were compared to native species and non-indigenous species had both stronger attachment and lower drag than similar native species, indicating they have the ability to remain attached to fast-moving marine vessels. Finally, a statistical analysis was conducted comparing environmental, demographic and vector variables in explaining the spatial distribution of non-native species. The results showed that recreational boating played a stronger role in the distribution of subtidal non-native species than the original introduction vectors, aquaculture and shipping. This body of research demonstrates that recreational boating is a significant vector for the introduction and spread of invasive species in this region and around the world. It is the first comprehensive study of the recreational boating vector in Canadian marine waters and the results have important implications for the prevention of new introductions and the preservation of biodiversity.

Preface

This is a statement of co-authorship, copyright and ethics

Co-authorship

Identification and design of research

I designed the settlement plate experiment with Glen Jamieson and Thomas Therriault. The boat survey and questionnaire research was designed in collaboration with Evgeny Pakhomov and Thomas Therriault. The biomechanics research (Chapter 5) was largely designed by Patrick Martone and myself, with assistance from Thomas Therriault. The drag experiment was conducted using the laboratory equipment and facilities of Patrick Martone. I designed the research in Chapter 4 and Chapter 6, consulting with Kai Chan and Thomas Therriault on the choice of statistical techniques.

Performing the research

I performed all of the research conducted for Chapters 2, 3, 4 and 5. The data for Chapter 6 was a combination of data collected during the settlement plate experiment (Chapter 2) and an expanded version covering more of the British Columbia coast conducted by Heidi Gartner as part of her master's thesis research at University of Victoria (Gartner 2010). I performed sample processing and species identifications for the settlement experiment with a significant amount of help from co-op students Heidi Gartner and Lindsay Orr and research assistants Jessica Yu, Angela Stevenson, Francis Choi and Trampus Goodman. I conducted the dive surveys as part of a SCUBA dive team. Underwater photographs were taken under my direction by Nicole Backe, Brandon Hill and Angela Stevenson. Dockside fouling assessments were performed by Jessica Yu, Megan Mach, Nicole Backe and Brandon Hill.

Analysing the data

I analysed all the data obtained for the five research chapters under the guidance of Thomas Therriault, Evgeny Pakhomov, Kai Chan and Christopher Harley.

Manuscript preparation

I wrote all seven chapters of the manuscript. All chapters were edited by Evgeny Pakhomov, Thomas Therriault, Kai Chan and Christopher Harley. Chapter 3 was published in the journal Diversity and Distributions in October 2011 and co-authored by Evgeny Pakhomov and Thomas Therriault. The citation for this paper is "Clarke Murray, C.; Pakhomov, E.A.; Therriault, T.W. (2011) Recreational boating: a large unregulated vector transporting marine invasive species. Diversity and Distributions 17 (6): 1161-1172". Chapter 4 has been submitted to a journal with coauthors Thomas Therriault and Evgeny Pakhomov. Chapter 5 has been published in the journal Biological Invasions with coauthors Thomas Therriault and Patrick Martone. The citation for this paper is "Clarke Murray, C.; Therriault, T.W.; Martone, P. (2012) Adapted for invasion? Evaluating attachment strength, drag and dislodgment for marine non-indigenous hull fouling species. Biological Invasions. 22 pp". Therefore anonymous reviewers and the journal editors also contributed to the editing of these manuscripts.

Copyright
I certify that I have obtained a written permission from the copyright owner(s) to include the above published materials in my dissertation.

Ethics
The boater questionnaire and marina owner/operator questionnaire were conducted under the regulations of UBC's Behavioural Research Ethics Board (BREB). Approval of the two questionnaires was granted in 2008 (Approval # H08-00967).

Table of Contents

List of Tables

List of Figures

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I would like to thank my family and friends for their endless support throughout my education. Finally, I could not have completed this without the understanding and love of my husband and sons throughout this entire endeavour.

Dedication

To my parents, Carol and Andrew,
for placing me on this path

and to my husband, Greg,
for sharing it with me

Chapter 1: Introduction to dissertation

1.1 Invasive Species

Biological invasions are a threat to biodiversity, ecosystem function, resource availability, human health, and economic growth (Carlton 1985; 2001; Elton 1958; Ruiz et al 2000; Sala 2000). The rate of invasions is escalating globally (Carlton 1999; Hewitt 2003; Ruiz et al 2000) and this trend seems to hold in Canada (Chapman *et al.* 2002; Claudi *et al.* 2002; de Lafontaine and Costan 2002; Levings *et al.* 2002). Claudi *et al.* (2002) estimated that 25% of Canadian plant species are non-indigenous, over 163 aquatic species were recorded as introduced to the Great Lakes (de Lafontaine and Costan 2002), over 120 species in estuarine and marine waters of British Columbia (Levings *et al.* 2002) and 17 species have been introduced to Atlantic Canada (Chapman *et al.* 2002). In the marine environment, many invasive species[1] are tolerant to a variety of environmental conditions, including temperature, salinity, and exposure (Darbyson *et al.* 2009a; Epelbaum *et al.* 2009) making much of Canada's marine habitat at risk of invasion (Herborg *et al.* 2008; Locke *et al.* 2007; Therriault and Herborg 2008).

A biotic invasion can be partitioned into a series of successive component stages (e.g., Carlton 1985; Lockwood *et al.* 2005; Mack *et al.* 2000) depicted in Figure 1. The invasion process begins with the engagement of propagules with a transport vector in a source location, for example planktonic organisms drawn into ballast water tanks or larval settlement on hulls. Those species which engage with the transport vector must then survive transport to a recipient location outside their native range. Upon arrival, propagules are released into the introduced habitat and may then become successfully established at the recipient location. Finally, population increase and natural spread to nearby locations may occur. Some introduced species have characteristics that cause impacts in the invaded range such as: excluding native species, occupying and/or

[1] There is much confusion surrounding the vocabulary of invasion (see Colautti & MacIsaac 2004 for review). For the purposes of this dissertation non-indigenous species (NIS) are those introduced by humans to an ecosystem where they did not historically evolve. Invasive species are a subset of non-indigenous species (NIS) that cause harm to ecology, economy or human health in their new location.

dominating habitat, affecting industrial activities or infrastructure, among others (Grosholz and Ruiz 1996; Johnson and Padilla 1996; Kado 2003; Parker *et al.* 1999; Pimm 1989; Ruiz 1999). It is these species which are referred to as "invasive". These stages of the invasion process are considered selective filters because the entire suite of species present at each stage will not have the ability to move on to the next stage of invasion (Fig. 1). Thus, only a small fraction of available species successfully navigate the entire process to become invasive.

In addition to primary introductions, secondary spread from the new invaded location and range expansion are additional concerns in invasion studies (Fig. 1). Spread within the introduced region can occur naturally through dispersal, for example, in marine environments spread can occur either through adult movement and migration or larval dispersal by drift. In most cases, little can be done to prevent natural dispersal following an introduction event but knowledge about a species' dispersal characteristics can serve to inform management decisions. For example, the length of larval period, potential spatial extent of dispersal, and timing of reproduction must all be taken into account when planning monitoring, mitigation, and control efforts for any non-indigenous species (NIS) of concern. The club tunicate, *Styela clava* (Herdman, 1881), has a relatively short larval duration and therefore limited ability to disperse naturally (Clarke and Therriault 2007) compared to the European green crab (*Carcinus maenas* Linnaeus, 1758), which has a long larval duration and hence the ability to disperse widely with ocean currents (Grosholz and Ruiz 1995). Spread also can occur through human-mediated vectors, either the original primary introduction vector or any number of additional secondary dispersal vectors. The relative importance of natural dispersal versus human vector transport will vary by species and area of introduction.

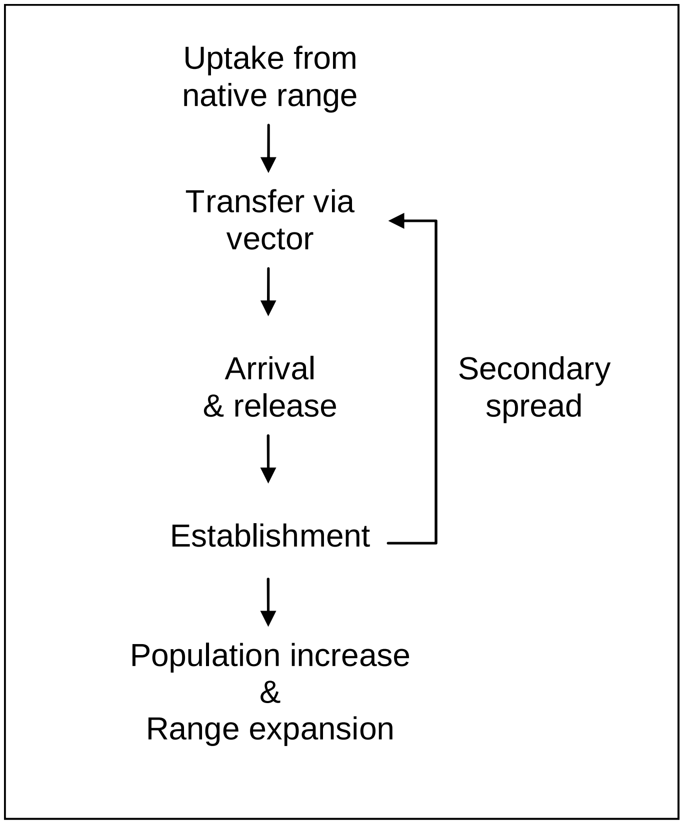

Figure 1: Successive stages of the invasion pathway, adapted from Lockwood *et al.* (2005).

1.2 Pathways and Vectors

There are many pathways that transport marine species outside their native ranges. These include commercial shipping, aquaculture activities, recreational boating, live animal and aquaria trade, research and teaching activities, and nursery and algal trade. Means by which species are transported within a pathway are called vectors. For example, vectors within commercial shipping include ballast water, hull fouling, and sea chest fouling.

The importance of individual vectors in the introduction of NIS has been subject to shifts over time that mirrors regional and global changes in human activities, notably transportation. The earliest known example of marine introduction is probably the shipworm, *Teredo navalis* (Linnaeus, 1758), a wood-boring bivalve with a cosmopolitan distribution. This species bores into the hulls of wooden ships and is subsequently transported throughout the world. Its transport has been so pervasive that it is difficult to identify its native range (Hoppe 2002). Dry ballast also was a historical introduction vector rarely seen in modern times with terrestrial and aquatic species associated with the movement of sand, cobble and rocks to new locations (Brawley *et al.* 2009; Fofonoff *et al.* 2003).

Improvements in technology, such as the advent of steel hulled vessels and steam engines allowing the shift from dry ballast to water ballast, development of protective chemical antifouling paints and ballast water management/treatment, may have decreased the relative importance of some vectors (Carlton 1985; Hewitt 2003; Hewitt *et al.* 2004; Ruiz *et al.* 2000). The International Maritime Organization's (IMO) ban of the highly effective and yet extremely harmful and toxic organotin compounds (commonly known as TBT) antifouling paints was adopted in 2001 (International Maritime Organization 2010). Following this ban there has been an increase in hull fouling which recently has potentially caused a resurgence of the relative importance of this vector in NIS transport (Fofonoff *et al.* 2003; Minchin and Gollasch 2003).

The implementation of guidelines and regulations limiting invasive species transport also has changed the relative importance of marine vectors. Policies such as the ICES Voluntary Code of Practice on the Introductions and Transfers of Marine Organisms in the 1970s reduced the number of species introduced intentionally for aquaculture or other purposes (International Council for the Exploration of the Sea (ICES) 2005). Today new introductions to Canada must undergo rigorous evaluation under the National Code on Introductions and Transfers of Organisms (Fisheries & Oceans Canada 2003). The IMO implemented mid-ocean exchange guidelines to reduce introductions by ballast water (International Maritime Organization 2006), however, the effectiveness of this measure has been questioned. Both the level of compliance by vessels and the effectiveness of this method for reducing the presence and density of coastal organisms remain unclear (Carlton 1985). In 2004, IMO adopted the International Convention for the Control and Management of Ships' Ballast Water and Sediments. This treaty, once it comes into force, will require the implementation of a ballast water management program for each ship in order to ensure that the vessel meets specified standards of ballast water management – either complies with open ocean ballast water exchange or meets discharge standards related to the density of organisms in ballast water (International Maritime Organization 2004).

Changes over time within a vector also affect its ability to transport NIS. Increases in shipping speed, which reduce voyage speed and increase the probability of survival of NIS during transport, have increased the probability of species transport both within ships' ballast and on the hull (Fofonoff *et al.* 2003). In addition, worldwide shipping traffic is escalating in volume so that the probability of species transport associated with this vector also is increasing (Levine and D'Antonio 2003). Today, the most important marine vectors responsible for species introductions are considered to be ballast water and hull fouling (Fofonoff *et al.* 2003; Fofonoff *et al.* 2003; Gollasch 2002; Hewitt 2002, 2003; Hewitt et al 1999, 2004, 2009; Minchin & Gollasch 2003; Ruiz *et al.* 2000).

The current work focuses on the uptake and vector transport stage of invasions. Vector transport occurs at two stages in the invasion process; primary introduction from the native range and secondary spread within the invaded range.

1.3 The Hull Fouling Vector

Hull fouling is the world's oldest vector of marine species introductions. Beginning in the 10th century, wooden ships began exploring the oceans with no protection from colonization by hull fouling organisms (Carlton and Hodder 1995; Hewitt *et al.* 2004). In fact, species with cosmopolitan distributions today may have been early invaders with undetected introductions (Hoppe 2002). Hull fouling has become more prominent in invasion research in the last few decades (reviewed in Godwin 2003). With the advent of modern marine transportation this vector rapidly has increased the magnitude and spatial scale of worldwide introductions.

1.3.1 Commercial Vessels

Hull fouling of commercial ships is recognized as an important vector for introduced species worldwide: including Canada (Drake and Lodge 2007), New Zealand (Coutts and Taylor 2004), Hawaii (Godwin 2003), the North Sea (Gollasch 2002), and even Antarctica (Lewis *et al.* 2004). Together with ballast water introductions, shipping is considered the most important pathway for NIS worldwide (Fofonoff *et al.* 2003). However, in contrast to ballast water, hull fouling remains largely unregulated. For example, in New Zealand, pending border clearance regulations will include a requirement for all merchant ships to meet a minimum hull cleanliness standard (MAF Biosecurity New Zealand 2011). Similarly, in the Port of Vancouver, British Columbia (BC) in-water cleaning is prohibited both to reduce contamination of marine waters by toxic chemicals and prevent release of potential invasive species (D. Moore, pers. comm. Facilities Manager, Port of Vancouver, Vancouver, Canada).

There is variability in the degree of hull fouling on individual ships, among commercial ship types and activity levels (e.g., Coutts and Taylor 2004). Hull fouling levels can range from 0% to close to 100% (e.g., Davidson *et al.* 2006; Sylvester *et al.* 2011). Sheltered areas (niche areas) of the hull are more likely to be colonised by fouling organisms; these areas have altered water flow in addition to inadequately applied or missing antifouling paint (Rainer 1995; Godwin 2003). In New Zealand, dry dock strips and sea chest grates had the highest levels of fouling (Coutts 1999). Vessels undertaking shorter distance trips had higher fouling levels than those on long distance routes (Godwin 2003).

The type of ship is an important indicator of invasion potential. The worst offenders are slow-moving vessels that spend long periods moored in one place between trips (Davidson *et al.* 2008). Towed obsolete vessels retain much of their hull fouling communities after transit and therefore have large potential to transfer non-indigenous species (Davidson *et al.* 2008). Slow-moving barges and towed dry docks have been implicated in a number of introductions worldwide. For example, fouling on a single floating dry dock towed from San Diego to Barber's Point Harbour, Hawaii in 1999 was the source for a species of non-indigenous macroalga, *Dictyota flabellata*, that was documented to survive and become established in a discrete area of this harbour (Godwin 2003). Also in Hawaii, *Chthamalus proteus* (Dando and Southward, 1980) most likely was introduced as larvae from adult barnacles on a vessel hull (Southward *et al.* 1998). The bivalve *Chama macerophylla* (Gmelin, 1791) and the sponge, *Gelliodes fibrosa* (Dendy, 1905), were found on the hull of a floating dry dock in 1992 that was brought to Pearl Harbor from the Philippines (DeFelice 1999 IN Godwin 2003). Both species were first recorded in 1996 (Godwin 2003). Finally, Apte *et al.* (2000) recorded the introduction of the blue mussel *Mytilus galloprovincialis* (Lamarck, 1819) from adults on the hull of the USS Missouri, towed from Washington to Pearl Harbor where settled juveniles were recorded three months later.

1.3.2 Recreational Vessels

1.3.2.1 Source of Primary Introductions and Secondary Spread

Smaller recreational vessels have the potential to act as primary vectors as well as facilitate secondary spread. Secondary vectors rarely have been investigated and are poorly understood. Fouling species settle and grow on all submerged surfaces of boats and also can become entangled in the propeller, propeller shaft, anchor, and fishing gear (Minchin *et al.* 2006). Similar to commercial ships, species transport by these vessels depends on the frequency and type of cleaning and the type, the duration and speed of travel, and the voyage history of the vessel. Species introduced and spread by the recreational boating vector come from diverse taxonomic groups, and include bivalves, bryozoans, ascidians, and algae.

Non-indigenous mussel introductions are notoriously harmful outside of their native range. Three well-documented examples include the black-striped mussel, *Mytilopsis sallei* Récluz, 1849 (Field 1999; Willan *et al.* 2000), green mussel *Perna viridis* Linnaeus, 1758 (Power *et al.* 2004), and zebra mussel, *Dreissena polymorpha* Pallas, 1771 (Buchan and Padilla 1999; Johnson *et al.* 2001; Padilla *et al.* 1996). The primary vector for the Australian invasion of black-striped mussel to Darwin harbour was believed to be an international yacht. Green mussel invasions have been attributed to recreational vessel movements in the southern east coast of the United States. Zebra mussels were thought to have been originally introduced to the Great Lakes by ballast water but are now spreading throughout North America via trailered recreational boats (Johnson *et al.* 2001; Padilla *et al.* 1996).

Algae introduced by recreational vessels include the marine macroalga *Undaria pinnatifida* Suringar, 1873 (Farrell and Fletcher 2006; Hay 1990) and *Codium fragile fragile* Hariot, 1889 (Bird *et al.* 1993). *Undaria pinnatifida* (Japanese kelp) was first observed in New Zealand in 1987 and is believed to have been introduced by fishing vessels from Asia. It has since spread to at least 15 other ports and harbours (Floerl and Inglis 2003). This species was detected on the hulls of 25% of the recreational vessels moored in a harbour in Wellington and its regional spread is attributed to the movement of vessels after periods of inactivity (Hay 1990). *Codium fragile* spp. *fragile* has been introduced to the Northeast Atlantic, Mediterranean, New Zealand and east coast of North America from its native range in Southeast Asia (Carlton and Scanlon 1985; Chapman 1999; Trowbridge 1995). Its introduction to Nova Scotia, Canada was attributed to an overseas yacht (Bird *et al.* 1993).

The bryozoans *Watersipora subtorquata* (d'Orbigny, 1852) and *Bugula neritina* (Linnaeus, 1758) are cosmopolitan invaders and well known hull fouling species (Floerl and Inglis 2005). They have a known tolerance to antifouling paint chemicals that allows them to facilitate the transport of other invasive species. Intolerant species grow on top of *W. subtorquata* that has settled on chemically protected hulls and are subsequently transported on surfaces unavailable to them without the assisting bryozoan (Floerl *et al.* 2004).

A number of invasive ascidian species introductions have been linked to recreational hull fouling (Lambert and Lambert 1998; Lutzen 1999). Ascidians are of particular interest because their short larval duration (< 24 hours) indicates that ballast water is not a likely vector (Lambert 2001). Instead, aquaculture product and equipment transfer and/or small craft hull fouling are the most probable vectors of both primary introduction and secondary spread. In Prince Edward Island, the primary introduction of solitary ascidian *Styela clava* was likely a slow moving barge but secondary spread has been attributed to both aquaculture transfers and hull fouling (Locke *et al.* 2007).

In many cases it can be difficult to determine which vector is responsible for an introduction event. There are countless additional examples where recreational vessels may have been responsible for introductions; however, it can be difficult to rule out other vectors. Recreational hull fouling has been inferred as a vector in places where there is no international shipping activity nearby (Wasson *et al.* 2001). In some cases, the species could have been introduced by either recreational hull fouling or aquaculture practices (Levings *et al.* 2002; Ruiz *et al.* 2011). Examples of introductions definitively attributed to the recreational boating vector are rare.

1.3.2.2 Marinas as Safe Harbours for Invaders

Marinas are ideal ports of entry for introduced species. The same properties that make marina and port sites safe for boats encourage settlement and establishment of non-indigenous species. In fact, ports and marinas are some of the most invaded marine areas in the world (Lambert and Lambert 1998) as a result of increased propagule pressure and altered environmental conditions. Local hydrologic and environmental conditions are altered to provide refuge for boats but in turn create favourable conditions for arriving non-indigenous species. Water velocities within marinas are considerably reduced compared to surrounding coastal water (Floerl 2003). There is an abundance and diversity of vertical and horizontal settlement substrates including pilings, docks, and breakwater walls; in addition to the boat hulls present with various stages of antifouling protection (Glasby *et al.* 2007). Much of the artificial habitat provided is floating and therefore protected from exposure that affects species in rocky intertidal habitats or predators common in benthic habitats. Artificial habitats and floating structures in particular, have been found to promote the dominance of NIS over native ones (Dafforn *et al.* 2009; Glasby *et al.* 2007). Supplementary artificial substrates increase the available habitat well beyond that in the local natural system. The continual renewal of antifouling paint and addition of ropes, equipment and boats to marinas provides a supply of new, unoccupied space, creating opportunities for colonisation in the absence of climax community structure that develops over time (Neves *et al.* 2007).

Fouling species growing on marina structures and resident boats have the potential to transfer propagules to visiting boats for subsequent spread to additional sites (Floerl and Inglis 2001). In northern Australia, Floerl and Inglis (2005) compared the species assemblages fouling boats with those within marinas. There was a positive relationship between fouling communities present on the boats and that in the marina but this relationship varied both with the age of the antifouling paint and the length of residency in that marina.

Once a NIS population is established within a port or marina it can then act as a secondary source for transport to uninfected sites. This type of secondary spread has been called stepping-stone introductions or hub-and-spoke introductions (Apte *et al.* 2000; Floerl *et al.* 2008). Coastal marinas are connected to each other to varying degrees by boating traffic. Boater movements used as a proxy for the probability of spread indicate a significant potential for recreational boaters to act as vectors in the southern Gulf of St Lawrence (Darbyson *et al.* 2009a; Darbyson *et al.* 2009b), Australia (Floerl and Inglis 2005), and New Zealand (James & Hayden 2000 IN Dodgshun *et al.* 2007; Floerl *et al.* 2005a).

1.3.2.3 Few Published Marine Studies

There have been only a few published studies on the recreational boat vector and many focus on freshwater trailered boating. Those studies that have been conducted sought to determine if the transport of invasive species by small boats is a significant threat. As a result, macroalgal transport by recreational boating was concluded to be an insignificant risk in France and Spain (Mineur *et al.* 2008). Trailered boats have been shown to transport zebra mussels and species of plants and algae (Johnson *et al.* 2001). Club tunicate (*S. clava*) can survive atmospheric exposure for at least 48 hours (Darbyson *et al.* 2009a), which means that they have the potential to endure extensive overland transport to new, uninfested waters. From a survey of recreational boater movements in eastern Canada, Darbyson and colleagues (2009b) estimated that recreational boats have the potential to spread invasive species, such as club tunicate and European green crab. Examination of the participants' boats during the same study failed to detect any invasive species, although sample size was low (Darbyson *et al.* 2009b).

The majority of studies of marine boaters have been limited by relatively low sample sizes (Ashton *et al.* 2006; Darbyson *et al.* 2009b; Mineur *et al.* 2008) and results can be difficult to compare because of variable sampling methods. For example, boat fouling levels have been evaluated visually from the dock only (Ashton *et al.* 2006), via video transect on pole-cams (Davidson *et al.* 2010; Floerl *et al.* 2005a), or were examined during snorkelling (Mineur *et al.* 2008). Despite differing methodology, there were consistent trends in their findings. Recreational boats showed high variability in the degree of hull fouling present (Ashton *et al.* 2006; James & Hayden 2000 IN Dodgshun *et al.* 2007; Floerl and Inglis 2005). Age of antifouling paint was consistently proposed to be the best predictor of the presence and extent of hull fouling (Floerl *et al.* 2005b; Floerl *et al.* 2008; Mineur *et al.* 2008). It also was found that hull fouling organisms have varying tolerances to antifouling compounds (Dafforn *et al.* 2008; Piola and Johnston 2009). Some species were able to colonise painted surfaces as early as three months post-application, with most appearing after seven months (Floerl and Inglis 2005). Results indicated that the effectiveness of modern antifouling paints may not decrease linearly over time (Christie and Dalley 1987).

Variations in boat travel behaviours also can affect the extent of hull fouling present and the probability of NIS transport. The boats found within a single marina can be divided into two categories. Resident boats are those paying for permanent moorage at a marina for at least part of the year. Many resident boats remain in the home marina or only take small day trips within the local area. Transient boats include boats visiting a marina from elsewhere. There are important differences between resident and transient boats (Darbyson *et al.* 2009b; Floerl and Inglis 2005). Although the former often have the highest amount of hull fouling (Floerl and Inglis 2005), they are not moving to other locations and as a consequence may pose only a modest risk of secondary dispersal.

Boating behaviours also are highly variable. Within each boating community there are a range of boating behaviours in terms of antifouling practices and trip histories that may be spatially different at global, regional, and local scales. Some places have limited boating seasons while other boating communities are active year-round. For example, ice cover excludes the majority of recreational boaters from Canadian Maritime waters in the winter months (Darbyson *et al.* 2009b). All these factors must be considered when evaluating and comparing the recreational boating vector in different systems.

1.3.2.4 Unregulated Vector of Unknown Magnitude

British Columbia (BC) has the highest number of pleasure craft vessels in Canada. The number of recreational boaters in BC has been estimated at over 400,000 (Georgia Strait Alliance 2009). The magnitude of the recreational boating vector in BC is comparable to other places worldwide where small craft fouling has become a priority area for detection and management, including Australia, New Zealand, and Hawaii. There are over 800 marinas in BC with more built or proposed each year. And yet, currently, there are no regulatory initiatives in Canada to reduce the dispersal by means of hull fouling (Transport Canada 2007). Under the current Canadian regulations, if a vessel would have been detected with a known invasive species present, there is no regulatory framework to force the owner to clean the boat or stop its movement either within the country or beyond international borders.

In contrast, both Hawaii and New Zealand have an unregulated code of practice to prevent fouled vessels from occupying their waters. The Australian Quarantine and Inspection Service (AQIS) has new regulations for boats less than 25 m entering Australian waters. The biofouling protocol went into full effect in 2006 and requires all small vessels' hulls and ancillary gear be clean upon arrival to Australian waters (Australian Quarantine and Inspection Services 2006). They recommend: 1) cleaning the boat's hull within one month of arrival in Australia; 2) applying antifouling paint within one year prior to arrival; or 3) booking the vessel to be hauled out and cleaned within one week of arrival. In-water cleaning of international vessels is not permitted in Australia.

A large number of known NIS are already present in BC waters, demonstrating the susceptibility of local waters to invasion (Levings *et al.* 2002; Lu *et al.* 2007). British Columbia is at risk both from direct introductions by international boats and from invaders brought in by other vectors and secondary spread by boating activities within the region. Asian trade routes plus, more historically, introduction through the aquaculture industry, have brought over 100 species of marine non-indigenous species to these waters. In addition, BC also faces the risk of secondary spread or stepping-stone invasions of species introduced and established in southern parts of the Pacific coast. San Francisco Bay was the site of original introduction of many species now seen in Oregon, Washington, and BC (Levings *et al.* 2002; Ruiz *et al.* 2011). Some of these species are now spreading north by natural dispersal (*e.g.,* European green crab) while others (*e.g.*, ascidians) are likely using secondary vectors or some combination of natural and human-mediated dispersal. A comprehensive examination of the recreational boating pathway for BC has not previously been completed. In order to prevent and manage marine invasions to the region, knowledge of this pathway is needed and will be the focus of the current study.

1.4 Research Objectives and Thesis Overview

This dissertation is the result of the first investigation of the recreational boating pathway in the marine waters of Canada. The overarching goal of the current research is to characterize the recreational boating pathway, both as a vector for original introduction between regions and as a vector for human-mediated spread within a region. In order to achieve this goal, the project is comprised of five research components (presented in Chapters 2-6).

In Chapter 2 I characterise the baseline complement of non-indigenous species present in BC marinas. Using settlement plates, the settlement and community development of native and non-indigenous species was monitored for one year at a number of marina sites in the southern Strait of Georgia (a location with a high number of NIS). This is the first quantitative investigation of subtidal marine non-indigenous species for the region and gives a unique look at their temporal and spatial patterns.

Chapter 3 evaluates the strength of the recreational boat hull fouling vector in BC and the complement of species associated with it. I performed dive surveys at marinas throughout BC examining the species presence and abundance on the hull and niche areas. Simultaneously, I conduct a boater questionnaire to characterize common boating behaviours. By combining the results of these two components, I examine whether the BC boating community is likely to transport hull fouling invaders into and within BC marine waters.

In Chapter 4 I build on the data from the dive survey and boater questionnaire to evaluate two commonly used rapid assessment tools. Using the two datasets, I present a statistical model based on BC boater behavioural characteristics that can be used to predict the presence of fouling species and by extension, the risk of invasive species transport. This tool may prove valuable to border management and monitoring activities in flagging risky boats that require further inspection for potentially invasive species.

In Chapter 5 I compare the biomechanical properties of attachment in non-indigenous and native species. I use biomechanical tools to experimentally test the hypothesis that non-indigenous hull fouling species are better adapted to hull fouling transport than their native congeners. I measure attachment strength and drag of common fouling species, with a focus on ascidians, in order to estimate maximum dislodgment velocity. There are distinct differences between non-indigenous and native species as well as between colonial and solitary biomechanical strategies. This research is a unique application of traditional biomechanical experimental techniques to answer a common question in vector research, probability of transport with a vector.

The sixth chapter combines the results of the settlement plate survey (Chapter 2) and boater data obtained from the boater survey (Chapter 3). The goal of this chapter is to examine and compare demographic, environmental and vector variables responsible for contemporary spatial patterns of non-indigenous species distribution. I then compare the variables that best able to explain the spatial pattern to the historical vectors of introduction.

In the seventh and final chapter I summarize the significant findings of this dissertation and discuss the implications for introduction and spread of non-indigenous and potentially invasive species in this region.

Chapter 2: Non-indigenous species in subtidal fouling communities of Strait of Georgia, British Columbia, Canada*

2.1 Introduction

Ports and marinas are some of the most invaded marine habitats in the world (Cohen and Carlton 1998; Lambert and Lambert 1998; Ruiz *et al.* 2000). Propagule pressure is high in these environments due to a number of human-mediated vectors including commercial shipping (Drake and Lodge 2004; Drake and Lodge 2007; Godwin 2003; Gollasch 2002; MacIsaac *et al.* 2002; Smith *et al.* 1999) and recreational boating (Acosta and Forrest 2009; Davidson *et al.* 2010; Floerl 2002; Johnstone *et al.* 1985; Mineur *et al.* 2008). Altered physical conditions, frequent disturbances, and higher abundance of artificial substrates favour the establishment of non-indigenous species (NIS) (Connell 2000; Connell 2001; Glasby *et al.* 2007; Leprieur *et al.* 2008). Ironically, the very same physical properties that make marina and port sites safe for maritime vessels encourages settlement and establishment of NIS.

To date, more than 120 marine and estuarine NIS have been documented in British Columbia (BC) waters, demonstrating the susceptibility of these waters to invasion (Gillespie 2007; Levings *et al.* 2002; Lu *et al.* 2007). Asian trade routes, in addition to historic aquaculture imports of both east coast and Asian species, can account for over 100 of these NIS. BC also faces the risk of secondary spread or stepping-stone invasions of species introduced and established in southern parts of the Pacific coast. Rapid assessment surveys performed on the West coast of North America showed that marinas have a high number of NIS present, including examples of highly invasive species such as intertidal cordgrass *Spartina* and club tunicate *Styela clava* (Cohen *et al.* 1998; Cohen *et al.* 2005; Mills *et al.* 1999; Pederson *et al.* 2005). San Francisco Bay was the site of original introduction of many species now recorded in Oregon, Washington, and British Columbia (Levings *et al.* 2002) and has been hypothesized to act as the gateway of

* This work is being prepared as part of a manuscript for publication with coauthors Glen Jamieson, Evgeny Pakhomov and Thomas Therriault.

introduction for California (Ruiz *et al.* 2011). Some of these species are now spreading north by natural dispersal (*e.g.,* European green crab) while others likely use secondary vectors or some combination of natural and human-mediated dispersal vectors.

Commercial shipping vectors such as ballast water and sediments, hull fouling, and sea chests often represent primary vectors that transport NIS over great distances. However, in addition to commercial shipping vectors, there is growing evidence that recreational vessels can serve as both primary and secondary spread vectors in both marine and freshwater environments. Freshwater NIS can become entangled in trailers and motors or are transported in live wells or bilge water. For example, the spread of zebra mussels *Dreissena polymorpha* (Bossenbroek *et al.* 2001; Bossenbroek *et al.* 2007; Johnson *et al.* 2001; Padilla *et al.* 1996) and Eurasian watermilfoil *Myriophyllum spicatum* (Buchan and Padilla 2000; Johnstone *et al.* 1985) have been attributed to trailered boats. The less studied marine recreational boating pathway also has been shown to transport NIS, although usually via hull fouling. A range of invertebrate and macrophyte NIS were found in small boat hull fouling communities and their introduction and secondary spread have been linked to recreational boats (e.g., Ashton *et al.* 2006; Davidson *et al.* 2010; Floerl 2002; Floerl *et al.* 2004; Floerl and Inglis 2005; Minchin and Sides 2006).

Fouling species growing on marina structures and resident boats have the potential to transfer propagules to visiting boats and may result in subsequent spread to new locations (Floerl and Inglis 2001). In northern Australia, Floerl & Inglis (2005) compared the species assemblages of fouled boats with those within marinas and found a positive relationship. Once a NIS population is established within a marina it potentially becomes a secondary source for transport to uninfected sites. This type of secondary spread has been called stepping-stone introduction or hub-and-spoke introduction (Apte *et al.* 2000; Floerl *et al.* 2008). Coastal marinas are inter-connected by varying degrees of boating traffic such that boater movements have been used as a proxy for the probability of spread. Boat movement patterns have indicated significant potential for recreational boats to act as dispersal vectors in the southern Gulf of St Lawrence (Darbyson *et al.*

2009a; Darbyson *et al.* 2009b), Australia (Floerl and Inglis 2005), and New Zealand (James & Hayden 2000 IN Dodgshun *et al.* 2007; Floerl *et al.* 2005a). The movements of BC boaters have not been documented and will be investigated further in Chapter 3.

In marinas, local hydrologic and environmental conditions are altered to provide safe refuge for boats but these alterations in turn often create favourable conditions for arriving NIS. Water velocities within marinas are considerably reduced compared to surrounding coastal waters (Floerl 2003) thus increasing the probability of NIS settlement and establishment. Further, coastal development can alter local circulation patterns (*e.g.,* the addition of jetties and breakwater walls) changing the natural dispersal and colonization patterns of native species as well as promoting the establishment of NIS (Bulleri and Chapman 2010). Chemical disturbances alter natural conditions and include pollution in the form of oil or diesel spills and leaks, agricultural and storm water run off, leaching from antifouling compounds, and sewage discharge from boats or nearby human settlements (Burgin and Hardiman 2011; Leon and Warnken 2008; Mack and D'Itri 1973; McGee *et al.* 1995; Piola and Johnston 2009). Physical disturbances may include water turbulence, sedimentation from dredging activities, and abrasion of artificial structures by minor boat collisions and from the influence of wave action on floating structures (Burgin and Hardiman 2011). Disturbance has been shown to promote NIS establishment by creating space, suppressing native communities and favouring disturbance-adapted NIS (Altman and Whitlatch 2007; D’Antonio *et al.* 1999; Hobbs 1992; Mook 1981; Piola and Johnston 2009).

The presence and introduction of artificial structures may also qualify as physical disturbance. In addition to boat hulls with various stages of antifouling protection, pilings, docks and breakwater walls in marinas significantly increase the amount of vertical and horizontal settlement substrates (Glasby *et al.* 2007). It is important to note, that much of the artificial habitat provided is floating and therefore protected from benthic predators and air exposure that affects similar species in intertidal habitats. In a recent study, it was shown that floating structures provided refuge for the invasive bryozoan *Bugula neritina* from subtidal predators (Dumont *et al.* 2011). Presence of shallow floating structures, such as floating docks, has been shown experimentally to promote NIS dominance because these species were better able to successfully exploit artificial floating structures (Dafforn *et al.* 2009). Temporally, a continual supply of new, unoccupied spaces creates new opportunities for colonisation in the absence of climax community structure that develops over time (Clark and Johnston 2005, 2009; Neves *et al.* 2007). Thus, both spatial and temporal elements of coastal marinas potentially favour NIS establishment.

Anecdotal evidence suggests that invasions of BC marinas are substantial but to date have not been well quantified. There has been no comprehensive inventory of the NIS present in BC marinas specifically or subtidal BC habitats in general. This study simulated the introduction of novel, artificial substrates into BC marinas. Following community development through time, this study focused on the presence and settlement patterns of NIS in the Southern Strait of Georgia, BC; an area of high marine/estuarine diversity and intense human use. The goal of this study was to characterize natural and NIS communities as well as examine the spatial and temporal development of fouling communities in BC marinas. The influence of native diversity on invasion patterns was investigated to determine if BC's high native species diversity could be a natural defence against further invasion (sensu Elton 1958; Levine and D'Antonio 1999; Stachowicz *et al.* 1999).

2.2 Methods

2.2.1 Study Location

The Strait of Georgia is a large temperate coastal basin located in southern BC. It lies between Vancouver Island and mainland BC with a major human population centre, Vancouver. Although the Strait of Georgia is bounded by a number of smaller tributary inlets, bays and sounds, Fraser River runoff largely drives its oceanographic dynamics (LeBlond 1983). The Strait of Georgia is connected with the Pacific Ocean via Juan de Fuca Strait to the south and Johnstone and Queen Charlotte Straits to the north (Harrison *et al.* 1983). Within the Strait of Georgia, nine study sites were chosen (Figure 2.1) although a high mussel settlement event at one site, West Vancouver, compromised settlement on the plates and resulted in no samples being processed from this site. The eight remaining sites are analyzed and discussed here.

2.2.2 Settlement Plates

One settlement plate array consisted of three black plastic lids, 30 cm in diameter with a 2 cm lip facing downwards. The lids were hung horizontally at increasing depths (A, B, and C), starting 0.5 metres from the surface and separated one metre apart (Figure 2.2). Each lid had four replicate plastic Petri dishes attached to the underside with cable ties. Each Petri dish was 56.5 cm^2 with a 1 cm high lip. Sixteen settlement arrays were suspended from floating docks at each of the nine sites from May 2006 through May 2007. Every four months (Fall: September 2006, Winter: January 2006 and Spring: May 2007), three randomly selected arrays were removed from the water at each site. Petri dishes were removed from the lids in the field, placed immediately in individual Ziploc bags and preserved in 4% formalin.

In the lab each Petri dish was processed by identifying each species present and recording its abundance/coverage. Abundance data about each species included either the number of individuals present for solitary species or percent cover for encrusting and colonial species. For percent coverage, a grid containing 152 points was overlaid on the Petri dish (minimum detectable area = 0.37 cm^2) and the number of points covering a species recorded. Morphotypes were sequentially removed from the canopy to reveal understory coverage. Percent cover was recorded for each morphotype present, including both canopy and understory species so that total percent cover per dish could potentially be higher than 100%. In total, 720 Petri dishes were processed from the eight sampling sites.

Species identifications were completed in the laboratory using taxonomic keys for the area. Morphotypes were vouchered and sent to taxonomic experts for identification or verification. Digital photographs of vouchered specimens can be seen at our laboratory reference website (www.bcbiodiversity.lifedesks.com) and physical specimens are archived at the Beaty Biodiversity Museum at the University of British Columbia.

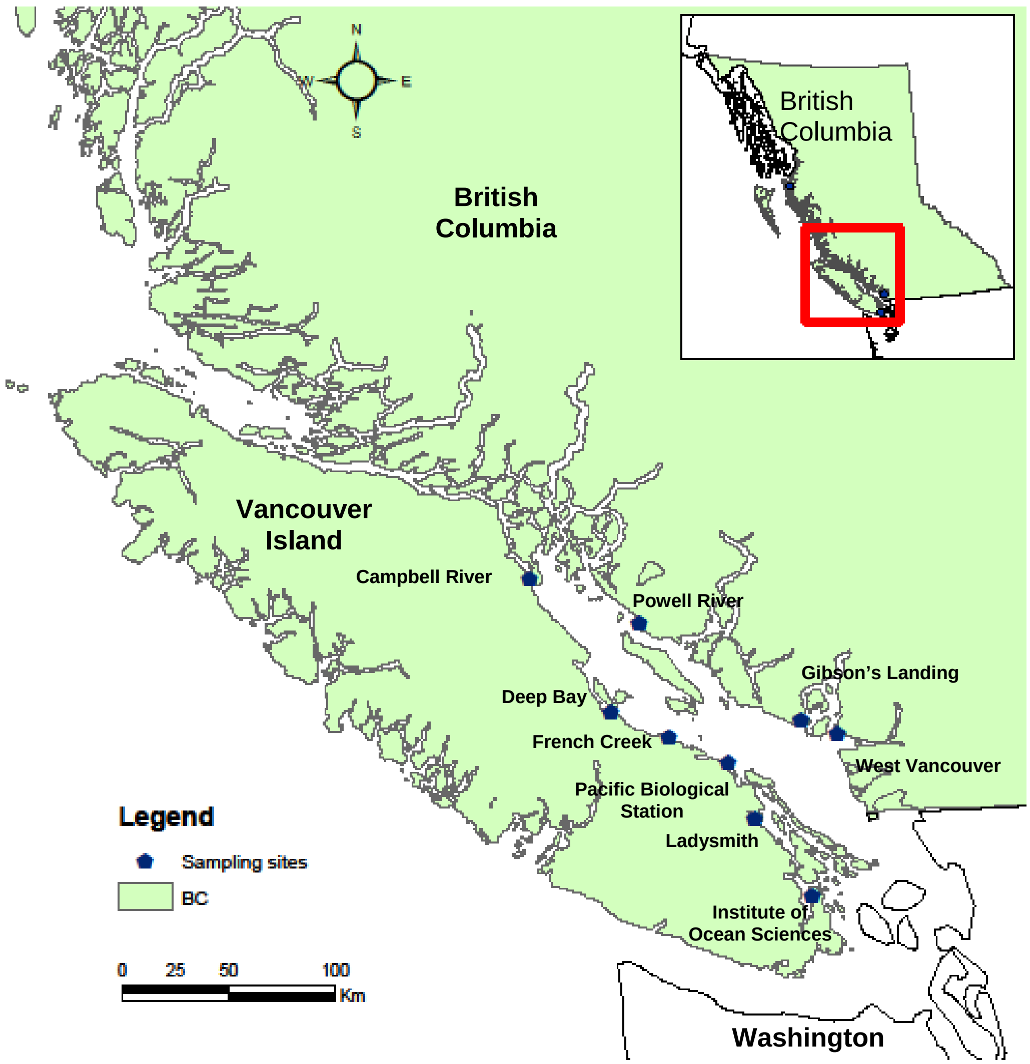

Figure 2.1: Map of sampling sites in Strait of Georgia, British Columbia, Canada. Site abbreviations used throughout the text: Campbell River (CR), Powell River (PR), Deep Bay (DB), French Creek (FC), Pacific Biological Station (PBS), Ladysmith (LDS), Institute of Ocean Sciences (IOS), Gibson's Landing (GIB) and West Vancouver (WVL). Inset shows location of study area within British Columbia, Canada.

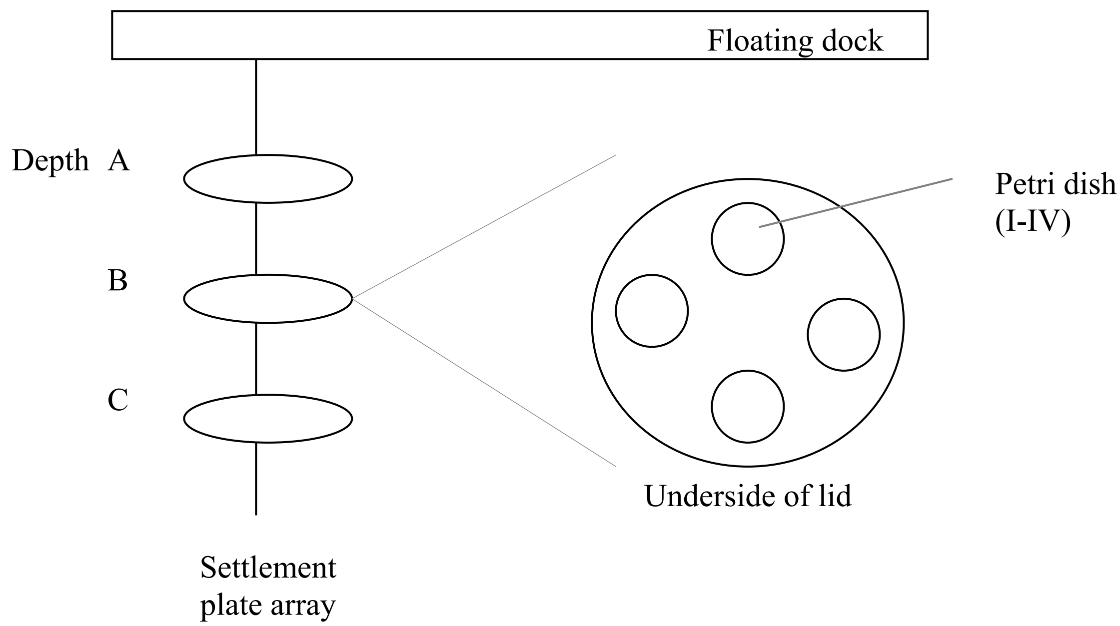

Figure 2.2: Sampling and settlement plate design

2.2.3 Data Analysis

Individual Petri dishes were treated as pseudo-replicates so percent coverage for Petri dishes (N=4) at each depth were averaged to yield three depth means for each array. The effect of depth, site and season on percent cover was tested using General Linear Model (GLM). The effect of depth was non-significant (GLM: $F = 1.222$, $df = 2$, $p = 0.298$) so depths were treated as pseudo-independent replicates and averaged to give mean values per array. The values from the three arrays were used to calculate means and measures of variance (standard error) for each site. Total species richness for a location was calculated as the accumulated number of species for all dishes on each array (all dishes and depths) at each sample period. The effect of site and season on total species richness was tested using General Linear Model.

The invasion status of each species identified was assigned for British Columbia based on the criteria by Carlton (Carlton 1989) as either native, cryptogenic, non-indigenous (NIS) or indeterminate. Cryptogenic species were those morphotypes that were definitively identified to species level but their invasion status was unknown in the study region. Morphotypes that were not identified to species-level were classified as indeterminate.

In order to investigate the relationship between NIS and native species richness overall, linear regression was performed on the species richness and mean percent cover (arrays as replicates, N=72). To determine if the relationship between NIS and native percent cover and richness differed by season, differences between the slopes were tested using the ANCOVA interaction term with season as the covariate. All statistical analyses were performed using SPSS 19 (SPSS: An IBM Company).

2.3 Results

2.3.1 Fouling Community Composition

In total, 394 morphotypes were processed and identified to the lowest taxonomic level possible. This included 222 native species, 11 cryptogenic species, and 19 non-indigenous species (Table 2.1 lists NIS and cryptogenic species). Of those morphotypes with definitive species identifications, 11.9% were NIS or cryptogenic. There were 142 morphotypes considered indeterminate as they could not be identified to species level. A complete list of species can be found in Appendix A.

Percent cover varied significantly by site (GLM: $F = 10.658$, $df = 7$, $p<0.001$) and season (GLM: $F = 78.630$, $df = 2$, $p<0.001$) but not by depth. Therefore percent cover and abundance values have been pooled across the three depths in all subsequent analyses. Species richness varied significantly by season (GLM Main effect $F = 6.928$, $df = 2$, $p = 0.008$) but not by site (GLM Main effect $F = 1.760$, $df = 7$, $p = 0.174$) and patterns differed among sites (GLM site*season: $F = 8.748$, $df = 14$, $p < 0.001$).

There was an overall positive relationship between native and NIS species richness (Figure 2.3a, Linear regression $F = 8.390$, $df = 1$, $p = 0.005$, $R^2 = 0.1098$). The trend was not significantly different by season (ANCOVA GLM native*season: $F = 1.477$, $df = 24$, $p = 0.128$). There was an overall negative but not significant trend between native and NIS percent cover (Figure 2.3b, Linear regression $F = 3.718$, $df = 1$, $p = 0.058$). There were no significant relationships between native richness and percent cover or NIS richness and percent cover (Linear regression $p > 0.05$).

Table 2.1: List of non-indigenous and cryptogenic species (taxa and taxonomic authority) identified from settlement plates

Species	**Taxa**	**Taxonomic Authority**	**Status**
Caprella mutica	Amphipod	Schurin, 1935	Nonindigenous
Monocorophium acherusicum	Amphipod	Costa, 1853	Nonindigenous
Monocorophium insidiosum	Amphipod	Crawford, 1937	Nonindigenous
Monocorophium uenoi	Amphipod	Stephensen, 1932	Nonindigenous
Botrylloides violaceus	Ascidian	Oka, 1927	Nonindigenous
Botryllus schlosseri	Ascidian	Pallas, 1766	Nonindigenous
Diplosoma listerianum	Ascidian	Milne-Edwards, 1841	Nonindigenous
Molgula manhattensis	Ascidian	De Kay, 1843	Nonindigenous
Styela clava	Ascidian	Herdman, 1881	Nonindigenous
Neotrapezium liratum	Bivalve	Reeve, 1843	Nonindigenous
Bugula neritina	Bryozoan	Linnaeus, 1758	Nonindigenous
Schizoporella japonica	Bryozoan	Ortmann, 1890	Nonindigenous
Crepidula fornicata	Gastropod	Linnaeus, 1758	Nonindigenous
Eulalia viridis	Polychaete	Linnaeus, 1767	Nonindigenous
Eumida sanguinea	Polychaete	Örsted, 1843	Nonindigenous
Hobsonia florida	Polychaete	Hartman, 1951	Nonindigenous
Melita nitida	Polychaete	Smith, 1873	Nonindigenous
Polydora cornuta	Polychaete	Bosc, 1802	Nonindigenous
Halichondria bowerbanki	Sponge	Burton, 1930	Nonindigenous
Incisocalliope newportensis	Amphipod	J.L. Barnard, 1959	Cryptogenic
Bowerbankia "gracilis"	Bryozoan	Leidy, 1855	Cryptogenic
Cryptosula pallasiana	Bryozoan	Moll, 1803	Cryptogenic
Obelia bidentata	Bryozoan	Clark, 1875	Cryptogenic
Chone duneri	Polychaete	Malmgren, 1867	Cryptogenic
Ctenodrilus serratus	Polychaete	Schmidt, 1857	Cryptogenic
Polydora limicola	Polychaete	Annenkova, 1934	Cryptogenic
Polydora websteri	Polychaete	Loosanoff & Engle, 1943	Cryptogenic
Leptochelia "dubia"	Malacostraca	Krøyer, 1842	Cryptogenic
Tubulanus pellucidus	Nemertea	Coe, 1895	Cryptogenic
Haliclona "permollis"	Sponge	Bowerbank, 1866	Cryptogenic

Table 2.2 NIS species mean percent cover and standard error (SE) by site and season

	Site	CR			DB			FC			GIB			IOS			LDS			PBS			PR		
NIS	**Season**	**Fall**	**Winter**	**Spring**	**Fall**	**Winter**	**Spring**	**Fall**	**Winter**	**Spring**	**Fall**	**Winter**	**Spring**	**Fall**	**Winter**	**Spring**	**Fall**	**Winter**	**Spring**	**Fall**	**Winter**	**Spring**	**Fall**	**Winter**	**Spring**
B. violaceus	Mean	1.48	0.42	3.47	1.17	1.19	5.56	12.61	3.09	2.60		1.01	3.73	15.15	0.62	1.39	41.48	8.44	3.51	1.55	10.36	0.26	9.47	1.17	5.96
	SE	0.38		1.43	0.48	1.51	1.74	2.65	1.03	1.75		2.08	1.90	4.43	0.41	2.11	3.82	1.99	0.92	1.43	6.90		2.10	0.42	1.92
B. schlosseri	Mean	0.29		0.18	7.69	0.62		3.23		1.57	0.20	5.79				0.04	3.67	3.27	1.21				19.46	0.07	0.88
	SE	0.14			3.14			1.54		0.68		5.86					2.18	1.26					3.76		1.35
B. neritina	Mean							0.02										0.02							
	SE																								
D. listerianum	Mean	18.00	0.05	2.89				0.05							0.84										
	SE	2.84		4.66											2.02										
H. bowerbanki	Mean	0.02		1.94	1.33	1.50		1.17	3.07	10.75						1.28	4.75		3.40	1.32		3.00		8.08	11.22
	SE			0.53		0.54			1.55	2.45						1.23	0.77		1.44	1.99		2.69		4.48	2.79
S. japonica	Mean	1.13		4.97	14.40	2.30	10.23	22.95	11.66	13.82		0.24		70.52	45.45	35.09	11.28	24.58	12.65	4.44	2.76	4.86	25.42	9.76	35.20
	SE	0.74		2.20	3.66	2.19	2.34	4.07	3.29	2.54		0.06		4.83	5.60	6.77	2.78	4.82	2.63	2.83	1.97	2.20	5.27	3.30	5.47

Table 2.3 NIS species abundance in mean number per site and season

	Site	CR			DB			FC			GIB			IOS			LDS			PBS			PR		
NIS	**Time**	**Fall**	**Winter**	**Spring**	**Fall**	**Winter**	**Spring**	**Fall**	**Winter**	**Spring**	**Fall**	**Winter**	**Spring**	**Fall**	**Winter**	**Spring**	**Fall**	**Winter**	**Spring**	**Fall**	**Winter**	**Spring**	**Fall**	**Winter**	**Spring**
C. mutica	Mean	1.14		0.06	6.78	0.56	0.17	9.83	1.17	1.83				5.39	0.03	16.56	7.42		17.33	0.08		1.44	12.06	3.19	39.83
	SE	2.47			2.30	0.31	0.17	2.44	0.49	2.07				1.20		15.26	5.76		4.36			0.69	5.32	1.58	15.19
C. fornicata	Mean						0.17																		
	SE						0.17																		
E. viridis	Mean														2.42										
	SE														0.31										
E. sanguinea	Mean						0.06								0.14										
	SE																								
H. florida	Mean																		0.11						
	SE																		0.00						
M. nitida	Mean											2.19	1.17												
	SE											1.05	1.28												
M. manhattensis	Mean																	0.53	0.11						
	SE																	0.25							
M. acherusicum	Mean				1.25		19.67	0.08		2.78	14.44			3.36			2.83	0.11	34.39	0.31			0.19	0.47	6.67
	SE				0.47		7.32			3.56	2.92			0.68			2.92	0.24	14.75				0.15	0.71	4.84
M. insidiosum	Mean				0.17			0.11			1.39			0.53									0.03		
	SE				0.24						0.39			0.54											
M. uenoi	Mean											20.22	39.94			40.94									
	SE											4.88	7.80			9.90									
N. liratum	Mean																							0.03	0.17
	SE																								
P. cornuta	Mean										0.03						0.06						0.03		
	SE																0.00								
S. clava	Mean			4.67	2.03	3.33	6.83	3.11	1.58	2.67				0.19		1.72	0.19	0.22	1.72			0.44		0.42	1.17
	SE			0.73	0.80	1.09	1.51	1.10	1.18	0.72				0.07		0.60	0.07	0.09	0.31			0.67		0.14	0.15

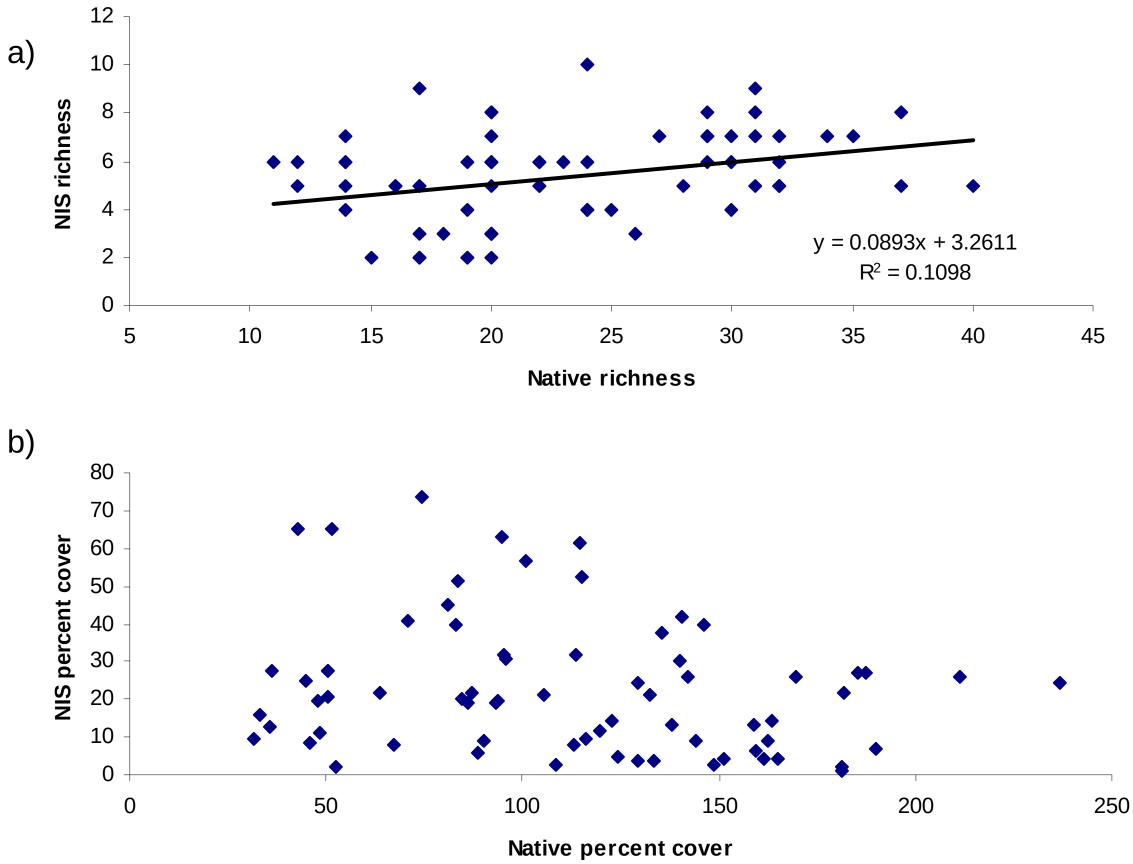

Figure 2.3: Relationship between native and NIS a) species richness and b) percent cover. Points represent total richness values from individual arrays for all sites and seasons (N=72).

2.3.2 Spatial Patterns

The NIS with highest percent cover were *Botrylloides violaceus,* which reached 41.5% coverage at Ladysmith in the fall, and *Schizoporella japonica,* which reached 35.2% coverage in the spring at Powell River (Table 2.2). The most abundant solitary NIS were the amphipods *Monocorophium uenoi* (mean 40.9 per dish or 7236.4 m^{-2}), *Monocorophium acherusicum* (mean 25.4 per dish or 4494.0 m^{-2}), and *Caprella mutica* (mean 39.8 per dish or 7029.3 m^{-2}).

Two species were found at all eight sites, *B. violaceus* and *S. japonica* (Table 2.2). The next most common species across sites were *Botryllus schlosseri, C. mutica, Halichondria bowerbanki, M. acherusicum,* and *S. clava,* all present at seven sites (Tables 2.2, 2.3). In contrast, six species were only found at a single site: bivalve *Crepidula fornicata,* polychaete *Eulalia viridis,* polychaete *Hobsonia florida,* polychaete *Melita nitida,* ascidian *Molgula manhattensis* and gastropod *Neotrapezium liratum* (Table 2.3). One species, the polychaete *Polydora cornuta*, was only found in the fall at three sites: Gibson`s Landing, Ladysmith and Powell River.

The highest mean percent cover of NIS (averaged across seasons) occurred at Institute of Ocean Sciences (38.2%), followed by Powell River (28.0%), and Ladysmith (26.7%) (Figure 2.4). The lowest NIS cover occurred at Gibson's Landing (2.5%) and Pacific Biological Station (6.3%). The highest native cover occurred at Campbell River (53.3%), followed by Institute of Ocean Sciences (48.7%), and Gibson's Landing (48.3%) (Figure 2.4). The lowest native cover occurred at Powell River (13.0%) and Ladysmith (24.1%). From a possible 19 NIS found at all sites, NIS richness was highest at Institute of Ocean Sciences and Ladysmith (both 12 species) while the lowest was Pacific Biological Station and Campbell River (6 and 7 species, respectively) (Figure 2.5).

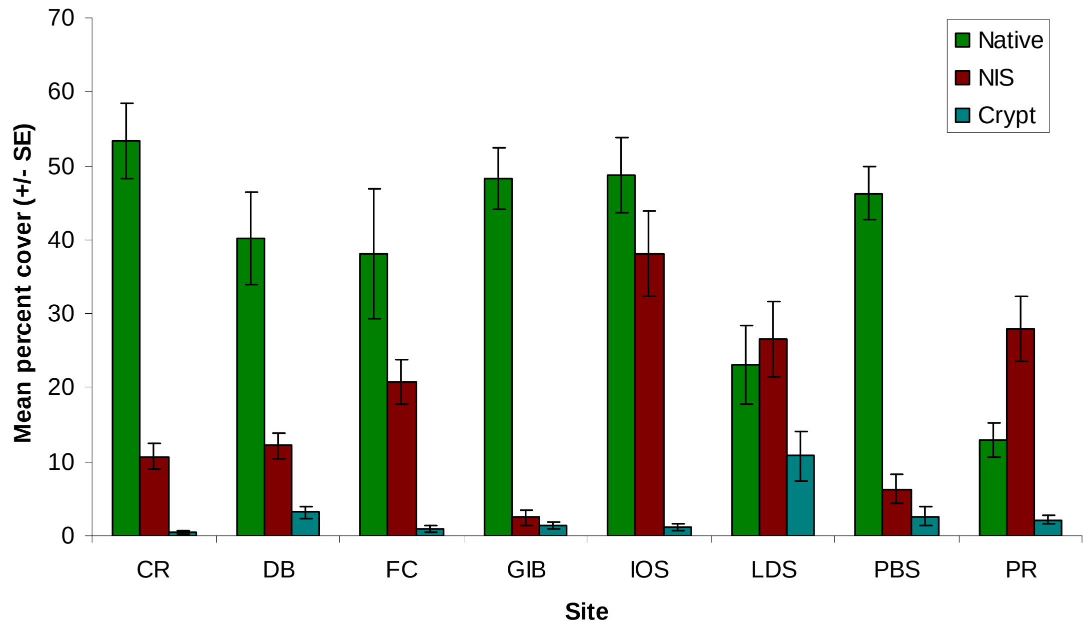

Figure 2.4: Mean percent cover (+/- standard error) averaged across three seasons by site for native (green), NIS (red) and cryptogenic (blue) species.

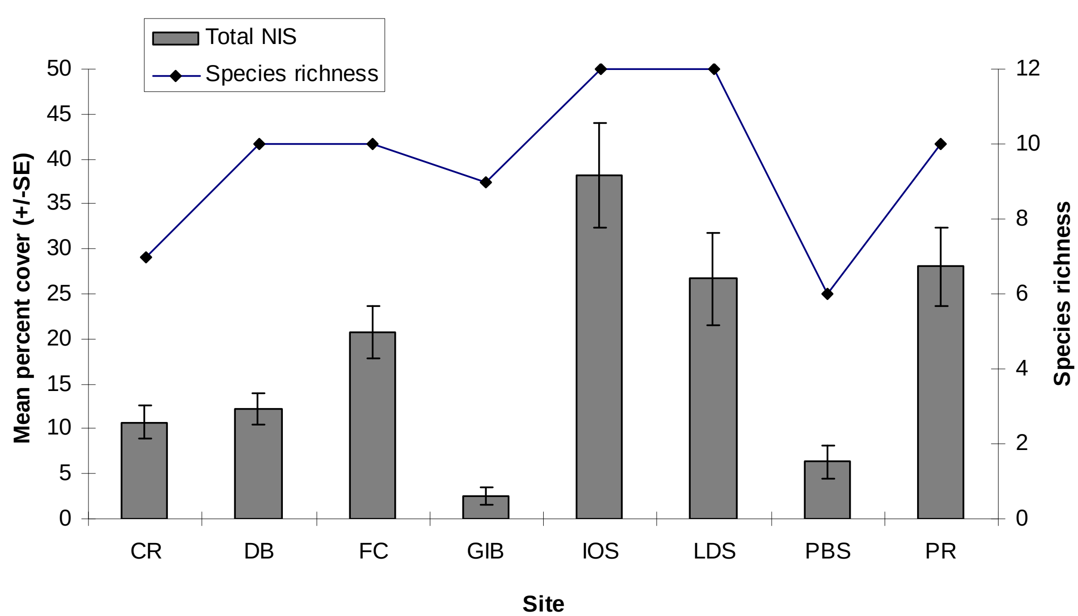

Figure 2.5: Non-indigenous species (NIS) percent cover (+/- standard error) (solid bars) and richness (points) by site.

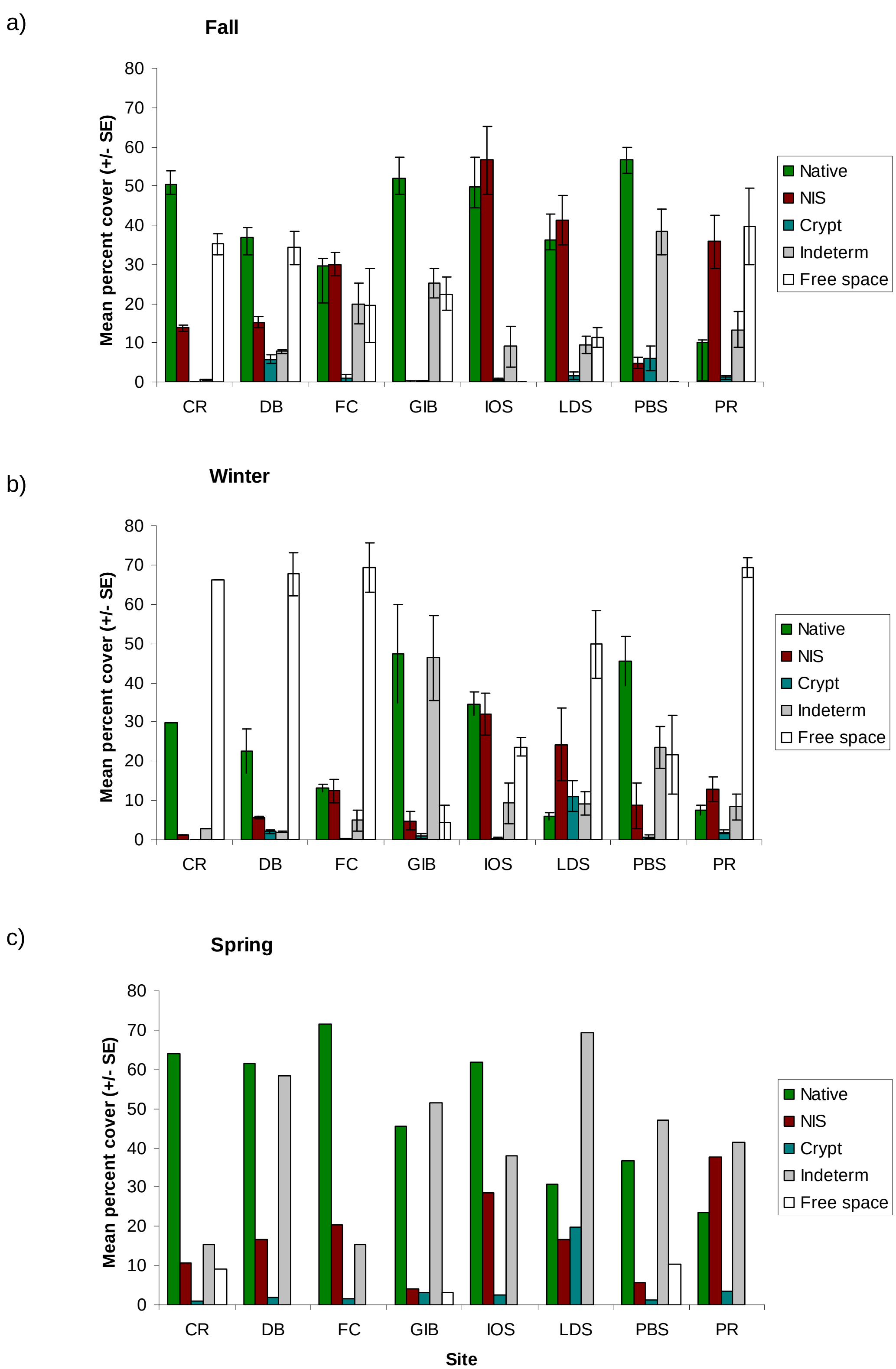

Figure 2.6: Mean percent cover by site of native (green), NIS (red), cryptogenic (blue), indeterminate species (grey) and free space (open) in the a) fall, b) winter and c) spring sampling periods.

2.3.3 Temporal Patterns

The temporal trends in percent cover of NIS varied by site, as demonstrated by the dynamics of *B. violaceus* (Table 2.2). This species was one of the few NIS found at all eight sampling sites and at some sites increased in percent cover through time, while at others it decreased or was highest in the winter sampling period.

The availability of free space was highest in the winter season (Figure 2.6). Overall, fouling cover was lowest in the winter season and both NIS and native species had the lowest cover during this time (Figures 2.6, 2.7). Native cover was highest in the spring sampling period while NIS was highest in the fall sampling period. The cover of NIS increased in the spring but not to the same levels as recorded during the fall.

The cover of NIS exceeded that of native species at four sites in the fall sampling (IOS, LDS, FC and PR), two sites in the winter (LDS and PR), and one site in the spring (PR) (Figure 2.6). Over the course of three seasons, native species gradually became more predominant within these communities (Figure 2.7).

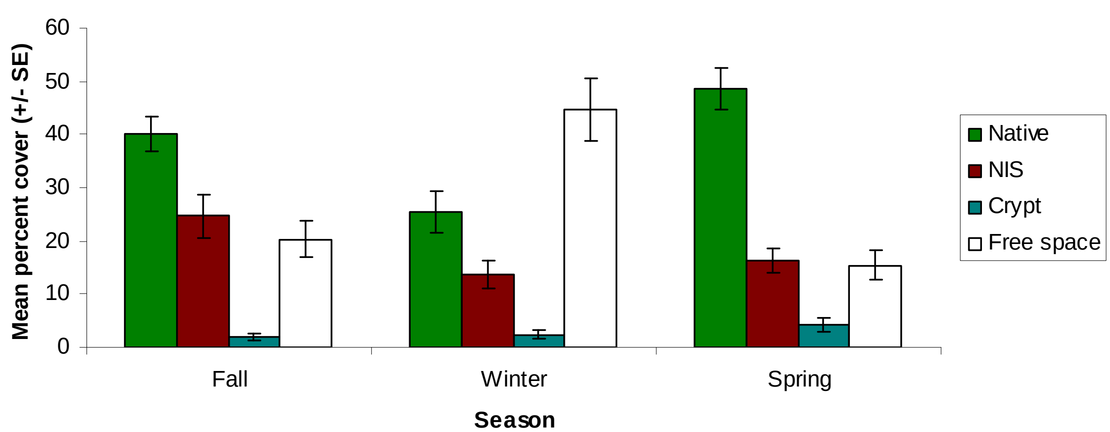

Figure 2.7: Grand means of percent cover by season of native, NIS, cryptogenic and free space (+/- standard error)

Most NIS were detected during the fall sampling at most sites (Tables 2.2 and 2.3). However some species settled after the first fall sampling (between Oct-Dec): *S. japonica* and *B. violaceus* at Gibson's Landing, *Diplosoma listerianum, E. viridis* and *E.*

sanguinea at Institute of Ocean Sciences, *H. bowerbanki, Neotrapezium liratum* and *S. clava* at Powell River, *M. uenoi* and *Melita nitida* at Gibson's Landing, and *M. manhattensis* at Ladysmith. A smaller number of NIS only settled before the third sampling period: *H. bowerbanki* and *M. uenoi* at Institute of Ocean Sciences, *H. florida* at Ladysmith and *S. clava* at Pacific Biological Station. An example of the development of the fouling community over time is depicted in Figure 2.8.

Figure 2.8: An example of the development of the fouling community on a settlement plate over time at Ladysmith.

2.4 Discussion

2.4.1 Characterizing the NIS Community

A number of well-known "invasive" species were documented settling within southern Strait of Georgia marinas. Ascidian species such as *S. clava* and *B. violaceus* have a history of invasion and impacts in other parts of Canada and the world (e.g., Carver *et al.*

2006; Clarke and Therriault 2007). The range expansion of the invasive caprellid amphipod *C. mutica* into BC waters was detected as part of the current survey and reported by Frey and colleagues (2009). NIS richness in the fouling community (19 species) was higher than that detected in intertidal and seagrass habitats in BC (Choi 2011; M. Mach, UBC, Vancouver, pers. comm.) but lower than that seen in other marina surveys on the Pacific coast of North America (Cohen *et al.* 1998; Cohen *et al.* 2005; Mills *et al.* 1999). This difference could be a result of a broader range of taxonomic expertise in the other surveys performed by a large group of taxonomists and/or the passive nature of the settlement plate survey (discussed below). In this survey, 36% of morphotypes were not identified to species level. However, high NIS diversity has been well-documented in Pacific coast hot spots, such as San Francisco Bay, California (Ruiz *et al.* 2011) and Coos Bay, Oregon (Cohen *et al.* 1998; Ruiz *et al.* 2000).

Non-indigenous species dominated some sites, representing more than 40% of percent cover of the fouling community. This level of dominance by NIS has significant implications for ecosystem health and function and these sites would likely experience some form of impact, such as loss of native biodiversity. Indeed, those sites with the highest NIS cover also had the lowest native cover and vice versa, although the overall trend was nonsignificant. The high level of NIS fouling also suggests that these sites may host large, reproductive populations that have the potential to become entrained in a variety of potential vectors and thus be transported to additional sites. Those sites with high NIS cover were dominated by the bryozoan *Schizoporella japonica* although little is known about this particular species, its vectors or its potential biotic interactions with other species.

The species sampled by the settlement plates are a sub-sample of the species present in the local subtidal fouling ecosystem. Results reflect the passive sampling nature, substrate and orientation and the pre- and post-settlement processes that occur before sampling was carried out. Settlement plates are passive sampling devices that have both advantages and disadvantages compared to active sampling such as dockside scrapings, quadrat digs, benthic grabs or plankton tows. Their passive nature allows a larger sampling size with relatively little field or observer time, potentially covering a larger spatial area over a longer time frame. However, because of their passive nature, they only sample a subset of individuals in the water column that physically come into contact with the plate and then successfully settle, survive and grow large enough to be effectively sampled. The numbers of individuals that contact the plate are governed by pre-settlement processes such as the local oceanographic conditions, species-specific larval traits such as energy stores and swimming ability, as well as larval settlement behaviours (Havenhand and Svane 1991; Koehl 2007; Osman and Whitlach 1995; Osman *et al.* 1989; Pawlik 1992). Between larval contact and sampling, individuals recruiting to the settlement plate are subject to a number of post-settlement biological processes. Newly settled juveniles can be subject to high mortality as a result of predation and competition (Brown and Swearingen 1998; Hunt and Scheibling 1997; Nandakumar *et al.* 1993). Competition for space also can increase as the community develops and becomes more complex leading to competitive exclusion through reduced settlement or increased post-recruitment mortality from overgrowth and/or starvation (Nandakumar *et al.* 1993).

The orientation and depth of the settlement plate also affects the species sampled. The settlement arrays had their experimental surface on the shaded underside of the plates. This selects for negatively phototactic, positively geotactic larvae of which many of the common fouling invasive species possess. In particular, the larvae of the suite of ascidian NIS present in Canadian Pacific waters have these larval characters (Carver *et al.* 2006; Clarke and Therriault 2007; Daniel and Therriault 2007; McHenry 2005). Light-limited species, such as some macroalgae, would be less likely to settle on the experimental surface of the settlement plates used and as a consequence we did not attempt to identify or report on the presence of algal species on the Petri dishes.

The settlement plates were suspended in the shallow surface waters in order to sample shallow subtidal species and therefore would not effectively capture the presence of deep fouling or benthic species. In fact, the invasive colonial ascidian *Didemnum vexillum* (Kott, 2002) was not detected on any of the settlement plates, despite its known presence in some of the marinas at deeper depths and fouling the benthos. This species is more commonly found on artificial structures within marinas and on compressed sandy bottoms (Daniel and Therriault 2007) though it has occasionally been observed fouling shallow floating docks (CCM personal observation). As a result of these selectivities, the species sampled in the current study are an underestimate of the complete diversity of this ecosystem and likely an underestimate of the total NIS as well. However, it gives us a unique look at the invasion and NIS community present in this community and its variation through space and time.

2.4.2 Spatial Variation

There was high among-site variability in both fouling cover and community composition of NIS. Two sites with high NIS fouling cover also had high NIS species richness and relatively low native fouling cover, suggesting that NIS could be competing for space with native species. In contrast, one site, Institute of Ocean Sciences, had both the highest NIS cover, richness and the second highest native cover which may suggest that this site is more productive and able to support more individuals and species. This site variability may suggest that site productivity may be an important variable governing the quantity of fouling cover in general, presence of NIS, and competition with native species.

At two sites, NIS cover was higher than native species cover, while at the remaining six sites native cover was higher than NIS cover. Sites dominated by NIS cover may experience reduced native settlement because of competition for available space and other resources (Stachowicz *et al.* 2002). The NIS *B. violaceus* was found at all eight sites, suggesting it has become widespread in the Strait of Georgia despite possible environmental differences between sites. In general, some NIS were common across sites while others would be considered rare. Observed spatial variation likely reflects a combination of varying physical conditions, chemical disturbances, biotic interactions, vector traffic (propagule pressure) and age of introduction. Sampled marinas differed in their orientation to currents, degree of flushing, freshwater run-off, and many other physical influences which could cause variation in settlement (Burgin and Hardiman 2011; Floerl 2003). The degree of boating activity, proximity to human settlement, and presence of pollution sources such as industrial activities and fuel docks may affect the water chemistry of the sites (Mack and D'Itri 1973). In addition, many of the sites were proximate to a number of potential NIS vectors, including aquaculture facilities, recreational boat marinas, shipping ports, and research vessels. The effect of these variables on NIS richness will be further investigated in Chapter 6. Lastly, some NIS (*e.g., D. listerianum*) are known for their spatial and temporal variability in population dynamics in other regions (Stachowicz *et al.* 2002).

2.4.3 Temporal Variation

The increased availability of free space in the winter season has implications for larval settlement timing, especially NIS that might have tolerances that allow settlement earlier than co-occurring native species. In North America, colonial ascidians *B. violaceus* and *B. schlosseri* reproduce twice per year, once in spring and a second time in late summer or early autumn (Grosberg 1988; Hewitt 1993; Whitlatch *et al.* 1995). The early cohort would be well-poised to exploit the availability of free space in the spring season. Native mussels and barnacles also have a spring settlement event but the fine-scale timing varies interannually in this region. In some years, at some sites, an early settlement of mussels can swamp all available free space, leaving little available for species competent to settle even a few days later (CCM, pers. obs.). Settlement patterns are species-specific and therefore depend not only on the season but local conditions as well (Caffey 1985; Underwood and Anderson 1994).

Not all NIS settled in the summer months (prior to the fall sampling). At some sites, certain NIS were first detected during the winter sampling period, although the pattern was not consistent for these species across sites. Presence in the winter sampling suggests a fall settlement event or late summer settlement that was not detected until the winter sampling because of size or taxonomic uncertainty which often is increased for smaller individuals. Reproductive and settlement timing is species-specific and some species may have multiple spawning events throughout the year if conditions allow. One site, Gibson's Landing, had relatively little percent cover of all species in the summer months as a result of a high freshwater input at this site. The temporal scale of the current study (four month intervals) does not allow us to investigate the fine details of reproductive timing of NIS versus native species. However, this is an area of future research that is important for understanding regional community dynamics and potential susceptibility to invasions.

Sampled NIS do not completely die off in the winter months in this study area and thus remain in the community to grow and possibly reproduce the following spring. This has been documented for NIS in eastern US where *B. violaceus* and *S. clava* both overwinter as small individuals (Grosberg 1988; Stachowicz *et al.* 2002). The spring sampling in the current study thus resembled a near climax community for this fouling assemblage, with a complex community of understory and canopy fouling species and increasing cover of native species. Adults from the previous year also may act as settlement cues for congeners if gregarious settlement occurs, similar to that documented for species of barnacles and mussels (Burke 1986; Havenhand and Svane 1991). Previously settled species also may provide habitat for subsequent settlers, increasing settlement rate (Bruno and Bertness 2001; Caffey 1985). In some systems, these interspecific relationships may provide an added advantage to incoming NIS species or it may simply increase abundance and/or diversity for all species in the ecosystem, both native and NIS.

2.4.4 Biotic Resistance vs. Biotic Acceptance

The positive relationship between native and NIS richness across the eight sampling sites supports the biotic acceptance hypothesis where sites that are able to support higher numbers of native species also support more NIS (Fridley *et al.* 2007; Leprieur *et al.* 2008). However, the trend of decreasing NIS percent cover with increasing native cover provides some support for the biotic resistance hypothesis. The limiting resource in fouling communities is usually space and so increased resource utilization (space occupation) by native species may prevent successful invasion by NIS. We would expect that measures of space occupation, such as percent cover, would support the biotic resistance hypothesis. This has been demonstrated experimentally using small fouling panels by Stachowicz (1999) where space occupation was directly related to species richness. The Petri dish level of the current study is similar in scale to that used in by Stachowicz (1999) but was conducted at multiple sites and over three time seasons.

The seeming contradiction between richness and percent cover could be attributed to scale (Byers and Noonberg 2003). Small-scale experiments, similar to the scale of the current study (small fouling panels or quadrats), seem to support biotic resistance because the species are interacting directly with one another (Lyons and Schwartz 2001; Naeem *et al.* 2000; Stachowicz *et al.* 1999; Tilman 1997) and this is reflected by the percent cover results. While large scale observational surveys (regional scales or across all sites), capture biotic acceptance because the diversity of microhabitats can support higher diversity of both native and NIS (Lonsdale 1999; Planty-Tabacchi *et al.* 1996; Shea and Chesson 2002; Stohlgren *et al.* 1999). Diversity in other habitats across the study region also support the biotic acceptance hypothesis; recent research in intertidal sandy habitats and seagrass beds showed a positive relationship between NIS and native diversity (Choi 2011; M. Mach, UBC, Vancouver, pers. comm.). High native diversity may provide BC's subtidal fouling community a certain level of protection from the potential impacts caused by some of the same species on the east coast of Canada (Aquaculture Association of Nova Scotia 2005; Locke *et al.* 2007). Nevertheless, the differing invasion history of the two coasts makes prediction of impact difficult. A more explicit experimental test of the influence of native species diversity on invasion processes in the study region is required to disentangle the processes underlying these relationships.

2.4.5 Implications for the Strait of Georgia

The implications for invasion into natural systems and potential impacts on native species remain largely unknown for this region. Of four non-indigenous ascidians tracked, only the colonial ascidians *B. violaceus* and *B. schlosseri* were found to move into nearby natural rocky habitats (Simkanin *et al.* submitted). The two species occurred at much lower densities in natural habitats than that observed on artificial marina structures. The infiltration of other species of NIS into natural ecosystems has not been investigated for this region and further studies are required.

Variability across sites and over time suggests there may be difficulties in prioritizing sites and seasons for monitoring and control effort. It was previously thought that little would settle in the winter months but, at least for the year studied, some NIS settled in the winter or spring at some sites and in some cases this was the only time certain species were detected. A longer term study, although beyond of the scope of this investigation, would be required to quantify variability in seasonal trends. In addition, the variability within species was relatively unexpected. The settlement of some species varied greatly across sites indicating the possible significance of environmental variability. An extreme example, at the Gibson's Landing site everything settled during winter. In the absence of prior baseline information, we cannot however discount the possibility of a new secondary invasion reaching sampled marinas during the duration of our study. Documented invasion histories of NIS in the study region vary widely. As an illustration, *S. clava* is considered a relatively new invader to the region, first detected in Deep Bay in 1998 (Clarke and Therriault 2007). At some sites during our study, it wasn't detected until the spring sampling period. This species is likely still spreading and could have arrived to at least some of the study sites during the study period (< 8 years after initial discovery).

The settlement plates used in this survey mimic the introduction of unprotected surfaces into marina habitats. These surfaces could include boats put into the water without antifouling paint applied, unprotected pilings and floating docks, or surfaces whose antifouling protection has ceased to function effectively. The results suggested that a number of NIS are present in BC marinas and they may become a significant component of marina ecosystems, covering high percentages of the available space and potentially exploiting other resources such as light and food. The presence and abundance of NIS in the Strait of Georgia showed wide variation spatially, temporally and by species, which is largely unstudied. Yet, the high cover of NIS suggests that marinas have the potential to act as both source and sink populations for further invasions into additional sites in the Strait of Georgia, BC in general and/or international waters given the connectivity to Puget Sound, Washington, USA. Studies conducted in New Zealand have shown that marinas can be highly connected by boat traffic and enable the secondary spread of fouling invasive species (Floerl *et al.* 2008; Goldstein *et al.* 2010). Many of the species seen on the settlement plates also were observed on the neighbouring marina structures and boats making recreational boats possible candidate vectors of introduction and subsequent spread of NIS (Ashton *et al.* 2006; Burgin and Hardiman 2011; Darbyson *et al.* 2009b; Davidson *et al.* 2010; Floerl 2002; Johnson and Padilla 1996; Minchin and Sides 2006).

Chapter 3: Recreational boating: a large unregulated vector transporting marine invasive species*

3.1 Introduction

Globally, non-indigenous species (NIS) are introduced intentionally or unintentionally to new locations by a variety of vectors (Elton 1958, Ruiz *et al.* 2000, Wonham *et al.* 2001). Over the past 30 years, efforts to control and manage introductions have focused on traditional and well-studied vectors such as ballast water and aquaculture imports. For example, the ICES Voluntary Code of Practice on the Introductions and Transfers of Marine Organisms in the 1970s reduced the number of species introduced intentionally for aquaculture or other purposes (International Council for the Exploration of the Sea (ICES) 2005). Today, intentional introductions to Canada must undergo a rigorous evaluation under the National Code on Introductions and Transfers of Organisms before approvals are granted (Fisheries & Oceans Canada 2003). Similarly, the International Maritime Organization (IMO) implemented mid-ocean exchange guidelines to reduce introductions by ballast water (International Maritime Organization 2006). More recently, IMO adopted the International Convention for the Control and Management of Ships' Ballast Water and Sediments. This treaty, once it comes in to force, requires the implementation of a ballast water management program for each ship that meets certain standards on the density of propagules (10 viable organisms per cubic metre) in ballast tanks (International Maritime Organization 2004). Despite ongoing debate about the effectiveness of this measure (Sutherland *et al.* 2001, Zhang and Dickman 1999), the intent is clear: by reducing the number of potential propagules the risk of invasion is reduced.

In contrast, a vast number of other vectors remain largely unmanaged. Small recreational watercraft hull fouling may be the largest unregulated vector for the introduction and spread of marine invasive species. Further, current restrictions on antifouling compounds like Tributyl Tin (TBT) are likely increasing the rate of invasion

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via this vector (Evans *et al.* 2000, Lewis *et al.* 2004, Nehring 2001). The IMO ban of the highly effective, and yet extremely toxic, TBT-containing antifouling paints was adopted in 2001 (International Maritime Organization 2010). Following this ban there has been an increase in hull fouling which recently has caused resurgence in the importance of this vector for NIS transport (Fofonoff *et al.* 2003).

Small recreational boats can travel long distances and the relatively low speeds of some boat types makes them ideal vectors for fouling species (Minchin *et al.* 2006). Pleasure craft have been implicated in the introduction of algae including *Undaria pinnatifida* (Farrell and Fletcher 2006, Hay 1990) and *Codium fragile* spp. *tomentosoides* (Bird *et al.* 1993, Carlton and Scanlon 1985, Chapman 1999, Trowbridge 1995) and several mussel species including *Mytilopsis sallei* (Field 1999), *Perna viridis* (Power *et al.* 2004), and *Dreissena polymorpha* and *D. rostriformis bugensis* Andrusov, 1897 (Buchan and Padilla 1999, Johnson *et al.* 2001, Padilla *et al.* 1996). Further, the bryozoans *Waterispora subtorquata* and *Bugula neritina* are cosmopolitan invaders and well-known hull fouling species (Floerl and Inglis 2005). They have a known tolerance to antifouling paint compounds that allows them to facilitate the transport of other invasive species by negating the need to make direct contact with vessel's hull. Species intolerant to antifouling compounds grow on top of *W. subtorquata* colonies that have settled on chemically protected hulls and are subsequently transported on surfaces unavailable to them without the assisting bryozoan (Floerl *et al.* 2004). Mobile species, such as the caprellid amphipod *Caprella mutica,* also have been observed in hull fouling communities and may be transported on small boats if macrofouling species serve as refuge areas (Frey *et al.* 2009).

Research on recreational boats has consistently revealed high variability in the degree of hull fouling present (Ashton *et al.* 2006, James & Hayden 2000 IN Dodgshun *et al.* 2007, Floerl and Inglis 2005). This pattern occurs despite large differences in sampling methods (Ashton *et al.* 2006, Mineur *et al.* 2008) and sometimes low sample sizes (Ashton *et al.* 2006, Darbyson *et al.* 2009b, Mineur *et al.* 2008); but see Floerl and Inglis (2003), Floerl and Inglis (2005) for exceptions. Studies of boat movements, as a proxy for the probability of spread, indicate a significant potential for recreational boaters to act as vectors in North America (Bossenbroek *et al.* 2007, Darbyson *et al.* 2009a, Darbyson *et al.* 2009b, MacIsaac *et al.* 2004), Australia (Floerl and Inglis 2005), and New Zealand (James & Hayden 2000 IN Dodgshun *et al.* 2007, Floerl *et al.* 2005). Previous research has equated NIS presence in hull fouling communities with transport of these species (Ashton *et al.* 2006, Floerl and Inglis 2005). However, stationary boats with high amounts of hull fouling would not pose a risk of NIS transport. Further, boats with little overall fouling but highly fouled niche areas could pose a greater risk than heavily fouled boats if travel frequency is high. Thus, to ascertain the level of risk posed by recreational boats, information on the amount and type of fouling and boat movements is required.

British Columbia's (BC) boating community is the largest in Canada, with an estimated 400,000 boats. In addition, the close proximity to Washington State and known connectivity to other US states on the coast make BC waters susceptible to primary international introductions. Further, southern harbours, such as the highly invaded San Francisco Bay (Cohen and Carlton 1998), may act as source populations for secondary introductions to BC through stepping stone invasions. Pleasure craft or other slower-moving hull fouling vectors (*e.g.*,, barges) are likely vectors for secondary invasions along the west coast of North America (Cohen *et al.* 1998, Cohen *et al.* 2005, Davidson *et al.* 2006, Davidson *et al.* 2008). Hub-and-spoke invasions of coastal marinas also may have occurred after primary introduction to centralized ports like the Port of Vancouver (Levings *et al.* 2004). In BC, as elsewhere, pleasure craft is a popular travel mode to visit pristine areas and protected marine parks; places that could be particularly vulnerable to invasion and that are largely removed from traditional vectors such as commercial shipping or aquaculture. Several NIS, including ascidians, are common in BC marinas and often dominate fouling communities (Chapter 2). Tolerance of these invasive species to varying environmental conditions suggests that much of BC's marine habitat could be at risk of invasion (Epelbaum *et al.* 2009, Therriault and Herborg 2008) but the role of specific vectors, including movements of BC boaters, have not yet been fully investigated (but see Herborg *et al.* 2008 for an exception).

The goal of the current study is to investigate the recreational boating vector in a previously unstudied temperate, Northern hemisphere system with high boating activity and characterize the complement of NIS being transported by recreational boating activities. In order to evaluate this vector, we pose three research questions: 1) Are NIS present in hull fouling communities of BC boats? 2) Are any of these NIS considered high-risk species with a history of invasion and impacts in other regions? and 3) Do fouled boats have travel or maintenance characteristics that make it likely they would transport hull fouling species?

3.2 Methods

3.2.1 Dive Survey

Hull fouling on recreational boats was surveyed using SCUBA at 24 marinas along the coast of BC (Figure 3.1) in two consecutive summers (2008-2009) with 10-30 boats examined and photographed at each marina. The first boat surveyed at each marina was selected randomly but sequential boats along the dock were surveyed to reduce the risk to divers from boat traffic. A checklist was developed that included 12 species of known NIS in BC waters, and six species of potential invaders noted from elsewhere (Table 3.1). Non-indigenous mussels *Mytilus galloprovincialis* and *Mytilus edulis* (Linnaeus, 1758) have hybridized with native *Mytilus trossulus* (Gould, 1850) making them indistinguishable without the aid of genetic testing. Thus, all *Mytilus* are grouped together as *Mytilus* spp. in this study and treated as cryptogenic. Species nomenclature was based on Carlton (2007) and references therein. For each boat, one diver searched the entire boat for known NIS and noted their presence on the checklist. The presence of general fouling taxa, such as barnacles, erect and encrusting bryozoans, and macroalgae, also was recorded. The second diver photographed (minimum of 10 photos per boat) the submerged surfaces of each boat including six replicate randomly-selected hull photos and one of each niche area (non-hull area) including the propeller, shaft, keel, vents, and water intakes. The field of view was standardized using a fixed 30.5 cm by 30.5 cm quadrat attached to the camera. Temperature and salinity data were recorded from each site using a hand-held YSI instrument (Xylem Brand).

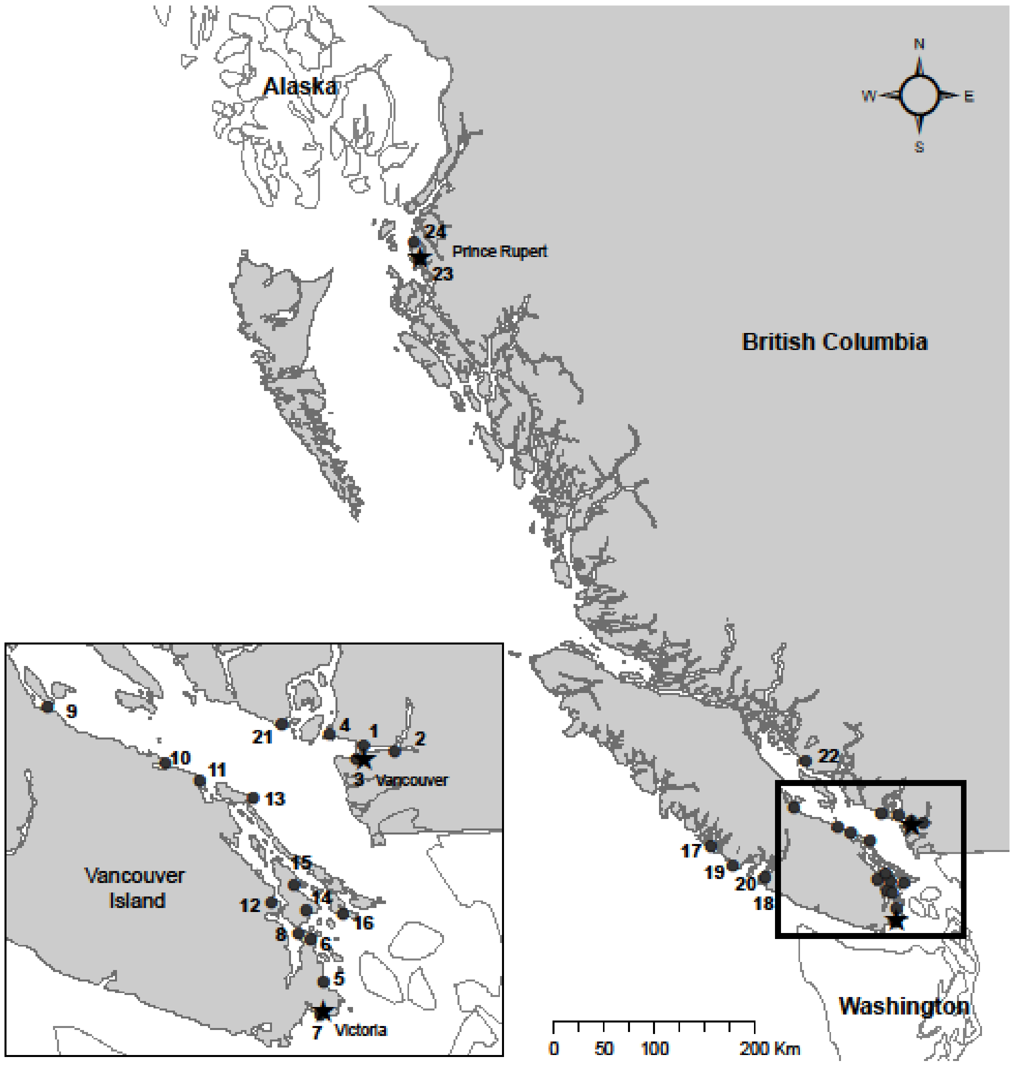

Figure 3.1 Map of British Columbia showing 24 marina study sites (black dots) sampled during the dive survey; stars mark major cities. Inset map shows close up of marinas sampled in Vancouver and on Southern Vancouver Island. Marina codes: 1 - Burrard Yacht Club, 2 - Reed Point Marina, 3 - Royal Vancouver Yacht Club Jericho, 4 - West Vancouver Yacht Club, 5 - Royal Victoria Yacht Club, 6 - Sidney North Saanich, 7 - Victoria Causeway Marina, 8 – Institute of Ocean Sciences, 9 - Deep Bay Marina, 10 - Fairwinds Nanoose Bay, 11 - Nanaimo Yacht Club, 12 - Maple Bay Marina, 13 - Silva Bay Gabriola, 14 - Fulford Harbour Saltspring, 15 - Saltspring Marina, 16 - Poet's Cove Pender Island, 17 - Tofino Fisherman's Wharf, 18 - Hawkeye Bamfield, 19 - Ucluelet Hemlock Basin, 20 - Poett Nook Bamfield, 21 - Gibson's Landing Marina, 22 - Powell River Marina, 23 - Rushbrooke Harbour, 24 - Port Edward Harbour.

Samples of species with uncertain identifications were collected and identified in the laboratory. A sub-sample of all surveyed boats was subjected to image analysis (N=207). Photographs were digitally overlaid with a 100-point grid to estimate percent cover overall and functional group or species, where possible. Image analysis was performed using Image J software (developed by Wayne Rasband, National Institutes of Health, Bethesda, Maryland, USA).

3.2.2 Boater Questionnaire

Concurrent with the dive survey, a boater questionnaire was distributed during boating outreach events and left on each boat examined as part of the dive survey, with a prepaid return envelope provided. The questionnaire consisted of three sections, asking about the boat, its antifouling practices, and its travel history (12 questions in total, see Appendix B). Boaters were asked to report their travel history for the previous 12 months and check off places they had visited from a list of 104 BC destinations or listing additional locations. They also were asked which trip types they undertook in the last 12 months: local or day trips (out and back to home marina in a single day), racing (trips made for the purpose of racing the boat), weekenders (trips of a few days duration visiting one or two different moorages), long trips (long haul travel to destinations further away, once there remain in a single moorage the entire time), and tours (long trips with multiple destinations along the way, staying in each moorage for only a few nights). Some respondents indicated ocean-crossing journeys (oceanic) in the other trip category and these were added to the data analysis as a separate category. The questionnaire was approved by University of British Columbia's Behavioural Research Ethics Board (Approval #H08-00967).

Table 3.1: Non-indigenous species (NIS) actively searched for during dive survey, percentage of boats with NIS attached or entangled, and number of marinas where they were found on boats. Asterisks (*) indicate species not known to occur in BC.

Species	**Common name**	**% boats**	**# marinas**
Mytilus spp.	Mussel	59.3	23
Styela clava	Clubbed tunicate	20.0	9
Botryllus schlosseri	Golden star tunicate	10.4	12
Botrylloides violaceus	Violet tunicate	9.8	15
Schizoporella japonica (=unicornis)	Horned bryozoan	9.0	13
Sargassum muticum	Japanese wireweed	7.5	10
Diplosoma listerianum	Colonial tunicate	3.1	6
Halichondria bowerbanki	Yellow sponge	1.6	8
Molgula manhattensis	Solitary tunicate	1.0	1
Didemnum vexillum	Colonial tunicate	0	0
Crassostrea gigas	Pacific oyster	0	0
Diadumene lineata	Orange-striped anemone	0	0
Musculista senhousia	Asian date mussel	0	0
*Clathria prolifera**	Red beard sponge	0	0
*Ciona intestinalis**	Vase tunicate	0	0
*Dreissena polymorpha**	Zebra mussel	0	0
*Caulerpa taxifolia**	Killer alga	0	0
*Undaria pinnatifida**	Kelp	0	0

3.2.3 Data Analysis

Mean percent cover of macrofouling for each boat was calculated by averaging percent cover of replicate quadrat measurements. Given expected differences between niche and hull locations, quadrat photographs from these different areas were averaged separately to calculate hull only and niche only means. Percent cover data was arcsine square root transformed to meet assumptions of normality and homogeneity of variance. Where transformation did not improve homogeneity, non-parametric analyses were performed. To assess the difference in fouling between hull and niche areas of the boat, Wilcoxon signed rank test was performed. To examine the variation in percent cover between marinas, a Kruskal Wallis test was used. Boater questionnaire variables were highly skewed so non-parametric statistical analyses were performed. The relationship between age of antifouling paint and travel type was examined using Spearman's rank correlation. To examine whether fouled boats move, Spearman's rank correlation analyses were performed on percent cover and age of antifouling paint (proxy for susceptibility to fouling colonization) and on the number of places visited in the last 12 months. All data analysis was performed using SPSS Version 10.0 (SPSS Inc.).

3.3 Results

3.3.1 Dive Survey

In total, 491 boats were surveyed with 65.7% having macrofouling species attached to the hull and/or niche areas. Over a quarter of boats surveyed (25.7%) were fouled with one or more NIS. The cryptogenic species complex, *Mytilus* spp. was present on 59.3% of boats surveyed, had the highest percent cover per boat, and was encountered on boats in 23 of 24 marinas.

Nine NIS were attached or entangled on the hull and niche areas of the boats surveyed. The *Mytilus* complex was encountered on almost 60% of the boats surveyed; however, we chose not to include *Mytilus* in further comparisons because of our inability to confirm the presence of invasive genotypes in this complex. The next most common NIS observed on surveyed boats was the solitary ascidian *Styela clava*, followed by the colonial ascidians *Botryllus schlosseri* and *Botrylloides violaceus* (Table 3.1). The NIS with the highest percent cover per boat were *Schizoporella japonica* and *Diplosoma listerianum* (Figure 3.2). The NIS found on boats at the most marinas surveyed were *B. violaceus* and *S. japonica* (Table 3.1). For all NIS, mean percent cover was 0.583 (+/- 0.138 SE). The invasive caprellid *Caprella mutica* was found incidentally in samples taken to the lab for identification of other species but since it is not possible to identify this species underwater, it was not surveyed quantitatively. Five fouling species known to be present in BC were not detected in our surveys including the ascidians *Didemnum vexillum* and *Ciona savignyi*, the bivalves *Crassostrea gigas* and *Musculista senhousia*, and the cnidarian *Diadumene lineata*.

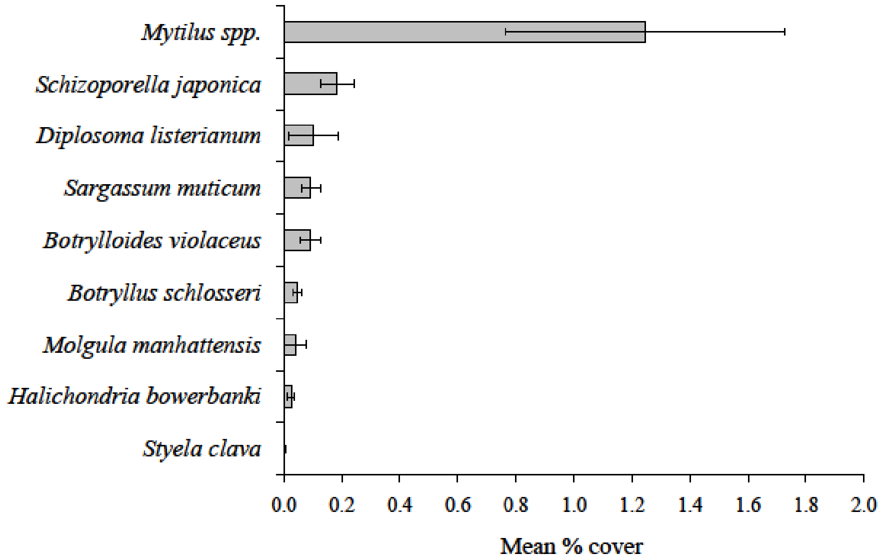

Figure 3.2 Mean percent cover of cryptogenic and non-indigenous species encountered on both hull and niche areas of boats examined during the dive survey. Error bars depict standard error.

Across all marinas, boats surveyed had a mean percent cover of 6.1 (+/- 0.84 SE) macrofouling that ranged from 0 to 79.78%. Seventy percent of boats surveyed had less than 5% macrofouling coverage, and niche areas (12.5%, +/- 1.34 SE) had significantly more macrofouling than hulls (1.2%, +/- 0.76 SE) (Wilcoxon Signed Ranks Z=-9.882, $p<0.001$). Percent cover of macrofouling differed significantly among marinas (Kruskall Wallis χ^2=39.521, df=22, p=0.012). The three marinas with the highest average fouling were Gibson's Landing on the Sunshine Coast (#21), Rushbrooke Harbour in the North Coast (#23), and Burrard Yacht Club, Vancouver (#1) (Figure 3.3). These also were the marinas with the highest variability in percentage cover. Three marinas, namely West Vancouver Yacht Club (#4), Institute of Ocean Sciences, Sidney (#8) and Poett Nook Bamfield (#20), had close to zero fouling (Figure 3.3).

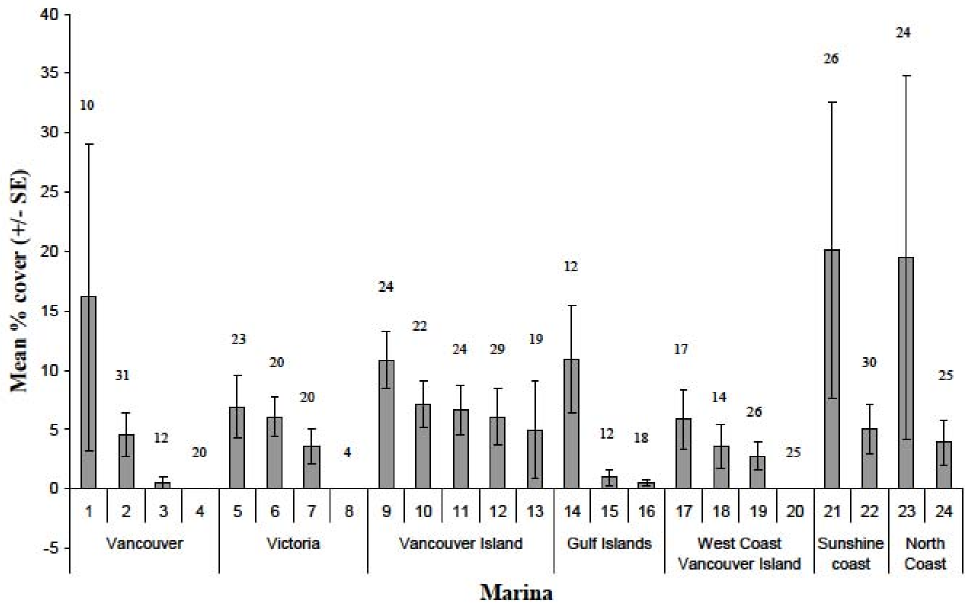

Figure 3.3 Percent cover (+/- standard error) of macrofouling on boats surveyed by marina, grouped by region. Marina numbers correspond to locations on Figure 1. Numbers above the bars represent the sample size for each marina. Marina codes: 1 - Burrard Yacht Club, 2 - Reed Point Marina, 3 - Royal Vancouver Yacht Club Jericho, 4 - West Vancouver Yacht Club, 5 - Royal Victoria Yacht Club, 6 - Sidney North Saanich, 7 - Victoria Causeway Marina, 8 – Institute of Ocean Sciences, 9 - Deep Bay Marina, 10 - Fairwinds Nanoose Bay, 11 - Nanaimo Yacht Club, 12 - Maple Bay Marina, 13 - Silva Bay Gabriola, 14 - Fulford Harbour Saltspring, 15 - Saltspring Marina, 16 - Poet's Cove Pender Island, 17 - Tofino Fisherman's Wharf, 18 - Hawkeye Bamfield, 19 - Ucluelet Hemlock Basin, 20 - Poett Nook Bamfield, 21 - Gibson's Landing Marina, 22 - Powell River Marina, 23 - Rushbrooke Harbour, 24 - Port Edward Harbour.

3.3.2 Boater Questionnaire

In total, 616 completed questionnaires were returned, with 164 of these from boats sampled during the SCUBA survey. The majority of respondents were Canadian residents (93.3%) and most were from BC (90.6%). There were slightly more powerboat (51.8%) than sailboat (42.5%) respondents and the average vessel length was 30.9 feet. Most respondents' home marinas were in southern BC and Washington State, reflecting a combination of population density and survey effort. Because survey effort was focused in marinas (rather than boat ramps) the majority of respondents had boats stored in water year-round (73.2%). Some respondents stored their boat in the water part of the year (9.3%), while the remainder trailered their boat between sites (13.8%).

Respondents had a high probability of utilizing antifouling practices. Most (80.5%) used antifouling paint and an even higher percentage used manual cleaning (83.3%). Manual cleaning often was used in combination with antifouling paint application (69.4%). Those boats reporting manual cleaning alone were those that trailered their boats overland and scrubbed their boats between uses. The age of antifouling paint ranged from 0 to 130 months, with a mean of 15.3 months. Maintenance typically was performed on dry land (52.8%), but cleaning also occurred in water (19.7%), in dry dock (12.8%) or on tidal grids (3.6%). One or more manual cleaning techniques were reported, including power-washing (60.8%), scrubbing (50.2%), scraping (24.1%), and a range of other alternatives.

Respondents exhibited a range of travel behaviours. The most common was local or day trips, followed by weekend trips and tours (Figure 3.4). The average amount of time boaters spent moored in marinas outside their home marina was 17.6 days, with a range from 0 to 330 days. Relatively few boats were never moored outside their home marina (~13%). Out of 104 possible travel destinations in BC, boaters traveled to an average of 8.3 destinations. The maximum number of places reported visited was 86. Roughly one-fifth of respondents had traveled to the US in the past 12 months (20.9%); most had traveled to Washington State, whose marine waters are contiguous with those of BC.

Antifouling practices varied among trip types, with boaters reporting racing trips the most likely to employ antifouling paint (97.8%) and manual cleaning (86.0%). Boaters reporting local trips had a slightly lower probability of using antifouling paint (81.3%) but similar manual cleaning rates (86.0%). Boaters that traveled frequently had newer antifouling paint; there was a significant negative correlation between age of antifouling paint and number of places visited, though the relationship was weak (Spearman's rho = -0.124, $p = 0.006$, r^2=0.014).

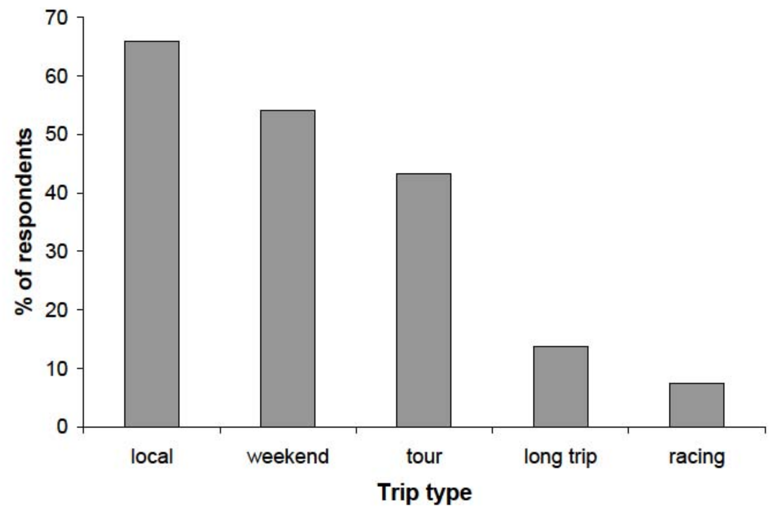

Figure 3.4 Percentage of respondents undertaking various trip types within the last twelve months.

3.3.3 Comparing Dive-Questionnaire Results

There were 164 boats with both dive surveys and questionnaires. Heavily fouled boats were under-represented in the questionnaire results – only 2 boats with macrofouling greater than 40% returned questionnaires while the dive survey sampled 33 heavily fouled boats (6.8% of the sample population). As a result, two heavily fouled boats appear as statistical outliers (percent cover >2 standard deviations from the mean) and were removed from correlation analyses (not included in regression) but are shown in Figure 5 as they represent a valid segment of boaters. The influence of antifouling paint age on percent macrofouling cover on boats surveyed varied by hull area (Figure 3.5). Age of antifouling paint was most strongly related to percent cover on the hull (Spearman's rho = 0.196, p=0.014, r^2=0.0999). There was a weaker, yet significant, relationship with overall percent cover (Spearman's rho = 0.258, p=0.001, r^2=0.1345) and no significant relationship was observed between niche fouling and antifouling paint age.

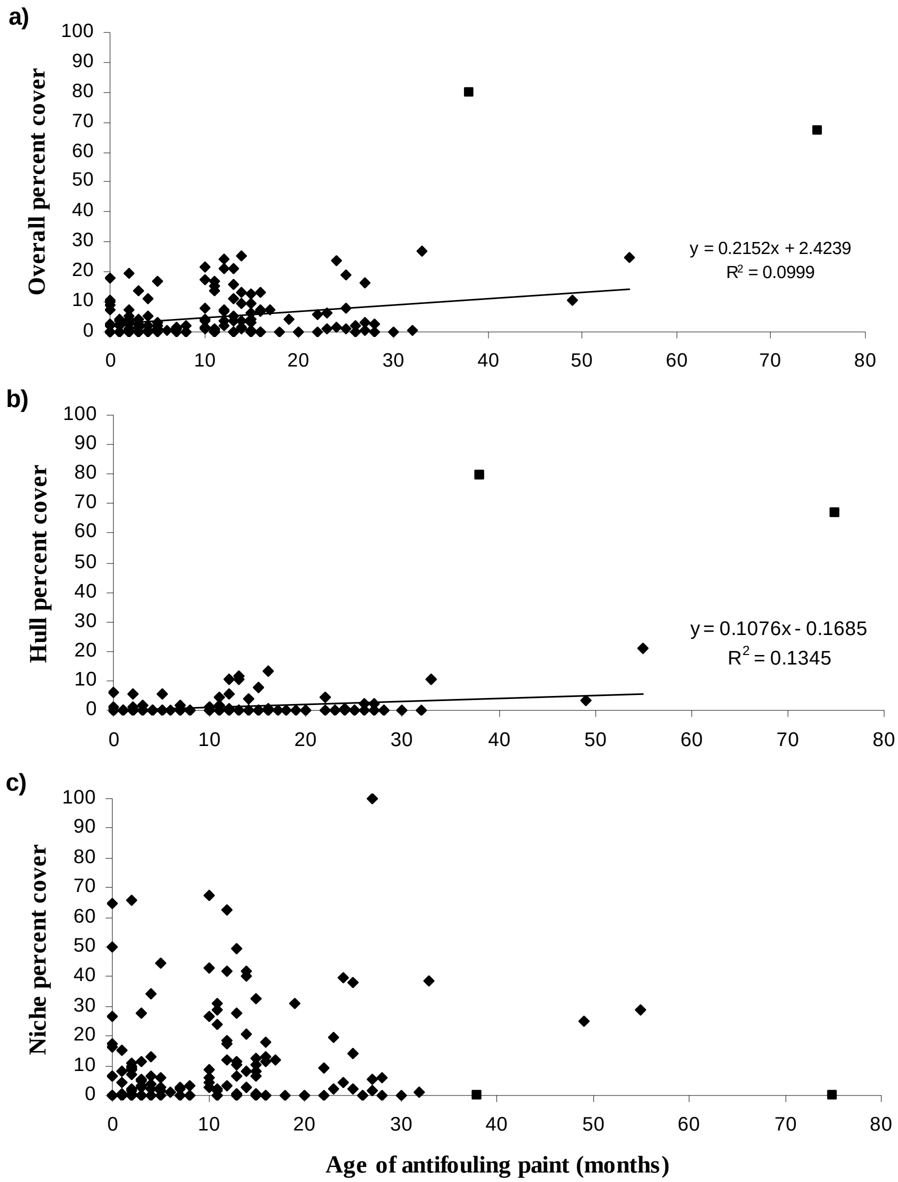

Figure 3.5 Relationship between age of antifouling paint (months) and a) overall percent cover, b) hull percent cover and c) niche percent cover. Diamonds represent individual boats, squares are statistical outlier points (percent cover >2 standard deviations from the mean) plotted but not part of regression fit line.

To assess whether fouled boats were traveling, we examined the relationship between percent cover and the number of places visited. In general, boats traveling to the most places had less fouling overall (r^2= 0.048) and on the hull alone (r^2= 0.0471), though the slopes were not significant. Fouling of niche areas had no relationship with traveling. For the subset of boats that were infected with NIS (N=44), most visited less than ten places in the previous twelve months (63.7%), though a small fraction (11.4%) could be considered frequent travelers making > 20 trips per year. Two of the three marinas with highest fouling cover (Gibson's Landing and Rushbrooke Harbour) had older antifouling paint on average (24.8 and 17.2 months respectively) and low number of visits or voyages (4.0 and 3.6 respectively. Burrard Yacht Club was the exception; it had high fouling cover but low age of antifouling paint (4.0 months) and high number of visits (20.3). The marinas with lowest fouling cover had mixed maintenance and travel characteristics. West Vancouver Yacht Club had medium aged antifouling paint (10.0 months) and a moderate number of visits in the previous 12 months (8.0). Poett Nook Bamfield had both low age of antifouling paint and low number of visits.

3.4 Discussion

3.4.1 Are Boats Fouled with NIS?

The threat of invasive species transport by recreational boats in northern temperate marine waters appears to be high. Our results demonstrate that NIS are present in the hull fouling communities of BC's recreational boats and that prevalence can be high with over one-quarter of the boating community fouled by NIS. The incidence of NIS was much higher than previously observed in Atlantic Canada (Darbyson *et al.* 2009b, Locke *et al.* 2007), the Great Lakes region (Johnson *et al.* 2001), or Europe (Mineur *et al.* 2008). Two-thirds of surveyed boats had macrofouling present, suggesting that boats are both susceptible to colonization by fouling species and potentially able to transport them between marinas. The proportion of boats examined with macrofouling present was much higher than previous studies in Scotland (59%) (Ashton *et al.* 2006), Italy (40%), and New Zealand (14.5%) (reviewed in Minchin *et al.* 2006), although all 70 boats examined in a northern Australian study were fouled (Floerl 2002). Fouling levels observed as measured by percent cover were similar to that of Australia and New Zealand (Floerl and Inglis 2003, Floerl and Inglis 2005). Although the magnitude of percent cover might appear small the actual introduction threat could be quite large. For illustrative purposes, if we consider the average boat size in the survey, a 9.1m sailboat with wetted surface area of ~84m^2, approximately 0.5m^2 would be covered by NIS not including mussels. Coupled with the high number of boats in BC, this indicates a level of propagule pressure similar to other regions where pleasure craft NIS introductions are believed to be high (Davidson *et al.* 2010; Floerl 2002; Floerl *et al.* 2005; Minchin *et al.* 2006).

Macrofouling cover varied both by marina and region of the coast surveyed and there was no relationship between fouling and environmental variables measured (temperature and salinity). The three marinas with boats having the highest percent cover were in different regions of the coast, confirming the observed high variability in fouling occurs across regions. This variability can be attributed to either differences in boater behaviours or variation in the community composition and/or fouling rate between marinas. If this variation were a result of boater behaviours we would expect that marinas with high boat-fouling would stem from the presence of boats with considerable fouling and either inactivity (low number of visits), older antifouling paint, or both. This holds true in some marinas; both Gibson's Landing and Rushbrooke Harbour had boats with older antifouling paint (24.8 and 17.2 months respectively) and low activity (4 and 3.6 visits in the previous year). In contrast, Burrard Yacht Club had boats with new antifouling paint and high activity, but contrary to expectations this marina also had high fouling. Boats in Poett Nook in Bamfield had very low fouling and new antifouling paint but had low number of visits. This marina community was unique in that boaters trailered their boat overland to the marina and then stayed for the summer fishing season making only day trips out to local fishing grounds. Their boats were stored on land over the winter months and therefore had low fouling and NIS presence suggesting they represent a relatively low risk of NIS spread. West Vancouver Yacht Club had low fouling but mid-level activity levels and antifouling paint protection. The varying results suggest that boater behaviours are not the sole variables responsible for variability among marinas. Anecdotal information from boaters suggests that some marinas have higher fouling rates than others which could be the result of community composition or environmental variables such as temperature, salinity and water flow.

Non-indigenous species observed on surveyed boats included examples of high-risk invasive organisms. The ascidians *Styela clava* and *Botrylloides violaceus* have caused severe impacts on shellfish aquaculture in eastern Canada (Darbyson *et al.* 2009a, Locke *et al.* 2007). Both species are native to the western North Pacific region. *Styela clava* was first reported from Newport Bay, California in 1933 (Abbott and Johnson 1972) and its original introduction has been attributed to live oyster imports for the aquaculture industry (Cohen 2005). It subsequently invaded Oregon and Washington and the earliest report in British Columbia was from Nanaimo in 1994 (Lambert and Lambert 1998). It remains unclear whether the BC introduction also resulted from live Pacific oyster importation or as a secondary stepping-stone introduction from southern populations. The first definitive west coast record of *Botrylloides violaceus* was in 1973 for San Francisco Bay (Cohen and Carlton 1995), though it was likely introduced much earlier but went undetected because of its similarity to the native California species *B. diegensis*. The first BC report for *B. violaceus* was in 1993 in marinas on the southern coast of Vancouver Island (Lambert and Lambert 1998) but again, this species could have been introduced much earlier.

Five of the nine NIS observed on boats in our study were ascidians. *Botryllus schlosseri* is native to the Mediterranean Sea (Berrill 1950). It is common in BC marinas but there have been no reports of negative impacts on shellfish aquaculture (Carver *et al.* 2006). Similarly, *Diplosoma listerianum* is native to Europe and although it is a common fouling species in marinas in California, little is known of its possible vectors or impacts. The solitary ascidian *Molgula manhattensis* was only found in a single marina in Prince Rupert, a small fishing community in northern BC with a recently constructed container port and active cruise ship terminal. This species is native to the western North Atlantic but the introduction vector is uncertain with hull fouling, oyster translocations, and possibly ballast water having been suggested (Cohen and Carlton 1995, Hewitt *et al.* 2004). Non-indigenous ascidians were often observed together (both within marinas and on boats) suggesting that they are being transported by similar vectors or ascidians are facilitating transport and establishment of other ascidians. These hypotheses are not necessarily mutually exclusive. The invasive bryozoan *Watersipora subtorquata* has chemical tolerances that allow it to settle early on copper-based antifouling paints, promoting its own transport as well as other species that settle on the bryozoan (Dafforn *et al.*, 2008, Floerl *et al.*, 2004) but it is unknown whether ascidians have similar chemical tolerances (Dafforn *et al.* 2008, Floerl *et al.* 2004).

The well-known ascidian invader *Didemnum vexillum* has been observed in BC marinas and harbours but was not detected fouling boats in the current study. This suggests that the vector of spread for this species in BC could be something other than recreational boating. However, additional research would be required to confirm *D. vexillum* is not able to utilize this vector. A species of *Didemnum* was observed fouling small boats in Ireland (Minchin and Sides 2006) but the complex taxonomy of this genus makes it difficult to determine if this is the same species. Previous studies have linked *D. vexillum* introductions to slow-moving barges (*e.g.,* Coutts 2002; Bullard *et al.* 2007) and this has been hypothesised as one of vectors for its introduction and spread in BC (Herborg *et al.* 2008).

Hull-fouling NIS observed were not always attached directly to boat surfaces; entanglement and refuge species also were sampled. Mineur *et al.* (2008) reported the marine alga *Sargassum muticum* (Fensholt, 1955) often entangled on the propeller or propeller shaft; a finding supported by our study. This species originated in the western North Pacific and was likely first introduced with live oysters imported for aquaculture activities (Quayle 1988). It is now widespread in British Columbia (White and Shurin 2007) and results of the current study suggest that its spread could be attributed to recreational boating. Plants could become entangled and transported to other locations, where dispersal occurs through fragmentation with propeller wash or maintenance activities.

The caprellid amphipod, *Caprella mutica,* recently recorded from British Columbia was collected incidentally in samples from hull fouling communities. This species is considered invasive, forming high densities and excluding native caprellid species (Ashton *et al.* 2007). It is unknown whether this mobile species is transported on boats.

However, it also has been sampled in commercial ships' sea chests (Frey *et al.* 2009) and it potentially seeks refuge in niche areas or amongst more complex hull fouling communities during transport.

There is relatively little information about the vectors and possible impacts of *Schizoporella japonica (=unicornis)* and *Halichondria bowerbanki*. The encrusting bryozoan *S. japonica* was fairly common in hull fouling communities and represented the highest percent cover per boat (after cryptogenic *Mytilus* spp.). This species is of Asian origin and believed to have been originally introduced to North America with oyster products (Carlton 1989). Its propensity for hull fouling lends to comparisons with the introduced bryozoan, *Watersipora subtorquata* and further study may reveal similar chemical tolerances for antifouling paint compounds. The sponge *H. bowerbanki* is native to the North Atlantic (Levings *et al.* 2002) and its original vector of introduction to North America also was attributed to oyster culture (Carlton 1989). Although it was the most common sponge species observed on boats and within marinas, there is little information regarding possible commercial or ecosystem impacts.

Cryptogenic mussels (*Mytilus* species complex) were found on a majority of boats examined, even those traveling frequently and/or long distances. On traveling boats, small *Mytilus* were found mainly on unpainted (and thus unprotected) niche areas, such as the crevice between the motor mount and the hull. These results suggest that this region is at substantial risk for introduction of notorious mussel invaders, like quagga mussels (*Dreissena rostriformis bugensis*) or zebra mussels (*Dreissena polymorpha*) now confirmed in California and reported in Washington State (Whittier *et al.* 2008).

As discussed earlier, five of the nine NIS species detected in this survey were likely introduced originally with the live oyster trade in the early 1900s. For the remaining species we do not have enough evidence to hypothesize about their original vectors of introduction. The high occurrence of these species on small boats leads us to the general conclusion that fouling of recreational boats is likely the major vector responsible for their regional spread today. Interpretation of the temporal and spatial patterns of introduction in this temperate marine region is confounded by close proximity to infected harbours further south. Most invasions were noted first in southern BC, which is the most populated area and experiences higher vector traffic. This area is both close to international shipping ports and historically experienced invasions via aquaculture introductions. Therefore, it is difficult to separate primary introductions direct from the western Pacific from secondary introductions from invaded southern harbours but recent analyses are helping to clarify some of these patterns. For example, genetic studies have been used to examine the invasion patterns of two botryllid ascidians in Canada (Lejeusne *et al.* 2011) and *B. schlosseri* in California (Stoner *et al.* 2002).

3.4.2 Do Fouled Boats Move?

It is important to distinguish between the presence of NIS on boats and the movement of these species. Previous studies have linked presence of NIS in hull fouling communities to transport, often without sufficient evidence that the species are actually carried to other destinations (Floerl and Inglis 2005, Mineur *et al.* 2008). A boat fouled with an invasive species may not necessarily move to other locations. In the current study, sedentary boats that had not moved in years had significant fouling, with often greater than 70% cover and usually more than one NIS present. Heavily fouled boats seen in the dive survey were underrepresented in questionnaire returns, as their owners probably do not use their boats often and thus would not find the questionnaires left for them. Although we cannot make predictions about the travel and maintenance patterns of heavily fouled boats based on available data, it is unlikely that they would be readily able to move any significant distance or even start their propellers if they have been neglected for a considerable period of time. Our results showed that traveling boats had lower fouling percent cover (albeit with high variability). Furthermore, boats infected with one or more known NIS were reportedly traveling to as many as 45 locations (or "destinations"). Although transoceanic boats were rare in the study, the few surveyed had small amounts of native barnacles attached in niche areas. This demonstrates that even traveling boats are both fouled and carry NIS, and as a consequence may act as potential vectors for NIS introduction and spread.

The final stage in the invasion process, colonization and establishment in new marinas has not been quantified in the current study. Upon arrival, transported NIS may be sloughed from boats and dislodged fragments re-grow or individuals on the boats may release gametes that successfully colonize the new habitat. Few studies address this part of the invasion process but regardless, the widespread occurrence of NIS in BC means that colonization and establishment occurs with some frequency. Further research is required to examine the connectedness of invaded marinas in BC and pinpoint hot spots of boater movements and possible hubs in the invasion process. These hubs could then be targeted for directed monitoring and management activities. These hypotheses are considered in further detail in Chapter 6.

Although percent cover was relatively low on average, niche areas hosted a disproportionately greater amount of fouling, as much as ten times the fouling cover observed on hull areas. Niche areas of commercial ships are susceptible to fouling because these areas often are overlooked or difficult to access during antifouling paint application (Coutts and Taylor 2004) and the same seems to be true for recreational boats. For example, boaters indicated that sailboats rest on the keel during painting on land and a single coat of antifouling paint is quickly applied to the keel as the boat is launched. Thus, the underside of the keel is inadequately protected and this area was often found fouled during the survey (CCM pers. obs.). Piola and Johnston (2008) demonstrated that even small areas without antifouling protection can become heavily fouled. It appears that niche areas become vulnerable to colonization long before the rest of the vessel and the lack of relationship between age of antifouling paint application and niche percent cover supports this conclusion. Other potential variables responsible for biofouling include other maintenance activities, boat activity patterns, and voyage characteristics, in addition to numerous environmental variables. However, previous studies consistently have shown that age of antifouling paint is the most consistent predictor of level of fouling on small boats (Floerl *et al.* 2005, Ashton *et al.* 2006); a finding that appears to extend to temperate marine waters.

In addition to lack of antifouling paint, niche areas may experience altered water velocities thereby promoting larval settlement and reducing dislodgement during voyages. Reduction in water velocity also may affect the performance of those antifouling paints that require water flow in order to release biocides. In contrast, niche areas that protrude into water flow may be exposed to higher levels of drag, wearing off antifouling paints faster than in other parts of the boat. Similar to dry docking support strips in large commercial ships (Coutts and Taylor 2004), niche areas of recreational boats may represent the transport mechanism for long distance introductions of hull fouling invaders. Future studies should incorporate a random stratified survey design in order to capture the fouling of niche areas. This is because if fouling is present, even in small patches, it will occur in niche areas prior to hull surfaces. In order to decrease movement of NIS, information outreach should target boaters to encourage them to increase antifouling protection of niche areas. In addition, vector inspections should specifically target niche areas to increase chances of detecting hull fouling invasive species.

The habits of the marine boating community in BC showed high variability in both trip type and frequency. A high percentage of the boating community used antifouling paint in conjunction with manual cleaning to prevent fouling; however two-thirds of the boats surveyed still had macrofouling species present. This suggests that current protection practices are insufficient to prevent the transport of NIS. Efforts to remove fouling from niche areas and more frequent reapplication of antifouling paint (within manufacturers' recommended limits) may reduce the fouling of boats in the BC community. Although boaters take steps to reduce fouling in order to improve fuel efficiency and speed (Minchin *et al.* 2006), this goal aligns conveniently with the need to reduce colonization by fouling NIS. However, since niche areas are often overlooked, because they do not affect the boat performance, public outreach is needed to elevate boaters' awareness on the issue of NIS transport.

3.5.1 Conclusions

This study demonstrated that NIS are both present on recreational boats and, perhaps more importantly, traveling on boats in British Columbia suggesting the risk posed to other temperate marine ecosystems could be high. Within the boating community we confirmed nine non-indigenous species, some of which are considered highly invasive, and many of these boats were visiting multiple marinas. Thus, the risk of spread of marine NIS in BC should be considered very high. Many of the NIS observed in hull fouling communities were likely introduced originally with live trade associated with Pacific oyster aquaculture. However, the current study provides evidence that the secondary spread of these species can likely be attributed to the recreational boating vector both in BC and in other regions as well. Fouling of niche areas is the most probable mechanism of introduction and spread as percent cover was not related to travel frequency or antifouling paint age. Transport may not be restricted to short distances as non-indigenous ascidians *Botrylloides violaceus* and *Molgula manhattensis* were found in marinas as far north as Prince Rupert. Boats undertaking frequent or long distance travel still had fouling on niche areas suggesting this region is at continued risk of primary introductions via recreational boats. In contrast to other historically important vectors such as shipping and aquaculture, there are no management actions in place today aimed at limiting introduction and spread by the recreational boating vector. Boating activities are on the rise worldwide, both in terms of number of boats, number of marinas, and connections between marinas, elevating the probability that NIS will be transported through this pathway into an increasing number of habitats, regions, and possibly countries.

Chapter 4: Rapid assessment of fouling on individual pleasure craft*

4.1 Introduction

Hull fouling of commercial and recreational vessels is a significant vector responsible for the re-distribution of aquatic invasive species worldwide (e.g., Clarke Murray *et al.* 2011; Coutts and Taylor 2004; Davidson *et al.* 2008; Fofonoff *et al.* 2003; Godwin 2003; Gollasch 2002; Minchin and Gollasch 2003; Wonham and Carlton 2005). Assessment of vessel biofouling risk has become a priority for many countries and government agencies (e.g., Coutts and Taylor 2004; Hayes and Hewitt 2000; Hayes 2002, 2003; Hewitt *et al.* 2009; Hewitt and Campbell 2010; Piola and Conwell 2010). To be effective in evaluating the risk presented by a vector and stopping the influx of invasive species, early warning rapid assessment tools must be a careful balance of resource use and tool precision. Rapidly assigned predictions would facilitate detailed inspection, the quarantine of individual vessels, and removal of possible non-indigenous species (NIS) from the vessel before they have a chance to establish and spread. Customs inspections commonly are used to prevent terrestrial introductions but comparable tools are not widespread in the marine realm.

In general, dockside assessments (or Rapid Assessment Surveys) are common for invasive species rapid assessments (Campbell *et al.* 2007). Subtidal NIS surveys often are conducted from the dock alone, without accompanying underwater surveys (Cohen *et al.* 1998; Cohen *et al.* 2005; Lu *et al.* 2007; Pederson *et al.* 2005). A study on NIS tunicates in Washington State suggested that dockside species surveys are similar in precision to underwater assessments of floating docks, with a significant savings in resources (Grey 2009). However, dockside assessments may not capture fouling in the entire three-dimensional marina environment or that occurring on recreational boats.

* A version of this chapter has been submitted for publication as a manuscript entitled "Rapid assessment of fouling on individual pleasure craft" with co-authors Thomas Therriault and Evgeny Pakhomov

Further, the presence of macrofouling has been used as a proxy for the threat of invasive species transport. A vessel with macrofouling present is insufficiently protected from fouling settlement and could be infected by NIS if propagules were encountered. To assess the recreational boating vector in Australia, Floerl (2002) developed a rapid assessment tool, commonly referred to as the Level of Fouling (LoF) index (Table 1). This index is a rank scale of the level of hull fouling based on observations of a vessel from the dock. The tool originally was calibrated against randomly placed photographic quadrats using a camera apparatus attached to a pole ("polecam") and results showed that the index was highly accurate for predicting the level of fouling. During more extensive trials on international yachts in New Zealand the LoF index correctly identified boats with fouling on the hull for 60% of those surveyed (Floerl *et al.* 2005a). This index has many advantages as a rapid assessment tool; requiring just a few minutes of observation and potentially producing high sample sizes with minimal resources as the LoF index does not require more than a single observer and is not training intensive.

Since its development, many invasive species researchers have utilized the LoF index with mixed results. It has been employed on thousands of yachts in New Zealand (Floerl *et al.* 2005a) and surveys in San Francisco Bay using the LoF index, calibrated using depth-stratified quadrat photographs of the hull and video of stern areas, showed a reasonable relationship between the index and observed fouling levels on the hulls (Davidson *et al.* 2010). However, further research has demonstrated that in New Zealand waters, at least, its accuracy seems to vary between vessel types and surfaces causing some to question its usefulness (Hopkins and Forrest 2010; Piola and Conwell 2010). The LoF has been employed in other regions but has not always been calibrated making further comparisons difficult. For example, Ashton *et al.* (2006) used the index in a survey of yachts in Scotland to assess risk of macroalgal introductions but without calibration of the index's accuracy with some form of underwater survey, precision cannot be evaluated.

In contrast to LoF type indices, human behaviour-based models use the characteristics of the vessel to explain the probability of transport of fouling in general or particular invasive species (Darbyson *et al.* 2009b; Drake and Mandrak 2010; Floerl *et al.* 2005a; Floerl *et al.* 2008; Johnson *et al.* 2001). Characteristics of potential interest include travel history and frequency, cleaning practices such as antifouling paint application, speed, and duration of time out of water or between uses. Data are collected by conducting behavioural questionnaires of vessel owners/operators and calibrated using biological surveys for the presence of live propagules or a specific invader on the vessel using some form of underwater survey.

Underwater assessments, such as SCUBA, snorkel or Remote Operated Vehicle (ROV) surveys, are far more resource intensive than either the LoF model or behavioural model. Underwater surveys using SCUBA are highly skill-dependent; requiring teams of divers working in adverse conditions, and training and equipment costs that can be significant. Further, most underwater assessments require additional laboratory processing and photographic analysis. This level of assessment requires significant resources and skill and may introduce a time lag between survey and implementation of any action to combat high risk vectors. These types of assessments cannot thus be considered a rapid assessment tool and but are crucial for their initial calibration.

The most extensive testing of fouling assessment tools has been performed in southern hemisphere waters and therefore it is unknown whether LoF, behavioural or other rapid assessment tools can be reliably applied in different regions of the world. A previous study characterized the occurrence of hull fouling species, including the presence of NIS, for a boating population in British Columbia (BC), Canada using dive surveys with accompanying photograph image analyses (Clarke Murray *et al.* 2011). British Columbia is a cold temperate region with a large active boating population and a history of marine invasive species introductions rivalling other global invasion hot spots (Levings *et al.* 2002). Macrofouling was frequently observed on vessels in this boating population (>65%) and NIS were present on one-quarter of the boats surveyed. In order to facilitate vector management and protection of marine biodiversity, the current study aimed to: 1) test whether the surface-applied Level of Fouling (LoF) rapid assessment tool provides similar results to diver-generated LoF scores and percent cover estimates, and 2) investigate whether boater behavioural characteristics can be used to predict hull fouling on pleasure craft. In essence, can we accurately and quickly predict the presence of fouling below the surface of the water without getting wet?

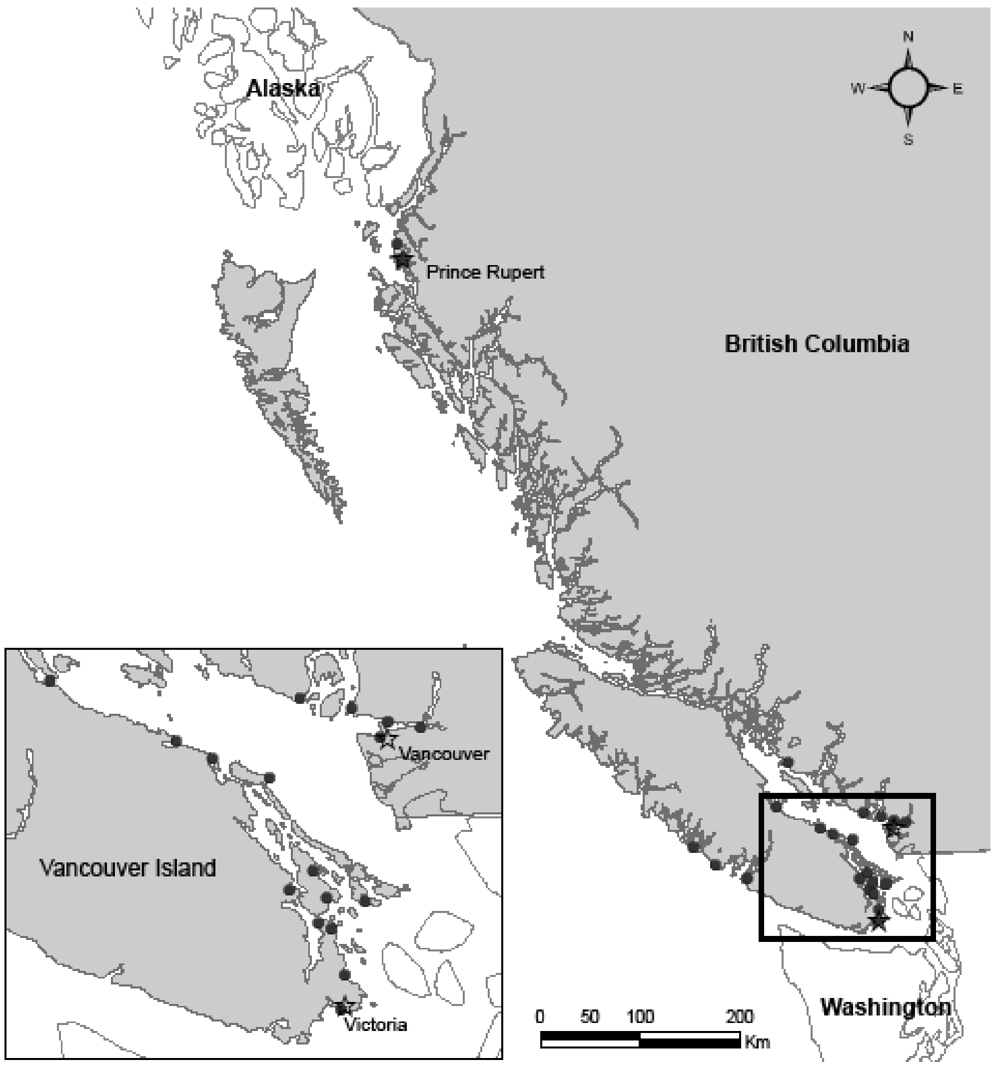

Figure 4.1 Map of British Columbia, Canada depicting marinas surveyed (black dots) and major cities (stars)

4.2 Methods

4.2.1 Dive Survey

In order to examine assessment techniques, twenty-four marinas in coastal BC, Canada (Figure 4.1) were surveyed during two consecutive summers (2008-2009). In the second year of sampling (2009), busy marinas with high levels of transient boater traffic were targeted specifically in order to obtain a more balanced sample of both resident and transient boats. For each dive, boats were surveyed by SCUBA sequentially along a marina finger from a random start point within each marina (10-30 boats per marina). For each boat, one diver photographed submerged surfaces of each boat using an underwater camera with a quadrat (0.25 x 0.25 m) attached to standardize photograph size. Waterline fouling was not photographed as it is often not fully attached to the hull. Six replicate quadrats were photographed on the hull in addition to one photograph of each niche area (*e.g.*, propeller, propeller shaft, keel, vents, knot meter, etc). A second diver actively searched the entire three-dimensional surface for hull fouling organisms and made note of the presence of a pre-determined list of NIS. The second diver also assigned the boat a LoF rank according to Floerl (2005a) (Table 4.1), hereafter called the "underwater rank". The safety diver on the dock walked the length of each boat in the dive survey and assigned a LoF rank according to the same scale, the "dockside rank". The same individual assigned the underwater rank for all boats surveyed while the dockside observer varied (4 in total). Prior to the study, all dockside observers underwent a training session where they practiced assigning LoF ranks until there was no difference in ranking of the same boats between observers. Temperature and salinity measurements were taken at each marina at 0.5 m and 3 m depth, measured using a hand-held YSI instrument (Xylem Brand).

Table 4.1: Level of Fouling (LoF) rank scale with descriptions of each level of fouling (adapted from Floerl *et al.* 2005)

Rank	Description	Visual estimate of fouling cover (%)
0	No visible fouling	0
1	Biofilm only. Absent of any macrofouling	0
2	Light fouling. Hull covered in biofilm and 1-2 very small patches of macrofouling (only one taxon)	1-5
3	Considerable fouling. Presence of biofilm, patchy macrofouling of one single or several different taxa	6-15
4	Extensive fouling. Presence of biofilm and abundant fouling assemblages consisting of more than one taxon	16-40
5	Very heavy fouling. Diverse assemblages covering most of visible hull surfaces	41-100

4.2.2 Boater Questionnaire

A boater questionnaire (adapted from Floerl *et al.* 2005a) was left with each boat examined as part of the dive survey, distributed during boating outreach events, and made available online. The questionnaire consisted of three sections, asking about their boat (*e.g.*, where it is stored, trailered, etc), antifouling practices (*e.g.*, paint or manual cleaning, time since last treatment, etc.), and travel history (twelve questions in total). Details of the full questionnaire are presented in a Appendix B. Boaters were asked to report their travel history for the previous twelve months including the types of trips taken and check off the places they had visited from a list of destinations. Trip types included local trips (out and back to marina in same day), weekend trips (trips of a few days duration visiting 1-2 different moorages), tours (long trips with multiple destinations along the way, staying in each moorage for only a few nights) and long trips (long haul travel to destinations further away, once there remain in a single moorage the entire time). The questionnaire and protocol was approved under University of British Columbia's Behavioural Research Ethics Board (Approval #H08-00967). In total, 616 completed questionnaires were returned, 164 of these from boats participating in the dive survey.

4.2.3 Data Analysis

A sub-sample of boats was subjected to image analysis (N=207) with photographs analysed using the image analysis software Image J (Sun Microsystems). Each photograph was digitally overlaid with a fixed grid of 100 points, equivalent to 18mm apart. Each point on the grid was assessed for fouling directly beneath to estimate total percent cover for the quadrat and record functional group or species, where possible. Niche areas were treated as two-dimensional and the same grid used to estimate percent cover, subtracting any empty space in the photograph. Percent cover was averaged over the replicate quadrats to obtain a percent cover and standard error estimate for each boat.

Dockside and underwater LoF rankings were compared using paired t-tests. Logistic regression was used to examine the relationship between dockside ranking and percent cover. To test for an observer effect, rank differences between underwater and dockside scores by observer were tested using ANOVA. Percent cover data was arc-sine square root transformed to meet model assumptions before analysis (Zar 1999). Statistical analyses were performed using SPSS (SPSS Inc.).

4.2.4 Model Development

The data were used to create two models: a "fouling model" to predict the presence of macrofouling and an "infection model" to predict the presence of NIS. For each model, surveyed boats were classified into groups. For the fouling model, boats were classified as "fouled" or "clean". Fouled boats were those that had any amount of macrofouling on underwater surfaces, either niche or hull areas, and correspond to a LoF rank greater than one (as determined by the diver-assigned rankings). The alternative classification was clean, where no macrofouling was present and LoF index was one or less. For the infection model, boats were classified as either "infected" or "non-infected". Infected boats included all boats that were infected with one or more recognized NIS (see Clarke Murray *et al.* 2011 for complete list) while non-infected boats could be either those without macrofouling or those with macrofouling species that did not include a known NIS. Boats fouled with cryptogenic species, such as the mussel, *Mytilus* species complex, were not included in the infected classification.

Discriminant function analysis is used to find a combination of variables that predict group membership (Francis 2001). Questionnaire results were converted into 57 separate variables, either continuous (*e.g.* age of antifouling paint) or discrete (*e.g.* sailboat vs. powerboat). We used discriminant function analysis to build the two models from the questionnaire variables that together discriminate between the two groups in each factor (*e.g.* fouled vs. clean or infected vs. non-infected). This analysis assumes equal variance and since Box's M statistic showed unequal variances, covariance matrices were used in model development (Francis 2001). Cross-correlations between variables in the predictive model were tested using Pearson's correlation and highly correlated (redundant) variables were removed from the model based on the lower correlation value and the analysis repeated. Model validation was performed using leave-one-out cross-validation analysis and an overall error rate calculated. Model Fisher's discriminant functions describe differences between groups using retained variables and these were used to construct a decision tree. The most accurate fouling model was applied to the remainder of the questionnaire dataset (questionnaires without accompanying dive surveys) and used to predict whether each boat would be fouled or infected. Model construction and validation was performed using SPSS (SPSS Inc.).

4.3 Results

4.3.1 Fouling Index

In total, 430 boats were surveyed by dockside and underwater observers. On an individual boat basis, the dockside LoF rank was not a good predictor of hull fouling. Overall, identical rankings were assigned by the dockside and underwater observers for only 26.5% of boats surveyed. Dockside rankings were significantly different than underwater rankings (Paired samples t-test, $t = 2.270$, $df = 429$, $p = 0.024$). The precision of underwater versus dockside rankings was best for rankings 2 and 5 (37.31 and 33.73% respectively; Table 4.2). Boats ranked 0 and 1 by the dockside observer still had a 36.44% and 64.59% chance of macrofouling being present (rank $\geq$ 2), respectively. Overall, there was no useful predictive relationship between dockside and underwater rankings and no consistent under- or over-prediction that would allow for easy correction.

Table 4.2: Percentage of each dockside rank assigned to each underwater rank by divers (according to the Floerl *et al.* (2005) fouling index). Identical rankings are in bold on the diagonal. Percentages above the diagonal signify under-predictions of LoF by the dockside observer, while those below the diagonal were over-predictions. Number of observations (N) for each dockside rank assigned given in N column

			Underwater rank observed (%)						
Dockside rank prediction		**N**	**0**	**1**	**2**	**3**	**4**	**5**	**Total %**
	0	106	**29.91**	33.64	32.71	2.80	0.93	0.00	100.00
	1	96	19.79	**15.63**	42.71	18.75	3.13	0.00	100.00
	2	67	2.99	26.87	**37.31**	22.39	5.97	4.48	100.00
	3	68	0.00	32.35	26.47	**26.47**	14.71	0.00	100.00
	4	44	0.00	18.60	37.21	27.91	**11.63**	4.65	100.00
	5	83	1.20	4.82	12.05	22.89	25.30	**33.73**	100.00

Dockside ranks also were not good predictors of percent cover, as derived from underwater surveys (Logistic regression $p>0.05$). Percent cover was highly variable compared to assigned dockside ranks (Figure 4.2a). Since each LoF rank corresponds to a pre-defined level of percent cover, rank 5 should include >41% fouling cover but the mean percent cover for dockside rank 5 was only 19.0% (ranged from 0 to 89.9%). All dockside rankings included large variation and outliers of percent cover, suggesting the dockside rank did not match the LoF percent cover definition. Macrofouling observed by the dockside observer was not included in the underwater ranking in only three of the surveyed boats; these boats were ranked 2 or higher by the dockside observer and yet were ranked 0 by the underwater observer. This was attributed to the presence of slime fouling at the waterline which was not included in underwater surveys or photographic quadrats. As expected, the underwater rank did better with fewer extreme and outlying values (Figure 4.2b); though, the means still did not match well with the percent cover definitions.

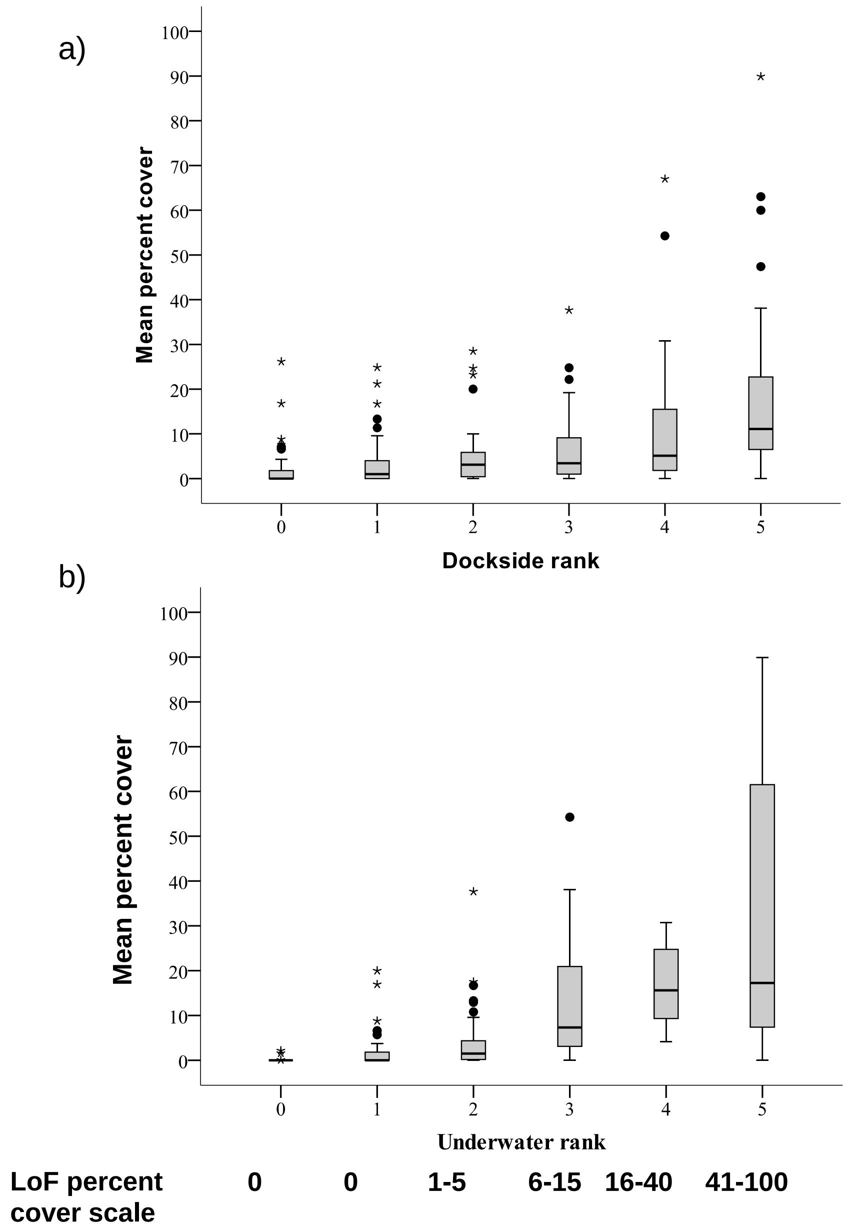

Figure 4.2 Boxplot of observed mean percent cover determined by photograph analyses vs. assigned a) dockside rank and b) underwater rank. Horizontal lines indicate mean value, black dots are extreme values outside the 95% confidence intervals, and asterisks indicate outliers (included in the analysis). The LoF percent cover values corresponding to the definition by Floerl *et al.* (2005) are shown below

There was no significant observer effect; all dockside observers were equally poor at predicting underwater ranking (Kruskal Wallis χ^2 p = 0.139). However, the precision of dockside ranking, as represented by the absolute value difference between dockside and underwater rankings, was significantly different among marinas (Kruskal-Wallis χ^2 = 49.316, df = 24, p = 0.002). The worst absolute value precision occurred in West Vancouver and Hawkeye Bamfield and the best in Rushbrooke Prince Rupert and Poett Nook Bamfield (Figure 4.3). There was no correlation between marina precision and environmental variables, including temperature, salinity and depth of thermocline and halocline (represented by the difference between measurements at 0.5 m and 3 m depth).

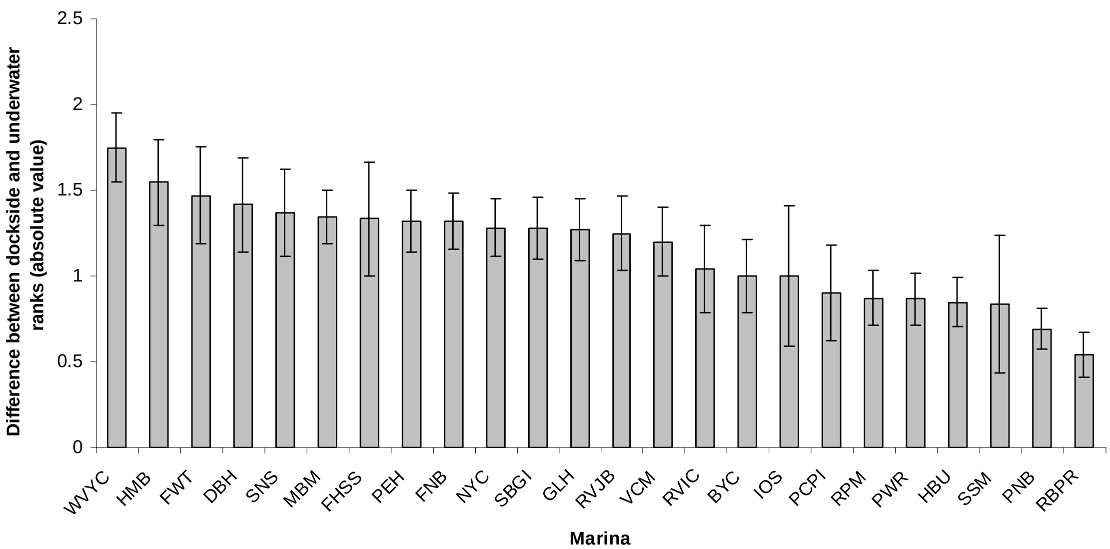

Figure 4.3 Mean difference (absolute value +/- standard error) between dockside and underwater fouling ranks, by marina. Marina codes: WVYC - West Vancouver Yacht Club, HMB – Hawkeye Marine Bamfield, FWT - Fisherman's Wharf Tofino, DBH - Deep Bay Harbour, SNS - Sidney North Saanich, MBM - Maple Bay Marina, FHSS - Fulford Harbour Saltspring, PEH - Port Edward Harbour, FNB - Fairwinds Nanoose Bay, NYC - Nanaimo Yacht Club, SBGI - Silva Bay Gabriola, GLH - Gibson's Landing Harbour, RVJB - Royal Vancouver Yacht Club Jericho, VCM - Victoria Causeway Marina, RVIC - Royal Victoria Yacht Club, BYC - Burrard Yacht Club, IOS – Institute of Ocean Sciences, PCP - Poet's Cove Pender Island, RPM - Reed Point Marina, PWR - Powell River Marina, HBU - Ucluelet Hemlock Basin, SSM - Saltspring Marina, PNB - Poet Nook Bamfield, RBPR - Rushbrooke Harbour

4.3.2 Predictive Model

Of 57 inputted questionnaire variables, four were retained in the best fouling model (Discrim function analysis: Cann. Corr 0.348, Wilks Lambda = 0.879, $p<0.001$). In order of importance, the predictor variables were: storage location, antifouling paint age, boat type, and incidence of long trips taken. Essentially, this fouling model predicts that boats stored in water full time, which do not undertake "long trips" and have antifouling paint older than an average of 12.61 (+/- 2.41 SE) months would be more likely to have macrofouling present. Both sailboats and powerboats with these characteristics would be likely to have macrofouling, but sailboats had a higher probability of fouling than powerboats (73% versus 60%). The corollary, sail and powerboats least likely to have macrofouling would be ones with antifouling paint less than 12.61 months old, which were stored in water only part of the year and had taken long trips in the past 12 months.

Model cross-validation showed that the fouling model correctly predicted case classification 71.2% of the time (Table 4.3). Fouled boats were correctly classified 76.6% of the time (true positives) and incorrectly classified 23.4% (false positives). Clean boats had a higher error rate with 40.4% incorrectly classified as fouled, indicating that the model was more likely to overestimate fouling than underestimate it. Applying the fouling model to the remainder of the questionnaire data set (N=329) revealed that 61.7% of surveyed boats were predicted to be fouled. This roughly corresponds to dive survey results, where 65.7% of boats surveyed had macrofouling.

Based on the fouling model, a decision rule model was constructed to aid in evaluation of boats for risk of macrofouling (Figure 4.4). Three questions would be used to determine whether a boat would be predicted to have macrofouling present and therefore poses a risk of invasive species presence: 1) Where is your boat stored? 2) When did you last apply antifouling paint? and 3) Have you taken long trips in the previous twelve months? Boats that fall within the shaded box are likely to have macrofouling and therefore should undergo secondary or detailed inspection.

Table 4.3: Cross-validation classification results matrix for fouling predictive model. Overall, correct classification = 71.2%

		Predicted Group Membership (%)	
		Clean	Fouled
Actual Group Membership (%)	Clean	59.6	40.4
	Fouled	23.4	76.6

A second discriminant function analysis was used to create an infection model, but an accurate model could not be defined due to the smaller sample size (N=44). Only 38.9% of fouled boats were infected with NIS and therefore the infection model does not have the same predictive power as that for fouling. However, the trends in the variables that predict fouling also hold for the infected group. Mean antifouling paint age for infected boats was higher than non-infected boats (12.02 and 10.97 months respectively) but the difference was not significant (ANOVA, p=0.632). Similarly, boats stored in the water full time and that had not undertaken long trips were more likely to be infected with NIS, but again the trend was not significant.

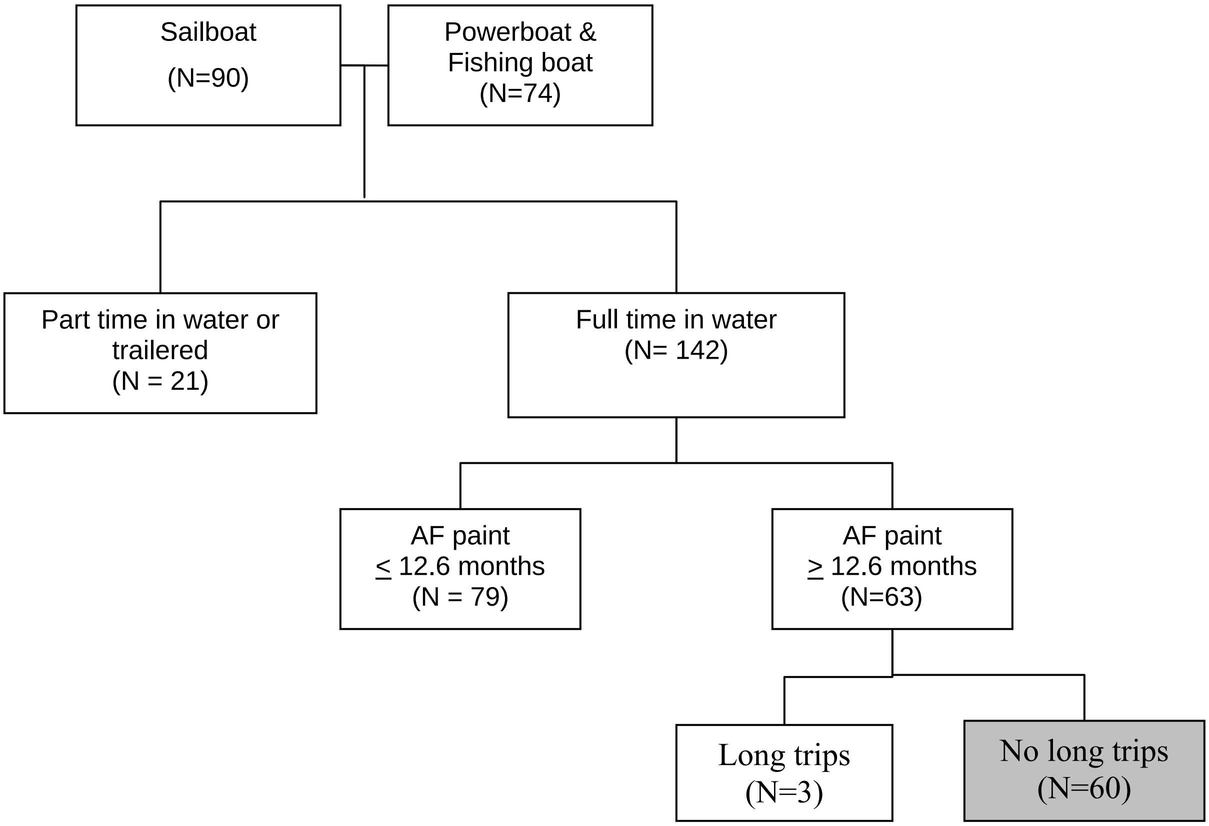

Figure 4.4: Illustration of discriminant function model for fouling. Three variables determine the risk of macrofouling for an individual boat and whether inspection is warranted. The model is the same for all boat types, although sailboats have slightly higher probability of fouling than powerboat or fishing boats. A boat that falls in the shaded box is likely to have macrofouling and therefore should undergo inspection for invasive species. Number of boats (N) in each variable type is indicated in brackets.

4.4 Discussion

The current study demonstrated that rapid assessment tools should be carefully chosen and calibrated to ensure their usefulness, especially when it comes to making management decisions. In BC, the dockside Level of Fouling (LoF) assessment had poor precision and no significant relationship to macrofouling estimated using either underwater observers or photographic quadrat analysis. The dockside LoF rankings both under- and over-estimated actual percent cover, with no consistent pattern. In contrast, the questionnaire-based fouling model was both effective in identifying fouled boats and relatively simple in its data requirements. The fouling decision rule model could be applied during routine border inspections to prevent new invaders or under quarantine situations in response to specific invaders. In Canada, recreational boaters mainly rely on radio to contact customs officials when entering Canadian waters and the decision rule model could easily be applied in these situations. Three questions identified by the model could be posed and risky boats ordered to dock for a secondary underwater inspection and subsequent removal of macrofouling, if required.

Other than those in Australia and New Zealand, there are no regulations aimed at reducing invasive species transport on recreational marine boats. Though internationally traveling boats were cleaner than local boats, they still carried small numbers of native barnacles and cryptogenic mussels in niche areas (Chapter 3), suggesting that there is an ongoing possibility of additional primary introductions, especially from invaded areas along the Pacific US coast like San Francisco Bay. International-origin and internationally-traveling boats made up a substantial portion of the boating community in BC (Chapter 3). The Canadian Border Services Agency (customs) routinely inspects vehicles and passengers crossing the Canadian-United States land border for known agricultural pest species but marine border crossings are subject to little physical monitoring. Vector research on freshwater, trailered boats have resulted in improved boater outreach, a voluntary code of best practices, and introduction of boating cleaning facilities in order to reduce the spread of freshwater invasive species such as zebra mussel (*Dreissena polymorpha*) (Bossenbroek *et al.* 2007) and spiny water flea (*Bythotrephes* spp.) (MacIsaac *et al.* 2004). The implementation of similar regulations in Australia, where international boats are required to provide proof that hull cleaning or antifouling paint application was undertaken within the last twelve months (Australian Quarantine and Inspection Services 2006; Floerl and Inglis 2003), would reduce the threat of hull fouling invasive species entering Canadian waters.

A myriad of environmental, social, and behavioural factors may affect the accuracy of rapid assessment tools. Spatial variation between marinas, boats, and even surfaces of individual boats may contribute to inaccuracy and inconsistency in dockside assessments, such as the LoF index. The current study surveyed 24 different marinas and most had significant thermoclines and haloclines at varying depths. This makes it difficult to see through the water for the whole depth of the boat and adds variability to scores among marinas. Though environmental variables were not significantly related to accuracy, they may contribute to the inconsistency of the LoF index within the current study region. The use of a Secchi disk to quantify water clarity may aid in quantifying variation among marinas and regions.

In addition to differences among marinas, the orientation of boats both to the sun and to the dock varies within and between marinas, as well as varying daily and seasonally. Usually a dockside observer only can see one side of the boat, and growth at the surface is related to degree of shading and orientation to the sun. Fouling at the waterline may cause the observer to overestimate the percent cover of fouling. Also, waterline fouling often is not firmly attached to the hull and may slough off when the boat begins to move. Macrophytes in particular can grow quickly on recreational boats at the level of the waterline (Mineur *et al.* 2008) and this situation would lead to overestimation of fouling below the surface, particularly under conditions of poor water clarity. Waterline fouling was not included in the underwater ranks or photographic quadrats and therefore the estimation of overall fouling may not reflect the entire submerged surface of the boat, though the waterline surface area reflects a very small amount of the total submerged area of a boat.

In contrast, fouling underestimation may occur if niche areas not visible from the surface are heavily fouled. Indeed, boats in San Francisco Bay were found to have increased fouling cover with depth (Davidson *et al.* 2010). Underwater areas of small boats are complex with unpainted niche areas, variations in paint application, and differing hydrodynamics. Niche areas have been found to host disproportionately higher amounts of fouling on both recreational (Clarke Murray *et al.* 2011; Davidson *et al.* 2010) and commercial vessels (Coutts 1999; Coutts and Taylor 2004; Gollasch 2002). Both situations likely contributed to the inconsistent accuracy of the LoF index in this study. Therefore, underwater inspections should always be stratified to include hull, waterline, and niche areas, as random sampling may miss fouling hot spots and thereby incorrectly assign risk of NIS presence (Davidson *et al.* 2006)

The LoF performed well in northern Australia, where it was developed, and in extensive tests in New Zealand but did not perform well in BC despite extensive operator training, suggesting that regional differences may affect its success and usefulness. Seasonal productivity patterns may drive differences in accuracy as higher latitude regions have more pronounced seasonality. Summer months in BC are characterized by high upwelling productivity and slightly warmer temperatures; further reducing water clarity (Harrison *et al.* 1983; Masson and Cummins 2007). In addition, regions with higher temperatures, such as the tropics, tend to have higher fouling rates (Minchin *et al.* 2006) and therefore dockside observers may be better able to estimate fouling cover. All of the boats surveyed in the original Australian LoF study had fouling present (Floerl 2002) and the majority of boats surveyed in San Francisco had higher levels of fouling (Davidson *et al.* 2010). In keeping with this trend, the current study showed that the tool seemed to be more accurate at assigning higher ranks when vessels had higher fouling levels. However, since NIS transport potentially occurs at even low fouling levels, this tool may not be appropriate for use across regions, especially in high latitudes with lower fouling rates.

The use of LoF rankings is useful in dockside observation to assess the presence and quantity of macrofouling on visible surfaces of the hull (approximately the first metre, dependent on water clarity). The current study showed that LoF estimates of overall percent cover are imprecise and gave no indication how many additional boats had fouling present (false negatives). Without calibration, LoF studies of this type cannot conclusively assign absences and even then, are unlikely to accurately predict fouling in unseen niche areas. In contrast, the fouling questionnaire model was slightly biased toward positive fouling predictions making it a more cautious or conservative assessment tool. For invasive species detection, conservative tools are preferred as the primary goal is to maximize the likelihood of predicting or detecting invasive species when present.

By increasing the chance of detecting invasive species on recreational boats, ideally before introduction to native systems, we can potentially reduce the number of successful invasions (Carlton and Ruiz 2005).

The four variables that comprise the fouling model: boat type, age of antifouling paint, time in water, and history of long trips, are consistent with findings of previous studies (e.g., Ashton *et al.* 2006; Floerl and Inglis 2005; Floerl *et al.* 2005b). Sailboats were more likely to have macrofouling present than powerboats or fishing boats and this is likely a result of their slower speed allowing macrofouling to remain attached. The three-variable decision rule flow chart applies to both powerboats and sailboats, although sailboats had slightly higher probability of having macrofouling than powerboats. In Scotland, presence of macrofouling was related to both age of antifouling paint and activity levels, where stationary boats were more heavily fouled (Ashton *et al.* 2006). Similar to Floerl and Inglis (2005), antifouling paint age was an important predictor variable of fouling on BC recreational boats. The average age of paint on boats surveyed in BC was 15 months, while most antifouling paint brands have a manufacturer's estimated lifetime between 9-18 months (Christie and Dalley 1987). The fouling model suggests that boats with paint older than twelve months were at greater risk of macrofouling and therefore the majority of BC boats surveyed have ineffective antifouling paint. This result is not surprising, given the high proportion of the BC boating population observed with macrofouling present. The 12-month threshold for fouling may be region-specific and care should be exercised in extrapolating these results to regions with differing environmental conditions and boater populations.

The time-in-water variable indicates that the longer boats are in the water, the more likely they are to have macrofouling present. Time in water was a significant factor in species richness and community assemblage on settling plates in Northern Australia (Floerl *et al.* 2005b), with more complex communities developing with time. In this study, boats kept in the water full time had greater chance of macrofouling than those that were trailered or stored in water for only part of the year. This variable reflects an important division within the BC boating community: full time moorage boats versus part-time and trailered boats. While fully marine boats are at greatest risk of transporting hull fouling invasive species, trailered boats are known to transport invasive aquatic plants and mussels by entanglement on propellers or trailers (Bossenbroek *et al.* 2007; Johnson and Padilla 1996; Johnson *et al.* 2001; Padilla *et al.* 1996) or larval stages or small species like spiny water flea in bilge tanks or other water holding tanks (MacIsaac *et al.* 2004). Therefore, the risk posed by each population is very different for invasive species introduction and spread.

The inclusion of the "long trip" variable in the fouling model is intriguing. Though it was weakly correlated with other significant model variables it may indicate dislodgement or reduced survival of fouling organisms on long trips. In contrast to touring trips (defined as "away from home marina for significant periods of time, with short distances between destinations" in Chapter 3), long trips would include substantial time in the open ocean with very different environmental conditions than experienced within marinas and coastal environments. Therefore, boats that undertake long trips may experience hydrodynamic and/or environmental conditions that reduce or prevent fouling (Coutts 1999; Coutts *et al.* 2010; Davidson *et al.* 2009). The drag experienced when a boat is moving likely increases fouling organisms' probability of dislodgement. Outside sheltered marinas, new settlement also may be reduced as water velocity affects larval settlement (Havenhand and Svane 1991). Additionally, long periods in open ocean conditions with differing temperature, salinity and resource availability may affect survival of fouling species. Though boats that undertake long trips were shown to have better maintenance practices (Chapter 3), more investigation of this variable and its relationship with fouling is warranted.

The dataset utilized by the statistical technique (Discriminant Function Analysis) to develop the behavioural fouling model was specific to the BC boating population. Although cross-validation was performed, no independent data set was available for model validation to be carried out so it cannot be implied that the BC model could be reliably extrapolated to other regions. Similar variables have been useful in explaining fouling in other studies (Ashton *et al.* 2006; Floerl & Inglis 2005; Floerl *et al.* 2005) but their relative importance and, in particular, quantitative thresholds are likely to be region-specific. Testing and validation of all potential variables should be performed if attempting to apply similar behavioural models to other boating regions.

Although useful for predicting fouling, the infected model developed from this dataset could not accurately predict the presence of NIS. The relatively low sample size of infected boats with accompanying questionnaires may be one reason, as trends in the two factors were similar. An alternate hypothesis is that infection is the result of more complex factors than general fouling, which can occur anywhere given sufficient time. Whether a boat becomes infected with a NIS is a combination of its susceptibility to fouling (fouling model variables such as antifouling paint age) and proximity to populations of NIS. In order to predict infection we likely need to model travel history in combination with boater behaviours and antifouling practices, similar to recent studies (Floerl *et al.* 2008; Johnson *et al.* 2001).

A variety of models have been used to estimate and predict boat movements. Modeling vector movements gives insight into the mechanism of spread and allows the prioritization of monitoring efforts (further investigated in Chapter 6). Epidemiological models have been utilized to predict which marinas are at greatest risk of NIS introduction because of their connectivity with other marinas (Floerl *et al.* 2008). Gravity models in particular have been shown to predict or simulate the spread of aquatic invasive species through networks of lakes and rivers (Bossenbroek *et al.* 2007; Drake and Mandrak 2010). Models of this type could be combined with questionnaire-based models to better predict infection of individual boats. In the current study, boats with macrofouling, though perhaps not infected, serve as potential hosts for new NIS. Fouled status demonstrates susceptibility to infection if they are moved to a location with proximity to NIS propagules. In addition, fouling communities may provide habitat for settlement of NIS. Therefore, the fouling model may be a more valuable tool for general vector management and prevention of new introductions in BC.

4.5 Conclusions

Our results suggest that rapid assessment tools must be carefully chosen and thoroughly tested before implementation, as their accuracy (and hence potential utility) appears to vary by region. The different methods used to assess fouling, and by extension risk of NIS transport, tested here represent varying degrees of resource investment. The lowest resource tool, the LoF index, was a poor predictor of macrofouling on BC boats likely due to varying environmental conditions and boater behaviours. Once verified, the three-question decision rule model was both the quickest assessment technique and proved to be effective in predicting fouling, although more testing is required. The current study showed that underwater surveys coupled with behavioural questionnaires, while initially resource intense, provide the highly detailed data required to develop effective predictive models. Behaviour-based questionnaire models calibrated in this way can significantly increase precision and effectiveness of rapid assessments. Researchers and government agencies might conduct trials and weigh the costs and benefits of each assessment method in order to determine the best choice for their region and purpose. The use of appropriate rapid assessment tools can serve as highly valuable protection against the introduction and spread of potentially invasive species.

Chapter 5: Adapted for invasion? Comparing attachment, drag and dislodgment of native and non-indigenous hull fouling species*

5.1 Introduction

Theories abound as to which traits make certain non-indigenous species become invasive. There is evidence that adaptation to disturbance (Altman and Whitlatch 2007; Hobbs 1992), wide environmental tolerances (Marchetti *et al.* 2004; McMahon 1996), rapid growth and/or high reproductive capabilities (Marchetti *et al.* 2004; McMahon 2002) assist non-indigenous species in establishment and subsequent spread (Kolar and Lodge 2001; Lodge 1993). These adaptations may have evolved in response to processes in their native range or may have been rapidly selected for within their newly invaded range. For example, in Australia cane toads at the leading edge of the invasion front have longer legs than those in older established populations, conferring a faster rate of spread (Phillips *et al.* 2006). Thus, adaptations of invasive species are not only of ecological interest but may inform conservation and management decisions.

Researchers have used physiological tolerances to predict invasion and range expansion of invasive species, examining temperature, altitude, depth, salinity, vegetation cover and many other variables (Dark 2004; Epelbaum *et al.* 2009; Herborg *et al.* 2008; Johnson *et al.* 2001). However, these types of analyses are only useful for explaining potential patterns of establishment, the final stage of invasion. The first step in the invasion process is uptake by a human-mediated vector either intentionally or accidentally (*e.g.,* settling on the hull of a freighter or being drawn into ballast water tanks) (Lockwood *et al.* 2005). Successful invaders must then survive the journey to the new location outside their native range. Previous modelling efforts to predict the

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potential distribution of an invader largely have not considered this uptake and transport stage explicitly.

Hull fouling is one of the earliest documented marine vectors. Wood-boring invaders, such as the shipworm bivalve *Teredo navalis*, have been travelling the world's seas as long as wooden ships (Hoppe 2002). Moreover, identity and quantity of hull fouling invaders have changed in concert with developments in the shipping industry. For example, antifouling paints and the subsequent ban of Tributyl Tin (TBT) have shaped the frequency and type of hull fouling invaders (Dafforn *et al.* 2008; Evans *et al.* 2000; Piola *et al.* 2009). The increase in size and speed of international ships has increased the surface area available for colonization, decreased transit time between ports, and is believed to have facilitated invasions (Carlton 1996; Levine and D'Antonio 2003). Some examples of long-distance hull fouling invaders include the black-striped mussel (*Mytilopsis sallei*) in Australia (Field 1999), the barnacle *Chthamalus proteus* in Hawaii (Southward *et al.* 1998), and the kelp *Undaria pinnatifida* in New Zealand (Floerl and Inglis 2005). In addition, there are many examples of secondary or regional spread attributed to recreational boating activities, including freshwater zebra mussels (*Dreissena polymorpha*) (Johnson *et al.* 2001) and the ascidian *Styela clava* in marine waters (Floerl and Inglis 2005; Locke *et al.* 2007). However, only a few studies have explicitly tested the ability of non-indigenous species to endure the voyage. For example, to evaluate the risk of overland transport by trailered boats, studies showed that zebra mussels can remain viable out of water for up to four days (McMahon 1996) while the ascidian *Styela clava* tolerated 48 hours of air exposure with low mortality rates (Darbyson *et al.* 2009).

Underwater hulls of boats are complex three-dimensional surfaces. Niche or non-hull areas, including the vents, propeller, and rudder, typically experience different flow regimes compared to smooth hull surfaces. Those surfaces in the lee of the keel, for example, experience reduced flow velocities while those protruding into flow may experience higher velocities. Fouling levels on recreational boats can be much higher in niche areas, not only due to differences in paint application or effectiveness but also due to localized reduction of velocity in these areas (Clarke Murray *et al.* 2011; Coutts 1999; Davidson *et al.* 2010). Similarly, fouling panels attached to commercial ships have demonstrated differences in community composition among different areas of vessels (Coutts *et al.* 2007). A study by Coutts and colleagues (2010) showed that fouling taxa respond differently to varying vessel speeds based on their growth form and morphology. These results suggest that hydrodynamics may be an important selective pressure for hull fouling species.

Hull fouling transport may be another example of a selection regime modification (Byers 2002) where hydrodynamic conditions experienced *en route* are substantially different than any experienced under natural conditions, potentially selecting for a suite of species able to withstand hull fouling transport. Thus, it is probable that hull fouling invasive species have adaptations that provide a survival advantage and allow them to remain attached to boat hulls long enough to reach new habitats. These adaptations may include superior attachment properties and/or drag reduction strategies. Barnacles and mussels are common hull fouling organisms whose spread has been linked to shipping activities worldwide (Kado 2003; Laird and Griffiths 2008; Pilsbry 1916; Schwindt 2007). For example, the Atlantic barnacle *Chthalamus proteus* was found fouling ships above the water line in Hawaii (Southward *et al.* 1998) and the introduction of Australian *Eliminius modestus* to Ireland was traced to hull fouling on transport ships during World War II (Lawson *et al.* 2004). Barnacles have a broad base that cements to the substrate, likely conferring superior biomechanical properties to remain attached to boat hulls. In contrast, mussels attach using flexible byssal threads, an extracellular, collagen-like material and attachment strength is related to the number of byssal threads and varies by season, wave exposure, and bed location (Bell and Gosline 1997; Carrington 2002; Hunt and Scheibling 2001; Witman and Suchanek 1984). Previous studies have suggested that water velocity is a highly selective force for mussel species (Schneider *et al.* 2005) and in Europe the abundance of *M. galloprovincialis* is positively related to wave exposure (Gosling and Wilkins 1981; Hilbish *et al.* 2002; Skibinski and Roderick 1991). Many mussel invasions have been linked to recreational boating as a vector including zebra mussels (*Dreissena polymorpha*) in the Great Lakes region of North America

(Bossenbroek *et al.* 2007; Padilla *et al.* 1996) and black-striped mussels (*Mytilopsis sallei*) in Darwin, Australia (Field 1999).

Beyond barnacles and mussels, little is known about attachment and drag of other fouling species. A previous study in British Columbia (BC), Canada documented a number of fouling species common to the submerged surfaces of recreational boats (Clarke Murray *et al.* 2011). Differing morphological types may affect their ability to endure hydrodynamic conditions of traveling boats and their ability to spread with this vector. Encrusting non-indigenous species such as *Botryllus schlosseri, Botrylloides violaceus, Didemnum vexillum* and *Halichondria bowerbanki* form mat-like colonies that grow horizontally across surfaces suggesting they may be less susceptible to drag and dislodgment. Solitary ascidian species, such as *Corella inflata* (Huntsman, 1912), *Styela gibbsii* (Stimpson, 1864) and *Styela clava* vary in body shape and size. The stalk of *S. clava,* a non-indigenous species in BC, can grow to 15 cm with a small attachment disc, whereas the native *S. gibbsii* is attached broadly at the base of the body and only grows to 6 cm. *C. inflata* has a broad base, is roughly rectangular in shape and compressed laterally, reaching 5 cm in length. Although all three species are common on floating docks and pilings of harbours and small craft marinas, only *C. inflata* and *S. clava* were observed attached to recreational boats (CCM unpublished data) suggesting that these species may differ in their ability to withstand the hydrodynamic conditions of hull fouling or tolerance for toxic antifouling paints. Therefore, non-indigenous species such as *S. clava* may be better adapted to the hydrodynamic environment experienced while attached to traveling boats, allowing it to successfully arrive in new environments.

We hypothesize that hull fouling invaders possess traits that allow them to settle and remain attached to vessels. Solitary species may then release reproductive gametes which settle on the same (boat hull) or different substrates (other boats, floating docks or pilings) in the new location while colonial species additionally may fragment and re-grow in the new habitat. Coutts and colleagues (2010) assessed the relationship between voyage speed and dislodgement but mechanical properties that allow fouling species to remain attached to marine travelling boats have not been quantified previously. A quantitative assessment of these mechanical properties may provide insight into vectors of introduction as well as patterns of invasion. In this study we investigate the biomechanical properties of eight common fouling species, both native and non-indigenous. We quantify dislodgment strengths and drag forces in order to estimate the velocity at which species will be dislodged from boat hulls, allowing us to compare to possible vectors of introduction and spread.

5.2 Methods

5.2.1 Attachment Strength

Recording spring scales were used to measure the force required to dislodge or break common fouling species (attachment strength, $F_{dislodge}$) measured in Newtons of force (N). We measured the attachment strength of the following species: native ascidians *S. gibbsii* and *C. inflata,* barnacle *Balanus glandula* (Darwin, 1854) and non-indigenous ascidians *S. clava, B. violaceus* and *D. vexillum*, sponge *Halichondria bowerbanki,* and the cryptogenic mussel *Mytilus* species complex. Species of *Mytilus* were grouped because the non-indigenous species *Mytilus galloprovincialis* and *Mytilus edulis* have hybridized with the native *Mytilus trossulus* making them indistinguishable without the aid of genetic testing. Measurements were taken in the field, on floating docks, pilings and rocks at four marinas: Institute of Ocean Sciences, Royal Vancouver Yacht Club Jericho, Royal Victoria Yacht Club and Thetis Island Marina in British Columbia, Canada. The spring scale was attached to individuals by a monofilament noose, rubber clip or recurved scraper and then pulled parallel to the surface in the direction of water flow (Figure 5.1). Effort was made to pull the spring scale repetitively in the same manner for all individuals and the same attachment device was used for all individuals tested within each species. Species identity, height, attachment area, failure location (stalk, fragment, byssus, shell, etc), and substrate type (wooden dock, piling, or cement) were recorded for all individuals. Height and attachment area were measured using calipers. The effect of species and marina location on attachment strength was tested using General Linear Model ANOVA (Attachment strength = Intercept + Species + Marina + Error). Only *Mytilus* was tested on more than one substrate type so the effect of substrate type on attachment strength of this species was tested using non-parametric Mann-Whitney U-test because the equality of variance assumption was violated. The relationship between attachment strength and height was tested using Pearson correlations.

To investigate the importance of attachment strength to presence/absence of species we compared the mean measured attachment strength of each species to their field occurrence from a dive survey of hull fouling communities of recreational boats at 24 marinas in British Columbia, Canada. Full details of the survey were presented in Chapter 3. We would predict that if attachment strength were a limiting factor in transport by this vector there would be a strong correlation with field occurrence on small boats, although the boat data represented both active and inactive boats that could affect the correlation. From the survey data, the percentage of boats with each fouling species present was recorded and this data used in Pearson correlation analyses with attachment strength. All statistical analyses were performed using SPSS (SPSS: An IBM Company) with α level set at 0.05.

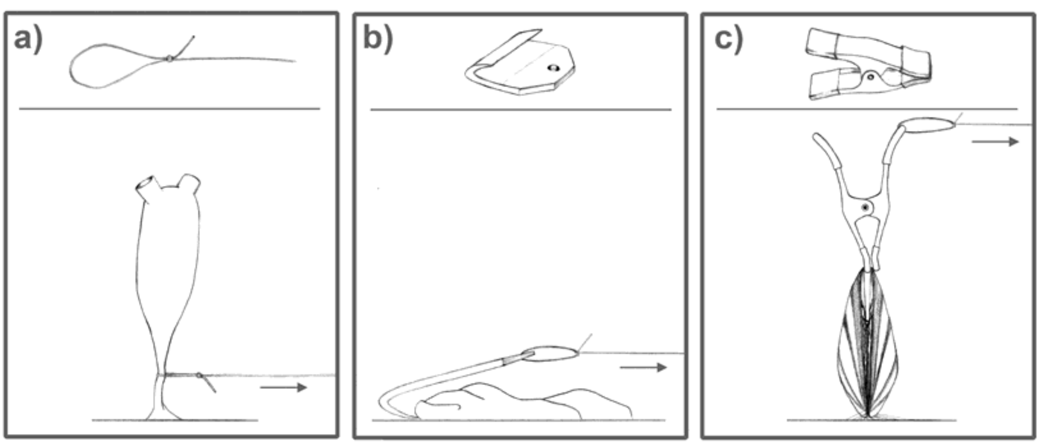


Figure 5.1: Illustration of attachment devices used with recording spring scale to measure force of attachment with examples of the devices in use: a) monofilament noose on solitary ascidian, b) recurved scraper on colonial ascidian, and c) rubber clip on mussel. Arrows indicate direction of force. Illustrations by Megan Mach.

5.2.2 Drag Force

Individuals of five species (*B. violaceus, C. inflata, D. vexillum, S. gibbsii,* and *S. clava*) were collected from two field sites and further tested in a custom high-speed recirculating flume (Ecological Mechanics, Rochester, NY). Individuals of solitary species were suspended from a quarter-inch screw at their natural attachment site using gel superglue. The test screw was inserted into a force transducer (FORT5000, World Precision Instruments, Sarasota, FL) and drag force was recorded at nine velocities (0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8 and 2.0 m/s). For comparison, maximum sailboat velocity is approximately 3.6 m/s and maximum powerboat velocity is approximately 20.5 m/s. Flow was turbulent at the velocities tested, as would likely be experienced under boat hulls. Colonial species were attached to circular compact discs (12 cm diameter) along the entire natural attachment area of the colony using gel superglue. Drag on colonial species was only measured at seven velocities due to their low drag profile and a comparable drag on the compact discs alone (0.2, 0.4, 0.6, 0.8, 1.0, 1.2 and 1.4 m/s). The baseline level of drag due to the experimental apparatus was determined using the test screw and disc without individuals attached. Height was recorded for each individual prior to testing. Digital photographs were taken of the specimen in the test chamber prior to water flow, and Software program Image J (developed by Wayne Rasband, National Institutes of Health, Bethesda, Maryland, USA) used to calculate maximum planform area of each specimen from the photographs.

5.2.3 Data Analysis

Species in hull fouling communities are subjected to drag forces, which can be calculated from the following equation:

$$F_{\text{drag}} = \tfrac{1}{2}\, \rho\, U^2 A\, C_{\text{d}} \qquad \text{(Equation 5.1)}$$

where F_{drag} is drag force, ρ is density of water, U is water velocity, A is maximum planform area, and C_{d} is drag coefficient. Note that A is maximum planform area (not projected area) and is therefore assumed to be invariant with water velocity. This assumption is standard practice in most hydrodynamic studies of flexible organisms, such as seaweeds, bypassing the difficulties of measuring area in turbulent flow and allowing drag coefficient to be the sole measure of flexible reconfiguration and reorientation (see

Carrington 1990, Gaylord *et al.* 1994, Koehl 2000, Johnson 2001, Martone and Denny 2008).

5.2.3.1 Calculating Drag Coefficients

Drag coefficients were calculated by re-arranging equation (5.1) to yield:

$$C_d = \frac{2\ \mathrm{F_{drag}}}{\rho\ U^2\ \mathrm{A}} \qquad \text{(Equation 5.2)}$$

For each individual tested, drag coefficients were calculated over the entire range of velocities and the mean C_d was plotted against velocity to produce a characteristic C_d-U curve for each species.

5.2.3.2 Predicting Dislodgment

Fouling organisms would be expected to dislodge when drag force (F_drag) experienced equals dislodgment force ($F_{dislodge}$). Dislodgment velocity was estimated by rearranging Equation (5.1) such that:

$$U_{dislodge} = \sqrt{\frac{2F_{dislodge}}{\rho\, A\, C_d}} \qquad \text{(Equation 5.3)}$$

Where A was the maximum planform area of each individual tested, species-specific drag coefficients C_d were calculated from Equation 5.2. For three species, *S. gibbsii*, *D. vexillum,* and *B. violaceus*, drag coefficient remained constant across velocities so the grand mean drag coefficient was used to calculate dislodgment velocity. For *S. clava* and *C. inflata* drag coefficient decreased with velocity. To calculate dislodgment velocity for these species, we used the drag coefficient measured at the highest velocity tested (2.0 m/s), following the method of Bell (1999 Extrapolation model B) and the average dislodgment strength measured for the species. In this manner, dislodgment velocity was calculated for each individual tested in the flume (using individual measured planform area), which yielded a range of minimum dislodgment velocities that vary with size. To determine whether each species would be carried on marine vessels, the range of estimated minimum dislodgment velocity was compared to reported maximum velocities of recreational marine vessels (powerboat and sailboat). If the estimated dislodgment velocity was lower than the vessels' maximum velocity, we predicted that the species would be dislodged by that vessel.

5.3 Results

5.3.1 Attachment Strength

The most strongly attached fouling species were the solitary species *B. glandula*, *Mytilus* sp. and *S. clava* (Figure 5.2). There were significant differences among species tested for attachment strength (GLM ANOVA: $F = 14.061$, $df = 5$, $p < 0.001$). The weakest attachment force was that of the solitary ascidian *C. inflata*, colonial sponge *H. bowerbanki,* and colonial ascidian *B. violaceus.* Attachment of *B. glandula* was significantly stronger than all other species. Attachment strengths of *Mytilus* sp. and *S. clava* were less than *B. glandula* but greater than other species and not significantly different from each other (Tukey's HSD post hoc, $p < 0.05$). The attachment of the non-indigenous ascidian *S. clava* was significantly stronger than the native solitary ascidians *C. inflata* and *S. gibbsii*. Marina location had no significant effect on attachment strength (GLM ANOVA: $F = 0.477$, $df = 2$, $p = 0.621$). Attachment strength of *Mytilus* spp. was significantly higher on wooden docks (Mean = 25.86 +/- 1.743 SE) than on rocks (Mean = 11.96 +/- 0.906 SE) (Mann-Whitney U-test: $Z = -2.846$, $p = 0.004$).

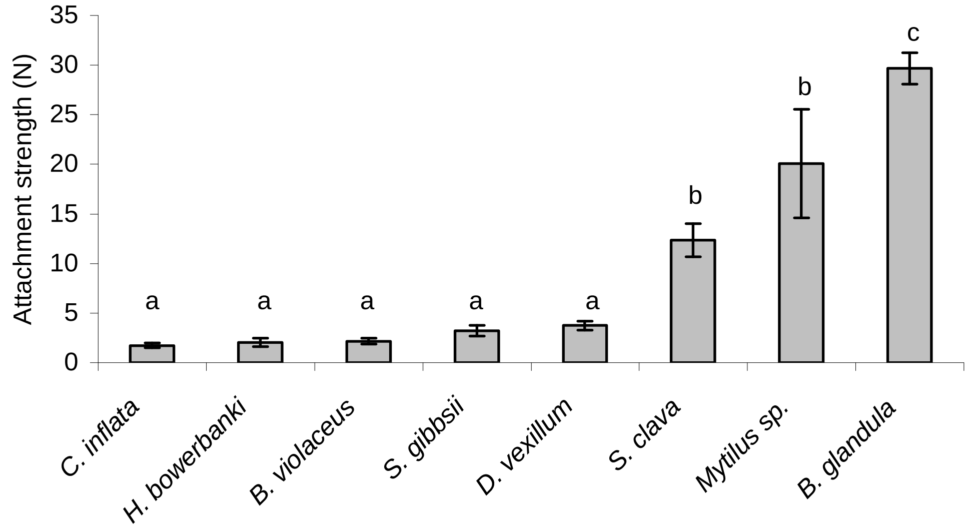


Figure 5.2 Mean attachment strength (N = Newtons) ± standard error for fouling species tested by recording spring scales. Letters denote statistically significant post-hoc groupings (One-way ANOVA, Tukey's HSD post-hoc test).

Species with highest measured attachment strength also were the most commonly observed in hull fouling communities of recreational boats (data from Clarke Murray *et al.* 2011). Attachment strength measured for each of the eight fouling species was strongly correlated to their incidence in boat hull fouling communities (Figure 5.3, Pearson's $r = 0.915$, $p = 0.001$), which included both hull and niche areas of the boats.

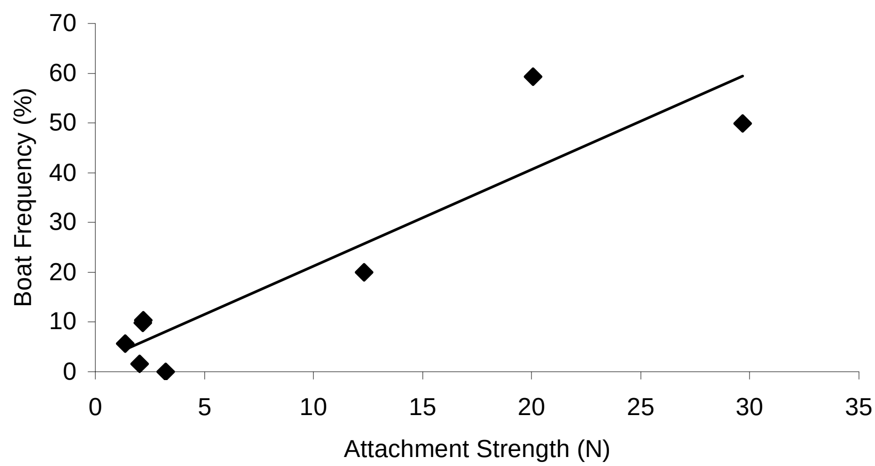


Figure 5.3 Correlation between mean attachment strength (N) of each fouling species and their corresponding frequency in boat hull fouling communities (boat frequency data from Chapter 3). Pearson's correlation $r = 0.915$.

Location of breakage differed between colonial and solitary species (Table 5.1). Breakage occurred at the base or stalk for solitary species (*B. glandula*, *S. clava*, *S. gibbsii*, and *C. inflata*). In contrast, for colonial species (*D. vexillum*, *B. violaceus* and *H. bowerbanki*), complete dislodgment was rare and most colonies simply fragmented under experimental conditions (*i.e*. part of the colony broke off but some always remained attached to the substrate). Attachment strength increased with individual height only for *S. clava* (Pearson correlation $F = 20.798$, $p < 0.001$, $R^2 = 0.510$) and *Mytilus* sp. (Pearson correlation $F = 37.624$, $p < 0.001$, $R^2 = 0.35$).

5.3.2 Drag Force

Drag coefficients of solitary species *C. inflata* and *S. clava* decreased as a function of velocity (Figure 5.4a). These two species demonstrated the ability to reconfigure at increasing velocities, *S. clava* bends at its flexible stalk (Figure 5.5) while *C. inflata* reconfigured to a lesser extent by bending its entire body and thus reducing its area projected into the flow. The other solitary ascidian, *S. gibbsii*, and colonial species *D. vexillum* and *B. violaceus* did not reconfigure in flow and their drag coefficients varied little with increasing velocities (Figure 5.4). Mean drag coefficients were used in further calculations for these three species (*S. gibbsii* = 0.156 ± 0.005, *D. vexillum* = 0.016 ± 0.003, *B. violaceus* = 0.093 ± 0.028). Variability in drag coefficient was higher for colonial species than solitary species tested (Figure 5.4).

Table 5.1: Dislodgment location for solitary (base, byssal threads, stalk, attachment disc or shell) and colonial species (fragment or colony dislodgment) and proportion for species tested by recording spring scales. NA = not applicable.

		Dislodgment location (%)				
Type	**Species**	**Base**	**Byssal threads**	**Stalk**	**Attachment Disc**	**Shell**
Solitary	*B. glandula* (n=30)	100	NA	NA	NA	0
	C. inflata (n=14)	100	NA	NA	NA	NA
	Mytilus sp. (n=47)	NA	100	NA	NA	0
	S. clava (n=28)	75	NA	16.7	5.6	NA
	S. gibbsii (n=21)	87.5	NA	12.5	NA	NA
		Dislodgment location (%)				
Type	**Species**	**Fragment**	**Colony dislodgement**			
Colonial	*B. violaceus* (n=23)	100	0			
	H. bowerbanki (n=7)	100	0			
	D. vexillum (n=20)	100	0			

5.3.3 Dislodgment Velocity

Dislodgment velocity was highest for *S. clava* (27.30 ± 4.04 m/s, N=12), followed by *C. inflata* (16.1 ± 1.0 m/s, N= 9), and *S. gibbsii* (15.4 ± 1.7 m/s, N=12) (Figure 5.6). Most individuals of *S. clava* had dislodgment velocities higher than powerboats and sailboats. In contrast, *S. gibbsii* and *C. inflata* had dislodgment velocities slower than powerboats but higher than sailboats (Figure 5.6). The dislodgment velocities of colonial species *D. vexillum* (9.7 ± 1.7m/s, N=6) and *B. violaceus* (6.8 ± 1.1 m/s, N=9) were much slower than powerboat velocity but still faster than maximum sailboat velocity.

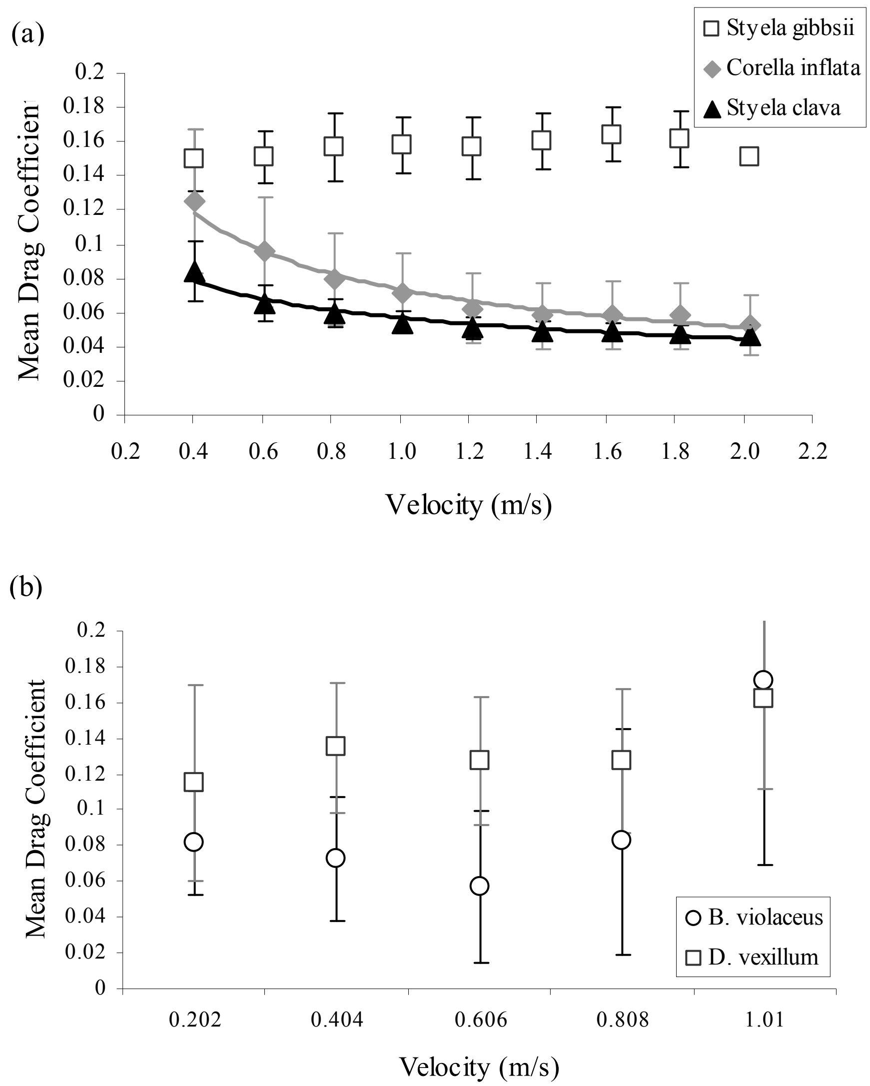


Figure 5.4 Drag coefficients (mean ± standard error) by velocity for a) solitary and b) colonial species. Regression curves: *S. clava* $y = 0.057x^{-0.3454}$, $R^2 = 0.9438$ and *C. inflata* $y = 0.0737x^{-0.5212}$, $R^2 = 0.9702$.

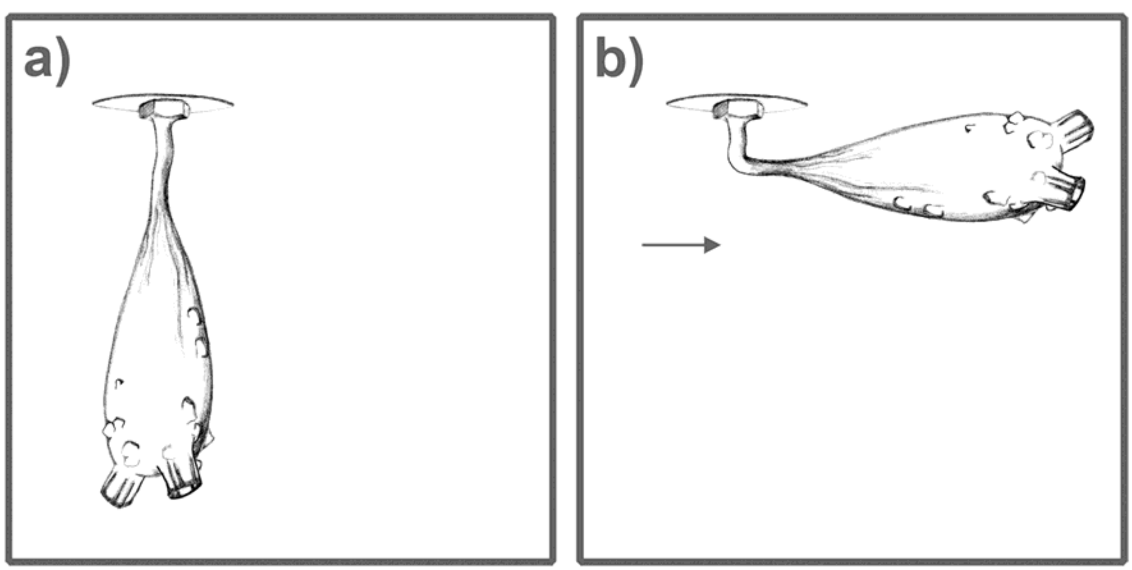


Figure 5.5: Illustration of reconfiguration of *Styela clava* in the flume a) at rest and b) in flow. Arrow indicates direction of water flow and scale bar, representing 2 cm. Illustration by Megan Mach.

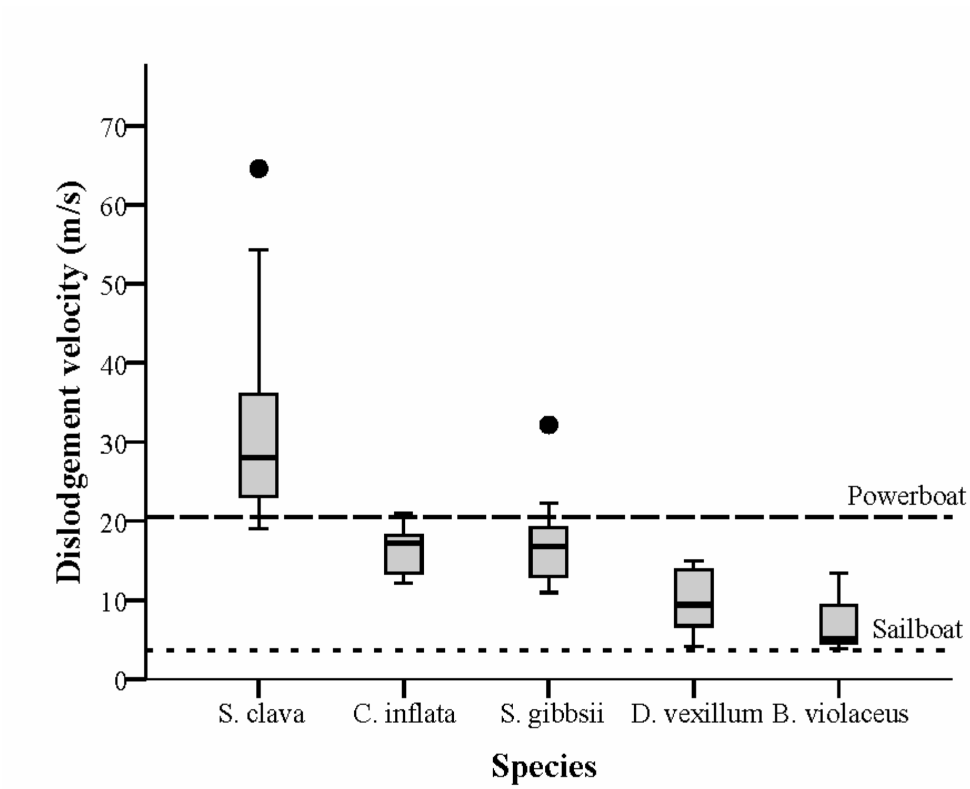


Figure 5.6: Boxplot of estimated minimum dislodgment velocity calculated based on observed sizes of individuals tested for each species. The top and bottom of the box represent the 25th and 75th percentile, the dark line is the median and whiskers are the extent of the 95% confidence intervals. Black dots indicate predictions outside the 95% confidence intervals. Dashed horizontal reference lines indicate top speed for sailboat (3.6 m/s) and powerboat (20.5 m/s). Any individuals below the line should be dislodged.

5.4 Discussion

Our results show that three common hull fouling invaders (*S. clava*, *D. vexillum* and *B. violaceus*) have the ability to remain attached to marine vessels. Although all five species tested are common fouling species and could be carried on sailing vessels, it appears that only *S. clava* would be regularly carried on the exposed hull surfaces of faster-moving powerboats. *S. clava* was the most common non-indigenous species encountered on the hull and niche areas of both sail and powerboats in a previous study (Chapter 3). This species was likely transported globally on naval ships in conjunction with the Korean War (Clarke and Therriault 2007). The timing of *S. clava* introduction to California (1933) occurred after initiation of widespread commercial traffic in the 1920s (Carlton 1979; Hewitt 1993) suggesting that the original introduction vector may have been ship hull fouling (Abbott and Johnson 1972). Further, its secondary spread has been linked to recreational boating activities on the east and west coasts of North America (Clarke Murray *et al.* 2011; Darbyson *et al.* 2009; Lambert and Lambert 1998). Here we show that this worldwide invader has a dislodgment velocity much greater than that of all vessel types – a consequence of its flexible reconfiguration, low drag coefficient, and high attachment strength. Flexible reconfiguration is a common strategy of many sessile marine organisms to reduce drag forces (Boller and Carrington 2006; Denny 1994; Koehl 1984; Vogel 1984). In sum, our results support the efficacy of boat-hull transport for this organism.

Despite their low attachment strength, the colonial ascidians tested (*B. violaceus* and *D. vexillum*) could be dispersed on slower-moving vessels such as sailboats and barges. Their low profile reduces drag, projecting a much smaller perpendicular area to water flow. In laboratory tests of a *Botrylloides* species from San Francisco, Edlund and Koehl (1998) found that the tissue strength of *Botrylloides* colonies was much stronger than their attachment to the substrate. To the contrary, our results suggest that colonies of *B. violaceus* and *D. vexillum* were more likely to fragment than to peel completely from the substrate. Such fragmentation may be an important survival strategy, reducing the probability that complete dislodgment occurs. Fragments of botryllid ascidians have the ability to reattach (Worcester 1994) and this may be an important part of the invasion process for these species. Rafting on broken eelgrass blades and subsequent reattachment has been shown to be a more efficient dispersal strategy over large distances than larval swimming (Worcester 1994). Such "programmed breakage" also has been observed in wave-battered corals and algae where fragments frequently break and regrow (Anderson *et al.* 2006; Highsmith 1982). Additionally, these ascidian species produce three-dimensional growth forms (lobes, strings, etc.) which could experience higher drag and increased risk of fragment dislodgment. Further testing is required to determine if there are differences in attachment strength among these vertical growth forms compared to horizontal colonies. Dislodgment of three-dimensional growths may be an effective dispersal strategy, such that the colony remains attached to the boat but pieces break off to establish new colonies in visited harbours.

The native solitary ascidians *S. gibbsii* and *C. inflata* had low attachment strength but experienced comparable drag to that of the larger *S. clava* despite their small size likely because they were unable to reconfigure in flow. On average, the dislodgment velocity for these species was slower than for powerboats, suggesting they could only be transported on slower-moving boats. However, dislodgment velocity is only one factor to consider in invasion dynamics: settlement preferences, larval behaviours, hydrodynamics, and early mortality dynamics may limit the presence of fouling species on boats (Koehl 2007; Pawlik 1992) thereby lowering their entrainment potential in the vector. Dislodgment velocity estimated using the current method assumes that fouling organisms are exposed to the maximum water velocity. Niche areas often harbour disproportionately higher amounts of fouling species than smooth hull surfaces likely because of the reduction in flow and an absence of antifouling paint application (Coutts et al 2007; Clarke Murray et al 2011; Davidson et al 2011). Thus, estimated dislodgment velocity may underestimate the probability of transport for individuals protected by niche areas. The model also assumes that these vessels reach maximum speed at least once during the voyage. For those vessels that remain below maximum speed, dislodgment for organisms attached to the hull also would be overestimated.

Two species tested in the current study, *S. gibbsii* and *D. vexillum* were predicted to be transported on slower-moving vessels but were not observed in the fouling communities of sailboat or powerboats despite being present on nearby floating structures (Chapter 3). Therefore, dislodgment velocity loses predictive power if organisms do not settle on vessels as a result of reproductive timing or settlement preferences. A comparison of the locations of species within boats (hull vs. protected niche areas) would provide an additional test of the accuracy of the dislodgment model as those species with low estimated dislodgment velocities should only be found on protected niche areas and not on the exposed hull.

Settlement preferences also may promote the transport of invasive species on marine vessels. For example, the invasive bryozoan *Watersipora subtorquata* is a fouling species with high tolerance to copper antifouling compounds applied to commercial and recreational boats (Floerl *et al.* 2004) and even prefers to settle on treated areas (Dafforn *et al.* 2008; Piola and Johnston 2009). It is unknown if invasive ascidians have similar adaptations to antifouling chemicals as these properties have not yet been tested.

Little information exists about the attachment of ascidians. The colonial ascidians *Perophora viridis* and *Amaroucium constellatum* attach initially using a viscous adhesive secretion laid down by the papilla (Grave & McCosh 1923 IN Lane 1973). The structure of the attachment disc of *S. clava* appears very different from the fibrous attachment of *S. gibbsii; S. clava* has a flat, disc-like attachment compared to *S. gibbsii* that had a bumpy, uneven attachment site (CCM pers. obs.). The solitary ascidian *S. clava* exhibited stronger attachment strength with height, but only *C. inflata* had a wider attachment site with larger size. This suggests *S. clava* either deposits more adhesive or lays down stronger adhesive as it grows.

Attachment strength was measured for *Mytilus* sp. on a subset of typical fouling substrate types: floating docks and natural rock. The presence of artificial structures has been shown to promote dominance of invasive species (Dafforn *et al.* 2009; Tyrell and Byers 2007) and substrate type was important in determining attachment strength for the cryptogenic *Mytilus* sp. complex here. Unfortunately, the other species studied were not found on more than one substrate but it is highly likely to be an important variable for other species as well. Further research is required to investigate the role of substrate in attachment of other hull fouling species. This is especially critical in addressing the attachment upon marine vessels protected by antifouling paints, not tested in the current work. While traditional antifouling paints employ toxic biocides that prevent invertebrate larval settlement, some types of antifouling paint are specifically designed to prevent species from remaining attached while a vessel is moving (Piola *et al.* 2009; Schultz *et al.* 1999). These ablative or fouling-release paints slough off the top layer with movement removing the associated fouling organisms.

Extrapolating drag forces from laboratory flume conditions (2 m/s) to environmentally relevant water velocities or boat speeds (20 m/s) may be problematic because of uncertainty in drag coefficient (*e.g.,* Bell 1999; Denny and Gaylord 2002; Gaylord *et al.* 1994). The colonial species tested had higher variability in drag coefficient than solitary species but this is likely a result of the higher drag of the disc apparatus used to suspend individual colonies within the flume. Mortality predictions based on dislodgment models often have high degrees of associated error (Denny 1995; Mach *et al.* 2007 but see Martone and Denny 2008). Recent studies show that data collected at higher speeds (up to 4 m/s) improves drag predictions for some hydrodynamically-stressed macroalgal species, but not for others (Patrick Martone, University of British Columbia, unpublished data). In this study, we acknowledge this uncertainty and have chosen to be conservative in our drag coefficient extrapolation for *S. clava* and *C. inflata*, assuming no decrease in C_d with increasing velocity above 2 m/s (see Bell 1999, Extrapolation Model B). Thus, dislodgment velocities calculated here represent minimum estimates for these species. If C_d continues to decrease with increasing velocity for these species, predicted dislodgment velocities would increase, suggesting that these species may be able to resist even faster boat velocities before being dislodged.

The drag coefficients recorded for the five ascidian species were relatively low. At comparable Reynolds numbers, solid spheres have drag coefficients of 0.6 and plastic sheets have drag coefficient of 0.015 (Gaylord *et al.* 1994). The mussel species *Mytilus californianus* had a recorded drag coefficient of 0.2 (Denny *et al.* 1985) while an anemone-shaped model without its tentacles had drag coefficient of 0.1. The reconfiguration in *C. inflata* and *S. clava* allows them to reduce their drag comparable to that of more streamlined shapes anemones and plastic sheets. However, *S. gibbsii* does not reconfigure with increasing velocity and still had a relatively low drag coefficient. The small *S. gibbsii* may be able to hide within the boundary layer and experience less drag from water flow in the experimental flume. Boundary layer size is affected by both water velocity and distance from the leading edge so that location on the vessel affects the probability of dislodgment (Koehl 1984). Further research is required to compare the boundary layer thickness on moving vessels compared to that experienced under experimental conditions in the flume.

An additional source of uncertainty in predicting dislodgment is the location of individuals within "beds" or dense fouling communities that may be primarily subjected to lift forces, rather than drag forces. For mussels, Bell & Gosline (1996) predicted that attachment measured parallel to the substratum (drag) is 53-57% of that measured perpendicular to it (lift). The angle of lift and drag forces experienced by the organism would vary with location on the boat, community composition, and fouling density as well as solitary versus encrusting growth forms (e.g. Coutts 2010). The development of complex fouling communities on vessels also can drastically reduce the drag experienced by individual members within a community, as previously shown for dense seaweed communities (Johnson 2001). In this manner, gregarious settlement, as occurs in barnacles and other marine organisms, can lead to sheltering thus reducing shear stress on individuals (Schultz *et al.* 1999). Complex communities also provide microhabitats for smaller sessile and mobile species, allowing them to be transported with travelling vessels, as has been hypothesized for a number of invasive species (Carlton 1979, 1989; Carlton and Hodder 1995; Frey *et al.* 2009; Hewitt et al 2004). Therefore, estimating dislodgment velocity based on single individuals may lead to an underestimation of the probability of transport to new habitats.

Single force application, such as the pull-to-break measure of attachment strength used here, may also lead to an underestimation of dislodgment. Previous authors found low probabilities of breakage compared to field observations (Gaylord *et al.* 1994; Gaylord 2000; Johnson and Koehl 1994; Kitzes and Denny 2005; Utter and Denny 1996 but see Martone and Denny 2008). Two additional mechanical properties may affect dislodgment over time: fatigue fracture and creep (Vogel 2003). Fatigue fracture, or repeated loading of smaller stresses, has been shown to be important for wave-swept organisms such as intertidal macroalgae (Mach *et al.* 2007; Mach *et al.* 2011). Fatigue fracture is not accounted for in the current dislodgment model and has not been well characterized in general (Koehl 1984; Mach *et al.* 2007). Creep, on the other hand, occurs when soft-bodied organisms slowly stretch or deform in response to constant force application (Koehl 1984). This has been well-studied in anemone mesogleal tissues and the same likely applies to solitary ascidians that also have hydrostatic skeletons. With constant force application, anemone tissues have been shown to reversibly deform and the degree of deformation depends on the time scale of force application (Koehl 1999; Koehl 1984). Attachment strength measured here is more representative of instantaneous forces, such as initial acceleration of a boat. However, creep experienced by organisms attached to a cruising boat has not been characterized but may lead to dislodgment at smaller forces during long slow boat voyages.

The three solitary species tested have the ability to retract their siphons when disturbed and they demonstrated this behaviour during experiments in the recirculating flume. This suggests that their ability to filter water and feed may be restricted when water velocity increases. New invasions can only be seeded by individuals that survive the voyage and reproduce in the new environment. Therefore, although they have the physical ability to remain attached, these species may not survive trans-oceanic voyages to invade new areas if filter-feeding time is critically limited. Similarly, environmental conditions within ballast tanks have been found to limit the survival of potential ballast water invaders (Flagella *et al.* 2007; Gollasch *et al.* 2000). Further study is required to determine effect of voyage duration, speed, and surface chemistry (such as antifouling paint) on survivorship of hull fouling invaders.

In conclusion, the non-indigenous species tested here demonstrated adaptations that allow them to remain attached to travelling vessels. Two successful strategies were observed for non-indigenous species, solitary species possessed low drag coefficients as a result of reconfiguration combined with high attachment strength. Colonial, asexually reproducing non-indigenous species had extremely low drag profiles but low attachment strength, which may contribute to fragmentation and dispersal. We demonstrated that the non-indigenous ascidians *S. clava*, *D. vexillum* and *B. violaceus* have the biomechanical ability to travel with marine vessels, suggesting that these species can be introduced and spread via hull fouling. Biomechanical properties, such as high attachment strength and reconfiguration in flow may contribute to the ability of hull fouling non-indigenous species to invade new habitats throughout the world. As little is known about these species in their native habitats, we can only speculate that high attachment strength and the ability to reconfigure evolved in response to high flow conditions. These adaptations may have been further selected for through the mechanism of selection regime modification of the hull fouling vector (Byers 2002). Only those individuals with the mechanical ability to withstand the hydrodynamic conditions of the human-mediated voyage would survive to invade the new region, except in niche areas protected from hydrodynamic stresses. Biomechanical parameters could be used to assess potential invaders for the ability to travel via hull fouling vectors and allow researchers to model species movements more effectively. In addition, this type of research can assist in the development of antifouling technologies and inform vector management in order to reduce the introduction and spread of invasive species.

Chapter 6: Assessing drivers of the spatial distribution of subtidal marine non-indigenous species*

6.1 Introduction

Examining spatial patterns of invasions gives us insight into the variables driving the introduction and spread of invasive species. This information can be valuable in the control, management, and prevention of invasion events (Carlton and Ruiz 2005; Drake and Mandrak 2010; Leung *et al.* 2005; Switzer *et al.* 2011). The current distribution of non-indigenous species (NIS) reflects the spatial pattern of introduction, establishment, and subsequent spread. The original introduction to a region may have been a single event to a single site, multiple introduction events to the same site through time or multiple introductions across the region. Establishment of NIS following introduction indicates that the environmental conditions of the new location are compatible with survival and reproduction. Post-establishment, secondary spread can occur via natural dispersal or human-mediated spread, which may occur via the same primary introduction vector or different vectors. Successful introduction and secondary spread ultimately depends on the habitat suitability of the local environment for each species introduced and potential dispersal vectors available to it. This complicated interplay of governing factors and processes means it can be difficult to explain and project the spatial pattern of invasions in a region.

The invasion history often follows region-specific patterns of settlement and trade. For marine ecosystems in British Columbia (BC) the primary introduction pathways include aquaculture import, commercial shipping, and recreational boating (Levings *et al.* 2002). Further, the aquaculture pathway has been suggested as the single greatest vector of introduction of NIS worldwide (Ruesink *et al.* 2005; Wasson *et al.* 2001). It includes both intentional imports (which may be subject to policy or regulation) and hitchhiking organisms. For example, commercial imports of live oyster seed and adults to the Pacific

* This work is part of a manuscript in preparation with co-authors Heidi Gartner, Kai M.A. Chan, Evgeny A. Pakhomov and Thomas W. Therriault

coast of North America began in the 1880s and continued unregulated until the 1930s (Wonham and Carlton 2005). This vector intentionally introduced *Crassostrea gigas* (Thunberg, 1793) from Asia and *Crassostrea virginica* (Gmelin, 1791) from Atlantic Canada and is believed to be responsible for dozens of associated hitchhiking introductions in BC, including the invasive oyster drill snail (*Urosalpinx cinerea* Say, 1822), mud snail (*Batillaria attramentaria* G. B. Sowerby I, 1855), and wireweed (*Sargassum mutica*), among others (Levings *et al.* 2002).

The shipping pathway is considered one of the most important pathways for NIS worldwide (Fofonoff *et al.* 2003). Propagules of NIS have been detected in ballast water and sediments (e.g., Flagella *et al.* 2007; Hayes and Hewitt 2000; Lavoie 1999; Levings *et al.* 2004; MacIsaac *et al.* 2002), hull fouling communities (e.g., Coutts and Taylor 2004; Drake and Lodge 2007; Godwin 2003; Gollasch 2002; Lewis *et al.* 2004; Sylvester *et al.* 2011), and associated with sea chest fouling (e.g., Coutts *et al.* 2007; Coutts and Taylor 2004; Frey *et al.* 2009; Godwin 2003). Biological studies of ballast water in ships entering Vancouver Harbour, BC showed that coastal organisms were still present after Mid-Ocean Exchange was performed (Levings *et al.* 2004). However, it has proved difficult to determine the magnitude of primary introductions associated with the shipping pathway in BC (Choi 2011; Lo 2009).

The importance of recreational boating as a pathway for marine NIS introduction and spread is less well understood than other NIS pathways. Small boats have been shown to be a possible vector for NIS in Scotland (Ashton *et al.* 2006), France and Spain (Mineur *et al.* 2008), Australia (Floerl 2002), New Zealand (Floerl *et al.* 2005a), and California (Davidson *et al.* 2010). Fouling species settle and grow on all unprotected submerged surfaces of boats and can become entangled in the propeller, propeller shaft, anchor, and fishing gear and subsequently transported to new locations (Davidson *et al.* 2010; Minchin *et al.* 2006). There is little direct evidence of introductions from this pathway and with the relatively new research focus it has been difficult to estimate its contributions to the invasion of a region (but see Chapter 3; Clarke Murray *et al.* 2011).

Regulatory and industry or market changes may have reduced the importance of earlier pathways, such as aquaculture. In Canada, policies such as the ICES voluntary Code of Practice on the Introductions and Transfers of Marine Organisms in the 1970s and Canadian Fishery Regulations reduced the number of species introduced intentionally for aquaculture or other purposes (Fisheries & Oceans Canada 2010; International Council for the Exploration of the Sea (ICES) 2005). Aquaculture transfer zones for movement of products within BC are governed by Fisheries & Oceans Canada (Fisheries & Oceans Canada 2010) and may limit the extent of secondary spread of these species and their associated hitchhikers, although more research is required. In contrast, other pathways continue to operate almost completely unregulated, such as recreational boating, and may still contribute to primary introduction and secondary spread within this region (Clarke Murray *et al.* 2011 Chapter 3).

Upon arrival with a vector in the introduced region, NIS propagules must survive, grow, and reproduce in order to establish a new population. The potential suitability of a newly invaded habitat depends on its abiotic conditions, biotic interactions, and stressors present. Species-specific physiological tolerances are among the best predictors of survival and reproduction of invaders and require a good match with the characteristics of the invaded habitat (Guisan and Thuiller 2005). A recent study by Roura-Pascual and colleagues (2011) found that climatic suitability, combined with human habitat modification, was the best predictor of the global distribution of an invasive ant species. In marine systems, temperature and salinity often are examined for their role in limiting survival and reproduction and data availability (Epelbaum *et al.* 2009). However, successful NIS with long invasion histories often have wide tolerance limits and these variables may not necessarily be useful (Epelbaum *et al.* 2009; Herborg *et al.* 2008), especially for informing potential management options (Therriault and Herborg 2008).

Examining the distribution of NIS provides an opportunity to explore the range of potential factors that contribute to their spatial distribution. There are a number of quantitative approaches to examining invasion patterns (reviewed by Drake and Mandrak 2010); including species-based, vector-based, and combination models. Ecological or physiological attributes have been used to predict future invaders at the species-level or at higher taxonomic groupings (Kolar and Lodge 2001; Marchetti *et al.* 2004; Ricciardi and Rasmussen 1998). While other studies have focused on the similarity or suitability of donor and recipient environments in order to estimate the probability of establishment (Ruesink 2005). Models focused on pathways or vectors have been used to predict introduction or spread (Floerl *et al.* 2008; Leung *et al.* 2006; MacIsaac *et al.* 2004) and commonly employ gravity or spatial interaction models (Bossenbroek *et al.* 2001; Bossenbroek *et al.* 2007; Leung *et al.* 2006; MacIsaac *et al.* 2004). Combining species and vector-based modeling approaches follows the entire invasion process: from introduction to establishment and spread (Bossenbroek *et al.* 2001; Herborg *et al.* 2007; Jacobs and MacIsaac 2009). Transport and environmental suitability models have been combined to model spread of zebra mussels (Bossenbroek *et al.* 2007), freshwater fish (Sharma *et al.* 2009), and macrophytes (Jacobs and MacIsaac 2009). Physiological tolerances and habitat suitability have been combined to predict distribution of aquatic species (Epelbaum *et al.* 2009; Herborg *et al.* 2008; Sharma *et al.* 2009). While these diverse models are very useful regarding the expected distribution of NIS they often do not compare the overall influence of the full range of factors on the actual spatial distribution of a species. Thus, as a result the potential distribution of an NIS could be an over- or under-prediction.

More recent modeling studies have attempted to compare the relative importance of biogeographic, climatic, economic, and demographic factors in terrestrial ecosystems. These studies have utilized statistical techniques at a variety of spatial and taxonomic scales. Drivers of the global distribution of individual NIS have been examined (Roura-Pascual *et al.* 2011). Continent-wide comparisons of NIS richness across a broad range of taxonomic groups have been performed (Essl *et al.* 2010; Pyšek *et al.* 2010). Smaller-scale regional studies of NIS richness also have sought to understand the drivers of invasion in California (Dark 2004; Higgins *et al.* 1999). Although contiguous with Pacific states, Canadian marine waters are governed separately and have been subject to their own patterns of vector traffic and slightly different environmental and biogeographic conditions. There has been no study of the regional pattern of invasion for

British Columbia or other coastal regions of Canada. This may be because of the lack of spatially-explicit data for the region and potential difficulties associated with biogeographical analyses.

Spatial analyses at regional scales introduce the problem of spatial autocorrelation. The very nature of spatial data often violates the basic assumption of linear regression; sampling units are no longer independent as closer sampling sites are more likely to be similar than distant ones. Several studies have demonstrated the importance of incorporating spatial autocorrelation when assessing species distributions (Dark 2004; Higgins *et al.* 1999; Jetz and Rahbek 2002; Lichstein *et al.* 2002). When data are spatially dependent, statistical tests lose some power compared to an independent sample of the same size (Anselin and Rey 1991). In order to ensure that the model chosen accurately reflects the scale at which the driving mechanisms operate spatial autocorrelation must be taken into account. Thus, it is essential to compare the spatial autocorrelation of the defined model to that of the original response variable to ensure spatial scale has been sufficiently captured by the variables included in the model.

This chapter presents an analysis of the distribution of subtidal marine NIS in BC using Geographic Information Systems (GIS) and multiple linear regression. The objective of this study was to examine the relationship between NIS spatial distribution relative to climate, vector, and demographic variables. In particular, recreational boating variables were included to compare the influence of this pathway on the current distribution of NIS alongside the better-known vectors, shipping and aquaculture-related activities.

6.2 Methods

6.2.1 Overview of Methodology

The distribution of NIS (response variable) was measured using settlement plates at marina and harbour sites throughout BC's marine waters. Predictor variables were compiled from a range of environmental, demographic, and vector traffic datasets.

Multiple linear regression with spatial autocorrelation was then used to characterize the influence of the predictor variables on the response variable.

6.2.2 Sampling Design

Suspended settlement plates were used to sample subtidal fouling species. Each plate consisted of a black plastic disc with four plastic Petri dishes attached to the underside to provide three-dimensional structure. The settlement plates were suspended from floating docks 1m below the surface, and fouling communities allowed to develop for approximately four months. The strings were weighted at the bottom to keep the lines vertical and the plates horizontal. In the first sampling season (2006), plates were placed at three depths on each string to check for depth effects (full sampling design can be seen in Chapter 2). In summer 2006, 16 tri-depth arrays were placed at each of nine sites in the Strait of Georgia. In summer 2007, single depth arrays (suspended at 1 metre depth) were placed at more than 50 sites throughout British Columbia (full details of the second sampling season methods can be found in Gartner (2010). After each sampling season the Petri dishes were removed from the disc, placed in individual Ziploc bags and preserved with 4.0% formalin in the field to prevent mobile species loss and predation. The dishes were processed in the laboratory to identify all individuals present to the lowest taxonomic level possible. Species were then classified as native, non-indigenous, cryptogenic, or indeterminate if species level identification was not reached according to criteria in (Carlton 1989).

6.2.3 Data Sources

The response variable was NIS occurrence at each site sampled, as extracted from the two settlement plate surveys described above. Species considered cryptogenic or indeterminate in BC were not included in the analysis.

Interpolation techniques were used to create raster surfaces for explanatory variables covering the entire study region. Variable surfaces were created using either Inverse Distance Weighting (IDW) or Kernel Density. Values for each of the sampling sites were extracted from the interpolated raster surfaces using point extraction. All spatial data analysis was performed using ArcGIS 9.1 (ESRI). Explanatory variables are summarised in Table 6.1.

Table 6.1 Overview of response and explanatory variables used in analyses; more detailed explanation of methods used for each can be found in relevant sections below

	Variable	**Description**
Response variable	Total NIS richness	#NIS per sampling location
Explanatory variables	Latitude	Latitude of sampling location
	Environmental	Temperature and salinity: minimum and maximum values
	Population density	Average number of people per km^2
	Recreational boating	Density: coastal density of marinas
		Marina propulsiveness: Probability of average resident boat traveling from that marina
		Marina attractiveness: Attractiveness of a marina based on its distance (home to visited marina) and reported visitation
		Marina visitors: Number of visitors and Shannon diversity of visitors
		Marina slip types: Number of resident and transient slips
	Commercial shipping	Port distance: distance from site to nearest port Arrivals: number of arrivals at nearest port, weighted by distance
		Ballast water: volume of water released at nearest port, weighted by distance
	Aquaculture	Aquaculture density: density of aquaculture sites, either all types, shellfish only, or finfish only

6.2.3.1 Environmental Variables

To examine environmental characteristics that might affect survival and establishment of NIS temperature and salinity variables were included. Temperature and salinity data were obtained from the lighthouse data set maintained by Fisheries & Oceans Canada. There are 18 lighthouse stations scattered along the coast of BC that record environmental data daily at the daytime high tide (Fisheries & Oceans Canada 2009a). The span of years for which data was recorded varies across stations so in order to maximize spatial coverage and control for the documented warming trend in the waters of this region (Masson and Cummings 2007) a common time series was used. Seven years (1979-1985) of monthly average temperature and salinity data were extracted and the maximum and minimum temperatures for the time series recorded. The recorded ocean temperatures ranged from 5.2 °C to 19.7 °C and salinity ranged from $18.5^{o}/_{oo}$ to $32.95^{o}/_{oo}$. The dataset was subjected to Interpolation by Distance Weighting (IDW) to create a surface layer for each of the four data series (maximum and minimum temperature, maximum and minimum salinity).

6.2.3.2 Population Density

Human population density information was obtained from the 2006 census data held by Statistics Canada by regional district (Province of British Columbia 2011b). Regional districts are the BC equivalent of census divisions and were created by the provincial government in the late 1960s for the delivery of certain services (Province of British Columbia 2011a). There are thirty regional districts in total and they range in size from 2,800 km^2 to 130,000 km^2, with larger districts where population is less dense. The Regional District population density (mean number of people per km^2) was assigned for each site in the response variable database. Population density was log-transformed to meet assumptions of homogeneity of variance.

6.2.3.3 Recreational Boating

There were three databases used to extract recreational boating data: marina spatial density, boater behaviour, and marina characteristics.

6.2.3.3.1 Marina Density

Marina spatial density was estimated from a database maintained by Parks Canada. There are 604 geo-referenced records in the original Parks Canada database which were checked and updated using the recent guides to marinas and harbours. GIS Spatial Analyst was then used to perform kernel density interpolation of locations of marinas to create a density layer.

6.2.3.3.2 Boater Behaviour

The second recreational boating data set was the boater questionnaire conducted with marine boaters of BC from 2008-2010. The questionnaire results gave information on travel patterns, cleaning behaviour, and physical locations of respondents' boats. The questionnaire included 104 possible 'destination marinas' (marinas visited) and each respondent was asked to check off those places they had visited in the previous 12 months. The full boater survey can be found in Appendix B. In total there were 616 respondents from 60 home marinas in British Columbia and Washington State. This dataset was used to extract three types of variables: marina propulsiveness, diversity of visitors, and marina attractiveness, which are all explained below.

Marina propulsiveness characterizes the propensity of boats to travel from a given home marina, as calculated by the total number of boats that reported travelling from the given home marina, divided by the total number of respondents from that marina. Diversity of visitors was calculated for each destination marina using the number of visitors hailed from each home marina using the Shannon diversity index to include both the richness of home marinas and their evenness (Equation 1).

$$H = -\Sigma\ [P_i * \ln P_i] \qquad \text{Equation 6.1}$$

where H is the Shannon diversity index and P_i is the proportion of travelers from each home marina.

Marina attractiveness was estimated using a well-known relationship between proximity, visitation and attractiveness commonly used to predict human movement in gravity models (Drake and Mandrak 2010; Leung *et al.* 2006; Schneider *et al.* 1998). Here, the attractiveness of a marina was estimated by analyzing the relationship between distance and probability of visitation. Distance was measured as the straight-line linear distance between home and each visited marina of each boater questionnaire respondent. To account for land mass barriers, half the circumference of Vancouver Island was used to penalize the distance for those marinas on opposite sides of Vancouver Island.

In this analysis we considered only the eight marinas with most respondents in order to ensure sufficient numbers of respondents from each home marina (N=238). In order to test if there was any difference between marinas in the relationship between distance and probability of visitation an ANCOVA was performed with home marina as the co-variate. There was no significant difference in the slope of the regression lines by home marina (ANCOVA (home mar*log D): $F = 0.628$, $df = 7$, $p = 0.733$). Therefore, the data from all eight marinas was plotted and the overall regression used to calculate the relationship between distance and probability of visitation. The resulting regression (log distance and probability: $y = -0.1621x + 0.945$, $F=302.907$, $p<0.001$, $R^2 = 0.283$) explained 28.3% of the variation in probability of visitation (Figure 6.1). The mean of the eight unstandardized residuals from the regression was used as an index of destination-marina attractiveness (Attractiveness Index minimum = -0.17 and maximum 0.32). If a marina was visited less than predicted by its distance, it was less attractive (negative average residual) and if visited more than predicted, it was more attractive (positive average residual). For example, Saltspring Island was a highly attractive marina while Port Alberni was an unattractive marina, since the former had more visitors than would be expected based on its distance and the latter fewer. Note that the behaviour of the attractiveness index is not perfect, as the regression relationship used does not allow negative residuals for marinas with distances further apart than approximately 600 km ($10^{5.8}$ m). The use of a different regression function may improve this index for future analyses.

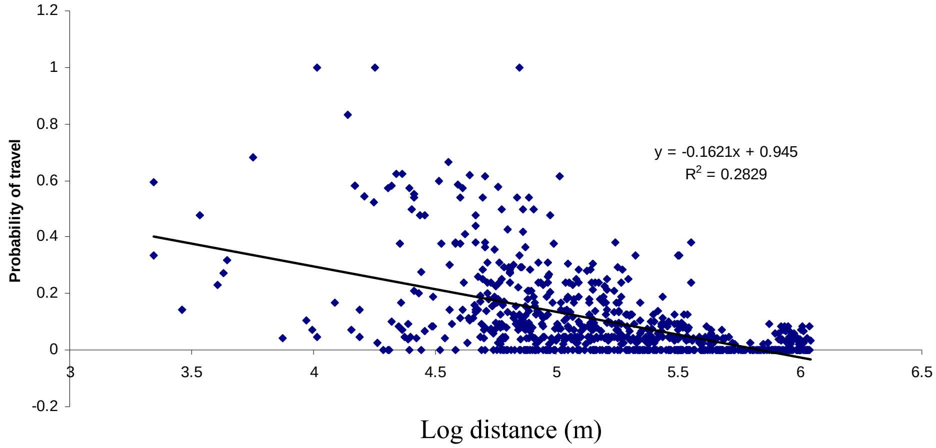


Figure 6.1: Relationship between distance between home and destination marinas (log-transformed) in kilometers and the probability of boat travel from one to the other (R^2 = 0.2839). Only the eight marinas with most respondents were considered as source marinas in this analysis.

6.2.3.3.3 Marina Characteristics

The third and final database used to extract recreational boating variables was the result of a marina owner and operator questionnaire. The complete marina owner and operator questionnaire can be found in Appendix C. It was approved under UBC Behavioural Research Ethics Board (BREB) authorization number #H08-00967. The questionnaire asked respondents to report their GPS spatial location, the number of resident versus transient berths available and an estimate of the percentage of residents that traveled outside the marina. In total, 277 respondents were included in the marina operator database. Variables used in the following analysis included the number of resident berths and number of transient berths. In some marinas, available transient moorage was given in dock length and this was converted to number of berths by dividing by the average boat size in the sample population – 30 feet.

6.2.3.4 Commercial Shipping

Shipping activity was represented by the locations of eight major ports in British Columbia and their corresponding number of ship arrivals and ballast water by volume taken from a Transport Canada database (Lo 2009). The database was compiled from ships logs submitted to Transport Canada and covers the years 2006-2007. A least-cost distance raster layer was created to measure the shortest marine distance to a port from each raster cell (straight-line distance). A land mask was created in order to simulate the effect of land mass barriers to diffusion. Land raster cells were assigned extremely large values so that the algorithm was forced to choose a least cost distance route between cells that circumvented the land. Least-cost distance was used to indicate that the lower the distance from port, the more influence the site has from port activities. The least-cost distance layer was created at a spatial resolution of 10 km^2 and resulted in those sites closest to a port having a value of 2.4 km. Raster cells with extremely high values indicate no influence from port activities.

The shipping pathway has two possible associated vectors: ballast water and hull fouling. The volume of ballast water is a measure of the potential propagule pressure from organisms associated with ballast water release. The number of ship arrivals represents the hull fouling vector, the more ships arriving to the ports equates to the increased possibility of hull fouling organisms arriving in the port. The average ballast water volume and number of arrivals across years was calculated for each port. Raster surface layers for ship arrivals and ballast water volume were created using Interpolation by Distance Weighting (IDW). The IDW function calculates the values of the output cell using the weights assigned (either ballast water volume or ship arrivals in this case) divided by the distance of the point from the raster cell (Childs 2004). Again, the greater the distance, the less influence the port activity has on the value of the raster cell.

Thus, three shipping layer types were created, (i) the least-cost distance raster layer, (ii) IDW layer for the influence of ballast water release at the nearest port and (iii) IDW layer for the number of ship arrivals at the nearest port. These three commercial shipping variables were log-transformed to meet assumptions of homogeneity of variance and to reduce the effect of the extreme high distance values.

6.2.3.5 Aquaculture

Aquaculture falls into two types in British Columbia: shellfish and finfish. Shellfish leases include intertidal beach leases as well as suspended oyster culture while finfish leases are coastal floating pen nets for rearing Atlantic salmon (Naylor *et al.* 2003; Quayle 1988). Locations of shellfish farms, finfish farms, and processing plants were provided by the BC Government. Further information on product volume or transfers between facilities of equipment or product was not available. Kernel density was used to create a raster layer (spatial resolution = 10 km^2) of shellfish farms, finfish farms and all facilities together for a total of three layers. The Kernel Density tool in ArcGIS creates a smooth curved surface fitted over each point where the surface value is highest at the location of the point and diminishes based on a quadratic kernel function from that point (Silverman 1986). The density of each cell is calculated by adding the values of the surface where they overlay the raster cell as bounded by the search radius (ESRI 2011). Essentially, Kernel Density calculates the density of points around each output raster cell. A circular search radius is used to define the neighbourhood search area which by default is set 1/30th of the extent of the input layer, approximately 20 km^2 in this case.

6.2.4 Data Analysis

The response variable (NIS richness) was square root transformed to meet assumptions of normality. Ordinary Least Squares stepwise multiple regression with spatial autocorrelation analyses (similar to Lichstein *et al.* 2002) was used to assess the relationship between NIS richness distribution and explanatory variables. Stepwise regression was performed where variables were included in the model only if associated F-values were significant at the $\alpha = 0.05$ level.

Calculation of Moran's I and spatial correlograms were used to check for spatial autocorrelation in the data (following the methods of Lichstein *et al.* 2002). CrimeStat 3.3 (Levine 2010) was used to test for the significance of spatial autocorrelation and, where present, the scale of spatial autocorrelation was determined by plotting spatial correlograms. Positive Moran's I indicates positive spatial autocorrelation where the closer two places are, the more similar their values (Lichstein *et al.* 2002). The response and each of the explanatory variables were tested for spatial autocorrelation. The residuals of the best regression model were analysed for the presence of spatial autocorrelation by calculating Moran's I, plotting a new correlogram and then comparing the results to the original variable.

6.3 Results

6.3.1 NIS Richness

The NIS settlement plate survey results identified 220 records of 12 NIS species (Table 6.2) at 60 sites in BC. Highest NIS richness was observed in the Strait of Georgia, between Vancouver Island and mainland British Columbia (Figure 6.2). The sites with the highest NIS richness were located on the southeast side of Vancouver Island. There were relatively few NIS in the northern region of British Columbia, although there were a few sites on the island of Haida Gwaii that had higher NIS richness.

Table 6.2: NIS included in the analyses, their native ranges, attributed vectors (O = oyster-associated introductions, H = hull fouling (commercial or recreational), B = ballast water, U = Unknown) and number of sampling sites at which they were present

Species	Taxon	Native Range	Vector	# Sites
Botrylloides violaceus	Ascidian	Asia[1]	O/H	23
Botryllus schlosseri	Ascidian	Europe[1]	O/H	15
Bugula neritina	Bryozoan	Cosmopolitan[2]	O/H	1
Caprella mutica	Amphipod	Northeast Asia[3]	O/H	26
Diplosoma listerianum	Ascidian	Europe	U	1
Halichondria bowerbanki	Sponge	Atlantic coast[4]	U	4
Melita nitida	Polychaete	Atlantic[5]	O	1
Monocorphium achersicum	Amphipod	Atlantic[5]	O/H/B	7
Monocorphium insidiosum	Amphipod	North Atlantic[6]	U	4
Polydora cornuta	Polychaete	Atlantic coast[7]	O/B	27
Schizoporella japonica	Bryozoan	Asia[5]	O	6
Styela clava	Ascidian	Asia	O/H	6

References: 1 - (Berrill 1950), 2 - (Carlton 1979), 3 - (Ashton *et al.* 2007), 4 - (Lee *et al.* 2007), 5 - (Carlton 1989), 6 - (Carlton 2007), 7 - (Çinar *et al.* 2005)

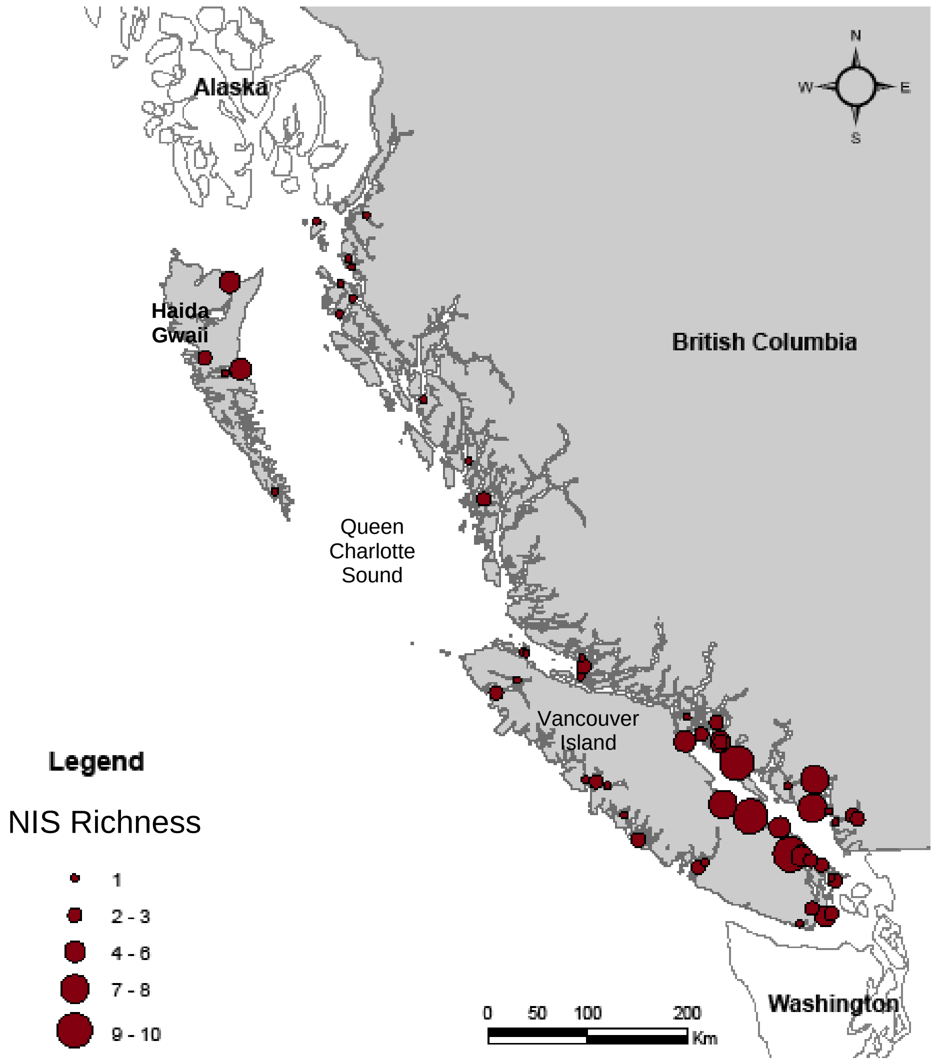


Figure 6.2: NIS richness at each of sampling sites. Size of the circles corresponds to the number of NIS at that site.

6.3.2 Spatial Autocorrelation

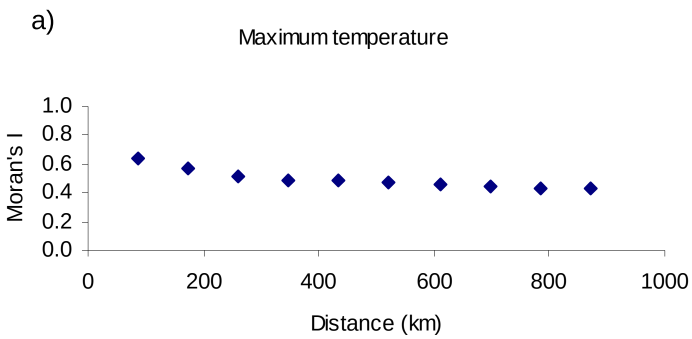
The response variable, NIS richness, had significant spatial autocorrelation (Moran's I = 0.408734, Z = 3.5324, p = 0.001). Autocorrelation was most prominent at the 0-100km range (Correlogram Figure 6.3a). Many of the explanatory variables also were spatially autocorrelated: temperature and salinity were autocorrelated, as expected (Figure 6.3b, max salinity: Moran's I = 0.395009, Z = 3.418549, p< 0.001; max temp: Moran's I = 0.43112, Z = 3.718145, p< 0.001). Human population density was positively spatially autocorrelated, most strongly at the 0-100km scale (Figure 6.3c, Moran's I = 0.364391, Z

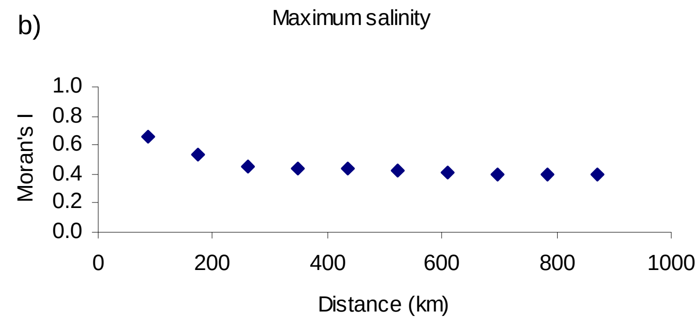
= 3.164471, p<0.001). Marina density was positively spatially autocorrelated at the 0-100 km range (Figure 6.3d, Moran's I = 0.323026, Z = 2.821212, p< 0.001). Aquaculture facilities were positively autocorrelated at the 0-200 km range (Figure 6.3e, Moran's I = 0.525772, Z = 4.503664, p< 0.001).

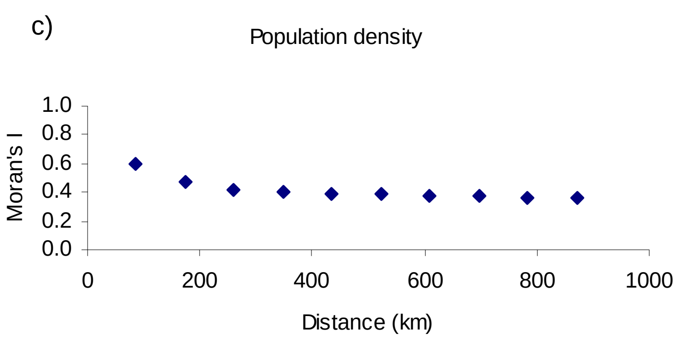
However, there was no autocorrelation detected in some of the variables. Both the number of permanent slips and transient slips at each marina were not spatially autocorrelated (permanent: Moran's I = -0.018102, Z = -0.121267, P>0.05; transient: Moran's I = 0.030181, Z = 0.0303245, p>0.05) reflecting the unique nature of marinas. Marina attractiveness and propulsiveness were not spatially autocorrelated (Figure 6.3f, g) likely reflective of individual boater biases. Finally, commercial shipping variables were not spatially autocorrelated. The ports are well separated geographically and we would not expect there to be a large influence on sites located between ports.

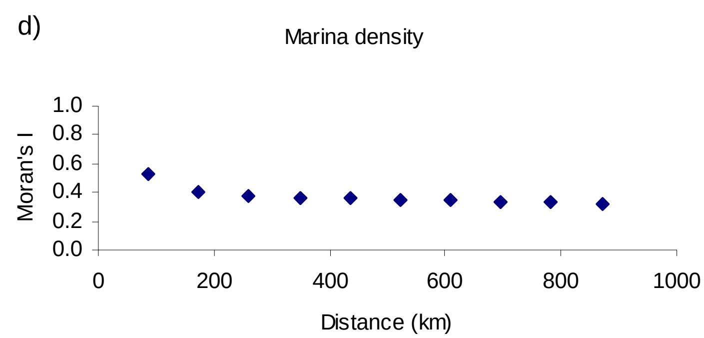
6.3.3 NIS Richness Model

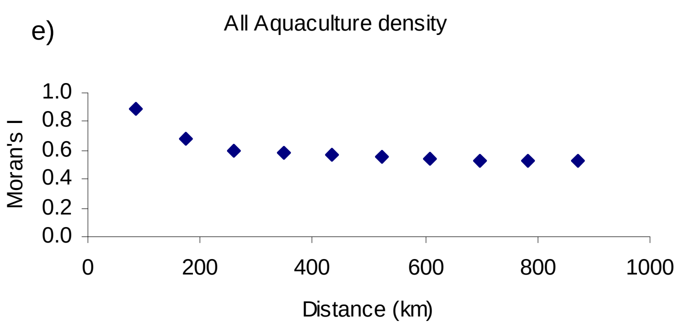
NIS richness ranged from zero to ten species with a mean of 2.02 +/- 0.302 SE. The suite of predictor variables was tested against this response variable to examine the major drivers of invasion in this region. The best stepwise regression model had three variables: minimum temperature, log-transformed population density, and marina propulsiveness (R^2 = 0.593, F = 9.238, df = 3, p = 0.001). NIS richness varied directly with all three variables, increasing with population density, marina propulsiveness, and minimum temperature (Figures 6.4, 6.5).

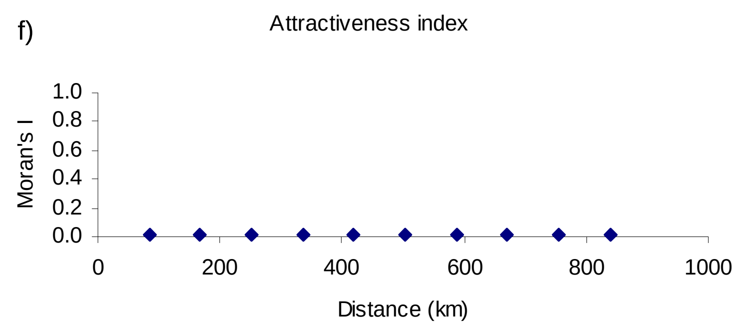












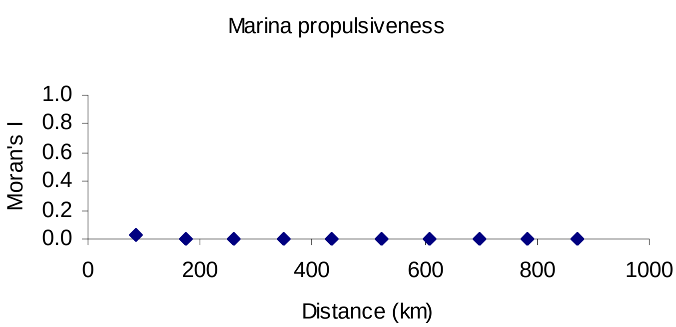


Figure 6.3 Spatial correlograms of explanatory variables showing how Moran's I varies by distance class for a) maximum temperature, b) maximum salinity, c) population density, d) marina density, e) aquaculture facility density, f) marina attractiveness, g) marina propulsiveness

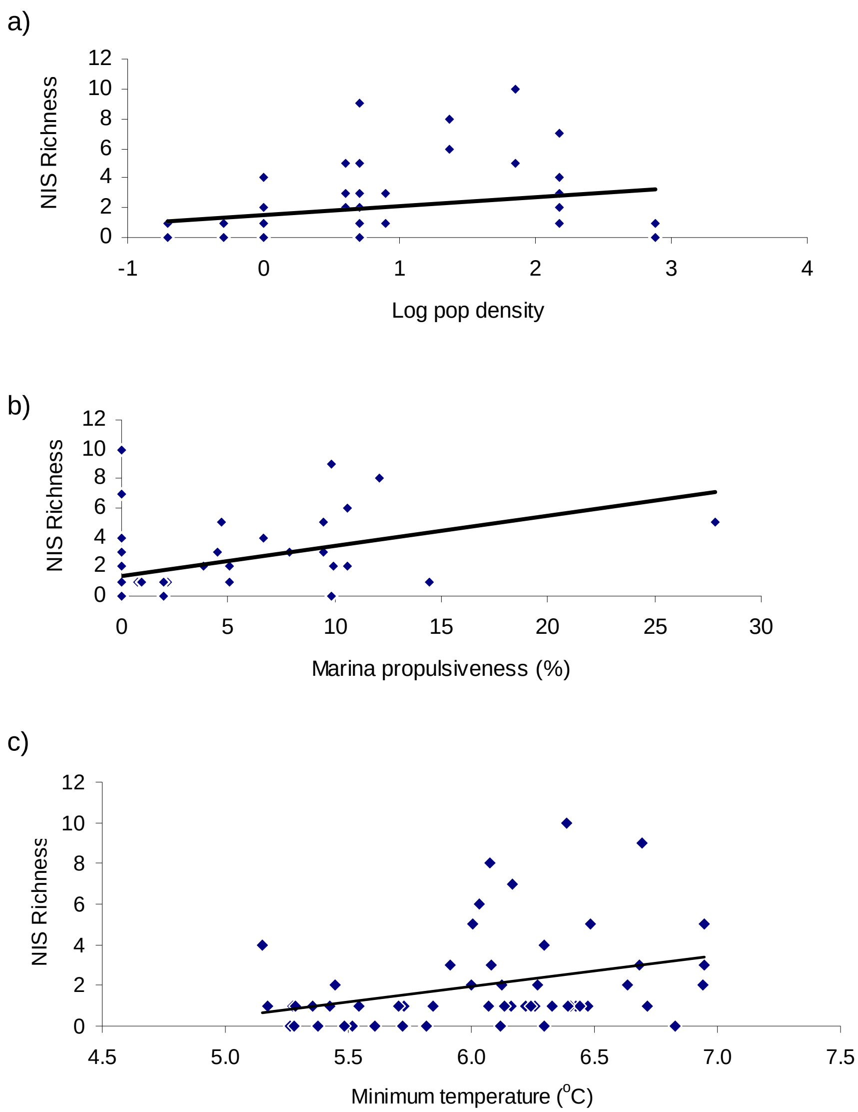
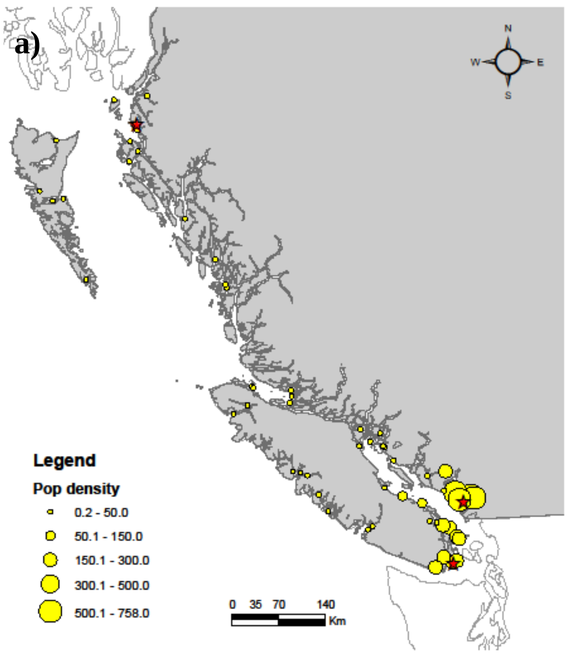
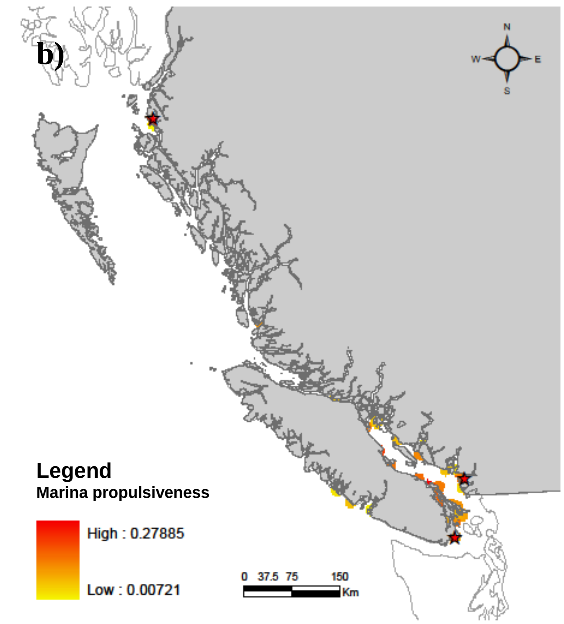


Figure 6.4 Scatterplots of NIS richness (presented untransformed for simplicity) and the three significant model variables a) log population density, b) marina propulsiveness (%) and c) minimum temperature (°C)





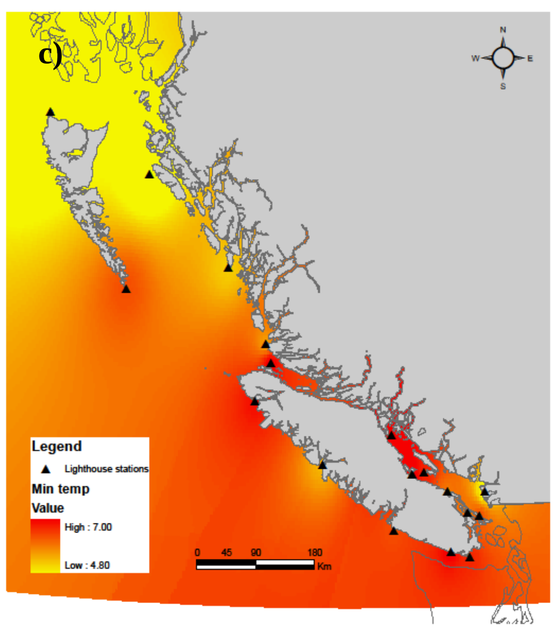


Figure 6.5: Spatial pattern of significant variables in NIS richness regression model a) population density (people/km^2), b) marina propulsiveness, and c) minimum temperature (°C). Stars represent the major population centres: from south to north Victoria, Vancouver and Prince Rupert. Triangles show the location of lighthouse stations.

The NIS richness model residuals were not spatially autocorrelated (Moran's I = 0.130631, Z = 0.564352, $p > 0.05$) and the corresponding correlogram no longer showed evidence of spatial autocorrelation (Figure 6.6b) suggesting that the explanatory variables in the model sufficiently capture the spatial scale of the predictor variable.

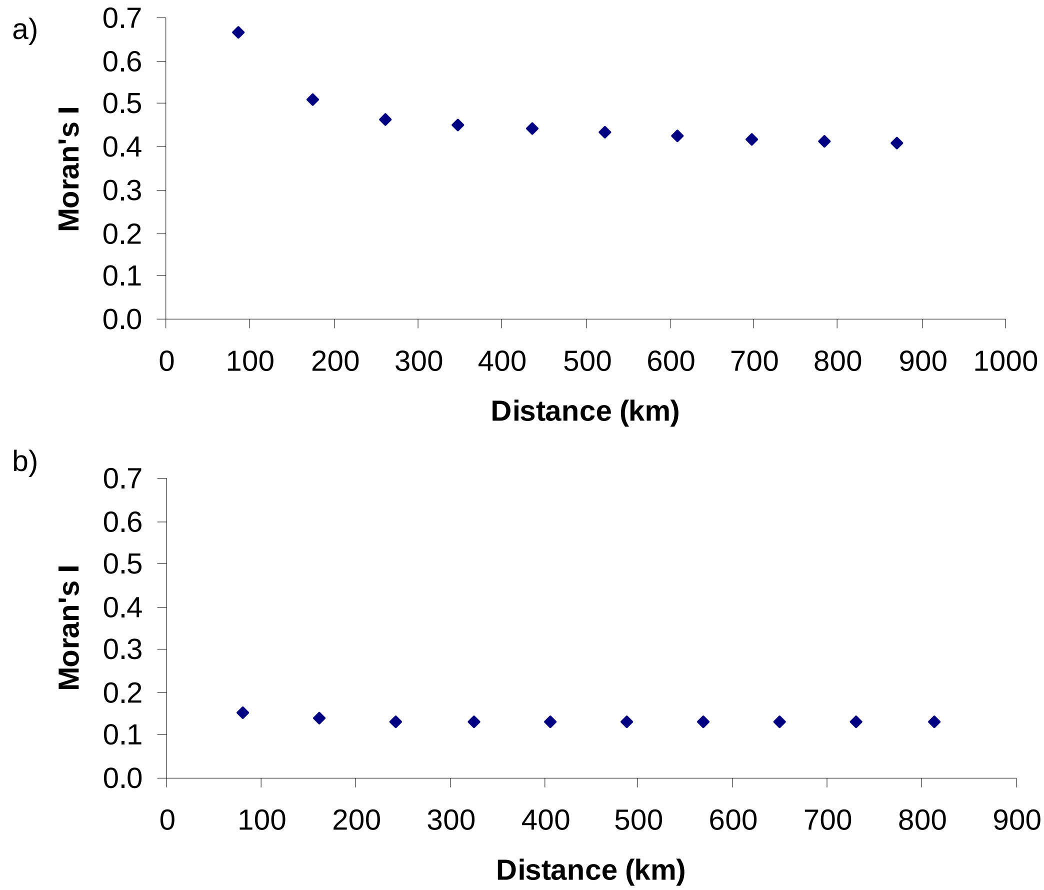


Figure 6.6: Spatial correlograms showing Moran's I at each of 10 distance classes of a) original SQRT NIS richness variable and b) OLS regression model residuals.

6.4 Discussion

6.4.1 Recreational Boating and Secondary Spread

Results suggest that small boat movements are important in explaining the current distribution of NIS, despite the original vector of introduction. Aquaculture importation, the suspected original vector for at least eight of the species included in the analysis, and commercial shipping variables did not explain the current distribution of BC's subtidal NIS. This suggests that the original vectors of introduction that brought these species to the region are not responsible for their secondary spread, and that significant secondary spread has occurred for these species. Instead, recreational boating is likely responsible for the spread of these species throughout the BC coast.

Many of the NIS in the current analysis have been specifically linked to hull fouling in general and recreational boating in particular. The original vector of the colonial ascidian *B. violaceus* has been hypothesized as oyster imports and/or hull fouling of small recreational boats or slow-moving barges (Carver *et al.* 2006; Lambert *et al.* 1987; Lambert and Lambert 2003). The sponge *H. bowerbanki* was introduced to San Francisco Bay from the US Atlantic coast (Lee *et al.* 2007) and although little is known of its original vector to BC its frequent observation on recreational boats and marina structures suggests hull fouling could be important (Chapter 3; Pederson *et al.* 2005). One of the few mobile species, the caprellid amphipod *C. mutica,* does not have a planktonic larval phase so its dispersal range is limited to a few kilometers (Ashton 2006). A previous study in the region showed that *C. mutica* is found in high densities on individual commercial vessels (Frey *et al.* 2009) so that propagule pressure may be high from even a small number of infected boats. Lastly, the bryozoan *S. japonica* was one of the most frequent NIS in hull fouling communities of small boats (Chapter 3).

The solitary ascidian *S. clava* is one of the more recent invaders to the system, first recorded from Nanaimo in 1994 (Lambert and Lambert 1998). Recent surveys have shown this species is common in shellfish aquaculture leases and marinas in southern BC (Chapters 2 and 3; Clarke and Therriault 2007) so it is likely that this species is still spreading in this region. The original vector of introduction to BC is unknown but in New Zealand, genetic data suggests that recreational boating is responsible for both primary introduction and secondary spread of this species (Goldstein *et al.* 2010). Its ability to withstand high water velocities (Chapter 5) is consistent with hull fouling, and in particular recreational boating, as the current vector of spread. Seven of the 12 species examined in this study have been noted in field studies of hull fouling communities of BC recreational boats (Chapter 3). The other five species were not part of the list of species searched for and many are too small to identify in field situations and therefore they may have been present but not detected. The importance of the recreational boating variable in the best NIS richness model, adds another layer of evidence that this vector is important to the secondary spread of subtidal marine NIS for this region.

6.4.2 NIS Species Richness

Distribution of NIS overall was related to the minimum temperatures recorded. There was a concentration of NIS in the Strait of Georgia, an area characterized by higher water temperatures than the rest of BC. Many of the NIS species sampled in BC also are found in California bays and harbours (Cohen *et al.* 2005; Lambert and Lambert 1998; Lambert and Lambert 2003) suggesting they survive and/or prefer warm temperatures. Laboratory testing conducted in BC where ocean temperatures range from 4.6 °C to 19.7 °C showed that the colonial ascidian *B. schlosseri* survives 10-25°C but attains its largest colony size at 15-20°C while *B. violaceus* survives 5-25°C but achieves largest size at 20-25°C (Epelbaum *et al.* 2009). The solitary ascidian *S. clava* is known to tolerate temporary extremes in salinity or temperature by closing its siphons (Lutzen 1999; Sims 1984) but reproduction was limited at less than 15°C (Eno *et al.* 1997). Since the distribution of NIS in the study region appears to be limited by minimum temperature forecasted, observed climate warming in this region (Masson and Cummins 2007) may have important implications for the northward spread of NIS in the future.

Habitat modeling for two botryllid species (*B. violaceus* and *B. schlosseri*) suggested that the entire coast of BC was susceptible to invasion, with potential hot spots on the west coast of Vancouver Island (Epelbaum *et al.* 2009). The current results suggest that temperature was a limiting variable for distribution of NIS and the warmer waters of the Strait of Georgia were therefore a hotspot of invasion. However, most modeling exercises use relatively coarse environmental data (satellite images of sea surface temperature, interpolated values), compared to that which organisms actually experience. While we tested lighthouse data, reflecting in situ temperature and salinity measurements, the GIS layer developed was still a relatively coarse interpolation of environmental conditions. Because marinas are small, semi-enclosed habitats that experience low mixing, peaks of freshwater run off, pollution, and elevated temperatures

(Floerl 2003) the marina environment is often very different from the surrounding coastline and may not be reflected in the lighthouse data measurements. Furthermore, it is possible that there are additional environmental variables (*e.g.*, oxygen, chlorophyll, etc) which may limit establishment and spread of NIS but the data are not available at an appropriate scale (marina-level).

Areas of higher human population density had higher NIS richness. Similar studies at larger scales also have found a relationship between measures of human activity and NIS species richness (Dark 2004; Leprieur *et al.* 2008; Vilà and Pujadas 2001) or the distribution of individual invaders (Sharma *et al.* 2009). On a country scale, Vila and Pujadas (2001) found that Human Development Index (HDI) and trade imports were the best predictors of alien plant density and indicators of human activity were found to be most related to NIS richness in a global comparison of fish species (Leprieur *et al.* 2008). Underlying elements of human population density may be related to NIS richness by affecting either the transport or establishment of NIS. The "human activity" hypothesis encompasses the facilitation of NIS establishment by disturbing natural ecosystems as well as increasing propagule pressure (Leprieur *et al.* 2008). For example, shoreline modification (physical disturbance) is intensified in areas of high population density in the form of breakwater walls, docks, and other artificial structures. Studies have shown that the presence of artificial structures, especially floating structures, may favour NIS establishment over native species (Connell 2000; Connell 2001; Glasby *et al.* 2007).

Multiple vectors operate in higher density areas (ships, boats, aquaculture, live food trade, etc) with increased frequency of transport. Areas of high population density also can indicate the presence of chemical and physical disturbances. Pollution disturbances occur in both single events (*e.g.,* oil spills) and continuous run off from agriculture, sewage, and storm drains. Marinas in particular are subjected to small but frequent oil and diesel spills as well as gray water dumping (Burgin and Hardiman 2011; Mack and D'Itri 1973). The colonial non-indigenous ascidian *B. schlosseri* has been found in extremely polluted sites in southern Spain (Naranjo *et al.* 1996) and Mexico (Lambert and Lambert 2003). This species may be pollution-tolerant and able to exploit sites unavailable to less tolerant species or may actively select polluted sites for some other reason.

In this study, the correlation between NIS richness and population density is more likely due to ecological and physical disturbance since the analysis included a number of possible vectors and only marina propulsiveness was significant. The effects of individual variables covered by human population density are difficult to disentangle and may have additive or multiplicative effects on NIS introduction and establishment. The use of finer scale data than regional district-level population data may provide additional insight into the specific disturbances contributing to invasions in the region. Despite the underlying cause of the relationship, the link between NIS and population density suggests that continued increases in population and development likely will lead to increased invasion rates in this region.

Marine propulsiveness was the final variable affecting the spatial pattern of NIS richness. In essence, marina propulsiveness represents the amount that recreational boats travel outside their home marina (Drake and Mandrak 2010). Boaters from marinas in the Strait of Georgia region were more likely to travel from their home marinas than other places on the coast. Highly mobile boaters may pick up NIS from other places and bring them back to their home marina, increasing invasions at the home marina. However, infection of boats while visiting other marinas is dependent on the type of species encountered (mobile or attached) and the length of time the boats spend moored in other locations. Colonization of boats by mobile species potentially occurs fairly rapidly as adults are able to move into boat hull fouling communities. Attached species would require visiting boats to be present during reproductive events and competent larvae present in the water column to settle on the boat. In BC, the peak boating season (spring-summer) coincides with peak reproductive season for many of the NIS encountered (Chapters 2 and 3). In contrast, marinas with relatively sedentary boater populations may not be subjected to the influx of "souvenir" NIS returning from other marinas with traveling resident boats.

In addition, these results suggest that visiting boats do not seem to have the same ability to cause infection of marinas as resident boats as variables related to visited marinas (attractiveness, visitor number or diversity) were not significant in the regression model. This may be because it takes some minimum amount of time for a fouling species to colonize a marina, which does not occur with visitation (hours to weeks) as opposed to residence within a marina (months to years). Marinas with populations of mature NIS may be able to infect visiting boats with short duration stays as they may be more likely to have at least some reproductively active individuals. It may be that NIS propagules acquired by traveling boats may then have time to mature and in turn infect home marinas with the longer time spent in residence. Alternatively, or additionally, there may be a lag between colonization of a marina and detection of the species in the marina which masks the influence of visiting boats. Further research is required to determine the mechanisms underlying these hypotheses however the difference between marinas, in terms of their travel characteristics, has important implications for management of the spread of NIS by this pathway.

Native species richness was not included as a variable in the current analysis and may improve the explanatory power of the model. There is evidence from small-scale experiments that native species diversity can act as protection from NIS by occupying space (Stachowicz 1999). However studies at larger regional scales suggest that regions with high native diversity also have high NIS diversity hypothesized to be the result of increased niche availability (Byers and Noonberg 2003). The current study was conducted at a regional scale and sites with higher native diversity would be expected to have more NIS. Results from settlement plates (Chapter 2) found that although there was a positive relationship between native and NIS, native richness explains relative little of the variation in NIS richness in the Strait of Georgia (~10%).

The explanatory variables used in the current analysis were created using a combination of interpolation techniques and density estimations (used for shipping, aquaculture and marina propulsiveness). The techniques chosen each have their own assumptions and behaviours and there are other methods available for estimating these values (Childs 2004; Haining 1990). For example, a different decay function could have been used for weighting distance for shipping arrivals that would have placed more or less influence on nearby port sites. Since there was not sufficient evidence to specifically choose different parameters and functions I chose to use default settings unless otherwise stated. A sensitivity analysis, while beyond the scope of the current work, could be performed to investigate the sensitivity and importance of the functions and parameter choices on the results of the regression analyses.

The spatial pattern of NIS richness is the result of long-term accumulation of NIS to the region, including both initial introduction events and secondary spread. The variables used in the current analysis are modern-day snapshots of potentially important processes. For example, data on the locations of aquaculture leases in the last ten years may not accurately reflect activities that have occurred for over a century. Previous studies have found that economic variables associated with historical activity were better predictors of NIS richness than contemporary data, resulting in a so-called "invasion-debt" (Essl *et al.* 2011). A finer scale assessment using variables at multiple time points may have shown a closer link between NIS richness and primary introduction vectors. As in the current study region, historical data is often difficult to obtain and its absence may explain why secondary spread seems to have been captured in the current analysis of NIS distribution, rather than primary introduction.

6.4.3 Spatial Pattern

It is interesting to note the differences between variables in their degree of spatial autocorrelation. While expected autocorrelation occurred with temperature and salinity variables, many of the marina variables were not spatially autocorrelated. The lack of relationship between proximate marinas indicates that boater behaviours and marina characteristics vary on a fine scale. For example, the values of marina attractiveness and marina propulsiveness were not similar among geographically close marinas. This result indicates that marina attractiveness may have more to do with individual marina facilities than the desirable aesthetic features of the region. This has important implications for management and monitoring of invasive species introductions. Identification of recreational boating hotspots must be done at the level of the marina (fairly small scale) as attractiveness and boater behaviour vary by marina and less so by region. Further research is required to determine how much secondary spread occurs between adjacent marinas as this likely depends both on the dispersal characteristics of the species of interest and the local circulation patterns.

6.4.4 Conclusions

The results of the analyses suggest that vector, demographic, and abiotic factors all play a role in defining the current distribution of NIS in BC. The original vectors of NIS introduction, aquaculture and shipping, did not significantly contribute to explaining the current distribution pattern of NIS in this region. Secondary spread of subtidal NIS common in BC's marina environments was best attributed to the recreational boating pathway. Although the current analyses only included very simple environmental variables (temperature and salinity) on a relatively coarse spatial and temporal scale, the establishment of these species may be limited by extremes in local physical conditions. The importance of both vector and abiotic variables should be noted for future modeling efforts. Further improvements in the predictive power of statistical models such as those presented here may benefit from the inclusion of biotic variables, such as native diversity or the presence of known predators/competitors, and more direct measurements of disturbance which may affect successful establishment. Linking the results of these models with an oceanographic model may improve our ability to predict further spread of those species with dispersive larval stages and untangle human-mediated and natural spread patterns. Understanding the factors driving invasion patterns contributes to better prioritization of research and management efforts as well as advancing our basic knowledge of invasion ecology.

Chapter 7: General discussion and conclusions

7.1 Evaluating the Recreational Boating Pathway

This research was the first comprehensive examination of the recreational boating vector in Canada's marine waters. The overarching goal of my dissertation was to investigate the role of the recreational boating pathway in invasion dynamics, notably transporting non-indigenous species (NIS). Based on the body of evidence gathered during this research I have concluded that recreational boating is an important pathway for secondary spread within British Columbia and likely for Canadian marine systems in general. Further, it is likely that this pathway has the potential to facilitate primary introductions from outside Canada. The continual, unregulated introduction and spread of potentially invasive NIS by the recreational boating pathway is a threat to the biodiversity, economy and ecosystem health of the region.

This research focused on the first two stages of the invasion process: uptake by a vector and transfer via vector to the introduced region (Chapter 1, Figure 1.1). In this capacity I sought to investigate both primary introductions into the study region and secondary spread within the region. I did not have the opportunity to investigate uptake in native regions directly but used vector uptake that occurs in the invaded range (secondary spread) as a means to gain insight into the process.

7.1.1 Uptake by the Vector

The first step in investigating the recreational boating vector was to look at marinas and characterize the presence of NIS in these habitats for BC. Boats spend the majority of their time within marinas and therefore would potentially uptake and deliver NIS in these areas preferentially. If recreational boating is an important vector in this region we would hypothesize that marinas, as the habitat of first encounter, would be highly invaded. Indeed, in Chapter 2 I showed that southern BC marinas had a number of NIS and cryptogenic species. These subtidal habitats had much higher NIS richness than other BC habitats (Intertidal: Choi 2011; Seagrass beds: M. Mach, UBC, Vancouver, pers. comm.) suggesting marinas are more invaded than other habitats. Although there was high variability among marinas some were dominated by NIS cover; with more than 40% of available space on the settlement plates occupied by NIS at some sites.

Those NIS present in marina fouling communities came from diverse taxonomic groups: species of ascidians, polychaetes, and amphipods were particularly prevalent but also included bryozoans, bivalves, sponges, and nemerteans. This taxonomic complement partially reflects the fouling life history strategy and the size of the species. The occurrence of the fouling life history strategy may not be evenly spread across all taxonomic groups. For instance, ascidian NIS are the predominant fouling organisms both on artificial structures and in the hull fouling communities of boats. However, other taxonomic groups are not as common in fouling communities (*e.g.,* bivalves, gastropods and crustaceans). However, larger organisms are easier to identify than smaller ones and there is a need in invasion ecology studies for an increased focus on taxonomic studies. An increased focus on taxonomy would improve our ability to access complete baseline species lists and better distinguish native from introduced species.

The presence of NIS in marinas is only circumstantial evidence that recreational boats may have been the vector of NIS transport. Therefore, the second step in my evaluation of the vector was to investigate the type and quantity of biota physically associated with the vector. What types of species have the ability to colonize artificial boat surfaces? Of the 19 NIS identified from the marina settlement plates (Chapter 2) eight of these species also were detected on recreational boats: The ascidians *Botrylloides violaceus, Botryllus schlosseri, Diplosoma listerianum, Molgula manhattensis,* and *Styela clava,* the amphipod *Caprella mutica*, the bryozoan *Schizoporella unicornis* and sponge *Halichondria bowerbanki* were found on BC boats (Chapter 3). The range expansion of the invasive caprellid amphipod *C. mutica* was detected as part of the settlement plate experiment (Chapter 2) and reported by Frey *et al.* (2009). The remaining NIS were not on the list of species searched for so it cannot be determined if they are unable to colonize boat surfaces or were simply undetected.

Boat fouling communities represent a subset of the resident fouling community at a marina. The longer the boat's duration of stay, the more its fouling community resembles the resident marina fouling community (Floerl and Inglis 2005). The best predictors of fouling on BC boats were duration in water and age of antifouling paint (Chapter 4). In this way, marinas may be acting as both source and sink populations for further spread. An even smaller subset of NIS is able to colonize natural habitat (Simkanin *et al.* Submitted). Only two of the ascidian NIS common in BC marinas were detected in nearby natural rocky habitats, *B. violaceus* and *B. schlosseri*. The ecological impacts of these species on natural systems in this region remain unknown suggesting that further research is needed.

7.1.2 Transfer by the Vector

The second stage of the invasion process, transfer by the vector, includes the behavioural characteristics of the vector and the ability of the associated organisms to endure the voyage. I examined characteristics of the BC boating population to gain an understanding of how boater behaviours in this region may contribute to introduction and spread of NIS (Chapter 3). A significant proportion of recreational boats in this region were infected with known NIS (Chapter 3). An even higher percentage had macrofouling attached or entangled, representing a susceptibility to colonization of fouling organisms and a risk of invasive species transport. It is highly likely that recreational boats are spreading NIS throughout BC marine waters. Although a smaller fraction of BC boaters reported international travel (17%), these boats often had small amounts of barnacles and mussels attached in niche areas. Species of barnacles and mussel represent some of the most widely introduced and invasive organisms globally (Bossenbroek *et al.* 2007; Johnson and Padilla 1996; Kado 2003; Schwindt 2007; Southward *et al.* 1998) and therefore these boats represent a potential for primary introductions of invasive species from other regions.

Although I did not find any completely new NIS on the boats surveyed, there is still potential for primary introductions via this vector. My search protocol could be expanded to include biological samples from each boat to search for new species introductions, especially smaller taxa that are under-represented in the current sampling design. There were a large number of unidentified morphotypes in the settlement plate experiment and an increased focus on taxonomy when surveying marinas (Chapter 2) and boats (Chapter 3) could have improved the probability of detecting new NIS not present in BC waters. More detailed searches could be performed on "high risk" fouled boats under the fouling model developed in Chapter 4. However, taxonomic expertise is very important to an enterprise of that magnitude and it takes time to process and identify samples to species level and determine their invasion status. More extensive sampling thus may not enable timely detection of novel invasions.

One could imagine that the submerged surfaces of recreational boats would be difficult places to inhabit and remain attached. Hull fouling organisms can be subjected to intense hydrodynamic conditions that may be well outside that normally experienced in their natural habitat. Chapter 5 asked the question what is it about hull fouling invaders that allow them to utilize this human vector so successfully? My results showed that not only do non-indigenous ascidians have stronger attachment strength than native species; they also have lower drag profiles. The combination of these two properties means that predicted dislodgment velocities of NIS are much higher allowing them to be easily transported by a range of marine vessels.

Hull fouling transport may represent an example of a selection regime modification by humans that creates an environment that favours NIS over native species (Byers 2002). The hull fouling vector itself creates a selective environment, only transporting individuals that possess adaptations allowing them to successfully endure the voyage. My results along with the work of recent authors (Coutts *et al.* 2010; Davidson *et al.* 2009) suggest an intriguing line of future research investigating the evolutionary implications of these adaptations. Will we be able to see NIS with evolving adaptations within the invaded range? What can be learned from their native environment about these biomechanical adaptations? Do these biomechanical abilities represent phenotypic plasticity or are they genetically-based? These questions are compelling to the ecological, physical, and evolutionary fields of research.

7.1.3 Strength of the Vector

The final stages of the invasion process include establishment, population increase and expansion, followed by secondary spread. The distribution of NIS in a region reflects the results of the entire invasion process and was further investigated in Chapter 6. Using the results of previous chapters, an evaluation of the strength of the recreational boating vector was performed by statistically comparing demographic, environmental, and vector variables against the spatial distribution of subtidal NIS in BC. Surprisingly, NIS invasion was found to be significantly related to recreational boating movements. The propulsiveness of a marina, or how much boaters travel from their home marina, was the vector variable that best explained the observed invasion pattern. Historical pathways of introduction, such as aquaculture, appeared to be less important to the modern day spread of subtidal NIS, at least in BC. Temperature and population density, speculated to represent anthropogenic disturbances, also were important in explaining regional NIS richness.

The southern Strait of Georgia had higher NIS species richness than other parts of the BC coast. My results suggested that this was a result of its warmer waters and high population density, coupled with intense and diverse propagule pressure in the form of recreational boats. There are both a large number of marinas and boats operating in this region making it high risk for invasion by subtidal hull fouling species. Thus, this area of BC should be a priority for monitoring and vector management activities in this region.

7.1.4 Impact of Invasions in British Columbia

The presence of NIS becomes a matter of concern only when there is evidence of impact on some aspect of the native ecosystem: either ecological, social, or economic. There are a limited number of examples of nonindigenous species in BC marine waters that have some form of impact and can be considered truly invasive. For example, the introduction and spread of Pacific oyster, *Crassostrea gigas*, has contributed to the decline of native Olympia oyster *Ostrea conchaphila* Carpenter, 1857 (Fisheries & Oceans Canada 2009b). The Olympia oyster was listed as a species of Special Concern under the Species At Risk Act (SARA) in 2003. In the absence of detected negative ecological impacts, many of the NIS in BC waters are considered to have had positive economic impacts. For example, Manila clam (*Venerupis philippinarum* A. Adams & Reeve, 1850) was originally introduced with Pacific oyster and is now a favoured aquaculture product that positively benefits BC's economy (British Columbia 2008).

Ascidian invaders in Atlantic Canada (including *Ciona intestinalis* Linnaeus, 1767 and *Styela clava*) have caused major impacts on the shellfish aquaculture industry (Bullard *et al.* 2007; Carver *et al.* 2003; Daigle and Herbinger 2009; Locke *et al.* 2007). Although ascidian NIS were prevalient within marinas and on boats and are likely being spread via recreational boating, the same scale of impact observed on the east coast of Canada has not been documented in BC. The high native diversity in BC may offer some protection from invasion impacts, which is consistent with the biotic resistance hypothesis (Elton 1958; Levine and D'Antonio 1999; Stachowicz *et al.* 1999). However, in the subtidal fouling community NIS species richness increased with native species richness (Chapter 2). The alternative hypothesis is biotic acceptance (Fridley *et al.* 2007; Leprieur *et al.* 2008). The BC marine environment is a highly productive system that may provide an increased capacity to support species, both native and NIS. With abundant resource availability, incoming NIS may be less likely to have noticeable impacts on the native ecosystem.

An additional, and not mutually exclusive hypothesis, is related to BC's different vector and invasion history. The Pacific coast has had a comparatively more recent onset of major international shipping, a differing set of source ports and different history of aquaculture importation and contemporary aquaculture activities than that on the Atlantic coast (Carlton 1979). In this regard, recent ascidian invaders such as *S. clava* and *D. vexillum* (detected in the last two decades) may represent early stages of the ongoing invasion process and major impacts on the shellfish aquaculture industry or elsewhere may occur in the future. It remains to be tested which of these hypotheses accurately explain the introduction, establishment, and impact of subtidal NIS in this region.

The absence of major impacts from current subtidal NIS in the BC region should not be seen as a reason to ignore the recreational boating vector. The advancing western spread of zebra mussel (*Dreissena polymorpha*) is a real threat to BC freshwater and estuarine habitats and primary introduction may come from an infected trailered or marine boat entering into BC. Mussels (species of *Mytilus*) were the most common taxa observed on traveling recreational boats surveyed in BC waters (Chapter 3) highlighting that this vector has the potential to transport such an invader. Impacts from NIS mussel introductions would certainly be high and therefore vigilance and action is required to stop new invasions.

7.2 Future Directions and Conclusions

Ultimately, vector management is undertaken in order to stem the introduction and spread of invasive species. With the results of the current research, vector interruption can be attempted and evaluated for its efficacy, the final component of vector management (Carlton and Ruiz 2005). Border inspections of incoming recreational boats could be implemented, utilizing the fouling model developed in Chapter 4. Outreach activities could be used to target risky boater behaviours. The boater questionnaire (Chapter 3) revealed that the majority of BC boats are operating with antifouling paint past the manufacturers` recommended limits. By decreasing the average age of antifouling paint on BC boats, for example, the risk of spread may be drastically reduced. Further research is required to examine these potential strategies and other possibilities for mitigating spread within BC. Eradication and control efforts are most effectively employed in areas where species are limited in local distribution. More extensive modeling of the potential spread of NIS in BC could be used to identify marina hotspots or hubs similar to studies performed in New Zealand (Floerl *et al.* 2008) in order to focus research and monitoring efforts. Treatment options are being developed that may allow control or eradication of fouling species in infected marinas and aquaculture facilities (Switzer *et al.* 2011).

In summary, based on the results of my dissertation there are a number of possible research directions I would suggest including, but not limited to, the following:

Non-indigenous species settlement and impacts

- A multi-year study of settlement of NIS in fouling communities for the region
- Monitoring for impacts from subtidal NIS in native environments as well as artificial habitats such as marinas and aquaculture facilities
- Explicit examination of why invasion impacts do not seem to be as extreme as other regions with the same invaders
- Experimental testing of the biotic resistance vs. acceptance hypothesis for the system studied

Recreational boating as a vector

- Biological sampling from boat hull fouling communities to monitor for new introductions
- More extensive testing of the fouling model developed and its effectiveness in predicting the presence of fouling
- Development and testing of species-specific models based on boater behaviour data, perhaps based on previous travel history to infected locations

Adaptation of hull fouling invaders

- An investigation of whether advantageous hull fouling biomechanical traits are genetically-based or a form of phenotypic plasticity
- Investigation of the influence of substrate type and antifouling paint on biomechanical properties

Regional trends

- Develop a model of spread in BC using recreational boat travel data

- Undertake an overview of invasion in BC and compare variables important for invasion across all marine habitats, in addition to subtidal marina ecosystems considered here

My evaluation of the recreational boating vector has shown that the potential for introduction and spread of invasive species in the study region is considerable. In BC the vector is of similar strength to that in other regions where comprehensive research has been undertaken including Australia (Floerl 2002), New Zealand (Floerl and Inglis 2003; Minchin *et al.* 2006), and California (Davidson *et al.* 2008). Proactive management and regulation, as has begun in these others regions should be implemented in BC and further research completed on other regions of Canada. Management of this vector is required to prevent potential loss of biodiversity commonly linked to invasions (Sala 2000), especially in a region, such as British Columbia, prized for its high marine biodiversity.

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Appendix A: Settlement Plate Species List

Complete species list by status: non-indigenous, cryptogenic, or native. Reference key: [1] USGS - NAS, [2] Lamb and Hanby 2005, [3] Carlton 2007, [4] Kozloff and Price 1987, [5] WORMS, [6] Coan *et al.* 2000, [7] J. Carlton, pers. comm., [8] ISSG, 9 IOBIS.org, [10] Blake *et al.* 1996, [11] Harbo 1999, [12] Bryozoa.net, [13] Zipcodezoo.com, [14] Sealifebase.org

Species	Phylum	Status	Reference
Eulalia viridis	Annelida	Non-indigenous	3
Eumida sanguinea	Annelida	Non-indigenous	3,4
Hobsonia florida	Annelida	Non-indigenous	5
Polydora cornuta	Annelida	Non-indigenous	3
Caprella mutica	Arthropoda	Non-indigenous	1
Melita nitida	Arthropoda	Non-indigenous	3
Monocorophium acherusicum	Arthropoda	Non-indigenous	3
Monocorophium insidiosum	Arthropoda	Non-indigenous	3
Monocorophium uenoi	Arthropoda	Non-indigenous	3
Botrylloides violaceus	Chordata	Non-indigenous	1
Botryllus schlosseri	Chordata	Non-indigenous	2
Diplosoma listerianum	Chordata	Non-indigenous	1
Molgula manhattensis	Chordata	Non-indigenous	3
Styela clava	Chordata	Non-indigenous	8
Bugula neritina	Ectoprocta	Non-indigenous	3
Schizoporella japonica	Ectoprocta	Non-indigenous	2
Crepidula fornicata	Mollusca	Non-indigenous	4
Neotrapezium liratum	Mollusca	Non-indigenous	6
Halichondria bowerbanki	Porifera	Non-indigenous	1
Chone duneri	Annelida	Cryptogenic	9
Ctenodrilus serratus	Annelida	Cryptogenic	3,4
Polydora limicola	Annelida	Cryptogenic	10
Polydora websteri	Annelida	Cryptogenic	3,4
Incisocalliope newportensis	Arthropoda	Cryptogenic	3
Leptochelia "dubia"	Arthropoda	Cryptogenic	3
Bowerbankia "gracilis"	Ectoprocta	Cryptogenic	3
Anobothrus trilobotus	Annelida	Native	3
Antinoella macrolepida	Annelida	Native	4
Aphelochaeta elongata	Annelida	Native	3
Arctonoe vittata	Annelida	Native	3,4
Armandia brevis	Annelida	Native	3
Artacama coniferi	Annelida	Native	13
Autolytus cornutus	Annelida	Native	3,4
Boccardia columbiana	Annelida	Native	13
Boccardia proboscidea	Annelida	Native	3
Boccardia tricuspa	Annelida	Native	9
Brania brevipharyngea	Annelida	Native	13
Ceratonereis paucidentata	Annelida	Native	4,5
Cheilonereis cyclurus	Annelida	Native	2
Chone minuta	Annelida	Native	2

Species	Phylum	Status	Reference
Circeis armoricana	Annelida	Native	3,4
Crucigera zygophora	Annelida	Native	4
Demonax medius	Annelida	Native	3
Dipolydora socialis	Annelida	Native	3,4
Dorvillea pseudorubrovittata	Annelida	Native	2
Emplectonema gracile	Annelida	Native	3,4
Eteone californica	Annelida	Native	3
Eulalia quadrioculata	Annelida	Native	3,4
Eunoe depressa	Annelida	Native	4
Eupolymnia heterobranchia	Annelida	Native	3
Eusyllis bloomstrandi	Annelida	Native	4
Exogone dwisula	Annelida	Native	3
Gattyana iphionelloides	Annelida	Native	4
Glycera tesselata	Annelida	Native	4
Glyphanostomum pallescens	Annelida	Native	3
Gyptis brunnea	Annelida	Native	3
Halosydna brevisetosa	Annelida	Native	2
Harmothoe fragilis	Annelida	Native	4
Harmothoe imbricata	Annelida	Native	2
Hemipodia simplex	Annelida	Native	3
Kefersteinia cirrata	Annelida	Native	4
Lepidasthenia longicirrata	Annelida	Native	3
Lepidonotus squamatus	Annelida	Native	3
Lysippe labiata	Annelida	Native	3
Micropodarke dubia	Annelida	Native	3
Myrianida inermis	Annelida	Native	3
Mystides borealis	Annelida	Native	4
Myxicola infundibulum	Annelida	Native	2
Nereis latescens	Annelida	Native	3
Nereis pelagica	Annelida	Native	4, 3
Nereis procera	Annelida	Native	2
Nereis vexillosa	Annelida	Native	2
Nicon moniloceras	Annelida	Native	4
Odontosyllis parva	Annelida	Native	4
Ophelina acuminata	Annelida	Native	3,4
Ophiodromus pugettensis	Annelida	Native	3,4
Paleanotus bellis	Annelida	Native	3,4
Phyllodoce williamsi	Annelida	Native	3,4
Pileolaria marginata	Annelida	Native	3
Pionosyllis gigantea	Annelida	Native	2
Pionosyllis magnifica	Annelida	Native	3
Platynereis bicanaliculata	Annelida	Native	3
Polycirrus californicus	Annelida	Native	3,4
Polydora pygidialis	Annelida	Native	3,4
Polydora spongicola	Annelida	Native	3,4
Pontogenia inermis	Annelida	Native	3,4
Pontogenia intermedia	Annelida	Native	3
Pontogenia rostrata	Annelida	Native	3,4
Potamilla neglecta	Annelida	Native	2

Species	Phylum	Status	Reference
Potamilla occelata	Annelida	Native	4
Pseudochitinopoma occidentalis	Annelida	Native	
Pygospio elegans	Annelida	Native	1
Rhynchospio glutaea	Annelida	Native	3
Salmacina tribranchiata	Annelida	Native	3,4
Schistomeringos caeca	Annelida	Native	4
Scolelepis squamata	Annelida	Native	3,4
Scolelepis tridentata	Annelida	Native	3
Serpula columbiana	Annelida	Native	4
Sige bifoliata	Annelida	Native	3
Spiophanes bombyx	Annelida	Native	4
Spirorbis bifurcatus	Annelida	Native	3,4
Subadyte mexicana	Annelida	Native	10
Syllis spongiphila	Annelida	Native	3
Syllis variegata	Annelida	Native	5
Thelepus hamatus	Annelida	Native	4
Thelepus setosus	Annelida	Native	10
Trypanosyllis gemmipara	Annelida	Native	2
Typosyllis alternata	Annelida	Native	3
Typosyllis pigmentata	Annelida	Native	2
Typosyllis pulchra	Annelida	Native	3
Aoroides columbiae	Arthropoda	Native	3
Aoroides intermedius	Arthropoda	Native	3
Apohyale pugettensis	Arthropoda	Native	3
Balanus crenatus	Arthropoda	Native	3
Balanus glandula	Arthropoda	Native	2
Bemlos concavus	Arthropoda	Native	3
Calliopius pacificus	Arthropoda	Native	3
Cancer oregonensis	Arthropoda	Native	2
Caprella alaskana	Arthropoda	Native	9
Caprella laeviuscula	Arthropoda	Native	9
Caprella striata	Arthropoda	Native	9
Chthamalus dalli	Arthropoda	Native	2
Cumella vulgaris	Arthropoda	Native	3
Eogammarus confervicolus	Arthropoda	Native	3
Gnathopleustes pugettensis	Arthropoda	Native	3
Gnorimosphaeroma oregonense	Arthropoda	Native	2
Haplostoma albicatum	Arthropoda	Native	3
Hemigrapsus nudus	Arthropoda	Native	2
Hemigrapsus oregonensis	Arthropoda	Native	2
Heptacarpus brevirostris	Arthropoda	Native	2
Mayerella banksia	Arthropoda	Native	3,4
Mimulus foliatus	Arthropoda	Native	2
Munna fernaldi	Arthropoda	Native	2
Paraclunio alaskensis	Arthropoda	Native	3
Petrolisthes eriomerus	Arthropoda	Native	3,4
Pontogeneia rostrata	Arthropoda	Native	3
Pugettia gracilis	Arthropoda	Native	2
Uromunna ubiquita	Arthropoda	Native	2

Species	Phylum	Status	Reference
Aplidium californicum	Chordata	Native	2
Aplidium solidum	Chordata	Native	3,4
Ascidia ceratodes	Chordata	Native	1
Bathypera feminalba	Chordata	Native	4
Chelyosoma productum	Chordata	Native	2
Cnemidocarpa finmarkensis	Chordata	Native	3
Corella inflata	Chordata	Native	2
Cystodytes lobatus	Chordata	Native	2
Distaplia occidentalis	Chordata	Native	11
Molgula pacifica	Chordata	Native	11
Perophora annectens	Chordata	Native	2
Pyura haustor	Chordata	Native	3,4
Styela coriacea	Chordata	Native	4
Styela gibbsii	Chordata	Native	3
Clytia hemisphaerica	Cnidaria	Native	3
Metridium senile	Cnidaria	Native	2
Obelia borealis	Cnidaria	Native	2
Urticina felina	Cnidaria	Native	2
Urticina piscivora	Cnidaria	Native	2
Dermasterias imbricata	Echinodermata	Native	2
Mediaster aequalis	Echinodermata	Native	2
Ophiopholis aculeata	Echinodermata	Native	3
Ophiopteris papillosa	Echinodermata	Native	3,4
Ophiothrix spiculata	Echinodermata	Native	3
Pisaster brevispinus	Echinodermata	Native	2
Pisaster ochraceus	Echinodermata	Native	2
Pseudocnus lubricus	Echinodermata	Native	3
Pycnopodia helianthoides	Echinodermata	Native	2
Strongylocentrotus droebachiensis	Echinodermata	Native	2
Strongylocentrotus purpuratus	Echinodermata	Native	2
Alcyonidium gelatinosum	Ectoprocta	Native	1
Alcyonidium polyoum	Ectoprocta	Native	1
Alderina brevispina	Ectoprocta	Native	12
Bugula californica	Ectoprocta	Native	2
Bugula pacifica	Ectoprocta	Native	3
Bugula pugeti	Ectoprocta	Native	3,4
Celleporella hyalina	Ectoprocta	Native	3
Conopeum osburni	Ectoprocta	Native	7,12
Conopeum reticulum	Ectoprocta	Native	7,12
Cribilina corbicula	Ectoprocta	Native	4
Cribrilina annulata	Ectoprocta	Native	4
Ellisina levata	Ectoprocta	Native	
Lichenopora novae-zelandiae	Ectoprocta	Native	2
Lichenopora verrucaria	Ectoprocta	Native	2
Membranipora fusca	Ectoprocta	Native	3
Membranipora serrilamella	Ectoprocta	Native	3
Reginella nitida	Ectoprocta	Native	3
Scrupocellaria varians	Ectoprocta	Native	3
Stomachetosella cruenta	Ectoprocta	Native	4

Species	Phylum	Status	Reference
Tegella horrida	Ectoprocta	Native	3
Tricellaria occidentalis	Ectoprocta	Native	3
Tubulipora tuba	Ectoprocta	Native	4
Barentsia gracilis	Entoprocta	Native	3,9
Barentsia parva	Entoprocta	Native	9
Aeolidia papillosa	Mollusca	Native	3,4,5
Aeolidiella chromosoma	Mollusca	Native	3
Alia gausapata	Mollusca	Native	2
Alia tuberosa	Mollusca	Native	3,4
Alvania compacta	Mollusca	Native	3,4
Amphissa reticulata	Mollusca	Native	4
Brachystomia angularis	Mollusca	Native	3
Callistochiton crassicostatus	Mollusca	Native	3
Cerberilla mosslandica	Mollusca	Native	3,9
Conchocele bisecta	Mollusca	Native	4
Crassadoma gigantea	Mollusca	Native	2
Cuthona cocoachroma	Mollusca	Native	3,4
Cyclopecten barbarensis	Mollusca	Native	14
Eubranchus rustyus	Mollusca	Native	2
Haminoea vesicula	Mollusca	Native	2
Hermissenda crassicornis	Mollusca	Native	2
Hiatella arctica	Mollusca	Native	6
Hima mendicus	Mollusca	Native	3,4
Lacuna marmorata	Mollusca	Native	3
Lacuna variegata	Mollusca	Native	2
Lacuna vincta	Mollusca	Native	2
Leukoma staminea	Mollusca	Native	6
Lirobittium attenuatum	Mollusca	Native	2,3
Lirularia succincta	Mollusca	Native	2
Littorina sitkana	Mollusca	Native	2
Lottia pelta	Mollusca	Native	2
Lottia scutum	Mollusca	Native	3
Lyonsia californica	Mollusca	Native	2
Mopalia lignosa	Mollusca	Native	2
Mopalia spectabilis	Mollusca	Native	2
Onchidoris bilamellata	Mollusca	Native	2
Onchidoris muricata	Mollusca	Native	2
Ostrea lurida	Mollusca	Native	3
Panopea abrupta	Mollusca	Native	4
Pododesmus macrochisma	Mollusca	Native	6
Solemya reidi	Mollusca	Native	2
Amphiporous imparispinosus	Nemertea	Native	3,4
Carinoma mutabilis	Nemertea	Native	3
Micrura alaskensis	Nemertea	Native	3
Ototyphlonemertes americana	Nemertea	Native	3
Tetrastemma nigrifrons	Nemertea	Native	4, 3
Hoploplana californica	Platyhelminthes	Native	3
Leptoplana chloranota	Platyhelminthes	Native	3
Notoplana sanguinea	Platyhelminthes	Native	4

Species	Phylum	Status	Reference
Pleioplana inquieta	Platyhelminthes	Native	3
Pseudoceros canadensis	Platyhelminthes	Native	3,4
Stylochoplana chloranota	Platyhelminthes	Native	3
Stylostomum album	Platyhelminthes	Native	4
Halisarca sacra	Porifera	Native	4
Leucosolenia nautilia	Porifera	Native	2

Appendix B: Canadian Boat Survey (Boater Questionnaire)

Date survey completed: ____/____/________ (dd/mm/yyyy)

1. Permanent residence information

Province/State_______________________________
Country ____________________________________

Part I: Your Boat

2. Type of craft:

    - o Sailboat
    - o Power boat
    - o Converted fish boat
    - o Personal watercraft (*e.g.,* Seadoo)
    - o Other (specify) ____________________

3. Hull type:

    - o Wood
    - o Aluminum
    - o Fibreglass
    - o Other (specify) ____________________

4. Size of craft: Length (in feet)________________________________
   and/or Displacement (in tonnes) ___________________

5. Where is your boat stored? Please check one of the following four choices.

    - o **In the water year-round.**
      What is the name and location of your home marina?
      Name _______________________________
      City or Town _________________________
      Province/State ________________________
      Country _____________________________

    - o **In the water only part of the year.**
      Which marina do you use ____________________________

How long was your boat stored in the water during the past 12 months? ____

- **Stored on land and trailered to launch site.**
  What is the boat launch you most commonly use? If unknown please write the closest city or town to the boat launch
  ____

- **Other (please specify)**
  ____

Part II: Antifouling

6. What types of antifouling practices do you employ on your boat? Please fill in all that apply.

- **None**

- **My boat is brand new and has not been cleaned yet**

- **I recently bought my boat and do not know its antifouling history**

- **Antifouling paint**:
  How often do you apply antifouling paint to your boat's hull (or have it applied?) eg. Once a year, Every two years, etc. ____________________
  ____
  What was the date of the last antifouling treatment you applied or had applied to your boat: ____/________ (month/year)
  What type* of antifouling paint did you apply during your last application treatment? If you can remember please enter the brand name of the paint in addition to the type. If you do not know the type of paint, you can enter the brand name only.

  - Ablative
  - Hard
  - Combination
  - I don't know
    Product brand name used (if known): _________________________

- **Manual hull cleaning** (brushing, scrubbing, pressure-wash, etc.):
  How often do you manually clean your boat's hull? ________________
  ____
  What was the date of your last manual cleaning?
  ____/________ (month/year)
  What methods of manual cleaning do you employ? Check all that apply.
  - Scrubbing

- Scraping
- Power washing
- Other (please specify) ____

Where do you perform your boat's manual hull cleaning? Please select all that apply.

- In water
- On tidal grid
- In dry dock
- On land
- Other (please specify) ____

Part III: Boat Movement

Please provide information on the use and movement of your craft within the **past 12 months**:

7. What types of trips did you take on your boat within the last 12 months? Check all that apply.
    - Locals – out and back to home marina in one day
    - Racing – trips made for the purpose of racing the boat
    - Weekenders – trips of a few days duration visiting 1-2 different moorages
    - Long trips – long haul travel to destinations further away, once there remain in a single moorage the entire time
    - Tours – long trips with multiple destinations along the way, staying in each moorage for only a few nights
    - Other (please specify) ____
    ____

8. In the last 12 months, what was the maximum amount of time you spent moored, tied up, or anchored in any single place outside your home marina?
    - Never moored outside my home marina
    - Unknown – it is a charter boat
    - 1 day
    - 2 days
    - 3 days
    - 4 days
    - 5 days
    - 6 days
    - 1 week
    - 2 weeks
    - 3 weeks
    - 1 month
    - 2 months
    - 3 months
    - 4 months
    - 5 months
    - 6 months
    - 7 months
    - 8 months
    - 9 months
    - 10 months
    - 11 months

9. Please check the names of **all the places** you visited **on your boat** within the **last 12months:**

√	**Lower Mainland**		**West Vancouver Island**		**North Coast & Queen Charlotte Islands**
	Vancouver		Tofino		Prince Rupert
	Horseshoe Bay		Ucluelet		Terrace
	Coal Harbour		Bamfield		Skidegate
	False Creek		Broken Islands Group		Masset
	Ladner		Port Alice		Queen Charlotte City
	Port Moody		Zeballos		Port Simpson
	Abbotsford		Winter Harbour		Kitimat
	Delta		Coal Harbour		Kemano
	Port Coquitlam		Tahsis		
	Richmond				
	South Vancouver Island		**Mid-North Vancouver Island**		**Central Coast**
	Berry Island		Nanaimo		Bella Coola
	Blind Channel		Protection Island		Bella Bella
	Pender Island		Nanoose Bay		Rivers Inlet
	Mayne Island		French Creek		Namu
	Galiano Island		Fanny Bay		Ocean Falls
	Thetis Island		Deep Bay		Klemtu
	Gabriola Island		Quadra Island		Kingcome
	Mudge Island		Cortes Island		Hartley Bay
	Saturna Island		Denman Island		Hakai Pass
	Saltspring Island		Hornby Island		Dawsons Landing
	Sidney		Comox		Dean River
	Saanich		Courtenay		Butedale
	Squamish		Campbell River		
	Sooke		Port McNeil		**Sunshine Coast**
	Victoria		Sayward		Gibson's
	Mill Bay		Alert Bay		Sewell
	Cowichan Bay		Telegraph Cove		Bowen Island
	Ladysmith		Port Alberni		Sechelt
	Crofton		Royston		Powell River
	Port Renfrew		Quathiaski Cove		Lund
			Port Hardy		Ladner
			Heriot Bay		Minstrel Island
			Kelsey Bay		North Broughton Island
			Hanson Island		Okeover Inlet
			Bull Harbour		Owen Bay
					Port Neville
					Refuge Cove
					Savary Island
					Simoon Bay
					Sointula
					Stuart Island
					Surge Narrows
					Passage Island
					Pender Harbour

10. Did you travel with your boat to any provinces outside of British Columbia within the last 12 months? Please check all provinces you visited.

- o Alberta
- o Manitoba
- o New Brunswick
- o Newfoundland
- o Nova Scotia
- o Ontario
- o Prince Edward Island
- o Quebec
- o Saskatchewan
- o Northwest Territories
- o Nunavut
- o Yukon

11. Did you travel to the US with your boat within the last 12 months? If yes, please check all states that you visited.

- o Alabama
- o Alaska
- o Arizona
- o Arkansas
- o California
- o Colorado
- o Connecticut
- o Delaware
- o Florida
- o Georgia
- o Hawaii
- o Idaho
- o Illinois
- o Indiana
- o Iowa
- o Kansas
- o Kentucky
- o Louisiana
- o Maine
- o Maryland
- o Massachusetts
- o Michigan
- o Minnesota
- o Mississippi
- o Missouri
- o Montana
- o Nebraska
- o Nevada
- o New Hampshire
- o New Jersey
- o New Mexico
- o New York
- o North Carolina
- o North Dakota
- o Ohio
- o Oklahoma
- o Oregon
- o Pennsylvania
- o Rhode Island
- o South Carolina
- o South Dakota
- o Tennessee
- o Texas
- o Utah
- o Vermont
- o Virginia
- o Washington
- o West Virginia
- o Wisconsin
- o Wyoming

12. If applicable, please write all the countries outside of Canada and the US that you visited on your boat in the last 12 months.

____

____

Appendix C: Marina and Yacht Club Questionnaire

UBC Marine Invasive Species

Marina and Yacht Club Questionnaire

As part of a graduate research project at the University of British Columbia focussed on invasive species and recreational boating we are gathering information about BC marinas and boaters. This research is supported in part by a grant from Environment Canada's Invasive Alien Species Partnership Program, a Government of Canada initiative and funding from Fisheries and Oceans Canada.

We recognize the information we collect could be considered sensitive and for this reason, your individual contact information will remain confidential. Information collected for this project is for scientific research only and by completing the following questions, you consent to participate in this research.

Information pertaining to your site may have been collected previously by the BC government and Parks Canada. We are cooperating with these agencies to update these databases. Please see the attached sheet to confirm the information previously collected is correct. If not, please update as needed.

PART A – CONTACT INFORMATION

Yacht Club/Marina Name: ____

Your Name: ______________________________ Your Position/Title: _____________

Facility Mailing Address:

____

Facility Coordinates: Latitude________________Longitude:____________________

Phone #: ( ) ______________ Fax: ( ) ______________

Email: _________________________ Website: _______________________________

PART B – GENERAL INFORMATION

1. Year facility was built: ____________ 2.Year facility began operation: __________

3.a) Age of floats or docks: ______________b) Last replaced (year): ________________

4. Rate of annual marina traffic (average number of visiting boats during peak season):

Low (<1 per day) Moderate (1-10 oer day) High (>10 per day)

5. Number of moorage slips: a) Resident _______b) Visitor _______ c) Total _______

6. Maximum length of slips (Ft): a) Resident _____ b) Visitor ______ c) Total ______

7. Total number of visiting boats (approximate):

a) **2007**: Canadian _________ b) **2008**: Canadian __________

International __________ International ___________

8. What type of trips do resident boat owners typically take from your facility (Please indicate the %, or approximate number of boats for each type of trip):

- Local Trips – out and back within a day_____
- Weekenders – trips lasting a few days _____
- Local Racing – racing that takes place visiting facilities within 50km in the waters immediately off the facility _____
- Long Trips – travel beyond 50km from ____
- Long Distance Racing – racing that facility requires overnight outings ______
- Other (please specify): ______________

9. Do you have a tidal grid: o Yes o No
Depth range (below datum): ________________

10. Facility services (please check all that apply):

O	Customs	O	Garbage	O	Launch Ramp
O	Power (amp.__________)	O	Recycling	O	Rails
O	Pumpout	O	Fresh Water	O	Crane
O	Fuel Dock	O	Repairs / Mechanical	O	Travel Lift

PART C – MAINTENANCE INFORMATION

1. Do you move floats/docks from other locations to your facility: o Yes o No

a) Where from: ____

b) Approximate time of year they are moved: __________________________________

2. Are the following cleaned at your facility:

a) Pilings: o Yes o No How often: ______________________________

b) Floats/Docks: o Yes o No How often: ______________________________

3. Do you use antifouling paint or other protective substances on facility structures:

o Yes o No

4. Do your members employ divers to clean hulls in-water: o Yes o No