Impacts and Interactions of Two Non-Indigenous Seaweeds *Mazzaella japonica* (Mikami) Hommersand and *Sargassum muticum* (Yendo) Fensholt in Baynes Sound, British Columbia

by

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MSc, University of Alberta, 2007
BSc, University of Alberta, 2004

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Supervisory Committee

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Abstract

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This thesis examines the interactions of two non-indigenous algae, *Mazzaella japonica and Sargassum muticum,* where they co-exist and their impacts on native species in their recipient habitats. Field and lab experiments were conducted to determine if they impact native seaweed communities, marine invertebrates, and supralittoral regions.

In situ studies conducted in areas where *Mazzaella japonica* exists without *Sargassum muticum* found that removal of *M. japonica* allowed for an increase of native seaweed abundance and richness growing in fully subtidal regions, but had no detectable impact on native seaweeds growing in intertidal regions. Additionally, at the intertidal site, removal of *M. japonica* resulted in the recruitment of *S. muticum*. In regions where the two non-indigenous seaweeds co-exist, removal of both non-indigenous seaweeds negatively impacted native seaweeds. The magnitude of this negative impact was greater in subtidal compared to intertidal regions. *M. japonica* removal had a greater impact on native seaweed recovery than did *S. muticum* removal in areas of co-existence.

Removal of *Mazzaella japonica* also allowed for a significant increase in percent cover of *Sargassum muticum* at both sites where these two seaweeds co-exist. An increase in percent cover of *M. japonica* was found at the subtidal site when *S. muticum* was removed. Though both species increased when reprieved from competition with the other non-indigenous species, removal of *M. japonica* had a far greater influence on the increase in cover of *S. muticum.* This suggests that *M. japonica* is the dominant competitor in the ecosystem outcompeting *S. muticum.*

Field surveys found *Mazzaella japonica* was the dominant wrack species washing up on beaches in Baynes Sound. Though *Sargassum muticum* is also a component of the wrack, it has a disproportionately large influence as a spatial subsidy on beach habitats. *S. muticum* decayed and decomposed at a faster rate than *M. japonica* and all native seaweeds tested except for *Chondracanthus exasperatus*. Additionally, *S. muticum* was colonized by significantly more invertebrates than either *M. japonica* or *Fucus* spp. Results from these studies are intended to provide information for resource managers making policy decisions regarding the fate of these two non-indigenous species.

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Dedication

For my Grandpa, Gordon Heye

Chapter 1: Introduction

Non-indigenous species (NIS) have become a significant threat to native species diversity in almost every ecosystem (Carlton 1996, Ruiz et al. 1997, Molnar et al. 2008). In many cases, NIS have detrimental impacts on native species diversity causing concern as it has been well established that biodiversity is essential for sustained ecosystem functioning (Naeem et al. 1994, Loreau et al. 2001). Marine NIS can have wide-ranging effects on recipient ecosystems from no detectable impacts to drastic shifts in community composition and ecosystem functioning. One such example is the lionfish (*Pterois volitans*) invasion in the Caribbean which decimated native reef fish populations (Albins and Hixon 2008, Albins 2015). As globalization increases, so too do the number of marine NIS with over 40 identified harmful non-indigenous species in coastal zones from Oregon to Vancouver alone (Molnar et al. 2008). This increase in reported invasive species is likely due both to increased transportation of NIS around the world and also to increased research efforts to identify these NIS (Ruiz et al. 2000).

There are some unsettling trends in invasive species research. More than twice the number of field studies that examined the impacts of invasive species are observational rather than experimental (Lowry et al. 2013). While observational studies have their place in ecology, manipulative experiments are essential. When conducted properly, they increase the potential that the treatment is eliciting the result rather than an undetected random factor (Shaffer and Johnson 2008). While much of the knowledge from field based studies relates to primary producers, the vast majority of this knowledge comes from terrestrial ecosystems (Lowry et al. 2013). Thus, it is essential to increase our understanding of the effect that marine NIS can have on recipient ecosystems, which may lead to better and swifter management initiatives.

Even though the disparity of knowledge of invasions is large between terrestrial and marine systems (Lowry et al. 2013), it is even more so for our understanding of macroalgal invasions (Schaffelke and Hewitt 2007). Non-indigenous marine seaweeds contribute 8-40% of the marine NIS in a given area (Ruiz et al. 2000, Castilla et al. 2004). However, much of our knowledge about the impact comes from only four algal invaders: *Caulerpa taxifolia*, *Codium fragile ssp. tomentosoides*, *Sargassum muticum*, and *Undaria pinnatifida* (see Schaffelke and Hewitt 2007, Davidson et al. 2015 for reviews) and has been gained from research done primarily in Australia, Europe, New Zealand, and the United States (Schaffelke et al. 2006). There are few manipulative studies examining the impacts of lesser-known, introduced seaweeds prompting a repeated call in the literature to expand scientific knowledge in this area (Thomsen et al. 2009, Maggi et al. 2015).

Non-indigenous seaweeds can have a wide variety of impacts on recipient communities and have become prominent members of coastal communities. *Undaria pinnatifida* and *Caulerpa taxifolia* have made it onto the list of the world’s top 100 invasive species (“Global Invasive Species Database” n.d.). Non-indigenous seaweeds can compete both directly and indirectly with native seaweeds (Verlaque and Fritayre 1994, Valentine and Johnson 2003, Britton-Simmons 2004), and have both negative and positive impacts on higher trophic levels through alteration of food sources and habitat structure (Maggi et al. 2015). They are also known to hybridize with native species (Coyer et al. 2007), and can have economic costs through loss of ecosystem function and through costs of control measures (Meyerson and Reaser 2002, Pimentel et al. 2005, Salvaterra et al. 2013). Impacts can also include alterations to both ecosystem structure and function as well as potential socio-economic impacts (Schaffelke and Hewitt 2007). As marine NIS become more common throughout the world, their influence reaches beyond the marine ecosystem.

Marine macroalgae also play an important role in supralittoral and terrestrial ecosystems as a ‘spatial subsidy’ (Polis et al. 1997). Spatial subsidies are defined as organisms or nutrients which cross habitat boundaries, thus providing an essential service or food source for populations in the recipient habitat (Polis et al. 1997). Sandy beaches worldwide receive inputs of seaweed from neighbouring rocky shores and offshore beds (Rossi and Underwood 2002, Dugan et al. 2003). These spatial subsidies are important for the ecology of otherwise nutrient depauperate beach habitats (Colombini and Chelazzi 2003). These seaweeds are used both as habitat and as food sources for a variety of invertebrates such as semi-terrestrial amphipods, isopods, and tenebrionid and staphlinid beetles (Inglis 1989, Colombini et al. 2000, Jedrzejczak 2002, Olabarria et al. 2007, Pelletier et al. 2011). Despite the importance of these spatial subsidies in coastal ecosystems, little attention has been paid to macrofauna communities utilizing these resources or to how those macrofaunal communities change with changing macroalgal community composition (Rodil et al. 2008). Accordingly, the introduction of macroalgae to subtidal systems translates into a change in seaweed community structure entering terrestrial systems as wrack (Jiménez et al. 2015a).

1.1 Multiple Invaders

There are three potential outcomes from the interactions of multiple invaders: superadditive, additive, or subadditive (Rauschert and Shea 2012). Superadditive interactions, also called the invasional meltdown (Simberloff and von Holle 1999), occur where multiple invasive species have positive impacts on one another. Two invasive species that have little impact on one another would be considered additive (Rauschert and Shea 2012). Subadditive or invasional interference occurs when both species perform less well together than when alone (Yang et al. 2011). However, invasional interference may still result in similar levels of impacts to native communities as a single invader because there are impacts from the invaders but these effects are not synergistic (Lohrer and Whitlatch 2002). Examples of these three outcomes are drawn largely from terrestrial literature. However, there have been some studies of multiple invasive marine invertebrates, mostly on the interaction of *Carcinus maenus* (European green crab) and *Hemigrapsus sanguineus* (Asian shore crab), where it was found that *H. sanguineus* has a negative impact on *C. maenus* (Lohrer and Whitlatch 2002, Griffen and Byers 2008, Griffen et al. 2011), and also on multiple invasive tunicates (Ramsay et al. 2008). The only studies examining multiple invasive algae have determined the interactions of *Caulerpa racemosa* and *C. taxifolia*. However, these two species do not seem to overlap where they have been introduced to the Mediterranean as most studies have had to use reciprocal transplant experiments to directly study competition (Piazzi and Ceccherelli 2002, Piazzi et al. 2003, Balata et al. 2004). Kuebbing et al. (2013) call for an increase in the study of multiple, overlapping plant invaders. Evidently, there is still a large knowledge gap in the literature regarding multiple algal invasions and their consequences.

The current state of Baynes Sound's ecosystem in British Columbia offers a prefect model to begin to explore the impacts of co-existing algal invaders. Two algae, *Mazzaella japonica* and *Sargassum muticum* were introduced to Baynes Sound and not only do they grow in the same range, but it is not uncommon for these species to grow together on the same rock (K. Pawluk, pers. obs.). This unique situation has allowed us to examine directly the competitive interaction of two introduced algae.

1.2 Mazzaella japonica

There is little information available regarding *Mazzaella japonica* Mikami (Hommersand) (Rhodophyta: Gigartinales). It is a red seaweed native to the western North Pacific specifically: Japan, Korea, and East Siberia (Hommersand et al. 1993). It was thought to have been introduced to BC via the aquaculture trade and confirmed as a NIS via genetic identification (Saunders 2009, Saunders and Millar 2014). *M. japonica* exhibits a tri-phasic life cycle which alternates between isomorphic haploid and diploid phases and is known to have high quantities of carrageenans, highly sought after for industrial purposes (Cai et al. 2013).

Little is known about the ecology of *Mazzaella japonica* in either its native range or its introduced range. Density estimates in its native range describe *M. japonica* as being anywhere from rare to abundant (Khotimchenko and Gusarova 2004, Skriptsova and Levenets 2012). To our knowledge, the east coast of Vancouver Island is the only known location of an introduced population of *M. japonica*, despite the fact that carrageenan harvesters are continually looking for new populations of isomorphic seaweeds (Cargill Inc. pers com). In some areas, *M. japonica* grows in a veritable monoculture (K. Pawluk, pers obs). Currently there is no information regarding the life history characteristics of *M. japonica* that make it an effective invader. This thesis is the first record of any examination of the invasion ecology of *M. japonica*.

1.3 Sargassum muticum

In comparison to *Mazzaella japonica*, much more is known about *Sargassum muticum* (Yendo) Fensholt (Phaeophyceae: Fucales) with over 650 published papers about its invasion (Engelen et al. 2015). Its native range extends from Japan to south eastern China where it is considered a minor component of the native macroalgal community (Engelen et al. 2015). *S. muticum* has been introduced to multiple continents and was first thought to have reached the BC coast as early as 1902 via the aquaculture trade. It was officially identified in BC in the 1940s (Scagel 1956) and was recorded as a major contributing species from Nanoose to Deep Bay (Haegele 1978). Its invaded range extends from Alaska to Baja California on the west coast of North America and from the UK and Denmark through the Mediterranean Sea and as far south as Morocco (Engelen et al. 2015) on the west coast of Europe.

Sargassum muticum possesses a number of life-history strategies and adaptations that make it a highly successful invader. It has a very high growth rate of 2-4 cm per day (Norton 1977), an early age of maturity and high fecundity (Norton 1976, Hales and Fletcher 1990), and employs multiple dispersal techniques including germling settlement and reattachment of drifting thalli (Deysher and Norton 1982). In addition, it can survive in a wide range of temperature regimes (Norton 1976, Viejo 1997). *S. muticum* also has a discoidal holdfast which can endure from year to year ensuring a reduction in the need to compete for space (Scagel 1956).

Though *Sargassum muticum* is one of the four most thoroughly studied invasive seaweeds due to its cosmopolitan nature (Schaffelke and Hewitt 2007), there are conflicting results regarding its impacts on native communities. Some studies have found that it has negative impacts on native seaweeds (DeWreede and Vandermeulen 1988, Britton-Simmons 2004). Others have found that there is little to no impact on native communities (Sánchez and Fernández 2005, Olabarria et al. 2009, Smith 2015). However, some of this difference appears to be due to varying densities of *S. muticum* as it often grows in higher densities in subtidal sites than intertidal sites (Ambrose and Nelson 1982, Sánchez and Fernández 2005). Studies examining the impact of *S. muticum* on higher trophic levels have also resulted in contradicting conclusions. *S. muticum* has been found to be eaten indiscriminately by some invertebrates (Cacabelos et al. 2010, Britton-Simmons et al. 2011) but was avoided by the urchin *Strongylocentrotus droebachiensis* (Britton-Simmons 2004) and herbivorous gastropods such as *Littorina littorea* and *Aplysia punctata* (Cacabelos et al. 2010). Beyond the marine system, once *S. muticum* senesces and washes onto beaches, it has impacts on the macrofaunal communities that utilize beach-cast wrack; with a greater abundance of invertebrates colonizing that native seaweed *Sacchoriza polyschides* over the non-indigenous *S. muticum* (Rodil et al. 2008).

1.4 Challenges Studying Invasive Species

Several challenges exist in studying non-indigenous species. Perhaps the most relevant to my research program is the lack of detailed baseline data regarding native seaweed communities and ecological processes pre-invasion (Wells et al. 2007). Basic maps of seaweed distributions in Deep Bay were created in the 1970s (Haegele 1978);

however, maps show areas dominated with different “colours of seaweed”, referring to the various seaweed phyla, with accompanying species lists without clear detail as to where specific species are present (Haegele 1978). Due to this lack of data, the preferred before-after-control-impact (BACI) design (Stewart-Oaten et al. 1986) can not be used to determine impacts. Another problem due to this lack of baseline data is not knowing what is being potentially replaced by alien species. It is possible that prior to the introduction of these two species few native seaweeds existed in the habitat; thus, there is no actual competition with native species to be concerned about. However, this seems unlikely given competition for hard substratum in marine ecosystems. Control sites comparing ecosystem processes with and without the two introduced species have been determined to be an inefficient way to evaluate the effect of an invasion. It is not possible to determine conclusively if the uninvaded site has yet to be invaded because the introduced species has not dispersed to that location or if the invader cannot physiologically exist there. Thus, to determine the impact of these two invasive species, I used non-indigenous species removal experiments and monitored the “recovery” of native species or, where possible, conducted experiments with both non-indigenous seaweeds and native seaweeds of similar functional groups to be able to draw comparisons. Removal of non-indigenous species has been widely used by others studying the ecological impacts of invasive seaweeds (Levin et al. 2002, Britton-Simmons 2004, Sánchez and Fernández 2005, Schmidt and Scheibling 2007, South et al. 2016).

1.5 Social Implications

A significant impetus to conduct this research was the issuing of a harvest license for *Mazzaella japonica* in 2010 by the BC Ministry of Agriculture. At that point, no research had been done to characterize either the distribution of this seaweed or its ecology; nor had an ecosystem impact assessment been conducted to determine the potential impacts of the harvest. Harvest has been considered as a potential management strategy for invasive seaweeds (Villanueva et al. 2010). Early implications of this work for management were to either 1) manage an "invasive" species or 2) manage the species as a sustainable natural resource. In some regards, these two management plans would have a similar mechanism, as removal of some amount of seaweed would be necessary to meet either goal. However, managing for an invasive species would require eradication and management of a natural resource would require leaving some proportion of the population to ensure a sustainable venture. The results of this research will be given to the BC Ministry of Agriculture to provide relevant ecological information that can be used for management of this species.

1.6 Overall Questions Being Addressed by this Research

The overall goal of this research is to begin to understand the impacts that a previously unstudied non-indigenous species (*Mazzaella japonica*) is having on the subtidal and intertidal ecosystem it has invaded and how it is interacting with a well-known and globally studied invasive seaweed (*Sargassum muticum*). With the Ministry of Agriculture's approved harvest in mind, I sought to develop a research program that would be policy relevant. Specifically I asked:

1. Has the introduction of *Mazzaella japonica* alter the native seaweed communities in areas where its population has become established?

2. Have the two most abundant non-indigenous seaweeds, *M. japonica* and *S. muticum*, which overlap in portions of their growing range in Baynes Sound, affected native seaweed communities?
3. Is there evidence for superadditive, additive, or subadditive interaction between these two non-indigenous seaweeds?
4. Do *M. japonica* and *S. muticum* act as spatial subsidies for supralittoral beach communities when they wash up as beach-cast wrack, in what capacity do they do this, and how does this compare to native species?

To address these questions I conducted a series of experiments in both the growing range of the two non-indigenous seaweeds and on beaches in Baynes Sound (Fig 1.1). In Chapter 2, I address the implications of the *Mazzaella japonica* invasion on its own by characterizing plant densities and biomass. I also used *in situ* removal experiments to determine if *M. japonica* has detectable impacts on native seaweed communities (Fig. 1.2). In Chapter 3, I examined the interaction of *M. japonica* and *Sargassum muticum* through a second *in situ* removal experiment to determine whether these two non-indigenous species potentially facilitated one another's success and determined their combined impact on native seaweed communities (Fig. 1.3). Chapter 4 moves away from the growing range of these two species and addresses the impact these species have on semi-terrestrial ecosystems as a spatial subsidy (Fig. 1.4). I conducted a wrack composition survey to determine the dominant algal species and proceeded to determine rates of decay (microbial action) and decomposition (microbial action and herbivory) of these abundant seaweeds. I also conducted a feeding preference assay of the two most abundant beach-dwelling amphipods. This paper is currently under review for Marine

Ecology Progress Series (co-author S. Cross). Chapter 5 more directly addresses how these two non-indigenous species act as a spatial subsidy in the high intertidal zone as compared to the native seaweed *Fucus* spp. I assessed how the various seaweed species were colonized over time and if the semi-terrestrial and terrestrial invertebrates inhabited these resources differently. In Chapter 6, I address the ecological implications of these two non-indigenous seaweeds, whether there is any interaction between the two, and potential management scenarios.

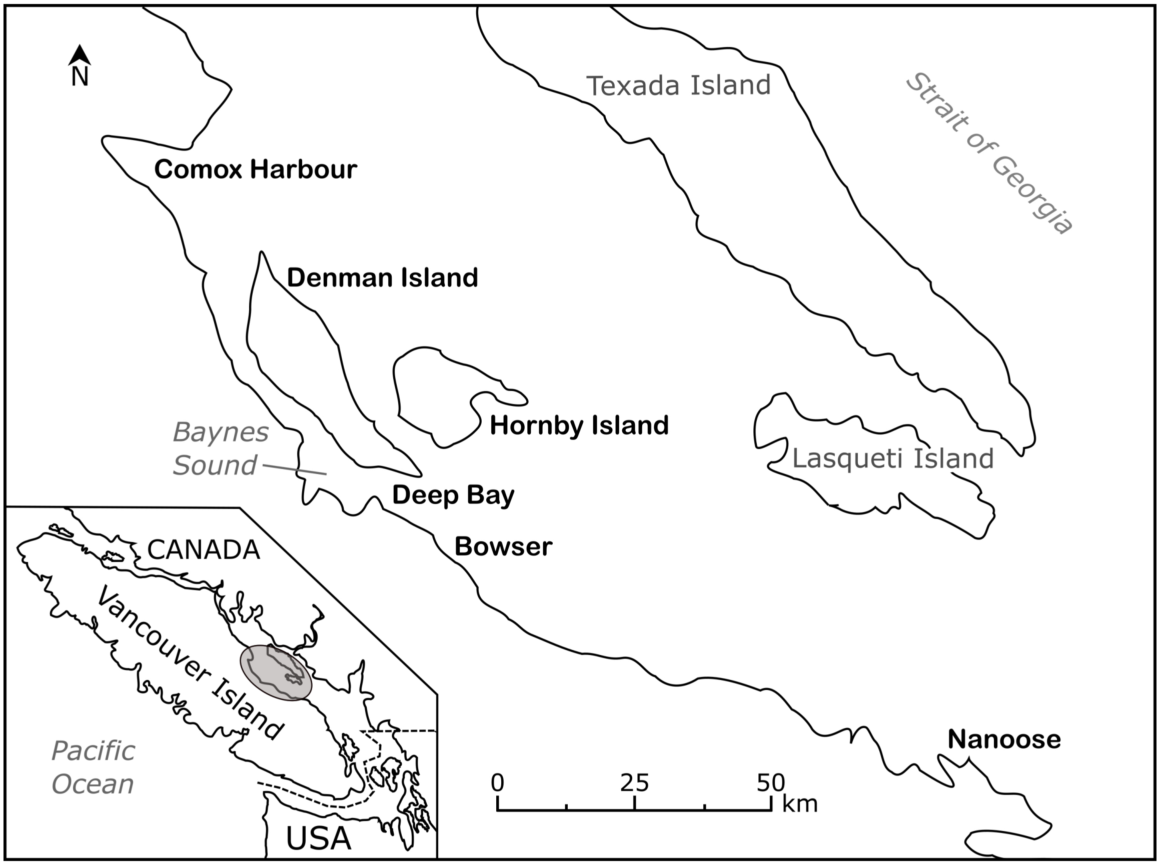

Figure 1.1 Baynes Sound, British Columbia where the research took place. Current confirmed populations of *Mazzaella japonica* are present from Comox Harbour to Nanoose. Genetic species confirmations by Saunders (2009) and Saunders and Millar (2014) confirm *M. japonica* in Comox Harbour and Savoie Rocks off Hornby Island. Species presence has also been confirmed in Deep Bay and Bowser (G. Saunders pers com) and visually confirmed in Nanoose (pers obs. K. Pawluk).

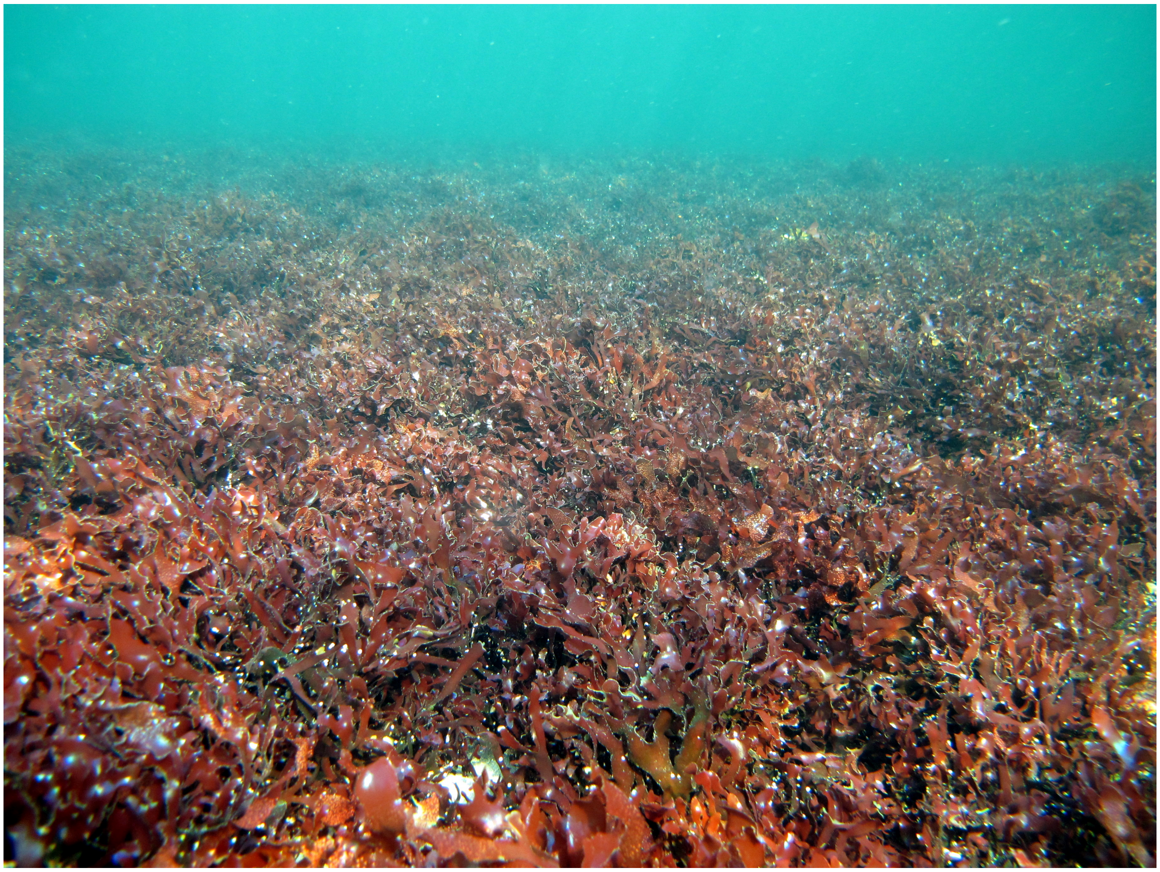

Figure 1.2 The shallow subtidal zone at Mailbox Beach where *Mazzaella japonica* is the dominant introduced seaweed present. Photo taken November 2014.

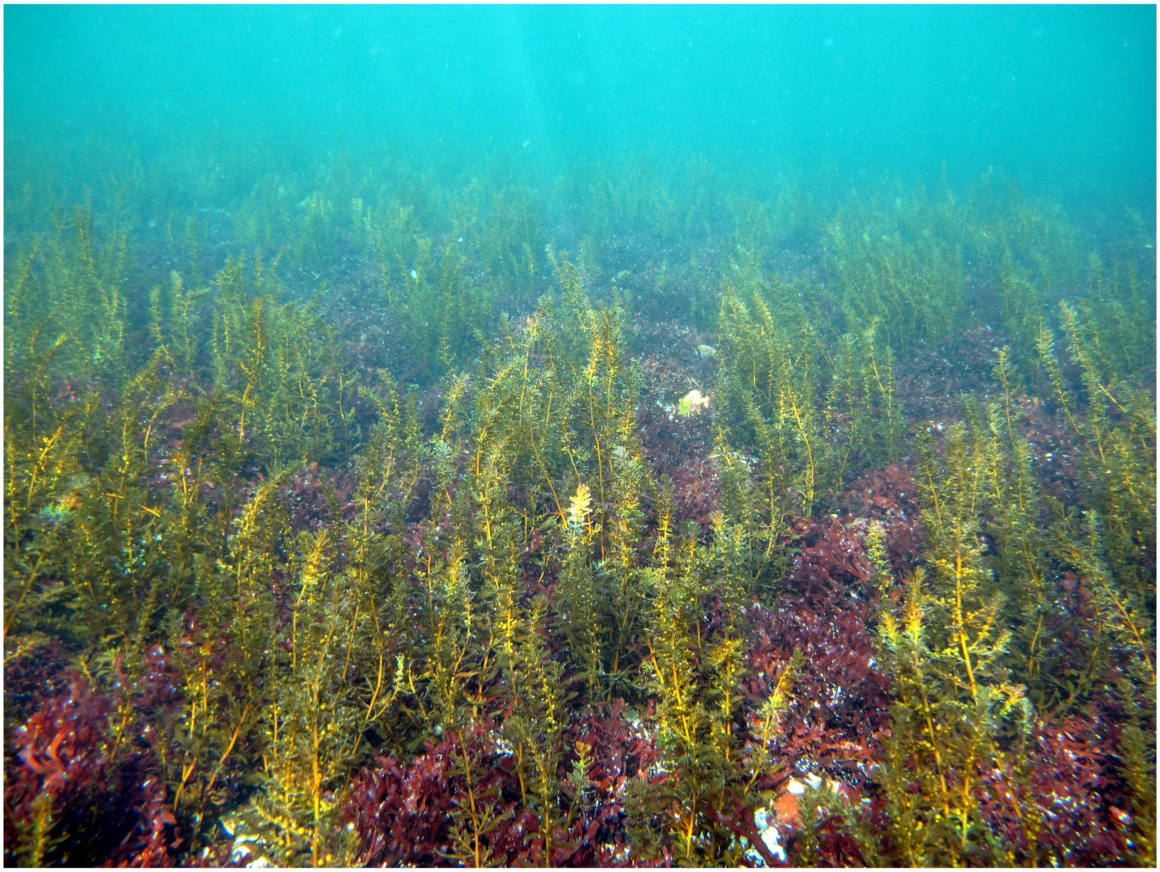

Figure 1.3 The shallow subtidal zone at Mailbox Beach where *Mazzaella japonica* and *Sargassum muticum* overlap in their growing range, often being found together on one cobble. Photo taken November 2014.

Figure 1.4 Beach-cast wrack in Baynes Sound, British Columbia. Photo taken November 2012.

Chapter 2 – Population Dynamics and Direct Impacts of the Non-Indigenous Seaweed *Mazzaella japonica*

2.1 Introduction

The introduction of non-indigenous species is modifying natural diversity worldwide (Vitousek 1990, Ruiz et al. 2000, Simberloff 2005, Molnar et al. 2008). Non-indigenous algae have become a prominent component of marine benthic ecosystems, with a myriad of ecosystem impacts resulting from their introduction (DeWreede 1996, Thomsen et al. 2009, Davidson et al. 2015). While the impacts of marine algae are understudied compared to terrestrial primary producers (Lowry et al. 2013), manipulative studies have shown that non-indigenous seaweeds can cause catastrophic shifts to communities (Santini-Bellan et al. 1996). The dominant negative impact that introduced algae has is due to direct competition with native seaweeds (Thomsen et al. 2014, Maggi et al. 2015).

Mazzaella japonica Mikami (Hommersand) is a recently discovered, introduced red alga on the east coast of Vancouver Island, British Columbia, thought to have been brought from northeast Asia as a “hitchhiker” via the aquaculture industry (Saunders 2009). There are no records to suggest when the first introduction occurred; however, it could have been first introduced in the early 1900s when the commercially important Pacific oysters (*Crassostrea gigas*) were introduced to Canada and continued until the mid-1960s when the habit of packing oyster spat in seaweed was ended (Scagel 1956). Vegetation surveys conducted by the Department of Fisheries and Oceans in Baynes Sound did not include *M. japonica* in lists of seaweed species sampled but an unidentified *Rhodoglossum* species was noted (Haegele 1978); *Rhodoglossum japonica* is the basionym for *M. japonica* (Guiry and Guiry 2016). Thus, it could have been first recorded in the 1970s.

Mazzaella japonica is in the family Gigartinales and has a tri-phasic lifecycle typical of the red algae with the sporophyte and gametophyte phases being isomorphic. Much of what is known about *M. japonica* pertains to its taxonomy (Lindstrom 2001, Saunders 2009, Hughey and Hommersand 2010, Saunders and Millar 2014) and other chemical properties of the species (Harada et al. 1997, Khotimchenko and Gusarova 2004). What little ecological information is known regarding this species comes from basic biodiversity studies in its native range in various areas of the Sea of Japan (Khotimchenko and Gusarova 2004, Kozhenkova 2009, Skriptsova and Levenets 2012) and Jeju Island, Korea (Kang et al. 2011). Descriptions of the seaweed population dynamics describes *M. japonica* as being rare (Skriptsova and Levenets 2012), associated with other species (Kozhenkova 2009), or commonly found with a biomass of up to 400 grams • m^{-2} (Khotimchenko and Gusarova 2004) and grows from chart datum to seven meters below chart datum (Khotimchenko and Gusarova 2004). *M. japonica* is also known to provide a substratum for herring spawn (*Culpea pallasii)* on Hokkaido (Hoshikawa et al. 2004). With regard to its introduced range, Saunders and Millar (2014) used DNA barcoding to confirm that *M. japonica* is present in the Comox Harbour, BC, off Savoie Rocks on Hornby Island, and at the field sites used in the present study (G. Saunder pers. com.).

Since the introduction of *Mazzaella japonica* to Vancouver Island, its distribution has not been monitored, but it has spread between the Comox Harbour to Nanoose Bay (Fig 2.1). Though it has likely been on Vancouver Island for over 50 years, this introduction garnered little attention until populations of *Chondrus crispus*, historically harvested for commercially valuable carrageenan on the east coast of Canada, began to decline (Sharp et al. 1993) and carrageenan extractors needed to find new populations to harvest. To date, nothing regarding the invasive capacity of *M. japonica* has been described. Thomsen et al. (2009), Davidson et al. (2015), and Maggi et al. (2015) all call for more manipulative *in situ* studies of the impacts of marine introduced algae to better understand how they impact native communities on both the same trophic level and at higher trophic levels.

The goal of this paper is to determine whether and to what degree *Mazzaella japonica* is impacting native communities in Baynes Sound using the criteria outlined by Blackburn et al. (2014). I have conducted preliminary studies to determine densities of *M. japonica* in its invaded range. As well, we conducted an *in situ* removal experiment to determine the effect of the introduction on native seaweeds and invertebrates. We expect that after *M. japonica* is removed, native species will be reprieved from space competition and shading and thus increase in abundance and richness.

2.2 Methods

2.2.1 Study Sites

This research was conducted on the east coast of Vancouver Island in Baynes Sound at two field sites with large beds of *Mazzaella japonica*. The two sites are Mailbox Beach (49°28’0.90”N, 124°43’27.40”W) and Buccaneer Beach (49°26’42.55”N, 124°40’51.88”W) (Fig 2.1). These two field sites both face north-east and are gradually sloping cobble beaches. However, there are some distinct differences between the two sites. Mailbox Beach is directly across from Denman Island and has low intertidal to shallow subtidal beds of *M. japonica* as the cobble substrate it lives on ends and is replaced with sandy substrate in deeper water. Buccaneer Beach has no sheltering from Denman Island, but rather, is exposed to the Strait of Georgia and has beds of *M. japonica* from the low intertidal to fully subtidal zones. *Mazzaella japonica* grows from the intertidal zone (~2 m above chart datum) to the subtidal zone (~7 meters below chart datum) where it exists in a veritable monculture (K. Pawluk pers.obs).

2.2.2 Population Density

At low tide on 25-26 November 2015, ten randomly selected 50 x 50 cm plots were sampled from along a 270 meter horizontal transect established at each of the two sites run parallel to the shore, and tidal height was noted for each plot. In each plot, the percent cover of *Mazzaella japonica* was estimated, the number of plants was enumerated by determining the number of holdfasts in a quadrat, and all plants were collected and brought to the lab. Once in the lab, all plants were rinsed in freshwater and dried at 60°C until they reached a constant weight. Dry plants were then weighed to determine biomass per plot.

2.2.3 Removal Study

All work for the removal experiments was done via SCUBA. In April 2013, 30-meter long permanent transects were laid out at the two separate sites (Fig. 2.1). The Mailbox Beach transect was at approximately 0.6 m above chart datum and the Buccaneer Beach site was at approximately 1 m below chart datum. Along each transect ten, 50 cm X 50 cm permanent plots were randomly demarcated using rebar pounded into the substratum in two corners of each plot. Plot placement was selected to ensure a high initial density of *Mazzaella japonica* in all plots. Plots were placed at least 2.5 meters apart to allow for a minimum of one-meter distance between plots with a 50 cm buffer zone around each plot. Plots were assigned to one of two treatments: control (no seaweed removed) and removal (*M. japonica* removed) employing a randomized block design. Each treatment was replicated five times at each site. Prior to removal of any *M. japonica*, all plots were sampled for the initial percent cover of all seaweed species (both non-indigenous and native). This was done by placing a 50 cm x 50 cm PVC quadrat (with various percent cover marked on it) around the two pieces of rebar and visually estimating percent cover of all seaweed species and visible substratum; all percent cover estimates were done by the author (Pawluk). Typical point measurements using strings across the quadrat (Sánchez and Fernández 2005) were not feasible in this system as placing the stringed quadrat down flattened the *M. japonica* and altered the species that were visible. After initial communities were sampled, *M. japonica* was removed from the appropriate quadrats; this was also done to 50 cm wide buffer zones to ensure that there was no influence of adjacent *M. japonica* plants on the treatment plots. Plots were re-examined at semi-regular intervals over two years. At each re-examination period, seaweed communities were again quantified and any *M. japonica* that had re-grown or had been carried into plots was removed when possible. Along with seaweed communities, motile macro-invertebrates were quantified. Re-examinations occurred in June 2013, August 2013, October 2013, December 2013, February 2014, April 2014, November 2014, and February 2015.

Onset®HOBO pendant® temperature and light data loggers were attached to one of the two rebar markers for each plot. Light (lux) and temperature (°C) were recorded every half hour from April 30, 2013 to June 10, 2013. Four data loggers were lost during this period leaving seven data loggers at Mailbox Beach (control n = 4, removal n = 3) and nine data loggers at Buccaneer Beach (control n = 5, removal n = 4).

2.2.4 Statistical Analysis

Population Density - Plant density (number of plants per quadrat) and plant biomass (grams per quadrat) were analyzed using linear regression as assumptions of normality were met.

Removal Study - Overall difference in algal community was compared using a repeated measures permutation analysis of variance (PERMANOVA) (Anderson 2001, Anderson and Walsh 2013) of percent cover community data (substrate was removed from the total percent cover) comparing the two treatments and all nine sampling periods. Resultant seaweed communities, native seaweeds and *Sargassum muticum* (*M. japonica* percent cover removed from the analysis), were then also compared using PERMANOVA separately at three time periods: pre-removal communities (April 2013), after one year (April 2014), and final sampling (February 2015). All PERMANOVA analyses were done in R (R Core Team 2015) using the vegan package (Okansen et al. 2016) and a Bray-Curtis distance matrix with 999 permutations. Data were visualized using non-metric multidimensional scaling (nMDS) using Bray-Curtis distance matrix and 1000 maximum random starts.

Average native species richness per plot and percent cover native species per plot were compared using separate repeated measures analysis of variance (RM-ANOVA) with treatment (two levels: control and removal) and time (nine sampling periods) as fixed factors and block (five levels) as a random factor. Data for species richness was log (x+1) transformed for both sites to meet the assumption of normality. Data for percent cover was square root transformed for Mailbox Beach and log (x+1) transformed for Buccaneer Beach. The change in percent cover for the most abundant native seaweed species and *Sargassum muticum* (Yendo) Fensholt were compared using RM-ANOVA. *Odonthalia* spp. and *Gastroclonium subarticulatum* (Turner) Kützing were each square root transformed and *S. muticum* was rank transformed (Conover and Iman 1981, Zimmerman and Zumbo 1993) for Mailbox Beach data. *Odonthalia* spp., *Constantinea subulifera* Setchell, and *Chondracanthus exasperatus* (Harvey & Bailey) Hughey were each rank transformed for Buccaneer Beach data. For all dominant species treatment (two levels: control and removal) and time (nine sampling periods) were fixed factors and block (five levels) was a random factor.

Abundance and species richness (number of species) of motile macro-invertebrates was compared for the two sites combined using RM-ANOVA with rank transformed data with treatment (two levels: control and removal) and time (eight sampling periods) as fixed factors and site (two levels: Mailbox Beach and Buccaneer Beach) as a random factor.

Average daily temperature was calculated for the 42 days that the data loggers were in the field. Daily average lux and daily maximum lux were also determined. Daily average lux was calculated only using values above one. All abiotic data were analyzed using RM-ANOVA with rank transformations to meet the assumption of normality. Treatment (two levels: control and removal) and time (42 days) were fixed factors and site (two levels: Mailbox Beach and Buccaneer Beach) was included as a random factor.

2.3 Results

2.3.1 Population dynamics

Mazzaella japonica grows in a veritable monoculture with plants filling from 67% to 99% cover within the 2500cm^2 area of the plots studied in November 2015 at both sites. There was no detected relationship between tidal height and number of plants per quadrat (p = 0.5550, estimate = -3.523, t-value = -0.602) (Figure 2.2a). However, biomass of dry plants per quadrat increased as tidal height decreased (p = 0.0022, estimate = -132.66, t-value = -3.573) (Figure 2.2b). Biomass per quadrat ranged from 177 $g \cdot m^{-2}$ up to 894 $g \cdot m^{-2}$ dry weight.

2.3.2 Removal Experiments

In pre-removal quadrats *Mazzaella japonica* constituted 90 ± 1.99 (mean % cover ± SE) at Mailbox Beach and 88 ± 2.64 (mean % cover ± SE) at Buccaneer Beach. Pre-removal total seaweed communities did not differ between the two treatments but were different between the two sites based on PERMANOVA (Fig. 2.3, Table 2.1); thus, remaining analyses of seaweed communities were completed separately.

Overall, removal of *Mazzaella japonica* had differing impacts on native seaweed communities at the two sites studied. Removal of *M. japonica* at Mailbox Beach did not result in a significant change of the native seaweed community as determined by repeated measure PERMANOVA (Table 2.2). Analyses from the final sampling period indicated no difference in native seaweed community based on PERMANOVA (Fig. 2.3, Table 2.3). However, there was a significant impact on native species richness and percent cover at various points throughout the experiment as determined by RM-ANOVA on both native species richness (Fig. 2.4a, Table 2.4) and percent cover (Fig. 2.5a, Table 2.4). Removal of *M. japonica* had a significantly negative impact on *Odonthalia* spp.

percent cover over time ending in a doubling of *Odonthalia* spp. in control plots compared to removal plots (Fig 2.6a, Table 2.7) but no detectible impact on *Gastroclonium subarcticum* (Fig 2.6b, Table 2.7). As of August 2013, *Sargassum muticum*, a known invasive seaweed in Baynes Sound appeared in quadrats where *M. japonica* had been removed and continued to be a component of the community for the remainder of the experiment (Fig. 2.6c, Table 2.5).

Conversely, when *Mazzaella japonica* was removed from Buccaneer Beach, significant impacts to overall seaweed community structure occurred as determined from PERMANOVA (Fig. 2.3, Table 2.3). There was a significant impact of removing *M. japonica* on both native species richness (Fig. 2.4b, Table 2.4) which increased four times as compared to control plots and percent cover which increased at least ten times compared to control plots as determined by RM-ANOVA (Fig. 2.5b, Table 2.4). Removing *M. japonica* also had significant positive impacts on the three dominant native species at that site: *Odonthalia* spp. (Fig 2.7a, Table 2.5), *Constantinea subulifera* (Fig. 2.7b, Table 2.5), and *Chondracanthus exasperatus* (Fig. 2.7c, Table 2.5) showed a two, six, and four time increase, respectively, as compared to control plots at the final sampling period.

Removal of *Mazzaella japonica* also positively impacted both abundance (Fig 2.8a, Table 2.6) and species richness (Fig 2.8b, Table 2.6) of motile macro-invertebrates within the two study sites. There was also a significant effect of removal on average light reaching the substratum in each plot (Fig 2.9a, Table 2.7) as well as maximum light (Fig. 2.9b, Table 2.7) but not temperature (Fig. 2.9c, Table 2.7) as determined by RM-ANOVAs.

2.4 Discussion

Overall, *Mazzaella japonica* is a major component of both the intertidal and subtidal ecosystems in Baynes Sound by covering up to 99% of the area in quadrats sampled. While there was no detectable impact of tidal height on *M. japonica* plant density, there was a positive effect of decreasing tidal height on plant biomass possibly indicating that *M. japonica* is better adapted physiologically or competitively to being submerged with less air exposure allowing it to grow larger. Biomass of *M. japonica* in its native range was reported as being 100 – 400 grams • m^{-2} (Khotimchenko and Gusarova 2004) which is half of what was found in Baynes Sound. This could suggest that either *M. japonica* is better adapted for the environmental conditions of Baynes Sound, is being reprieved from herbivory as theorized by the 'enemy release hypothesis' (Elton 1958), or is a competitively dominant species as, other than *Sargassum muticum,* it is the tallest seaweed in the system and receives the most light (K. Pawluk, pers obs.).

Removing *Mazzaella japonica* had different effects on native seaweed communities at the two sites. Removal of *M. japonica* at the intertidal site had both positive and negative impacts for the dominant native seaweeds. *Odonthalia* spp. was more prevalent in plots where *M. japonica* remained though mostly only in the final sampling period and there was no detectable impact of removing *M. japonica* on *Gastroclonium subarcticum*. At the subtidal site, removal of *Mazzaella japonica* allowed for the increased percent cover of all dominant native seaweeds for the majority of the experimental monitoring, including *Odonthalia* spp. which showed the opposite trend at the intertidal site. This supports our hypothesis that *M. japonica* is outcompeting native seaweeds through either competition for space or light or both. The relatively quick increase in native species richness at Buccaneer Beach is likely due to the presence of a native seaweed "seed pool" in the general vicinity of the plots which allowed for fast recruitment or growth or species.

There are several potential reasons for why removal of *M. japonica* had differing effects at the two sites. The Mailbox Beach transect was placed in the low intertidal zone as compared to Buccaneer Beach which was fully subtidal. These two sites also had different initial native community compositions, and it is possible that the different native seaweeds inhabiting the two depth profiles had varying abilities to respond to the removal of introduced seaweeds. Removal studies of the invasive seaweed *Sargassum muticum* have shown than when growing in subtidal areas it has negative impacts on native seaweed communities (Britton-Simmons 2004), but when growing in the intertidal there are few or no detectable impacts (Sánchez and Fernández 2005, Olabarria et al. 2009). There are three hypotheses that may explain the observed patterns. 1) It is possible that fewer native seaweeds have the physiological tolerance to exist in the intertidal range as compared to the subtidal range. As a result, the removal of *M. japonica* had little effect on native seaweed recruitment. 2) Native seaweeds may require more time to colonize intertidal areas as opposed to the more abiotically constant subtidal zone (Witman and Dayton 2001); thus, the 22-month duration of the experiment was not long enough to allow for recovery. 3) The final possibility is with regarding the density of *M. japonica* being lower in the intertidal. Though the density studies were only conducted in the intertidal range of the seaweed, there was a strong relationship between tidal height and biomass of seaweed within the quadrats. Even though *M. japonica* has roughly the same percent cover at both the intertidal and subtidal sites, *M. japonica* had a higher biomass at lower tidal ranges having a greater resultant impact on native seaweeds; a trend which has been noted with other introduced seaweeds (White and Shurin 2011). Further studies need to be conducted to tease apart these hypotheses.

Interestingly, removal of *Mazzaella japonica* allowed for the recruitment of *Sargassum muticum* at the intertidal site (Mailbox Beach) but not at the subtidal site (Buccaneer Beach). This suggests that in the subtidal range, *M. japonica* may be outcompeting *S. muticum* or perhaps *S. muticum* is excluded due to limited physiological tolerance or dispersal. Due to the recruitment and subsequent survival of *S. muticum* in multiple intertidal quadrats, it is presumed that *S. muticum* is not limited in its physiological tolerance in this range; but rather, is limited due to competition. To better understand this interaction, knowing the arrival timing of the two invasions would be helpful. This leads to two hypotheses regarding the interaction of these two species: 1) if *S. muticum* was introduced first it was then outcompeted by *M. japonica* or 2) if *M. japonica* arrived first then *S. muticum* was unable to successfully recruit in deeper areas because *M. japonica* was already occupying the space. Either hypothesis suggests that *M. japonica* is a dominant competitor for space. A transplant experiment moving *S. muticum* to deeper areas where *M. japonica* is dominant and *S. muticum* currently does not grow should be conducted to determine whether physiological intolerances or dispersal limitations bar *S. muticum* from surviving in the deeper subtidal regions of Baynes Sound.

Removal of *Mazzaella japonica* also had a positive impact on both the abundance and richness of motile macro marine invertebrates. While this provides us with only a crude idea of the impact that this introduced seaweed could be having on other trophic levels within Baynes Sound, it does suggest that *M. japonica* could be having negative impacts on large bodied, motile invertebrates. More detailed studies examining both macro and micro invertebrates as well as the transfer of nutrients through the food chain need to be conducted before we can determine if this non-indigenous seaweed is having a bottom up effect on higher trophic levels.

Not surprisingly, removal of *Mazzaella japonica* positively affected both the average daily light and the maximum light able to reach the substratum. Shading is a known impact that non-indigenous seaweeds can have on ecosystems (Crooks 2002, Britton-Simmons 2004, Arenas et al. 2006) and is thought to be one of the mechanisms through which non-indigenous seaweeds outcompete native seaweeds (Schaffelke and Hewitt 2007, Davidson et al. 2015). Where percent cover of native species increased in plots but species richness did not increase, could have been due to the reduction in shading by *M. japonica* thus allowing for expansion of existing native species. Temperature was unaffected by removal of *M. japonica* indicating that the shading action of the seaweed is not great enough to cause temperature changes. The increase in temperature over time was expected as the recording period began in April and ended in June when air temperature was also increasing.

Ultimately, the goal of this paper was to characterize a new introduced seaweed species in an attempt to increase knowledge about non-indigenous seaweeds as has been called for in the literature (Thomsen et al. 2009, 2011, Newton et al. 2013, Davidson et al. 2015). We sought to determine if the non-indigenous species *Mazzaella japonica* was having detectable impacts on native species within Baynes Sound and to use the classification from Blackburn et al. (2014) to determine its impact magnitude. According to impact class 1: competition (Blackburn et al. 2014), *M. japonica* ranges from minimal impact (negligible level of competition) to major (resulting in local or population extinction with community changes which are reversed when the introduced species is removed). Clearly, further study is required to better understand the impact that *M. japonica* is having on native seaweed communities. As the majority of the beds are subtidal (K. Pawluk, pers. obs.), more replicated removal studies throughout the *M. japonica* growing range need to be conducted to determine the magnitude of effect in deeper waters. However, the current results show that *M. japonica* has a negative impact on subtidal native seaweeds in Baynes Sound. Ultimately, this study demonstrates the myriad of impacts that non-indigenous species can have on multiple trophic levels and the importance of manipulative studies in recipient habitats.

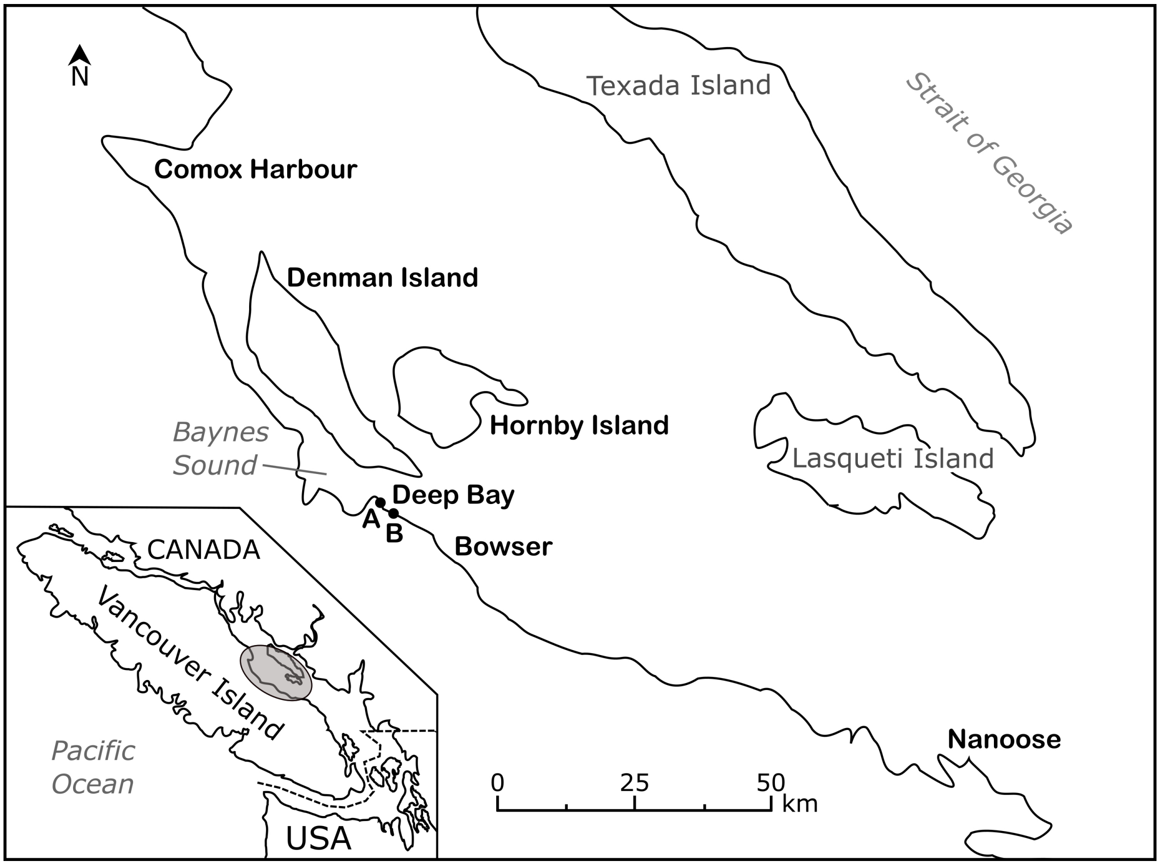

Figure 2.1 Site map of the population extent of *Mazzaella japonica* and the two field sties where the studies took place A) Mailbox Beach and B) Buccaneer Beach. *M. japonica* is known to be present from the Comox Harbour to Nanoose Bay. Genetic confirmations of *M. japonica* come from Comox Harbour, Savoie rocks (off Hornby Island), and the two field sites from this study.

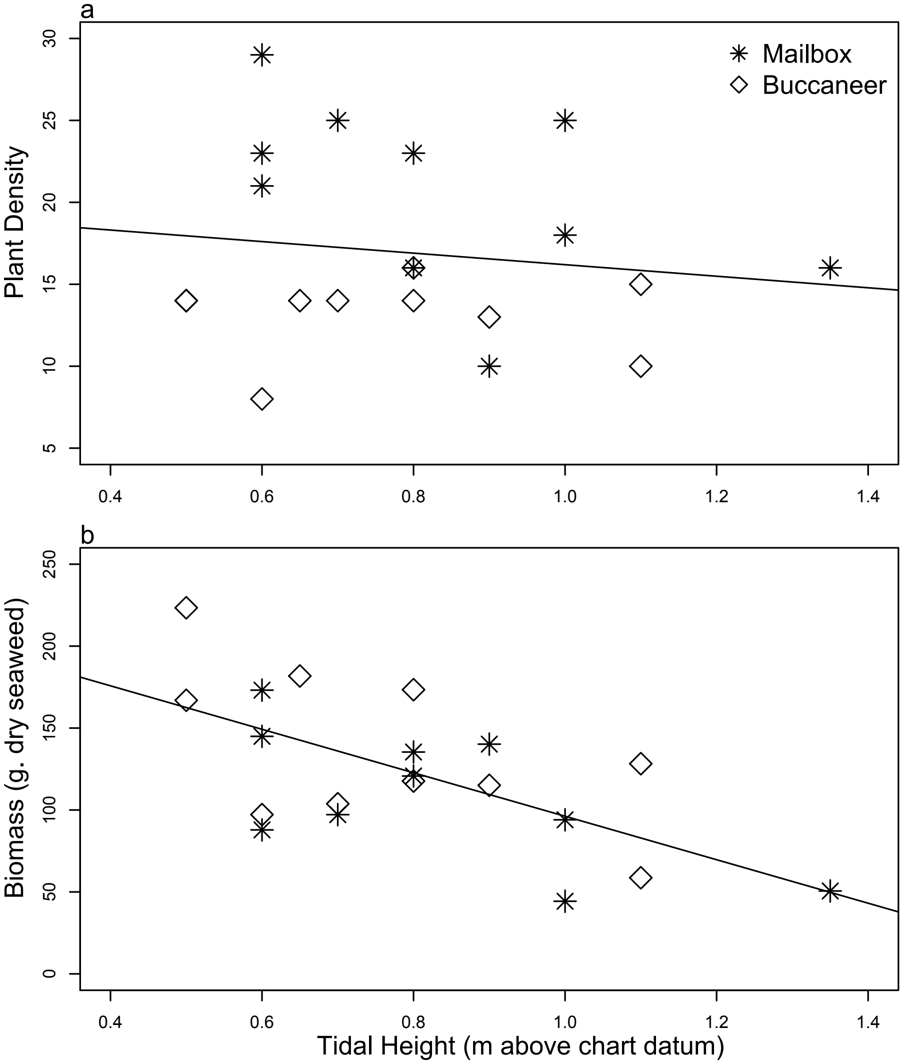

Figure 2.2 *Mazzaella japonica* population abundance a) number of plants per 2500 cm^2 quadrat at the sampled tidal heights and b) biomass of dry plants per 2500 cm^2 quadrat at the two study sites in Baynes Sound. Samples taken on November 24 and 25, 2015. Lines represent the results of a linear regression for the different plant metric, R^2 (number of plants) = 0.0197, R^2 (biomass) = 0.4149.

Table 2.1 Pre-removal comparison of native seaweed communities at the two sites for the two treatment types: control and *Mazzaella japonica* removal, results of PERMANOVA. Samples taken April 2013, n = 5 per treatment per site. Bold values indicate statistical significance.

	df	SS	MS	Pseudo-F	P
Treatment	1	0.321	0.321	0.912	0.473
Site	1	1.823	1.823	5.186	**0.001**
Treatment*Site	1	0.262	0.262	0.754	0.661
Residuals	16	5.625	0.352		

Table 2.2 Effect of *Mazzaella japonica* on algal communities. Results of repeated measures permutation analysis of variance (RM-PERMANOVA) testing the effect of removal of *M. japonica*, n = 5 per treatment for all nine time periods. Bold values indicate statistical significance.

	df	SS	MS	Pseudo-F	P
Buccaneer Beach					
Treatment	1	11.157	11.157	87.994	**0.001**
Date	8	3.920	0.490	3.864	**0.001**
Treatment*Date	8	3.701	0.463	3.649	**0.001**
Residuals	72	9.129	0.127		
Mailbox Beach					
Treatment	1	9.942	9.942	69.490	**0.001**
Date	8	4.203	0.525	3.673	**0.001**
Treatment*Date	8	4.087	0.511	3.571	**0.001**
Residuals	72	10.301	0.143		

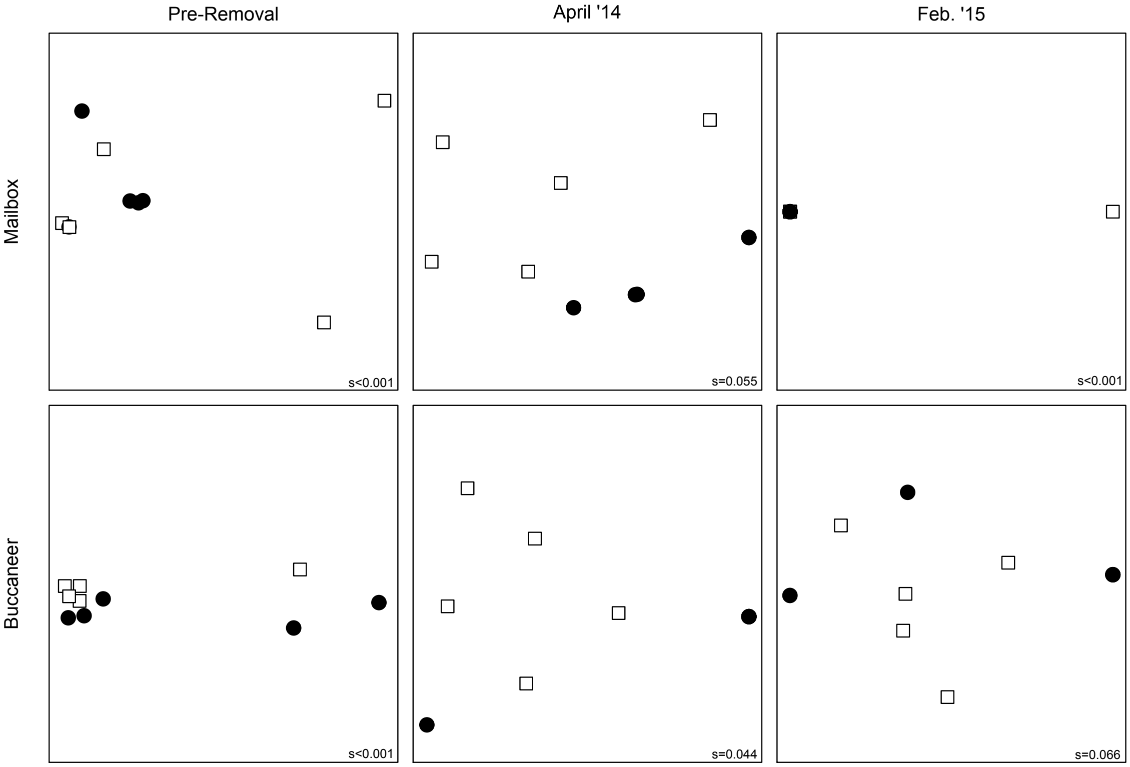

Figure 2.3 nMDS ordination showing temporal changes in seaweed communities not including *Mazzaella japonica* (native seaweeds and *Sargassum muticum* only) from the pre-removal assemblages (April ‘13) to the final sampling period (February ‘15) at Mailbox Beach and Buccaneer Beach. Filled circles are control plots (●) and open squares (□) are removal plots, n = 10 for all plots, where fewer than 10 symbols are visible indicates that communities within plots were so similar they directly overlap. Stress values are indicated for each panel.

Table 2.3 Summary of PERMANOVA results of seaweed communities not including *Mazzaella japonica* (native seaweeds and *Sargassum muticum* only) at chosen time intervals, n = 5 for each treatment at each site per time period. Bold values indicate statistical significance.

	df	SS	MS	Pseudo-F	P
Mailbox April '13 (Pre-removal)					
Treatment	1	0.853	0.853	2.246	0.088
Residuals	8	3.040	0.378		
Mailbox April '14					
Treatment	1	0.730	0.730	2.076	0.06
Residuals	8	2.815	0.352		
Mailbox February '15					
Treatment	1	0.282	0.282	0.745	0.606
Residuals	8	3.032	0.379		
Buccaneer April '13 (Pre-removal)					
Treatment	1	0.111	0.111	0.404	1
Residuals	8	2.200	0.275		
Buccaneer April '14					
Treatment	1	0.841	0.841	2.814	**0.036**
Residuals	8	2.391	0.299		
Buccaneer February '15					
Treatment	1	0.703	0.703	2.075	**0.046**
Residuals	8	2.710	0.339		

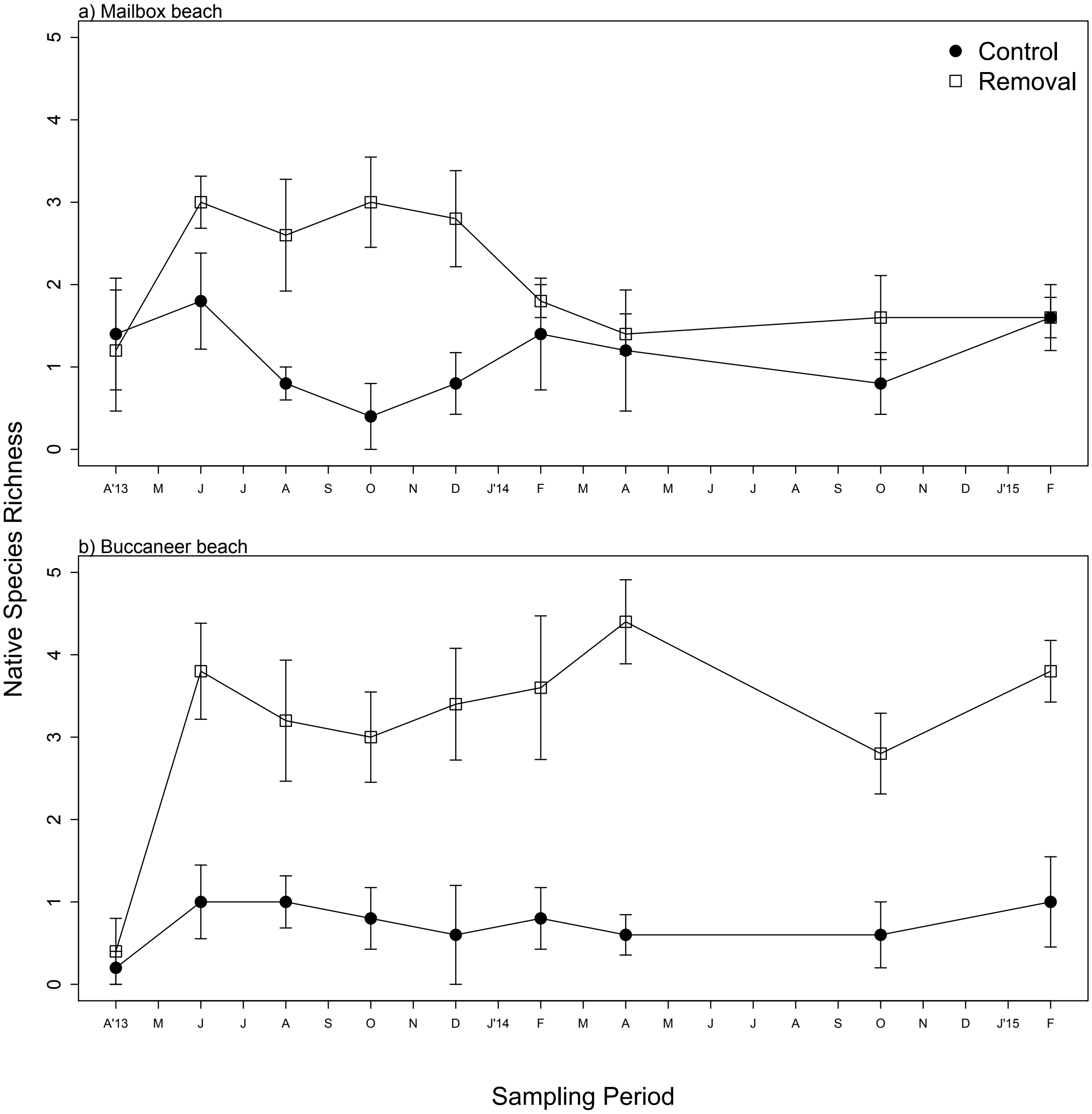

Figure 2.4 Native species richness (mean ±SE) in control and *Mazzaella japonica* removal plots (n = 5 per point) at a) Mailbox Beach and b) Buccaneer Beach. The first data point (April 2013) in each series is a pre-removal sample. Note: when found, *Sargassum muticum* was included as it was not the target non-indigenous seaweed.

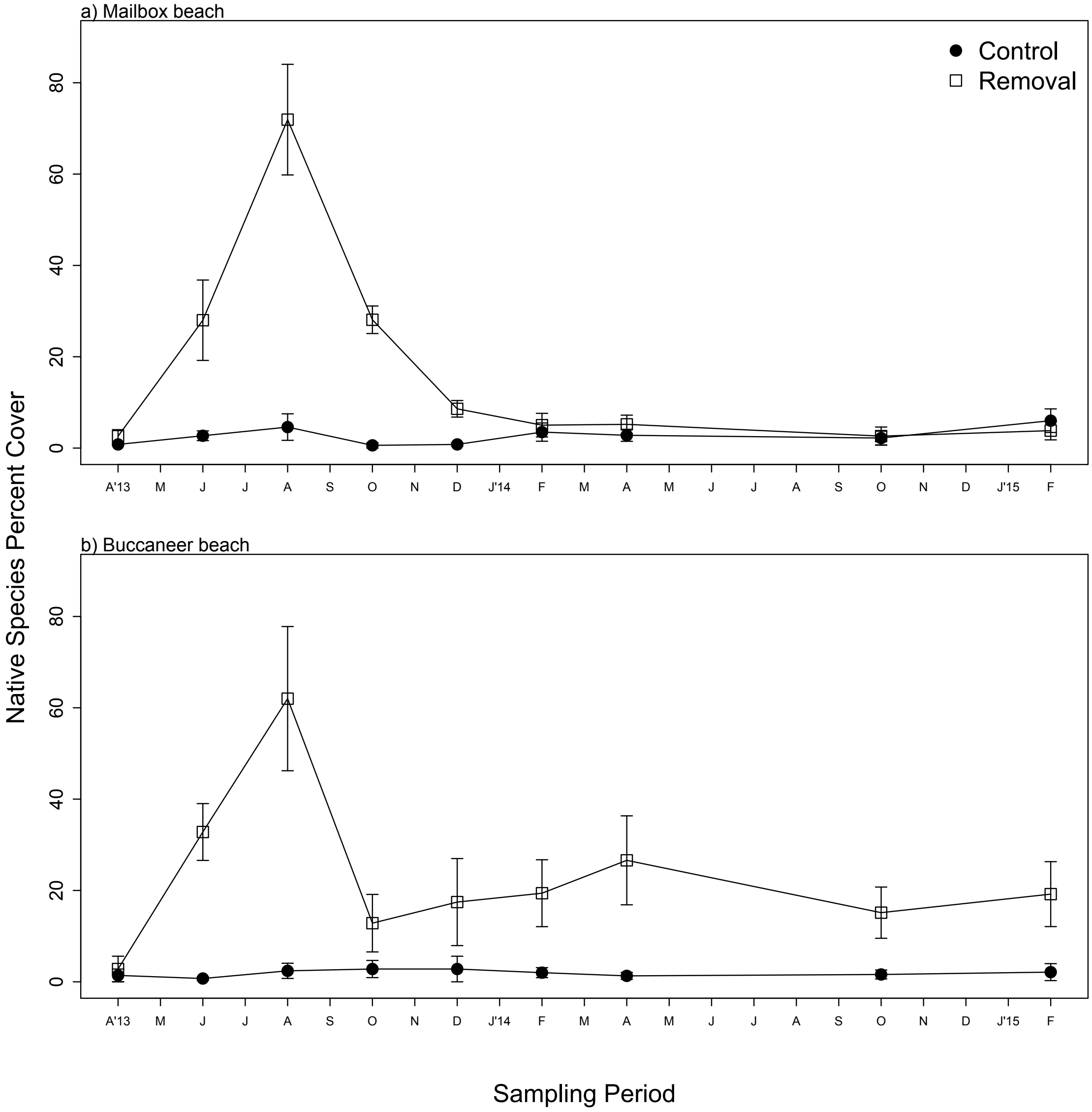

Figure 2.5 Native species percent cover (mean ±SE) in control and *Mazzaella japonica* removal plots (n = 5 per point) at a) Mailbox Beach and b) Buccaneer Beach. The first data point (April 2013) in each series is a pre-removal sample. Note: when found, *Sargassum muticum* was included as it was not the target non-indigenous seaweed.

Table 2.4 Effect of removal of *Mazzaella japonica* on native seaweed richness and native seaweed percent cover. Results of RM-ANOVA testing the effects of *M. japonica* removal, n = 5 per treatment for each of the nine time periods. Bold values indicate statistical significance.

	df	F-value	p
Species Richness			
Mailbox Beach			
Treatment	1	24.3624	**<0.0001**
Date	8	1.3488	0.2351
Treatment*Date	8	2.2714	**0.0323**
Buccaneer Beach			
Treatment	1	107.5798	**<0.0001**
Date	8	4.6992	**0.0001**
Treatment*Date	8	1.7434	0.1041
Percent Cover			
Mailbox Beach			
Treatment	1	24.3624	**<0.0001**
Date	8	1.3488	0.2351
Treatment*Date	8	2.2714	**0.0323**
Buccaneer Beach			
Treatment	1	143.7887	**<0.0001**
Date	8	4.3327	**0.0003**
Treatment*Date	8	3.2539	**0.0034**

Table 2.5 Effect of removal of *Mazzaella japonica* on the most abundant seaweed species at Mailbox Beach and Buccaneer Beach. Results of RM-ANOVA testing the effects of *M. japonica* removal, n = 5 per treatment for each of the nine time periods. Bold values indicate statistical significance.

	df	F-value	p
Mailbox Beach			
Odonthalia spp.			
Treatment	1	7.5014	**0.0079**
Date	8	2.7117	**0.0118**
Treatment*Date	8	0.9089	0.5145
Gastroclonium subarcticum			
Treatment	1	1.0674	0.3052
Date	8	1.4006	0.2122
Treatment*Date	8	0.7500	0.6473
Sargassum muticum			
Treatment	1	16.3263	**0.0001**
Date	8	0.9003	0.5214
Treatment*Date	8	0.9003	0.5214
Buccaneer Beach			
Odonthalia spp.			
Treatment	1	20.7008	**<0.0001**
Date	8	3.7256	**0.0012**
Treatment*Date	8	1.4241	0.2025
Constantinea subulifera			
Treatment	1	37.2282	**<0.0001**
Date	8	3.3519	**0.0027**
Treatment*Date	8	1.7525	0.1021
Chondracanthus exasperatus			
Treatment	1	37.1136	**<0.0001**
Date	8	3.6286	**0.0014**
Treatment*Date	8	2.4814	**0.0201**

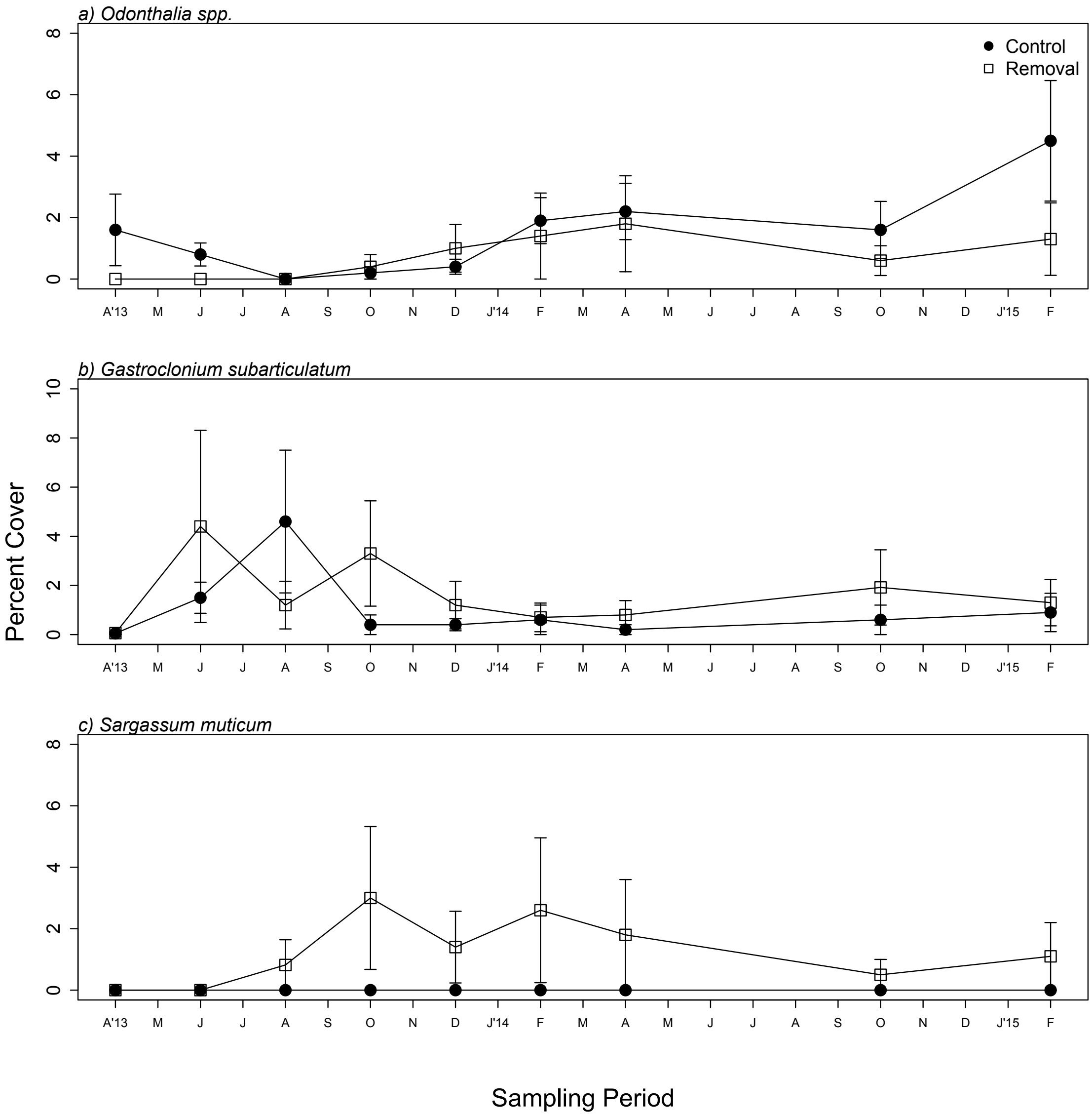

Figure 2.6 Percent cover (mean ± SE) of a) *Odonthalia* spp., b) *Gastroclonium subarticulatum*, and c) *Sargassum muticum* in control and *Mazzaella japonica* removal plots (n = 5 per point) at the Mailbox beach site. The first data point (April 2013) in each series is a pre-removal sample.

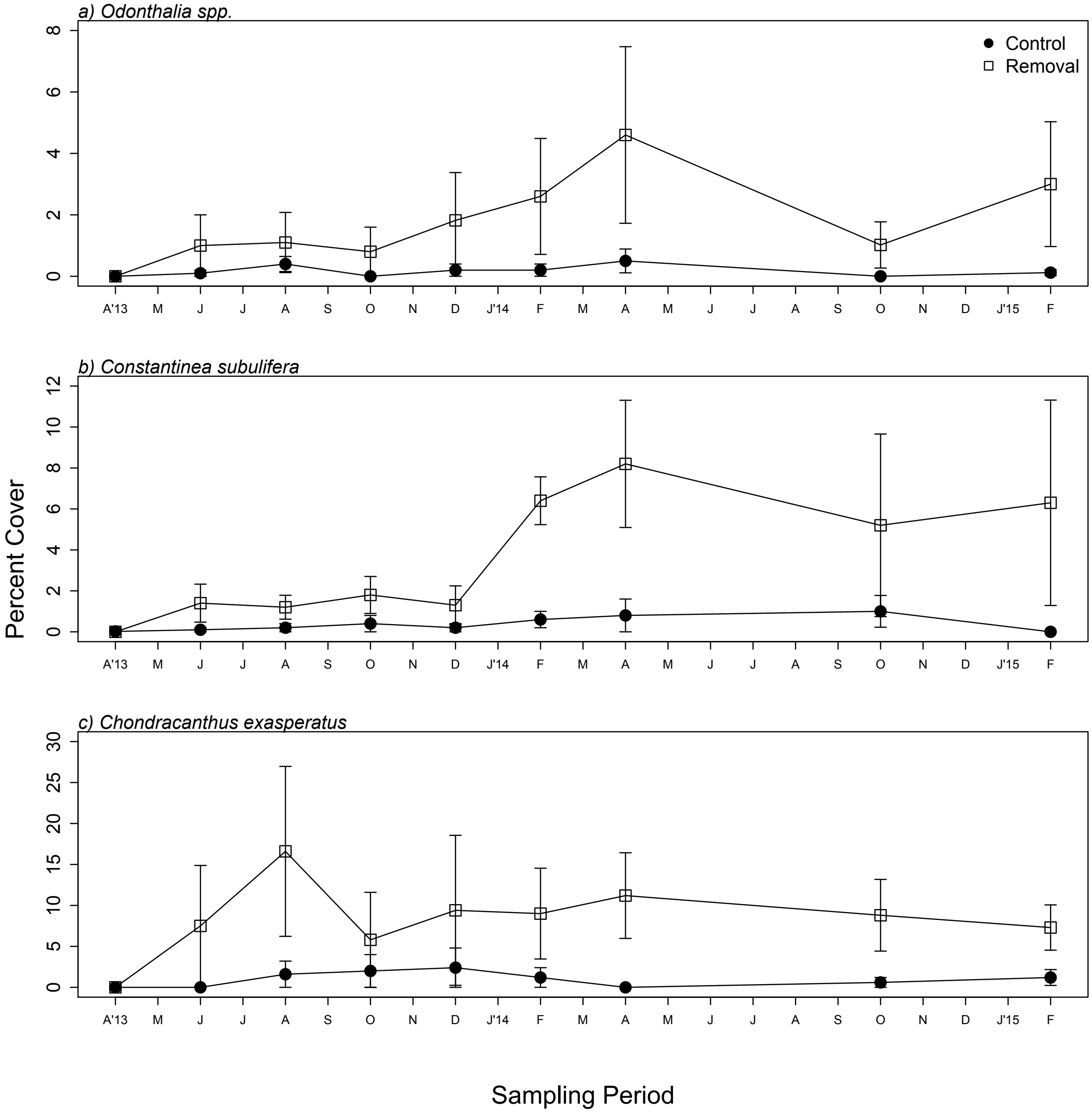

Figure 2.7 Percent cover (mean ± SE) of a) *Odonthalia* spp., b) *Constantinea subulifera*, and c) *Chondracanthus exasperatus* in control and *Mazzaella japonica* removal plots (n = 5 per point) at the Buccaneer beach site. The first data point (April 2013) in each series is a pre-removal sample. Note difference in y-axes.

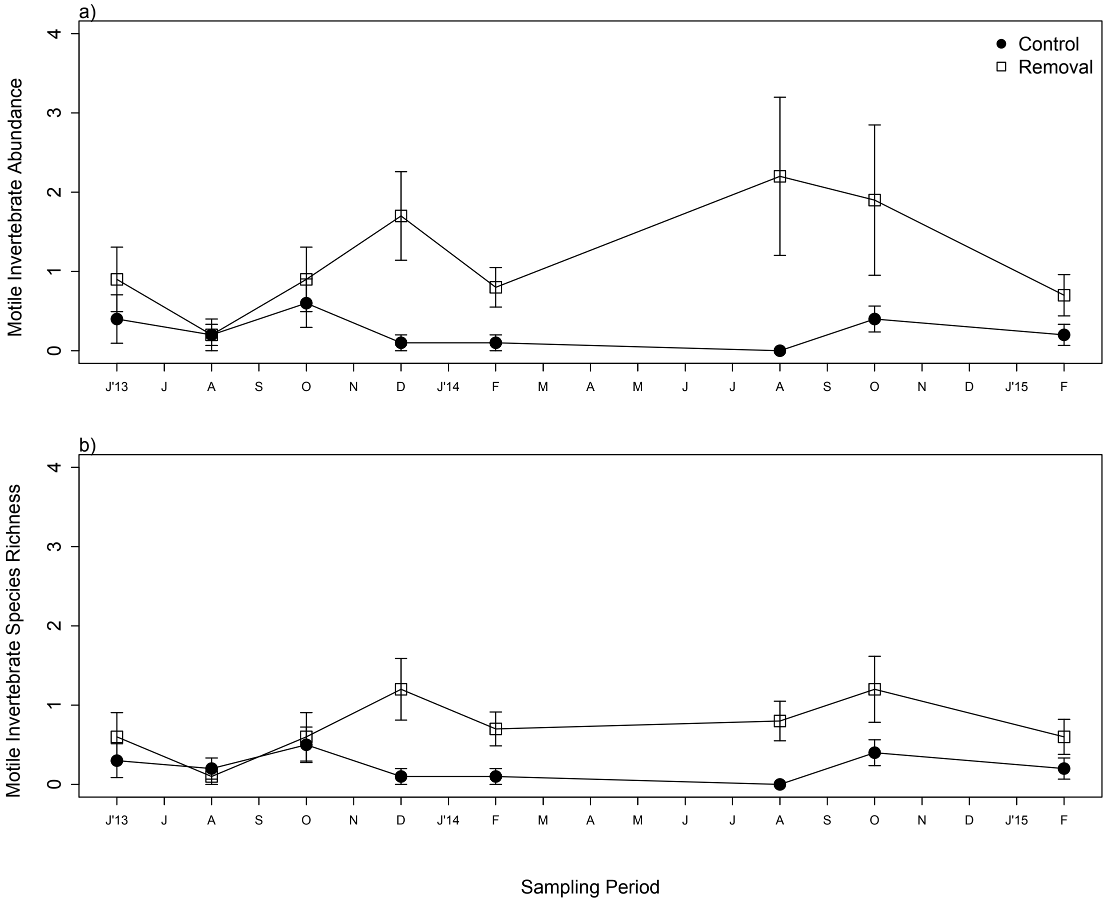

Figure 2.8 A) Total number (mean ± SE) and b) species richness (mean ± SE) of motile invertebrates in control and *Mazzaella japonica* removal plots (n = 10 per point) at the two sites combined. The first data point (June 2013) is from the first time period after seaweed removal.

Table 2.6 Effect of *Mazzaella japonica* on total number and species richness of motile invertebrates. Results of two-way RM-ANOVAs testing the effects of *M. japonica* removal, n=10 per treatment for each of the eight time periods. Bold values indicate statistical significance.

	df	F	p
Species Richness			
Treatment	1	20.0861	**<0.0001**
Date	7	1.3554	0.2288
Treatment*Date	7	1.8456	0.0830
Total Individuals			
Treatment	1	23.1880	**<0.0001**
Date	7	1.1992	0.3070
Treatment*Date	7	1.7221	0.1083

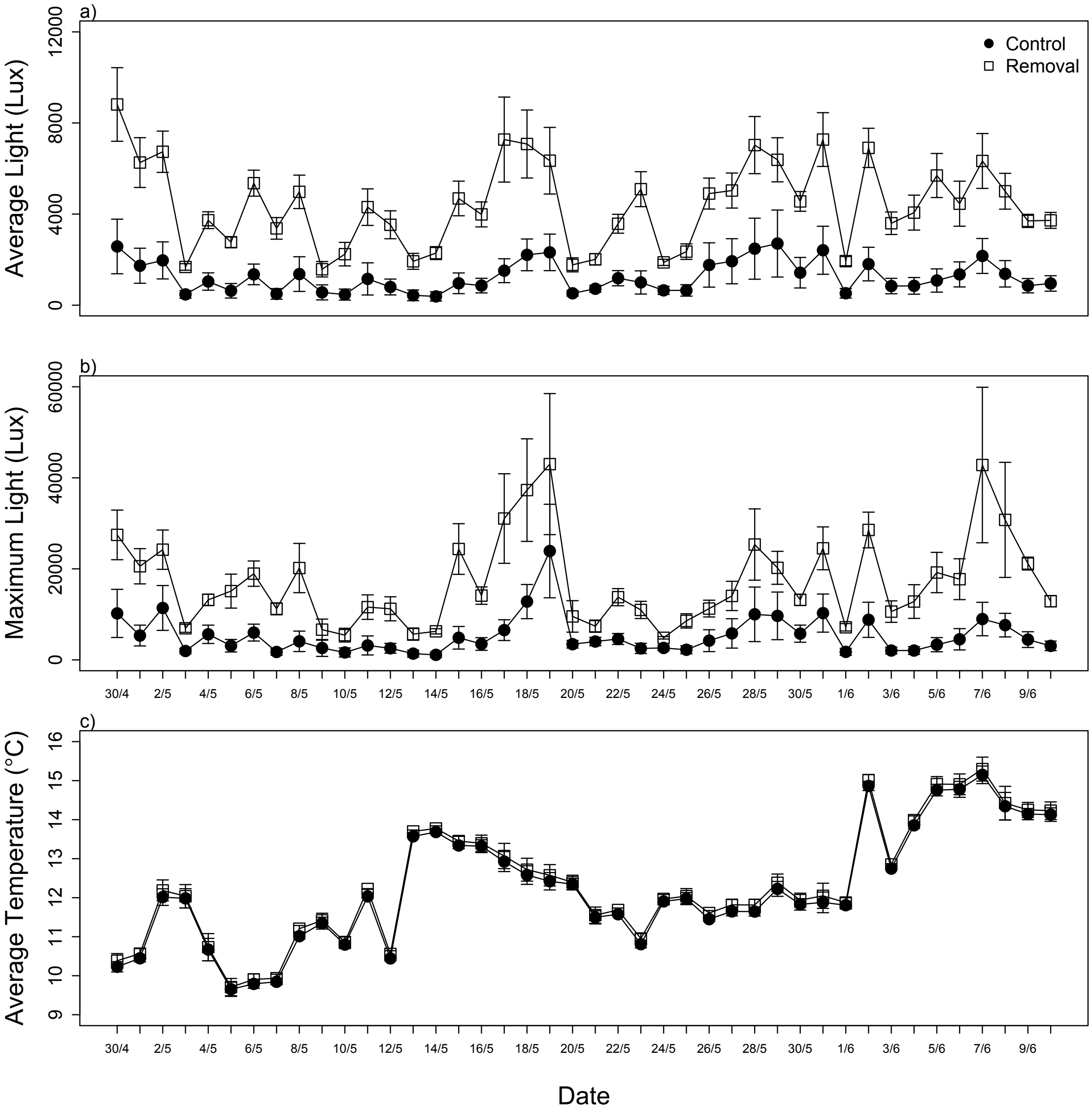

Figure 2.9 Daily a) average light (lux ±SE), b) maximum light (lux ±SE), and c) average temperature (°C ±SE) per day from April 30 to June 10, 2013 for control and *Mazzaella japonica* removal plots. Both sites are combined, n = 9 per control point, n = 7 per removal point. Note differences in y-axes.

Table 2.7 Effect of *Mazzaella japonica* on abiotic factors in plots including average and maximum light (lux) and average temperature (°C) per day. Results of repeated measures ANOVA the effects of *M. japonica* removal, n = 9 per control plot and n = 7 for each removal plot for each of the nine time periods. Bold values indicate statistical significance.

	df	F-value	p
Average Light			
Treatment	1	27.5150	**0.0001**
Date	41	14.8824	**<0.0001**
Treatment*Date	41	0.7175	0.9061
Maximum Light			
Treatment	1	20.9730	**0.0004**
Date	41	13.5126	**<0.0001**
Treatment*Date	41	1.9292	0.6000
Average Temperature			
Treatment	1	4.039	0.0641
Date	41	411.931	**<0.0001**
Treatment*Date	41	0.245	1

Chapter 3 - A Tale of Two Aliens: *Mazzaella japonica* Outcompetes the Global Invader *Sargassum muticum*

3.1 Introduction

Introductions of non-indigenous species to coastal ecosystems have accelerated on a global scale threatening native biodiversity and ecosystem integrity (Carlton 1996, Ruiz et al. 1997, Bax et al. 2003, Olden et al. 2004, Byrnes et al. 2007, Molnar et al. 2008). Understanding the role that non-indigenous species play in their recipient habitat is an important first step for determining what the long-term implications of an invasion are and what, if any, management actions are needed and would be valuable. Knowledge of non-indigenous seaweed interactions has lagged behind that of other taxa (Schaffelke and Hewitt 2007). Non-indigenous seaweeds can have impacts on native seaweeds through direct competition for space (Valentine and Johnson 2003, Britton-Simmons 2004, Schaffelke and Hewitt 2007) and light (White and Shurin 2011), and alteration of abiotic factors in an ecosystem (Crooks 2002). They can also impact organisms on different trophic levels by providing a new food source (positive) or reducing the abundance of palatable foods (negative) (Lyons and Scheibling 2007, Britton-Simmons et al. 2011), through the 'enemy release hypothesis' (Elton 1958) which states that non-indigenous organisms will experience a reprieve from herbivory due to its unfamiliar nature.

Most of the studies examining the ecological role of non-indigenous seaweeds on native seaweeds have focused on the impact of one non-indigenous species on native communities in the recipient region, which is the region to which the alien species has been introduced (Levin et al. 2002, Schmidt and Scheibling 2007, Gagnon et al. 2011, Salvaterra et al. 2013, Newton et al. 2013). Most habitats, however, are not subject to only one non-indigenous species at a time; thus, it is important to conduct manipulative experiments on multiple non-indigenous species to understand the potentially additive and interactive effects they can have (Preston et al. 2012, Jackson 2015). The 'invasional meltdown', first proposed by Simberloff and von Holle (1999), postulates that one non-indigenous species may facilitate the invasion of a second non-indigenous species and/or that two non-indigenous species may have positive impacts on each other, facilitating their population growth. Much of the research regarding interacting invasive species examines how disturbance caused by one invasive species allows for the success of a second (Scheibling and Gagnon 2006). Facilitation by multiple non-indigenous species has been previously demonstrated in the literature for invertebrates (Wonham et al. 2005, Griffen and Byers 2008, Preston et al. 2012) but these types of interactions are thought to be underrepresented in the literature. None, to our knowledge, have been examined for non-indigenous seaweeds (Simberloff and von Holle 1999). There are several possible outcomes for interactions between multiple species such as: positive interaction or superadditive impacts whereby the two species facilitate each other, little interaction or additive, or negative interaction or subadditive where the two species inhibit each other but can still have negative impacts on native communities (Rauschert and Shea 2012).

Separating the effects of multiple invaders is challenging as they can interact with native species and/or other invaders with both direct (e.g. competition for space) and indirect effects (e.g. reduction of a food source) (Preston et al. 2012). Thus far, the only studies to look at the impacts of multiple non-indigenous algae have used transplant experiments to determine how *Caulerpa taxifolia* and *C. racemosa* could potentially compete in the Mediterranean (Piazzi and Ceccherelli 2002, Balata et al. 2004). However, very few (Preston et al. 2012) (and none, to our knowledge, in the marine system) have looked at the interaction of multiple non-indigenous algae within the same habitat of the recipient region and how they interact with each other and with native species.

Sargassum muticum and *Mazzaella japonica* are both native to the coastlines of East Asia and are thought to have been introduced to the west coast of North America via the aquaculture trade as 'hitchhikers' on introduced oysters (Scagel 1956, Saunders 2009); *S. muticum* populations have been found almost everywhere that non-indigenous oysters have been introduced (Engelen et al. 2015). Though there is little evidence to suggest when these species were introduced to BC it could have been as early as 1902 (Saunders 2009) and continued up to the 1960s when the practice of shipping non-indigenous oysters in seaweed ended. There is no information regarding which species was introduced first but the presence of *S. muticum* was noticed initially in the 1940s (Scagel 1956) followed by *M. japonica* which was confirmed in the late 2000s (Scagel 1956, Saunders 2009). Unfortunately, this does not serve as an indicator of the actual timeline as both species could have been introduced at the same time but as *S. muticum* is one of the four most globally invasive seaweeds (Engelen et al. 2015), it is likely to have been detected earlier than *M. japonica*. Thus far the only known population of *M. japonica* found outside its native range has been on the east coast of Vancouver Island from the Comox Harbour to Nanoose and is most abundant in Baynes Sound which is where these two species were mostly likely initially introduced. It is here that these two species overlap in the recipient habitat, even growing on the same cobble.

Previous studies examining *Sargassum muticum* have found conflicting results regarding its impacts on native communities. Work done off the west coast of the Pacific Northwest has found that *S. muticum* does have negative impacts on native seaweeds through competition (DeWreede 1983, Britton-Simmons 2004); however, studies done in Europe have found the opposite effect (Sánchez and Fernández 2005, Olabarria et al. 2009). Studies have also shown that marine invertebrates have varying preference for eating *S. muticum*. Some studies have supported the enemy release hypothesis and found that urchins and various gastropods do not feed on *S. muticum* (Britton-Simmons 2004, Monteiro et al. 2009, Cacabelos et al. 2010) but other species of urchins and gastropods preferentially consumed it (Cacabelos et al. 2010, Britton-Simmons et al. 2011).

Thus far the only study to examine the impacts of *Mazzaella japonica* was conducted by Pawluk in Chapter 2 of this thesis, *M. japonica* was found to negatively affected native communities in a subtidal habitat but had no detectable impact in an intertidal area and that overall the removal of *M. japonica* had a positive effect on marine invertebrate abundance or richness.

The objectives of this study were to determine the effect that *Sargassum muticum* and *Mazzaella japonica* have on native seaweed communities and if they interact with each other using simultaneous *in situ* removal experiments. The two seaweeds have the capacity to compete with each other and native species through the same mechanisms: shading and space competition. In addition to this, through a lab study, we sought to determine if the herbivore *Strongylocentrotus droebachiensis* would have a preference for native over non-indigenous seaweeds in support for the enemy release hypothesis. We predicted that both *M. japonica* and *S. muticum* would have competitive interactions with native seaweeds and that removal of one non-indigenous species would allow for further growth of the other invasive species indicating a subadditive interaction. Furthermore, we predicted that the urchin feeding preference would conform to the 'enemy release hypothesis' and would demonstrate preference for the kelp *Saccharina latissima* over all other species and then when kelp is not available it will prefer other native seaweeds to non-indigenous ones.

3.2 Methods

3.2.1 Study Sites

This research was conducted on the east coast of Vancouver Island at the southern edge of Baynes Sound at two field sites where the two non-indigenous species *Mazzaella japonica* and *Sargassum muticum* co-exist. The two sites were Mailbox Beach (49°28'0 N, 124°43'27 W) and Buccaneer Beach (49°26'42 N, 124°40'51 W) (Fig. 3.1). These two field sites both face northeast and are gradually sloping cobble beaches both of which are exposed to the Strait of Georgia and therefore to strong currents and extensive wave force. Both non-indigenous seaweeds extend from the low intertidal to fully subtidal beds where *S. muticum* presence ends around one meter below chart datum but *M. japonica* beds extend further to at least seven meters below chart datum at the Buccaneer Beach site (K. Pawluk, pers obs).

3.2.2 Removal Experiment

To address the impacts of these non-indigenous seaweeds on native seaweed communities we conducted a removal experiment done via SCUBA. A 60-meter permanent transect was laid out at each of the field sites parallel to waterline. The Mailbox Beach transect was at approximately 0.8 m above chart datum and the Buccaneer Beach transect was at approximately 0.8 m below chart datum. Along each transect 20, 50 cm X 50 cm permanent plots were demarcated by hammering rebar into two corners of each plot. Plots were placed at least 2.5 meters apart to allow for 50 cm buffer zones around each plot and a minimum of one meter un-impacted area between buffer zones. Buffer zones had the same removal applied as the plot they surrounded and were included to ensure that seaweeds directly around the plots did not impact the effect of the treatments. Plots were selected to ensure that high initial densities of both *Sargassum muticum* and *Mazzaella japonica* were present prior to removal. Plots were assigned to one of four treatments: control (no removal), *M. japonica* removal (-Mj), *S. muticum* removal (-Sm), or both *M. japonica* and *S. muticum* removal (-Mj&Sm). Each treatment was replicated five times at each site and placement was determined by using a randomized block design for a total of five blocks per site. Prior to removal of any introduced species, all plots were sampled for the initial percent cover of all species and any visible substrata. All percent cover estimates were completed by placing a 50 cm x 50 cm PVC quadrat with the area of one percent cover demarcated on it around the two pieces of rebar. Appropriate removals were then applied to each plot and to the buffer zones. Plots were re-examined at semi-regular intervals over a year and a half from April 2013 to November 2014. Re-examinations occurred in June 2013, August 2013, October 2013, December 2013, February 2014, April 2014, and November 2014. At each re-examination period, seaweed communities were again quantified and any introduced seaweeds that had re-grown or washed in were removed. Motile macro-invertebrates (e.g. crabs, sea stars, nudibranchs etc.) were also counted and identified in each plot during the re-examination periods.

Onset®HOBO pendant® temperature and light data loggers were attached to one of the two rebar markers for each quadrat. The data loggers recorded light (lux) and temperature (°C) every half hour from midnight May 3, 2013 to midnight June 10, 2013. Three data loggers were lost during their deployment resulting n = 9 for control, *Mazzaella japonica* removal (-Mj), and *Sargassum muticum* removal (-Sm) but n = 10 for the two species removal (-Mj&Sm).

3.2.3 Urchin Feeding Preference

On July 27, 2015, 30 *Strongylocentrotus droebachiensis,* green urchins, between 12 and 24 grams, were collected by hand from Norris Rocks off Hornby Island (49°29’0 N, 124°38’ 55 W). *Mazzaella japonica, Sargassum muticum*, and *S. droebachiensis* co-occur here. Urchins were transported back to the Deep Bay Marine Station where they were housed in a flow through sea table until the experiment commenced. Urchins were fed to satiation on mixed seaweeds and were then starved for three days before the experiment began. On August 3, 2015, the first day of the experiment, snorkelers collected five species of live seaweeds for the feeding trial: *M. japonica*, *S. muticum*, *Chondracanthus exasperatus*, *Ulva* spp., and the kelp *Saccharina latissima*. *S. latissima* was the dominant kelp in this ecosystem (Haegele 1978) but its populations have declined over the past few decades likely due to a number of factors especially rising sea surface temperature (Simonson et al. 2015). To determine the feeding preference of *S. droebachiensis* we conducted two feeding assays where urchins were provided with large quantities of each seaweed with equal access to all species to allow for a determination of which seaweed was preferred (Peterson and Renaud 1989). Urchin feeding containers were made of 2L plastic storage containers with the sides cut out and replaced with 1 cm x 1 cm mesh sides to allow for water flow. The first feeding experiment included all five species of seaweeds in roughly equal quantities (~4 g/species) and the second included all species except for *S. latissima* (~5 g/species). This was done as we were concerned that if all treatments included *S. latissima*, we would be unable to determine a preference between the four other species which are more abundant in the ecosystem. In both assays most seaweeds consisted of an individual piece except for *Ulva* spp. which was typically multiple pieces to make up the necessary weight. All seaweeds were placed around the bottom of the rectangular treatment containers with urchins placed in the middle to ensure there was equal access to all seaweeds. The containers were small enough so that urchins most likely touched multiple species at once. Each preference setup (with and without kelp) was replicated 10 times and had five control (no urchin) replicates to determine autogenic change of seaweed. At the beginning of the experiment, urchin test diameter and wet weight was measured. To standardize the wet weight of seaweed, each species was spun in a salad spinner for 20 seconds, weighed to the nearest 0.01 gram, and placed in its respective container. Urchin treatment and control containers were dispersed throughout a single large, circular flow-through tank using a randomized block design. Urchins were allowed to feed for five days until August 7, 2015 when seaweeds were removed from containers and re-weighed following the same procedure used for the experimental set up. Half way through the experiment all treatment containers were checked to ensure urchins were alive. During this period, all urchins were moved within their containers likely affecting the seaweed species they were next to.

3.2.4 Statistical Analyses

All data were analyzed using the R statistical software (R Core Team 2015). Native Seaweed Community Analyses - Permutation analysis of variance (PERMANOVA) was used to determine if the pre-removal seaweed communities differed between the four treatments, the two sites, and the interaction between treatment and site (Anderson 2001, Anderson and Walsh 2013). Substratum was removed from analyses. As the pre-removal communities at the two separate sites were statistically significantly different, all further analyses were run for the two sites separately. PREMANOVA was also used to look at the effect of the different removals over time specifically for pre-removal sampling, April 2014, and November 2014 examination periods. As there is no post-hoc test for PERMANOVA, paired PERMAOVA tests were run to compare all treatment for the final sampling period (November 2014) for the two separate sites. All PERMANOVA tests were run using the vegan package (Okansen et al. 2016) with a Bray-Curtis distance matrix and 999 permutations. Data were visualized using non-metric multidimensional scaling (nMDS) using a Bray-Curtis distance matrix and 1000 maximum random starts.

To more fully examine how each non-indigenous species impacted native seaweed communities, repeated measures analysis of variance (RM-ANOVA) were used to determine how removal of the different non-indigenous seaweed combinations affected the richness and percent cover of native species over time. Treatment (four levels: control, -Mj, -Sm, and –Mj&Sm) and time (eight sampling periods) were fixed factors and block (five levels) was used as a random factor. None of the data were normal, and thus were rank transformed (Conover and Iman 1981, Zimmerman and Zumbo 1993) to meet assumptions of normality and equal variance. Post-hoc summary comparisons were made on significant results.

Interactions between *Mazzaella japonica* and *Sargassum muticum* – The interaction of these two species was determined by examining how one non-indigenous seaweed responded to the removal of the other (e.g. *M. japonica* percent cover in –Sm plots compared to control plots). Data were analyzed using RM-ANOVA with rank transformation to meet assumptions of normality using percent cover of the remaining introduced seaweed as compared to the control. Treatment (two levels: control and non-indigenous seaweed removal) and time (eight sampling periods) were fixed factors and block (five levels) was included as a random factor. Post-hoc summary comparisons were made on significant results.

Animal and Abiotic Data – RM-ANOVA with treatment (four levels: control, -Mj, -Sm, and –Mj&Sm) and time (seven sampling periods) as fixed factors and site (two levels) as a random factor were used to analyze data for total invertebrate abundance and species richness, daily average light, maximum light, and temperature. None of these data were normal and were therefor rank transformed. Post-hoc summary comparisons were made on significant results.

Urchin Feeding - Prior to data analysis the average percent change in mass of each seaweed species in control containers was calculated to determine autogenic change of each seaweed species during the experiment (Peterson and Renaud 1989). Average autogenic change was then multiplied to the initial seaweed mass for each species. Total grams eaten of each seaweed was then calculated by subtracting the final seaweed weight from initial weight accounting for autogenic change. Seaweed preference was determined using Manly's alpha index. This type of experiment has been show to be an effective method of determining feeding preference (Dudas et al. 2005). To determine which seaweed the urchins preferred, Manly's α, an index of preference (Manly 1974) was calculated using the following equation (Krebs 2009):

$$\alpha_i = \frac{r_i}{n_i} * \frac{1}{\sum^m (r_j/n_j)}$$

Preference is determined by:

$\alpha_i = 1/m$=no preference for species *i*

$\alpha_i > 1/m$=preference for species *i*

$\alpha_i < 1/m$=avoidance of species *i*

where m = number of prey types available. For the with kelp assay α = 0.2 thus, α -values above 0.2 indicate preference, and less than 0.2 indicate avoidance. For the no kelp assay α = 0.25 thus, α -values above 0.25 indicate preference, and less than 0.25 indicate avoidance of seaweeds.

3.3 Results

3.3.1 Seaweed Communities

There was no interaction between treatment and site for pre-removal seaweed communities and they did not differ between the four treatment types but were statistically significantly different between the two sites (Table 3.1). As sites have statistically significantly different initial communities, all further analyses were conducted on each site separately (Britton-Simmons 2004).

Non-indigenous species removal has differing impacts on the subsequent seaweed species composition (Fig 3.2, Table 3.2) at both sites. Based on the nMDS at both Buccaneer and Mailbox Beaches the control plots were similar in seaweed community composition to the -Sm plots for the post-removal time periods (Fig. 3.2). These observed trends were confirmed by the paired PERMANOVA analyses from the final examination period (November 2014) at Mailbox Beach where control plots were statistically significantly different from –Mj&Sm and the –Mj plots but not –Sm plots (Fig 3.2, Table 3.3). The –Mj&Sm plots were significantly different all other plot types (Fig 3.2, Table 3.3). Additionally, the –Sm plots were significantly different from the –Mj plots (Fig. 3.2, Table 3.3). At Buccaneer Beach, seaweed communities in the final examination period showed the same statistical trends as those at Mailbox Beach where control plots were statistically significantly different from –Mj&Sm and the –Mj plots but not –Sm plots (Fig 3.2, Table 3.4), –Mj&Sm plots were significantly different all other plots types (Fig 3.2, Table 3.4), and –Sm plots were significantly different from the –Mj plots (Fig. 3.2, Table 3.4).

Overall, there were significant interactions between seaweed removal and date for both richness and percent cover of native seaweeds (Fig. 3.3, Table 3.5). There was similar species richness but double the percent cover of native species at Buccaneer Beach as compared to Mailbox Beach (Fig. 3.3). As compared to control plots, post-hoc comparisons showed that -Mj&Sm allowed for more than twice the richness of native species at Mailbox Beach (p=0.001) (Fig 3.3, Table 3.5) and four times the native species richness (p=0.003) at Buccaneer Beach (Fig 3.3, Table 3.5). Control plots were also significantly different than –Mj plots (p=0.0195). Similar trends were seen for percent cover of native species. Native species percent cover was eight times greater for –Mj&Sm plots than control plots at Mailbox Beach and over ten times greater at Buccaneer Beach (Fig 3.3, Table 3.5).

Interaction of *Mazzaella japonica* with *Sargassum muticum*

Percent cover of both *Mazzaella japonica* and *Sargassum muticum* was much higher at the Mailbox Beach site than it was at the Buccaneer beach site throughout the entire experiment. Removal of *S. muticum* had a positive effect on the growth of *M. japonica* at Buccaneer Beach (the fully subtidal site) but not at Mailbox Beach (the site that was exposed to air when the tide was lower than 0.8 m above chart datum) and overall percent cover changed over time (Fig 3.4, Table 3.6). At the eighth re-examination period (November 2014) percent cover of *M. japonica* was not statistically different between the *S. muticum* removal plots and the control plots at either site (Fig. 3.4) based on post-hoc analyses (p>0.150 for both comprisons).

However, removal of *M. japonica* resulted in a significant increase of *S. muticum* at both sites (Table 3.6) but more so at Mailbox Beach where *S. muticum* increased to approximately 40 percent cover in plots compared to only 15 percent cover at Buccaneer Beach (Fig. 3.4). At the final re-examination period in November 2014, *S. muticum* increased four times at the Mailbox Beach and 1.5 times increase in percent cover at Buccaneer Beach as compared to control plots.

3.3.2 Motile Marine Macro-invertebrates

The total abundance of invertebrates and species richness were significantly affected by the seaweed removals but were not affected by time (Fig. 3.5, Table 3.7). For both of these metrics, -Mj&Sm removal resulted in the greatest total abundance and species richness of invertebrates followed by -Mj plots, -Sm and control plots had the lowest total abundance and species richness of invertebrates throughout the experiment. For total invertebrate abundance, –Mj&Sm was significantly different from control (p=0.0035) and –Sm plots (p=0.0463) but not from –Mj plots (p=0.2871) based on post-hoc analyses. The trend was similar for invertebrate species richness as –Mj&Sm plots were significantly different from control (p=0.0024) but not from –Mj or –Sm plots (p>0.05 for both contrasts).

3.3.3 Abiotic Factors

Daily average light and maximum light were significantly affected by removal of the non-indigenous seaweeds (Fig. 3.6, Table 3.8). This was most pronounced in the –Mj&Sm plots (p=0.0368) and –Sm plots (p=0.0055); however, there was also an increase in the amount of light that reached the substratum in the –Mj plots (p=0.0346) as compared to control plots. Control plots received the least amount of daily average and maximum light. There was no significant effect of treatment on temperature on the plots but there was an effect of time (Fig. 3.6, Table 3.8).

3.3.4 Urchin Feeding Preference

Strongylocentrotus droebachiensis had slightly different feeding preferences when the kelp *Saccharina latissima* was either present or absent (Fig. 3.7). When *S. latissima* was available *S. droebachiensis* significantly preferred *Sargassum muticum* over all other seaweeds and avoided *S. latissima*. When the kelp was not available, *S. droebachiensis* preferred both *S. muticum* and *M. japonica* and avoided *Chondracanthus exasperatus* and *Ulva* spp.; however, none of these preferences were statistically significant.

3.4 Discussion

The removal of the non-indigenous seaweeds, *Mazzaella japonica* and *Sargassum muticum*, had direct impacts on native seaweed and invertebrate community structure; however, these effects were dependent on the species removed. Though not directly tested, these results were most likely due to competition for space between *M. japonica* and other species.

3.4.1 Effect of Introduced Seaweed Removals on Native Seaweeds

The pre-removal communities differed between the two sites most likely due to the fact that the transect at Mailbox Beach was in an intertidal site and the experimental communities experienced exposure to air when the tide is lower than 0.8 meters above chart data as compared to the subtidal transect at Buccaneer Beach which was never exposed to air; thus, physiological tolerance could have caused differences in species present at the two sites (Witman and Dayton 2001).

While the initial seaweed communities were different between the two sites, we were more interested if the pattern of impact from the removals were consistent between the two sites.

Removal of the different non-indigenous seaweed species had significant positive impacts on native seaweed communities. This was confirmed by the RM-ANOVAs at both sites for native species percent cover and richness. Unsurprisingly, the -Mj&Sm removal plots had a greater positive impact on the native seaweed communities than the –Mj or -Sm removals as compared to the control plots which could partially be due to an overall greater amount of seaweed being removed from those plots. This suggests that for native seaweed communities to recover, both introduced species would need to be fully eradicated.

One surprising discovery was the lower density of both non-indigenous species at the subtidal site (Buccaneer Beach) as compared to the intertidal site (Mailbox Beach). Both of these species have extensive subtidal beds (Britton-Simmons 2004, K. Pawluk, pers. obs.) and *S. muticum* typically has higher densities in subtidal locals (Britton-Simmons 2004). Non-indigenous species typically have greater impacts when their densities are higher (White and Shurin 2011, Jackson et al. 2015). However, we found the opposite here where there were greater increases in native species richness and percent cover at the subtidal sites of Buccaneer Beach even though density of the non-indigenous species was lower than at the intertidal Mailbox Beach site. This could potentially be due to the increased competitive interactions that typically occur in subtidal area due to the decreased impact of abiotic factors (Witman and Dayton 2001).

3.4.2 Interaction of *Mazzaella japonica* and *Sargassum muticum*

Interestingly, *Mazzaella japonica* appears to have the capacity to outcompete *Sargassum muticum* at these sites suggesting it has a subadditive impact on *S. muticum*. The increase in percent cover of *S. muticum* at both field sites when *M. japonica* was removed suggests that *M. japonica* may be outcompeting the global invader for space. It could also suggest that *M. japonica* is a shade-tolerant species as shading is one of the common ways *S. muticum* impacts communities it has invaded (Britton-Simmons 2004, White and Shurin 2011). This result is surprising as it is theorized that *M. japonica* could have been introduced to all the areas that *S. muticum* has been translocated to as they have the same introduction vector (Saunders 2009). Yet *M. japonica* has, to our knowledge, only been found in British Columbia. This could suggest that either *M. japonica* was only introduced to Baynes Sound, it has particular habitat preferences found only in Baynes Sound, or lower propagule pressure has limited its anthropogenically mediated dispersal in other potential recipient habitats preventing colonization. *S. muticum* is known to have wide physiological tolerances for both salinity and temperature (Norton 1976, Viejo 1997) and is an excellent disperser as it can disperse both through reproductive structures and fragmentation of fronds (Deysher and Norton 1982). Little is known about the physiological tolerances of *M. japonica* or its dispersal capacity, though it is known that red seaweeds generally have poor dispersal abilities due to their heavy, non-motile spores (Norton 1992, Reed et al. 1999). Not knowing *M. japonica*'s dispersal ability or physiological tolerances presents a gap in our understanding of this species as these are two key features of a species when attempting to determine its invasive capacity.

Mazzaella japonica also increased in percent cover as compared to control plots, when *Sargassum muticum* was removed but only at the Buccaneer Beach site. This indicates that, though not as strong, *S. muticum* also has the capacity to inflict negative competitive impacts on *M. japonica* and that removal of *S. muticum* may also facilitate further invasion of *M. japonica* as opposed to having a positive effect on native seaweeds.

3.4.3 Motile Marine Macro-invertebrates

Removal of non-indigenous seaweeds allowed for a greater abundance and richness of motile marine macro-invertebrates especially sea stars and caenogastropods. This is possibly due to an increase in space allowing large sea stars like *Pisaster ochraceus* and *Dermasterias imbricata* to move into the removal plots. It is possible that smaller, cryptic invertebrates such as *Pugettia producta* were not all enumerated in the *Mazzaella japonica* beds as they can often be a similar colour as the *M. japonica* (K. Pawluk, pers. obs.)or could be hiding within the dense fronds. These results were not consistent with other seaweed removal experiments where it was found that removal of either *S. muticum* (Britton-Simmons 2004) or *Undaria pinnitifida* (South et al. 2016), both brown seaweeds, had no impact on invertebrate abundance or richness as compared to control plots. However, the non-indigenous study species in both of these studies grow from stipes off the substratum and would allow for easier movement as compared to the shaggy nature of *M. japonica*.

3.4.4 Abiotic Factors

It was unsurprising that removal of *Sargassum muticum* (-Sm) and both non-indigenous seaweeds (-Mj&Sm) had the greatest effect on the average and maximum amount of light that reached the substratum as *S. muticum* is the dominant canopy forming seaweed in this particular ecosystem. Some *Saccharina latissima* exists but is in very low abundance and generally deeper, around six meters below chart datum (K. Pawluk, pers. obs.). These results are similar to what Britton-Simmons (2004) found. It is unsurprising that there was no difference in temperature between the different plot types as the constant water flow would mitigate any micro-temperature differences due to shading.

3.4.5 Urchin Feeding Preference

Urchins are known to be generalist herbivores (and carnivores and detritivores) and previous research has shown urchin preference for kelp over other seaweeds (Vadas 1977). Interestingly, our study showed the opposite. When kelp was available, *S. droebachiensis* significantly preferred the invasive seaweed *Sargassum muticum* and significantly avoided *Saccharina latissima*. One possible reason that the urchins avoided the kelp could be due to its physical structure. The kelp was the thickest seaweed presented to the urchins (K. Pawluk, pers obs) and small urchins were chosen for this experiment and they may have found the kelp more difficult to eat or perhaps are not cued to eat *S. latissima*. When the kelp was not present, the urchins did not show any significant preferences or avoidances of the four remaining seaweeds. This is in direct opposition to predictions based on the enemy release hypothesis and previous studies which have found that urchins prefer kelp over invasive species such as *S. muticum*, *Codium fragile*, and *Caulerpa racemosa* (Scheibling and Anthony 2001, Monteiro et al. 2009, Cacabelos et al. 2010, Tomas et al. 2011). This preference could indicate that if urchins were able to inhabit areas with *M. japonica* and *S. muticum* they could have potentially acted as native agents of biological control if consumption rates were high enough. This urchin preference not necessarily surprising as Parker and Hay (2005) and Britton-Simmons (2011) found that not all introduced seaweeds experience reprieve from herbivores. While juvenile green urchins have been seen in *M. japonica* beds (K. Pawluk, pers. obs.) they are not abundant members of the invertebrate community. Though urchins eat the two non-indigenous species, this does not mean they are energetically and reproductively beneficial food sources for urchins. Future work should examine if single species diets of the various seaweeds impact rate of urchin test growth and gonad growth to determine the potential impacts of these introduced seaweeds on the food chain.

3.4.6 Implications for management

As *Sargassum muticum* is a well known non-indigenous species in Baynes Sound, local citizen science groups (e.g. Nile Creek Restoration Society) have discussed a removal effort of *S. muticum* to attempt to return the ecosystem to its natural state, hoping for a greater abundance of the formerly common kelp species *Saccharina latissima* (Haegele 1978). Based on the results of this study, it is possible that removal of *S. muticum* could lead to an expansion of *Mazzaella japonica* by further freeing space for it to colonize rather than having the intended positive effect on native seaweeds. Additionally, the actual eradication of *S. muticum* would be challenging, as all traces of the seaweed would need to be removed to ensure that the area is not re-colonized. Previous seaweed removal studies have demonstrated how difficult eradications are unless done early in the invasion process (Myers 2003, Anderson 2007, Klein and Verlaque 2011). Prior to a removal attempt, more studies need to be conducted to determine if there is a threshold of eradication needed to reduce introduced seaweed populations enough to allow recovery of native seaweeds. Studies should also include re-colonization observations after a single introduced species removal (as opposed to continual removal) of a large area to determine dispersal capacity of the two species as part of our understanding of how effective eradication will be.

It is evident from the experiments that overall these two species are having a subadditive impact on each other though *Mazzaella japonica* is having greater negative impact on native algal and invertebrate communities in Baynes Sound as compared to the impacts of *S. muticum* alone. This is likely due to the overall greater competitive abilities of *M. japonica* in the study area due to its greater abundance and its larger, more resilient, encrusting holdfast as compared to those of *S. muticum*. While we now know how *M. japonica* impacts native seaweed communities and *S. muticum* in the study area, we still need to determine the relevant life-history characteristics of *M. japonica* to better understand it competitive abilities.

As globalization continues, habitats are going to be consistently invaded by multiple NIS which have to potential to interact with each other and native species. This is the first study to directly examine how two non-indigenous seaweeds can impact native seaweed communities and interact with each other, showing that though NIS may have subadditive effects on each other, there may still be catastrophic effects on native communities. It highlights the need to broaden the scope of studies examining the impacts and interactions of multiple NIS within a community context.

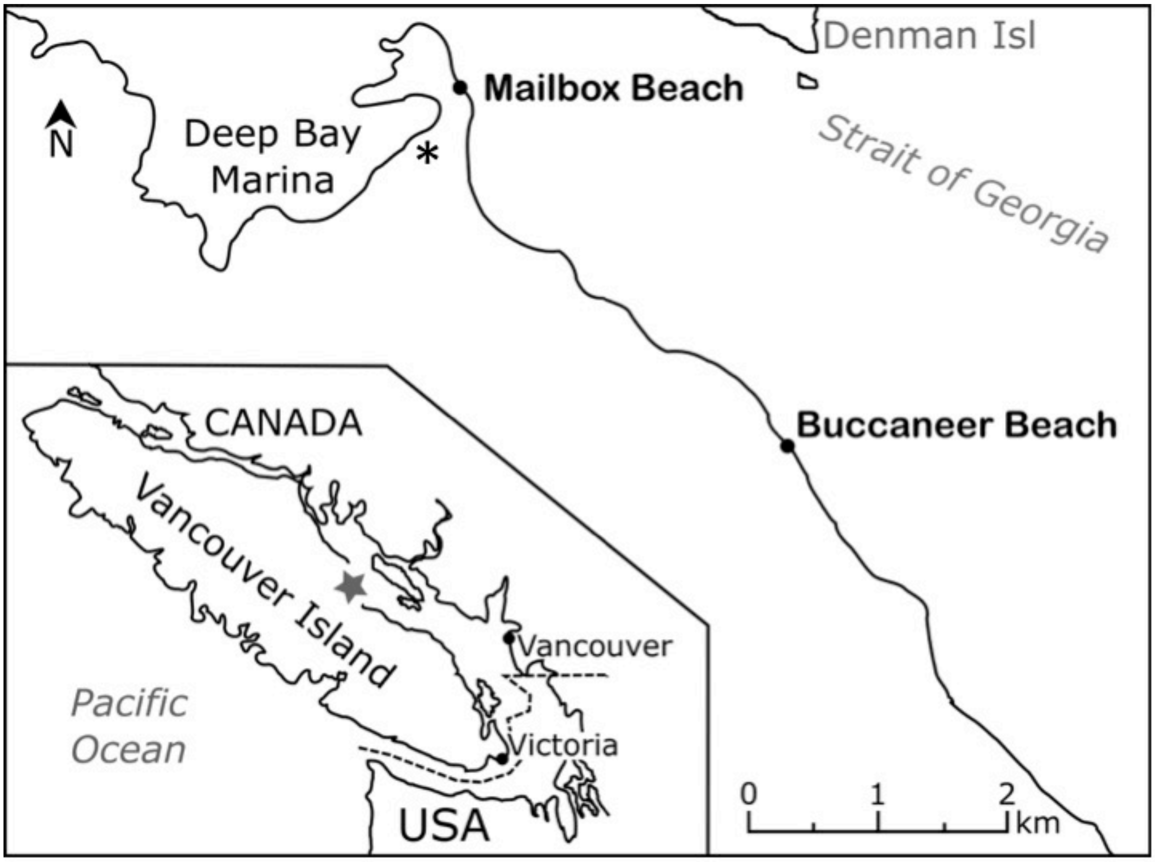

Figure 3.1 Study sites in Baynes Sound, British Columbia. Seaweed removal *in situ* studies were conducted on the two beaches indicated. Urchin feeding studies were conducted at the Deep Bay Marine Field Station (indicated by *).

Table 3.1 Pre-removal comparison of seaweed communities at the two sites for the four treatment types: control, *Mazzaella japonica* removal, *Sargassum muticum* removal, and both *M. japonica* and *S. muticum* removal. Samples taken April 2013, n=5 per treatment per site. Bold values indicate statistical significance.

	df	SS	MS	Pseudo-F	p
Treatment	3	0.129	0.043	0.914	0.463
Site	1	1.231	1.231	26.080	**0.001**
Treatment*Site	3	0.164	0.055	1.157	0.314
Residuals	39	1.510	0.047		

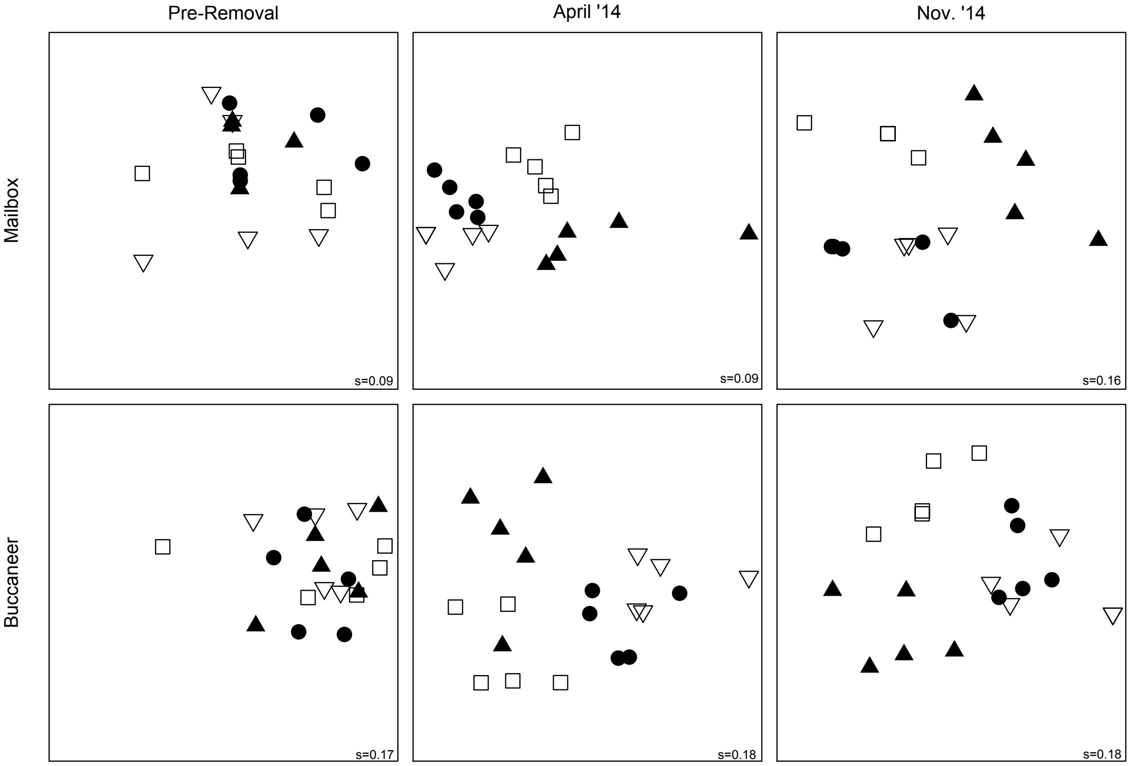

Figure 3.2. nMDS ordination showing temporal changes in seaweed communities from the pre-removal assemblages (April 2013) to the final sampling period (November 2014) at Mailbox Beach and Buccaneer Beach. Filled circles are control plots (●), open squares *Mazzaella japonica* removal (□), open upside-down triangles *Sargassum muticum* removal (▽), and filled triangles both *M. japonica* and *S. muticum* removal (▲). Sample size = 20 for each panel, where fewer than 20 symbols are visible indicates that communities within plots were so similar they overlap. Stress values are indicated for each panel.

Table 3.2 Summary of PERMANOVA results of seaweed communities at chosen time intervals, n = 5 for each treatment at each site per time period. Bold values indicate statistical significance.

	df	SS	MS	Pseudo-F	P
Mailbox April '13 (Pre-removal)					
Treatment	3	0.087	0.029	0.961	0.432
Residuals	16	0.485	0.030		
Mailbox April '14					
Treatment	3	4.646	1.549	15.088	**0.001**
Residuals	16	1.642	0.103		
Mailbox November '14					
Treatment	3	4.599	1.533	17.469	**0.001**
Residuals	16	1.404	0.088		
Buccaneer April '13 (Pre-removal)					
Treatment	3	0.206	0.069	1.070	0.388
Residuals	16	1.024	0.064		
Buccaneer April '14					
Treatment	3	3.221	1.074	8.622	**0.001**
Residuals	16	1.992	0.125		
Buccaneer November '14					
Treatment	3	3.990	1.33	10.217	**0.001**
Residuals	16	2.083	0.130		

Table 3.3 Summary of post- hoc PERMANOVA comparisons of seaweed communities at the final time interval (November 2014), n = 5 for each treatment at Mailbox Beach. Bold values indicate statistical significance, * indicates significance for bonferroni corrected p-values (α = 0.008).

	df	SS	MS	Pseudo-F	P
Control vs. Both Removal					
Treatment	1	1.838	1.838	12.413	**0.012**
Residuals	8	1.185	0.148		
Control vs. *Mazzaella* Removal					
Treatment	1	1.659	1.659	97.162	**0.014**
Residuals	8	0.137	0.017		
Control vs. *Sargassum* Removal					
Treatment	1	0.056	0.056	1.933	0.126
Residuals	8	0.233	0.029		
Both vs. *Mazzaella* Removal					
Treatment	1	1.678	1.678	11.465	**0.009***
Residuals	8	1.171	0.146		
Both vs. *Sargassum* Removal					
Treatment	1	1.680	1.680	10.607	**0.009***
Residuals	8	1.267	0.158		
***Mazzaella* Removal vs *Sargassum* Removal**					
Treatment	1	2.286	2.286	83.326	**0.008***
Residuals	8	0.219	0.027		

Table 3.4 Summary of post- hoc PERMANOVA comparisons of seaweed communities at the final time interval (November 2014), n = 5 for each treatment at Buccaneer Beach. Bold values indicate statistical significance, * indicates significance for bonferroni corrected p-values (α = 0.008).

	df	SS	MS	Pseudo-F	P
Control vs. Both Removal					
Treatment	1	1.577	1.577	9.102	**0.008***
Residuals	8	1.386	0.173		
Control vs. *Mazzaella*					
Treatment	1	1.559	1.559	17.383	**0.011**
Residuals	8	0.717	0.090		
Control vs. *Sargassum* Removal					
Treatment	1	0.019	0.019	0.377	0.768
Residuals	8	0.409	0.051		
Both vs. *Mazzaella* Removal					
Treatment	1	1.166	1.166	5.573	**0.012**
Residuals	8	1.674	0.209		
Both vs. *Sargassum* Removal					
Treatment	1	1.644	1.644	9.630	**0.01**
Residuals	8	1.366	0.171		
***Mazzaella* Removal vs *Sargassum* Removal**					
Treatment	1	2.015	2.015	23.141	**0.012**
Residuals	8	0.696	0.087		

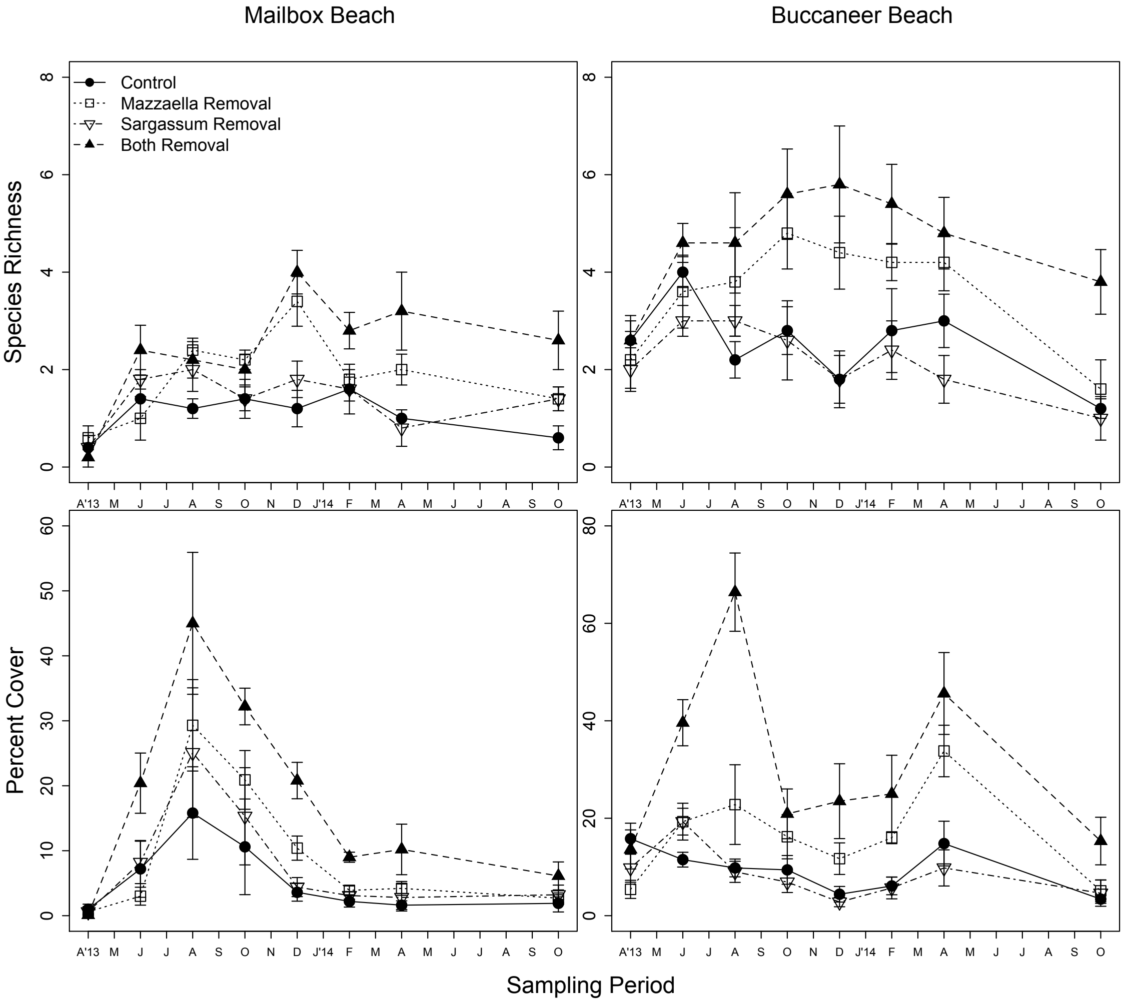

Figure 3.3 Native species richness and percent cover (mean ±SE) in control, *Mazzaella japonica* removal, *Sargassum muticum* removal, and both removal plots (n = 5 per point) at Mailbox Beach and Buccaneer Beach. The first data point (April 2013) in each series is a pre-removal sample.

Table 3.5. Effect of removal of *Mazzaella japonica* and *Sargassum muticum* on native seaweed richness and native seaweed percent cover. Results of RM-ANOVA, n = 5 per treatment for each of the eight time periods. Bold values indicate statistical significance.

	df	F-value	p
Native Species Richness			
Mailbox Beach			
Treatment	3	20.5900	**<0.001**
Date	7	11.866	**<0.001**
Treatment*Date	21	2.3595	**0.0019**
Buccaneer Beach			
Treatment	3	24.371	**<0.001**
Date	7	6.520	**<0.001**
Treatment*Date	21	1.268	0.2101
Native Percent Cover			
Mailbox Beach			
Treatment	3	11.316	**<0.001**
Date	7	22.027	**<0.001**
Treatment*Date	21	2.008	0.1413
Buccaneer Beach			
Treatment	3	35.791	**<0.001**
Date	7	11.498	**<0.001**
Treatment*Date	21	2.204	0.2791

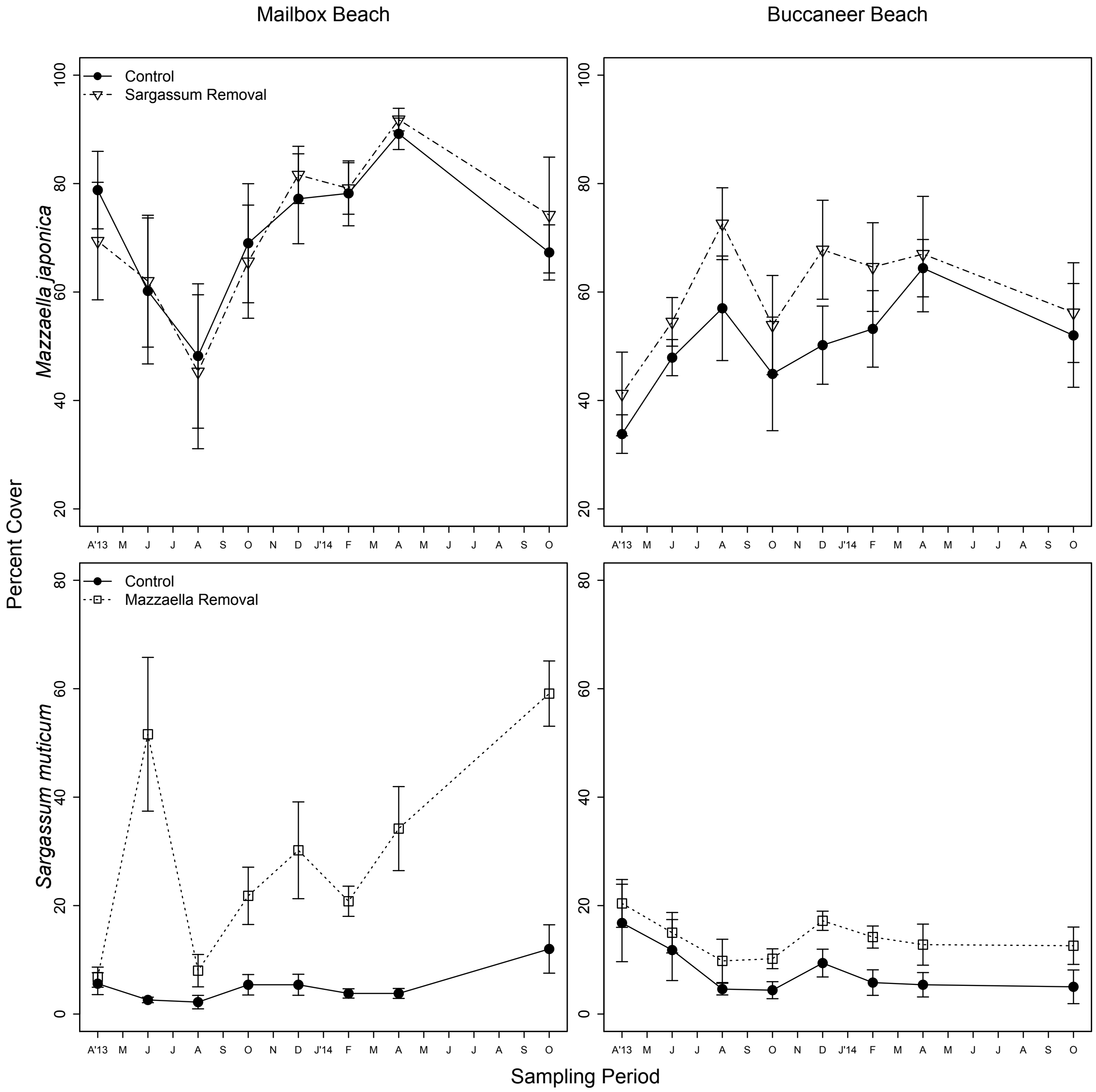

Figure 3.4 The effect of removing either *Mazzaella japonica* or *Sargassum muticum* on the percent cover (mean ±SE) of the remaining introduced seaweed at Mailbox Beach and Buccaneer Beach as compared to control plots (n = 5 per point). The initial point (April 2013) is for pre-removal data.

Table 3.6. Effect of removal of *Mazzaella japonica* or *Sargassum muticum* on the remaining non-indigenous seaweed as compared to control plots. Results of RM-ANOVA, n = 5 per treatment for each of the eight time periods. Bold values indicate statistical significance.

	df	F-value	p
S. muticum **percent cover after** ***M. japonica*** **removal**			
Mailbox Beach			
Treatment	1	6.079	**0.017**
Date	7	3.532	**0.003**
Treatment*Date	7	0.558	0.787
Buccaneer Beach			
Treatment	1	15.603	**<0.001**
Date	7	3.162	**0.007**
Treatment*Date	7	0.205	0.983
M. japonica **percent cover after** ***S. muticum*** **removal**			
Mailbox Beach			
Treatment	1	0.001	0.976
Date	7	6.247	**<0.001**
Treatment*Date	7	0.239	0.974
Buccaneer Beach			
Treatment	1	7.564	**0.008**
Date	7	3.714	**0.002**
Treatment*Date	7	0.304	0.949

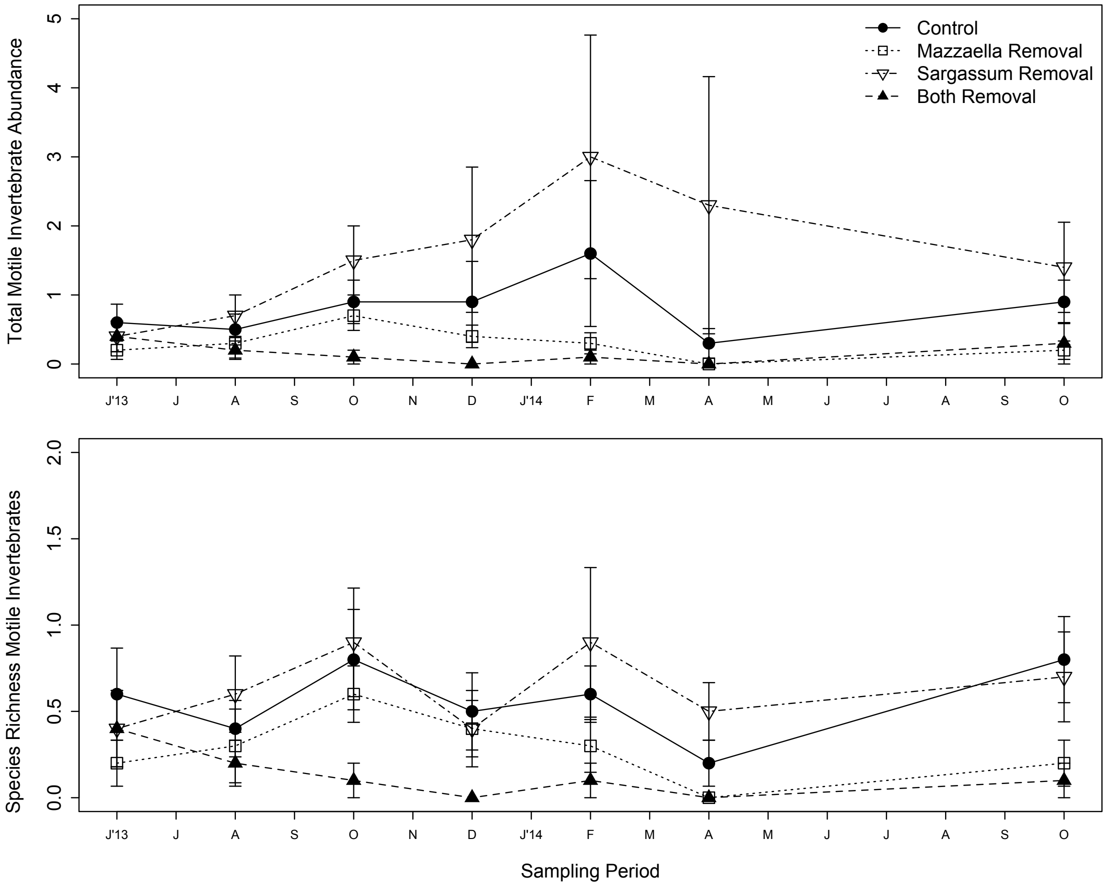

Figure 3.5 Total number (mean ± SE) and species richness (mean ± SE) of motile invertebrates in control, *Mazzaella japonica* removal, *Sargassum muticum* removal, and both seaweed removal plots (n = 10 per point) at the two sites combined. The first data point (June 2013) is from the first time period after seaweed removal.

Table 3.7 Effect of removal of *Mazzaella japonica* and *Sargassum muticum* on richness and total individuals of marine macro-invertebrates. Results of RM-ANOVA, n = 10 per treatment for each of the seven time periods after the initial implementation. Bold values indicate statistical significance.

	df	F-value	p
Total number of motile invertebrate individuals			
Treatment	3	7.657	**0.0015**
Date	6	0.848	0.5111
Treatment*Date	18	0.629	0.8552
Richness of motile invertebrates			
Treatment	3	9.509	**<0.001**
Date	6	4.595	0.149
Treatment*Date	18	0.727	0.782

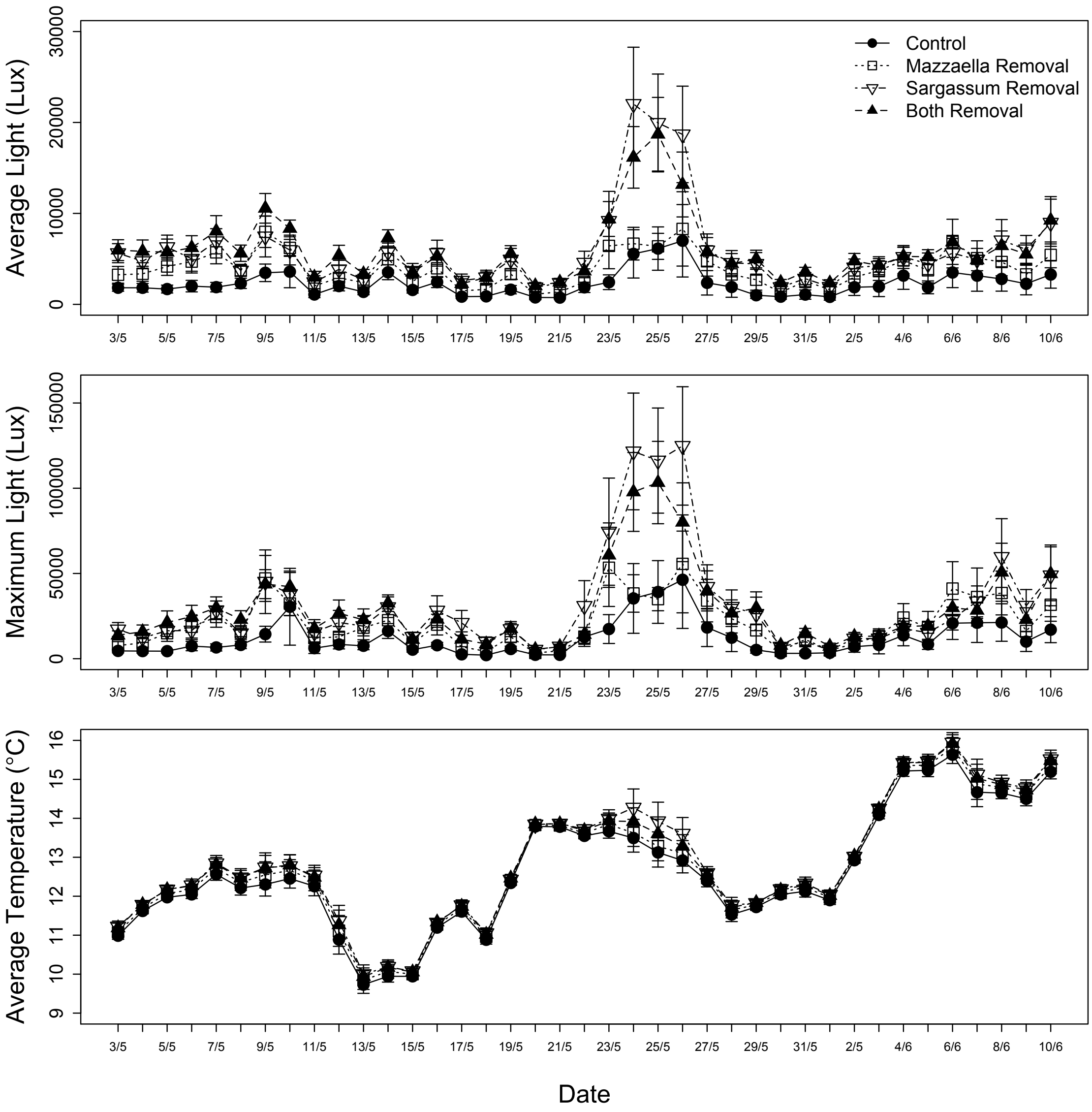

Figure 3.6 Light (average lux ±SE), light (maximum lux ±SE), and temperature (average °C ±SE) per day from May 3 to June 10, 2013 for control (n = 9), *Mazzaella japonica* removal (n = 9), *Sargassum muticum* removal (n = 9), and both seaweed removal (n = 10) plots. Both sites are combined. Note differences in y-axes.

Table 3.8. Effect of *Mazzaella japonica* and *Sargassum muticum* on abiotic factors in plots including average and maximum light (lux) and average temperature (°C) per day. Results of RM-ANOVA, n = 10 for both seaweeds removal and n = 9 for all other treatments for each of the 39 time periods. Bold values indicate statistical significance.

	df	F-value	p
Average Light			
Treatment	3	127.672	**0.0186**
Date	38	13.733	**<0.001**
Treatment*Date	114	0.408	0.9521
Maximum Light			
Treatment	3	94.988	**0.0311**
Date	38	18.545	**<0.001**
Treatment*Date	114	0.490	0.9335
Average Temperature			
Treatment	3	12.908	0.7429
Date	38	537.987	**<0.001**
Treatment*Date	114	0.308	1

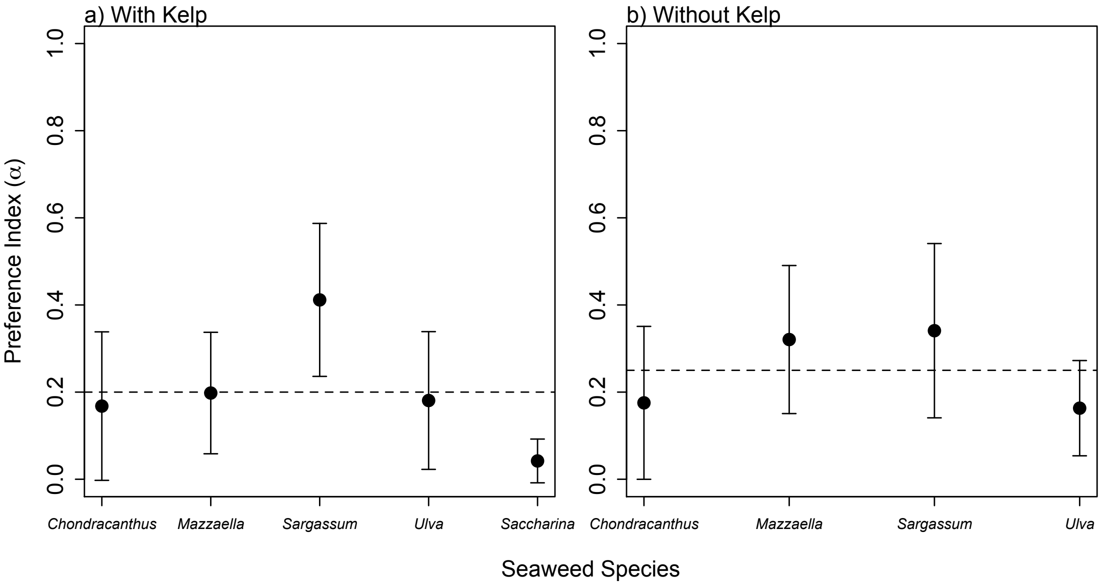

Figure 3.7 *Strongylocentrotus droebachiensis* feeding preference for the dominant seaweeds a) with the kelp *Saccharina latissima* or b) without *S. latissima*. Dashed line denotes value of zero preference (a)α = 0.2, b) α = 0.25), values above denote prey preference, values below, avoidance. Bars represent 95% CI, where CI does not overlap the dashed line preference or avoidance is significant, n = 10 per feeding assay.

Chapter 4 - Native and introduced seaweeds show variable rates of breakdown as beach-cast wrack in Baynes Sound, British Columbia

Submitted to Marine Ecology Progress Series – Currently in Review

4.1 Abstract:

Allocthonous seaweeds that wash up on beaches are important ephemeral spatial subsidies for the semi-terrestrial invertebrates and microorganisms that inhabit these otherwise nutrient-limited coastal habitats. In turn, these semi-terrestrial organisms play a role in recycling the nutrients trapped in the macroalgae. Invasive seaweeds growing in intertidal and subtidal ecosystems can affect the nutrient cycling if there is a shift in species washing up as wrack. To determine how two invasive seaweeds present in Baynes Sound could be influencing terrestrial organisms, we completed a three-part study to determine: the dominant wrack species, the seaweed preferred by herbivores, and the decomposition and decay rates of those seaweeds. Least preferred by detritivores, though constituting >89% of the wrack composition, was the introduced red seaweed *Mazzaella japonica*. The invasive seaweed *Sargassum muticum* was the preferred seaweed by amphipod herbivores and had one of the highest rates of decomposition. *Chondracanthus exasperatus* had the highest rate of *in situ* decay and decomposition suggesting that the influence of native red seaweeds may have been previously overlooked in seaweed spatial subsidy experiments. We demonstrate that different introduced seaweed species play unequal roles in the provisioning of nutrients to beach ecosystems.

4.2 Introduction:

The coastal interface, where the oceans and the land interact, comprises approximately eight percent of the earth's surface (Ray and Hayden 1992) and experiences a dynamic, bi-directional flow of nutrients and organic content between these seemingly separate ecosystems (Burke et al. 2001). To date, the majority of our wealth of knowledge of how marine nutrients impact terrestrial systems comes from our understanding of the seasonal migration of marine fishes from coastal environments to nearshore marine and terrestrial ecosystems (Hocking and Reynolds 2011, Fox et al. 2014). Receiving far less attention, is the role that marine seaweeds or phytodetritis play as a 'spatial subsidy' (Polis et al. 1997).

Seaweeds growing in intertidal and subtidal zones are typically considered nutrient sinks as they sequester large quantities of nutrients (Hanisak 1993); however, when not fully grazed, seaweeds are eventually detached from benthic substrates. These detached seaweeds are either washed onto coastal beaches (Kirkman and Kendrick 1997, Colombini and Chelazzi 2003) or are exported offshore (Kirkman and Kendrick 1997, Orr et al. 2005). In temperate zones, the quantity of wrack typically increases during winter months after the natural senescence of the plants or after strong storm systems (Ochieng and Erftemeijer 1999, Barreiro et al. 2011). Once deposited on beaches, seaweeds breakdown and organic content and sequestered nutrients are released (Dugan et al. 2011). These seaweeds are essential for supporting beach-dwelling invertebrate communities (Rossi and Underwood 2002, Crawley et al. 2009).

Wrack, as a spatial subsidy, provides both habitat and food sources for beach dwelling invertebrates (Bustamante et al. 1995, Dugan et al. 2003, Rodil and Lastra 2004, Rodil et al. 2008, Rossi et al. 2010). The role that wrack plays in the ecosystem is largely dependent on the species composition of the wrack, the amount that is deposited, and its spatial distribution (Orr et al. 2005, Rodil et al. 2008). Though thought to be opportunistic feeders (Porri et al. 2011), several studies have shown that detritivorous amphipods show strong seaweed preference based on species specific properties (Pennings et al. 2000, Mews et al. 2006, Duarte et al. 2010, Poore and Gallagher 2013). Microbial decay of seaweed has also been shown to be species specific (Mews et al. 2006). Thus, the species composition of wrack is important to the way in which sequestered nutrients enter subsidized coastal systems.

Introductions of non-indigenous marine seaweeds have been shown to alter the near-shore communities of native seaweeds through competition and space monopolization (Schaffelke and Hewitt 2007, Davidson et al. 2015), which can result in an alteration of wrack species composition (Newton et al. 2013). This shift in wrack species composition may have bottom up effects on beach communities by either acting as a new food source or by reducing an important food source depending on whether native herbivores and detritivores recognize and subsequently feed on these introduced seaweeds. Native herbivores typically prefer species that have evolved in the ecosystem with them (Cacabelos et al. 2010, Tomas et al. 2011) and non-indigenous species are thought be reprieved from being fed on as per the ‘enemy release hypothesis’ (Elton 1958). While it has been demonstrated that beach-dwelling organisms use both native and introduced wrack species, they do so differently (Rodil et al. 2008). To our knowledge, there has been no research to determine how rates of decay and decomposition vary between native and invasive seaweeds on beaches and ultimate use of this resource.

Wrack lines along beaches within Baynes Sound, BC, are a regular occurrence throughout the year (K. Pawluk, pers. obs.). Baynes Sound also hosts several species of introduced seaweeds, most notable are the global invader *Sargassum muticum* and a novel introduced species *Mazzaella japonica* (Saunders 2009). Both species were thought to be transported to British Columbia from North East Asia via the oyster aquaculture industry (Saunders 2009) and therefore could have arrived as early as 1902. As both species are observed in the standing wrack lines, we aimed to determine if and how these seaweeds were being assimilated back into the ecosystem and whether this rate differed from the native seaweeds in the ecosystem.

Using a series of observational studies and experiments, we sought to understand what seaweeds could be potentially acting as a spatial subsidy on beaches in Baynes Sound, BC and if and how the sequestered nutrients in the seaweeds were being returned to the coastal ecosystem. More specifically, we sought to determine: (i) the species composition of wrack washing up on beaches in Baynes Sound; (ii) how the dominant species comprising the wrack lines breakdown over time; and (iii) the feeding preference of the most abundant beach detritivores. We predicted from previous field observations that *Mazzaella japonica* would be the dominant species found in the wrack line at all sites. We further predicted that the amphipods would prefer native seaweeds over the introduced seaweeds, *Sargassum muticum* and *M. japonica,* and that the processes of decay (microbial action only) and decomposition (decay with the addition of herbivory) would be greatest for native seaweed species.

4.3 Materials and Methods:

The study site was on the southern edge of Baynes Sound on eastern Vancouver Island, British Columbia (Fig 4.1). Field sites were selected because they are within the known range of the two introduced seaweeds *Mazzaella japonica* and *Sargassum muticum*. The four sites chosen for the wrack composition study all face northeast and are long, flat stretches of either sand or cobble and are all known to receive large quantities of wrack input during winter months (Kingzett et al. unpublished data). Sites chosen for the decay and decomposition were a subset of the wrack composition sites.

4.3.1 Wrack composition:

Standing load of wrack was sampled after a winter storm on December 3, 2013 from four beaches within Baynes Sound: RV park, Shoreline Drive, Oceanside Drive, and Buccaneer Beach (Fig. 4.1). Five replicate wrack samples were collected from each beach by removing a 20cm x 20cm randomly selected section from wrack patches along the beaches where both *Mazzaella japonica* and *Sargassum muticum* grow (total n = 20). Samples were returned to the lab and frozen before being thawed and sorted. Samples were sorted to species level where possible or lowest identifiable taxon when species could not be determined. Terrestrial inputs (e.g. leaves, twigs, beach grass) were grouped together as their input was relatively low. All wrack species were then dried in a 60°C oven until they reached a constant weight. Plant materials were then weighed to the nearest 0.01 g.

4.3.2 Amphipod feeding assays:

Simultaneous feeding preference assays were conducted on the five dominant seaweed species as determined from the wrack composition study. The five dominant species were chosen as those with the greatest percent composition at all sites (see results and Fig. 4.2): *Mazzaella japonica, Sargassum muticum*, *Chondracanthus exasperatus*, *Fucus* spp*.,* and, *Ulva* spp. Though there was a greater biomass of *Saccharina latissima* than *S. muticum,* it was not included in this component of the study as it was found at only two of the four sites and in two out of the 20 samples. The two native amphipod genera, *Megalorchestia californiana* and *Traskorchestia spp.,* were used as they are common detritivores in the ecosystem. Ten replicate feeding preference experiments were completed for *Traskorchestia* spp. and six replicates for *M. califoriana* as well as five control replicates to control for autogenic change during the feeding trial. Samples of each seaweed were spun in a salad spinner for 20 seconds, weighed, and placed in a container. Ten individuals of each amphipod genera were added to appropriate containers and left to feed for 72 hours. Remaining seaweeds were removed from the containers, rinsed with freshwater to remove any debris or feces, spun in a salad spinner for 20 seconds, and reweighed.

Percent change in mass of each seaweed species in control containers was calculated and applied to each individual in treatment containers to account for autogenic changes in the seaweed over time (Peterson and Renaud 1989).

4.3.3 Decay and decomposition:

The five dominant seaweed wrack inputs were also used for the decay and decomposition experiment. Living samples of *Mazzaella japonica, Sargassum muticum*, *Chondracanthus exasperatus*, *Fucus* spp*.,* and *Ulva* spp. were collected from the site at low tide on July 10, 2014 to complete an *in situ* decomposition and decay experiment. Two treatments were employed, consisting of Nitex mesh and plastic mesh bags of either 355µm or 10mm openings; all bags were 100 mm^2. The 355µm litter bags allowed exposure to microbial agents, excluding the dominant herbivorous and detritivorous invertebrates, to determine the amount of decay over time whereas the 10mm litter bags accounted both for decay and allowed herbivores access to the seaweed, thus, allowing for a decomposition rate to be calculated. Plants were held in seawater and taken back to the lab where they were shaken of excess water, spun in a salad spinner for 20 seconds, weighed to the nearest 0.01 g, and then sealed in the appropriately sized mesh bag. Five replicates of each seaweeds species per mesh size were deployed at three sites (n = 5 for each seaweed species, per mesh size, per site). On July 11, 2014 each sample was re-wetted with salt water, buried under the freshest wrack line and secured in place by attaching mesh bags to rebar pounded into the ground. Mesh bags were left for approximately 72 hours and then collected. After collection the bags of seaweed were returned to the lab and frozen until they could be processed. Samples were then rinsed with freshwater to remove any extraneous particles (e.g. sand, gravel, other organic materials), and dried at 60°C in a drying oven until they reached a constant weight and were re-weighed. As this experiment was conducted in the summer, the seaweed samples were desiccated as well as consumed in the field. To account for the desiccation, a wet to dry mass ratio constant was calculated. Fresh samples of each species of wrack seaweed being tested were collected and were processed in the same manner as the samples deployed in the field (spun of excess water and weighed) and were then immediately placed into a drying oven at 60°C until a constant weight was reached. This was replicated seven times for each species and an average constant value was calculated. This ratio was used as a standardization for the initial seaweed samples to be comparable to being deployed in the field. After drying at 60°C to a constant weight, ash-free dry weight was determined by combusting blades at 500°C for three hours to determine percent organic content.

4.3.4 Data Processing and Statistical Analyses:

R software was used for all data analyses and production of all figures (R Core Team 2015).

Wrack composition

Species composition was determined by calculating the percentage of each species relative to the total sample of seaweed dry weight. Seaweeds not determined to be one of the five most abundant wrack species as well as seagrasses and terrestrial inputs were grouped for graphical purposes as they were minor components of the wrack.

Amphipod Feeding Assays

Average autogenic change was calculated for each seaweed species and then applied to the initial seaweed mass by multiplying initial seaweed mass by mean percent autogenic change. Total grams consumed of each seaweed was then calculated. As these data were normally distributed, amount consumed was analyzed using a two-way ANOVA comparing seaweed species and amphipod genus then followed with a post-hoc Tukey HSD test. To determine which seaweed species the amphipods preferred, Manly's α was used as an index of preference (Manly 1974). This index has been used by several other studies to determine feeding preferences (Pattinson et al. 2003, Dudas et al. 2005). Manly's α using the following equation:

$$\alpha_i = \frac{r_i}{n_i} * \frac{1}{\sum^m (r_j/n_j)}$$

Preference is determined by:

$\alpha_i = 1/m$=no preference for species *i*

$\alpha_i > 1/m$=preference for species i

$\alpha_i < 1/m$=avoidance of species i

where m = number of prey types available. For the five species of seaweeds being tested $\alpha = 0.2$ thus, α -values above 0.2 indicate preference, and less than 0.2 indicate avoidance.

Decay and decomposition

Initial dry weight of each sample was calculated by multiplying each initial wet weight by the constant determined from the wet to dry mass ratio prior to being used for data analyses. Data were not normal and could not be transformed thus a separate Kruskall Wallis test was performed for decay and decomposition. Post-hoc Wilcoxon Rank Sum tests were conducted with bonferroni adjustments. Mixed effects models were run separately for each seaweed species to compare the percent organic content from the ash-free dry weights in the decay and the decomposition samples with mesh size as the fixed factor and site as the random factor.

4.4 Results

4.4.1 Wrack Composition

The majority of the seaweed composing the wrack lines was *Mazzaella japonica* (minimum 89% per site) as it was found in all samples at all sites (Fig. 4.2). *Fucus* spp., *Chondracanthus exasperatus*, *Ulva* spp., and *Sargassum muticum* were found at all sites, though not in all samples at Shoreline drive, and constituted the largest proportions of the remaining wrack (from most abundant to least abundant) (Fig. 4.2, Table 4.1). These five species were then used for the decay and decomposition study as well as the amphipod feeding assays. Close in proportion to *S. muticum* was the kelp *Saccharina latissima* and while overall it constituted a greater proportion by dry weight than *S. muticum*, it was only found at two sites and only in four of the twenty samples. Buccaneer Beach and Ocean Trail had the greatest number of total taxa (Table 4.1), followed closely by the RV park site. Shoreline drive had the greatest percent of *M. japonica* but the fewest number of other taxa (Table 4.1). Shoreline drive had the greatest biomass of combined wrack during the sampling period followed by Ocean Trail and Buccaneer and Shoreline (Table 4.1).

4.4.2 Amphipod Feeding Assays

The amount of seaweed eaten was significantly different between the various species of seaweed, but the genus of amphipod did not affect the amount eaten nor was there an interaction between the species of seaweed and the species of amphipod (Table 4.1). Both genera of amphipods preferred *Sargassum muticum* over all other species offered (Fig. 4.3). *Chondracanthus exasperatus* and *Mazzaella japonica* were significantly avoided (Fig. 4.3) but *Fucus* spp. and *Ulva* spp. were neither preferred nor avoided.

4.4.3 Decay and Decomposition

Overall, no more than 60 percent decay (Fig. 4.4) and 70 percent decomposition (Fig. 4.5) of total mass was noted for any of the seaweed species tested over the course of the experiment. There were statistically significant differences in amount of decay (Kruskal Wallis, df=4, X^2=41.321, $p<0.0001$) and decomposition (Kruskal Wallis, df=4, X^2=20.807, $p=0.0003$). For both mechanisms of seaweed breakdown, *Chondracanthus exasperatus* showed the greatest decrease in mass followed by *Sargassum muticum* (Fig. 4.4 and 4.5). The amount of decay and decomposition between these two was not statistically significantly different for either breakdown process (Fig. 4.4 and 4.5).

Mazzaella japonica underwent statistically significantly less breakdown than *C. exasperatus* and *S. muticum*, but around the same amount as *Ulva* spp. for both processes. *Fucus* spp. was least affected by both decomposition and decay. Overall, the addition of herbivory to the process of decay (decomposition method of seaweed breakdown) had little additional effect on the breakdown of any of the seaweed species.

Chondracanthus exasperatus showed the greatest percentage of organic content and *Fucus* spp. the least (Table 4.3); however, there were no significant differences in the percentage of organic content between decay (microbial action only) and decomposition (microbial action and herbivory) for any of the five seaweed species examined (Table 4.4).

4.5 Discussion

Our findings reveal the dominance of an introduced seaweed species in the wrack on beaches in Baynes Sound and confirm that there are species-specific decay and decomposition rates, a finding which corroborates previous studies (Orr et al. 2005, Mews et al. 2006). These results also support our hypothesis that wrack species composition affects the rate of nutrient supply into the coastal ecotone.

Mazzaella japonica is the dominant seaweed present in the wrack and may; therefore, have the greatest potential spatial subsidy for semi-terrestrial organisms inhabiting the beaches adjacent to existing subtidal *M. japonica* beds. However, *M. japonica* is not preferred by the dominant detritivores nor quickly processed by microbes as evidenced by its intermediate rates of both decay and decomposition. While eventually, *M. japonica* must decay or decompose it does not appear as though these processes occur readily on beaches in Baynes Sound. These data support the enemy release hypothesis and partially support our own hypotheses regarding nutrient release from seaweed. The seaweed in greatest abundance in the coastal ecotone has one of the slower rates of return into the terrestrial system. Additionally, decay and decomposition studies were conducted in the summer when metabolic rates of both invertebrates and microbes would be highest due to warm temperatures as compared to winter months when the majority of the wrack would accumulate on the beaches. Based on the multiple lines of evidence, we can conclude that though *M. japonica* is least preferred by multiple amphipod species and has an intermediate rate of decay and decomposition is likely still an important spatial subsidy to the Baynes Sound coastal ecosystem due to the overwhelming dominance of the species in the wrack.

Sargassum muticum was the least abundant of the five dominant wrack species but was the only seaweed that was significantly preferred by the amphipods and decayed and decomposed at a greater rate than all of the other species other than *Chondracanthus exasperatus.* These results do not support our hypothesis as we predicted that there would be less herbivory due to the 'enemy release hypothesis' and previous studies (Britton-Simmons 2004, Cacabelos et al. 2010); however, *Sargassum* sp. in its native range have been shown to be a preferred food source by herbivores (Poore and Gallagher 2013). Additionally, though it supports different communities of macroinvertebrates, *S. muticum*, is an important wrack component (Rossi et al. 2010) and is preferred by some herbivorous snails (Britton-Simmons et al. 2011) even in its invasive range. Combined, these results indicate that *S. muticum* populations in Baynes Sound are not released from their terrestrial predators. Our results from the amphipod feeding assays and the decay and decomposition study demonstrate the relative importance of *S. muticum* as a spatial subsidy.

It is evident that these two invasive species play different roles in the ecosystem. *Sargassum muticum* was the most preferred species by detritivores but also had one of the fastest rates of decay as opposed to *Mazzaella japonica* which was least preferred by the amphipods and decayed slower than *S. muticum* and a native red algal species. Nutrients from *S. muticum* are readily reincorporated into the ecosystem providing a spatial subsidy while the greater proportion of nutrients in *M. japonica* remain sequestered within the seaweed for longer periods of time. Therefore, it is important to address species-specific impacts and to determine the effects that invasive seaweeds have in an ecosystem.

Interestingly, though significantly avoided by the amphipods during the feeding assays, *Chondracanthus exasperatus* had the highest rate of decay and decomposition. While we expected that amphipods would not prefer *C. exasperatus* (Pennings et al. 2000, Mews et al. 2006); to our knowledge, there is no previous research to indicate how native red seaweeds breakdown on sandy beaches. Our findings suggest that native red seaweeds such as *C. exasperatus* may be a more important spatial subsidy than previously thought due to its high rate of decay and inferred microbial preference. This also brings to light the concept that native red seaweeds may play a greater role as a spatial subsidy in coastal ecosystems than has been previously addressed in the literature.

Ulva spp. was the only seaweed to breakdown at a different rate between the decay and decomposition study (Fig. 4.4, 4.5). The relative importance of herbivory on *Ulva* spp. was unexpected as it was not a preferred species of seaweed in the amphipod feeding assays. However, previous studies have found varying results with regard to invertebrate preference of *Ulva* spp. Pennings et al. (2000) found *Ulva* spp. to have variable levels of preference between different wrack invertebrates with *Traskorchestia* spp. not preferring it when compared to native kelps. Similar to our study, Mews et al. (2006) found that *Ulva* spp. was readily consumed when used in field decomposition studies but not in laboratory-based feeding assays. The low rate of decay of *Ulva* spp. could be due to high levels of phytotoxins known for a similar species *U. intestinalis* which have antimicrobial properties (Rizvi and Shameel 2005). How *Ulva* spp. is incorporated into coast beach dynamics needs further investigation. *Fucus* spp. also had relatively low rates of decay and decomposition which corroborates Mews et al. (2006) who also found a low mass loss in field studies and also supports the finding of Pennings et al. (2000) who found that *Fucus* spp. was not preferred by herbivores. It is possible that the high level of phenols in *Fucus* spp. could be negatively impacting its rate of decomposition and decay (Targett et al. 1992).

The relatively low impact of the addition of herbivory in the decomposition bags as compared to the decay-only mesh bags was surprising. Mews et al. (2006) conducted a similar study on the west coast of Vancouver Island and found the addition of herbivory to the decay treatment to have a far greater impact on the rate of seaweed breakdown. However, this pattern has not held it other locations as seagrasses studied in Poland were also not greatly affected by the addition of herbivory (Jedrzejczak 2002). There are two potential reasons for this reduced effect of herbivory. First, it is possible that the decay packages (355 µm) allowed microbes in but also possibly trapped these microbes; thus, artificially increasing the rate of decay as compared to the decomposition packages. Second, herbivory, and therefore decomposition is not the main driver of seaweed breakdown in Baynes Sound which, instead, is mostly influenced by microbial action. This reduced level of herbivory could help explain some of the differing patterns between those found in the amphipod feeding assays and the decomposition packages. Overall, the rate of seaweed breakdown seen in Baynes Sound on the east coast of Vancouver Island is far less that observed rates of breakdown on the west coast of Vancouver Island (Mews et al. 2006) suggesting that either agents of seaweed nutrient recycling are less prevalent in the environment or seaweed washes up in greater quantities swamping the system.

From our results, it is evident that species composition of wrack is important for determining the rates of decay and decomposition of seaweeds. Not all introduced species are re-incorporated into the ecosystem at the same rate highlighting the need to conduct species-specific experiments when attempting to determine the role that introduced seaweeds play in their host environment. Still yet to be determined is the rate of decay and decomposition of seaweeds during winter months when these organisms are at peak abundance in the coastal ecotone. The potential spatial subsidy that native red seaweeds represent for an ecosystem may have thus far been overlooked in other studies and also warrant further attention.

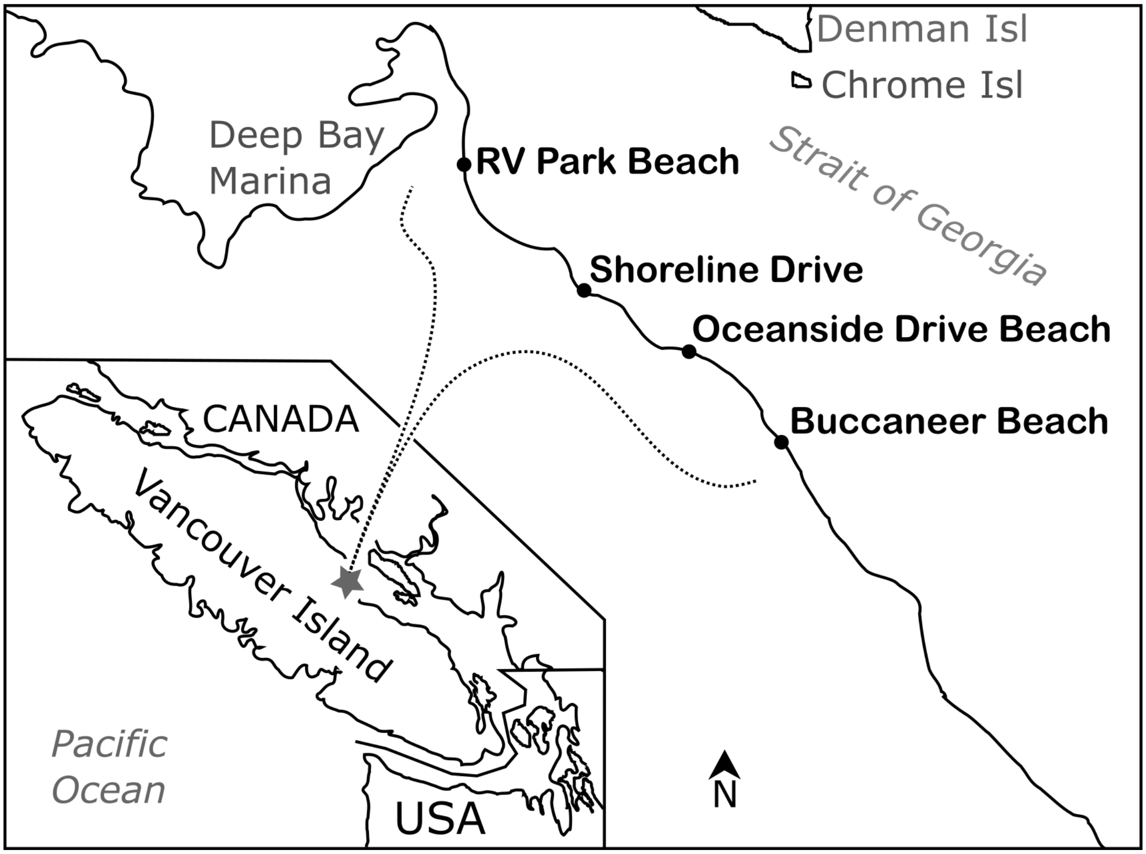

Figure 4.1. Study sites in Baynes Sound, British Columbia. All sites were sampled during the wrack composition study. All sites but Shoreline Drive were part of the *in situ* decay and decomposition study.

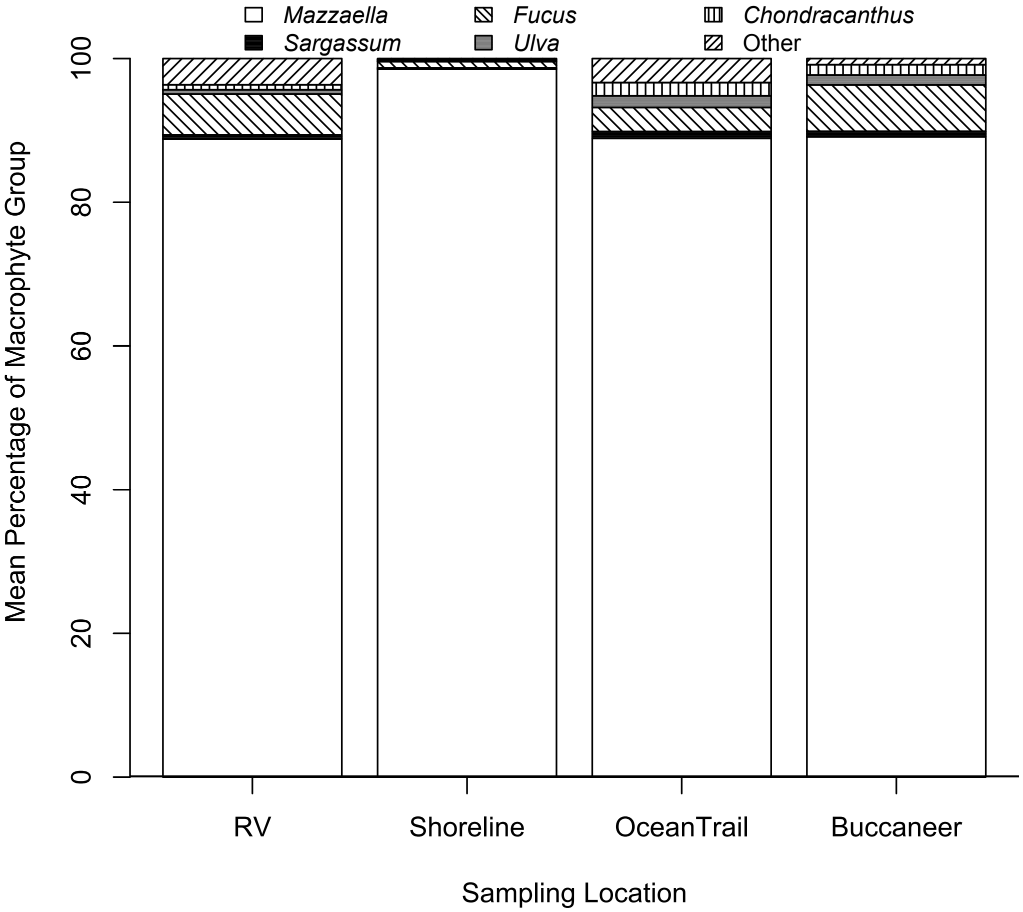

Figure 4.2. Species composition of standing wrack load at beaches in the study site in Baynes Sound in December 2013. Data are mean percentages of five samples within a 400 cm^2 plot. Other, includes all other species found including several species of red seaweeds, *Saccharina latissima*, seagrass, and terrestrial inputs. Sites are listed from North to South.

Table 4.1. Mean dry weight in grams (±1SE) of the five dominant wrack species and all other groups as well as the total average gramps per site (bold) collected from five replicate 400cm^2 quadrats sampled from four sites in Baynes Sound, British Columbia. Ave. species is the average number of distinguishable species per quadrat and max. species is the maximum number of distinguishable species found in a quadrat per site. *Mazzaella japonica* and *Sargassum muticum* are two of the introduced seaweeds inhabiting Baynes Sound, *Fucus* spp., *Ulva* spp., and *Chondracanthus exasperatus* are native seaweeds. Sites are listed from north to south.

	RV Beach	Shoreline Drive	Ocean Trail	Buccaneer Beach
Mazzaella	76.15 (6.27)	154.73 (7.80)	83.73 (13.52)	74.95 (4.86)
Sargassum	0.50 (0.19)	0.25 (0.21)	0.90 (0.17)	0.65 (0.18)
Fucus	4.88 (1.33)	1.37 (0.56)	3.15 (0.99)	5.42 (1.70)
Ulva	0.51 (0.19)	0.19 (0.12)	1.51 (0.43)	1.16 (0.23)
Chondracanthus	0.60 (0.25)	0.33 (0.26)	1.74 (0.49)	1.21 (0.71)
Other	3.14 (1.03)	0.17 (0.08)	3.16 (1.37)	0.73 (0.14)
Total grams	**85.77 (7.13)**	**157.94 (8.42)**	**94.18 (13.49)**	**84.12 (4.48)**
Avg. Species	12.17	7.33	13.00	12.00
Max. Species	16	11	17	17

Table 4.2. Summary of the two-way analysis of variance for the amount of dominant wrack seaweed (*Chondracanthus exasperatus*, *Fucus* spp., *Mazzaella japonica*, *Sargassum muticum*, *Ulva* spp.) eaten by the two terrestrial herbivores (*Megalorchestia californiana* n=6 and *Traskorchestia* spp. n=10) and for the interaction between seaweed and amphipod species. Bold values indicate statistical significance.

	df	SS	MS	F	p
Seaweed	4	0.259	0.065	10.185	**>0.001**
Amphipod	1	0.011	0.011	1.678	0.199
S X A	4	0.010	0.002	0.373	0.827
Residuals	70	0.445	0.006		

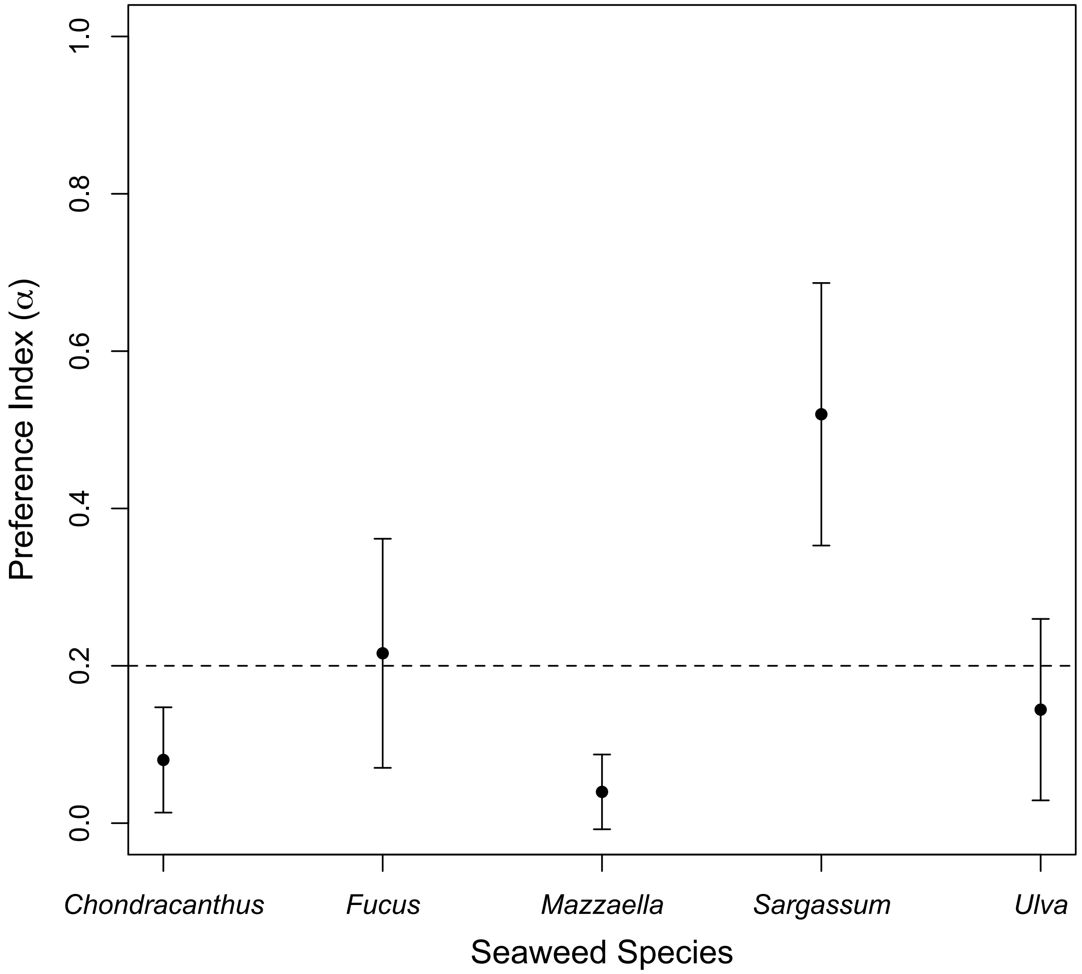

Figure 4.3. Amphipod feeding preference of the dominant wrack component species. Dashed line denotes value of zero preference (0.2), values above denote prey preference, values below, avoidance. Bars represent 95% CI. Where CI does not overlap the line preference or avoidance is significant, n=16.

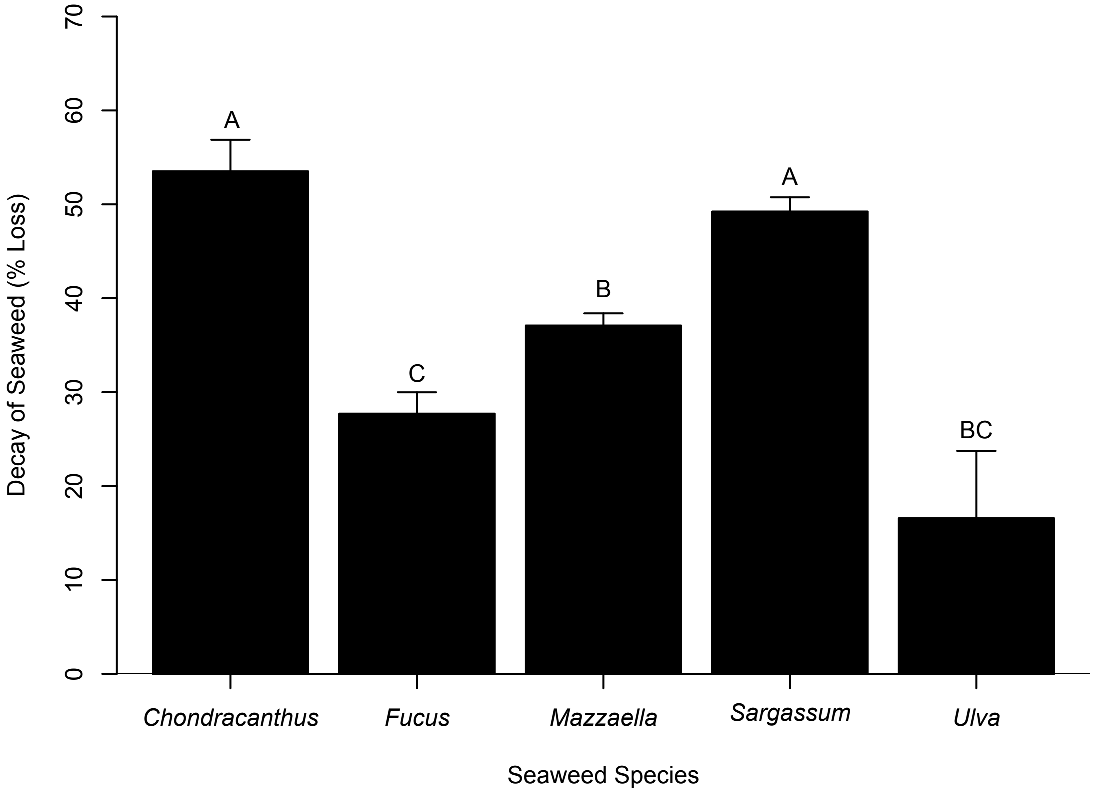

Figure 4.4. Mean percent decay of the five seaweed species in standing wrack lines over 72 hours in July 2014. Bars represent standard error. Letters are significantly different from one another (Wilcoxon Rank Sum Tests with bonferroni adjustment p<0.05).

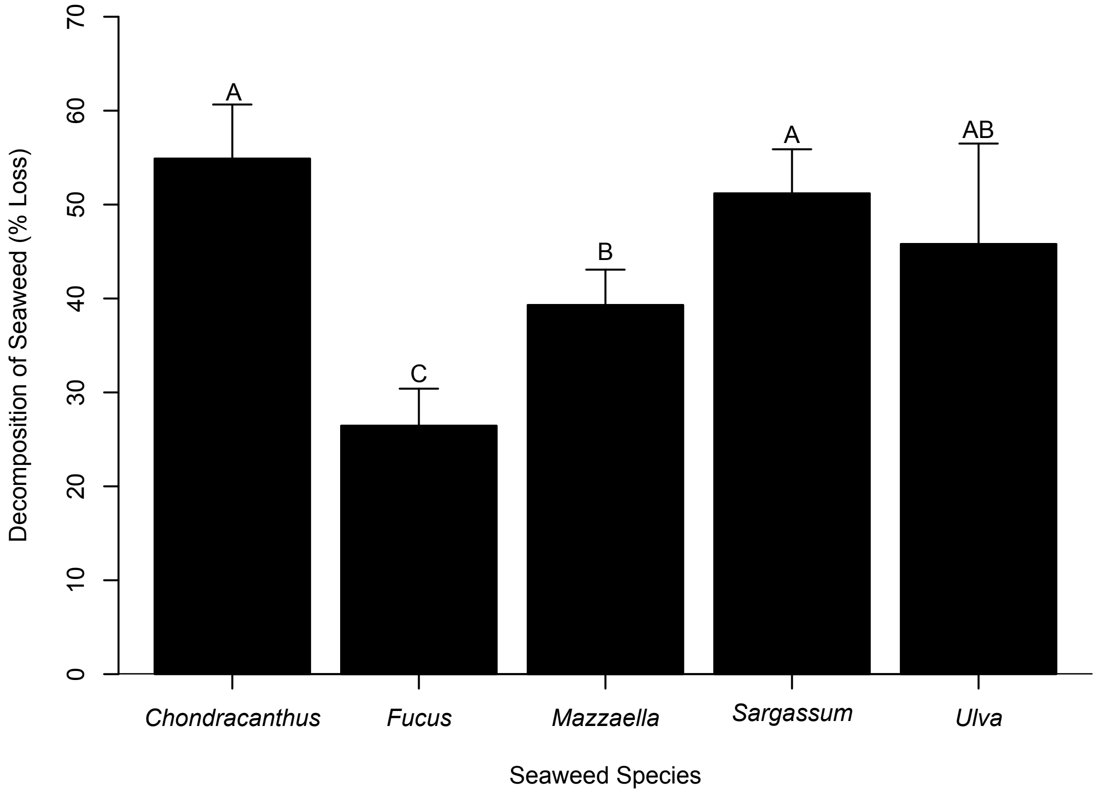

Figure 4.5. Mean percent decomposition of the five seaweed species in standing wrack lines over 72 hours in July 2014. Bars represent standard error. Letters are significantly different from one another (Wilcoxon Rank Sum Tests with bonferroni adjustment $p<0.05$).

Table 4.3. Summary data for the percent organic content after the different methods of seaweed breakdown and each standard error (SE). Decay is microbial action (355 µm mesh), decomposition is the combination of microbial action and herbivory (10mm mesh). N=15 for each process per seaweed species.

Seaweed	**Breakdown Process**	**Mean Percent Organic Content**	**SE**
Sargassum	Decay	74.54	1.87
	Decomposition	69.56	1.92
Mazzaella	Decay	71.22	1.72
	Decomposition	71.26	1.46
Fucus	Decay	68.98	1.80
	Decomposition	68.48	2.19
Ulva	Decay	70.92	1.96
	Decomposition	74.15	1.69
Chondracanthus	Decay	74.89	2.22
	Decomposition	78.14	2.58

Table 4.4. Summary of the mixed effects models comparing the percentage organic content for *Chondracanthus exasperatus*, *Fucus* spp., *Mazzaella japonica*, *Sargassum muticum*, and *Ulva* spp. between the two methods of seaweed breakdown decay (microbial action only) and decomposition (decay and herbivory), n=15 for each process per seaweed species.

Seaweed	Value	df	t-value	p-value
Chondracanthus	3.246178	26	1.514549	0.142
Fucus	-0.503194	26	-0.228903	0.8207
Mazzaella	0.072231	26	0.021227	0.9832
Sargassum	-4.983089	26	-1.867108	0.0732
Ulva	3.228299	26	1.228927	0.2301

Chapter 5 – Variable influence of multiple non-indigenous seaweeds on beach invertebrates communities.

5.1 Introduction

Beaches around the world receive significant inputs of marine algae and seagrasses which have been dislodged from neighbouring algae beds that grow on rocky shorelines or in soft sediment (Inglis 1989, Jedrzejczak 2002, Rossi and Underwood 2002, Jiménez et al. 2015b). Influxes of marine plants occur throughout the year (Dugan et al. 2003, Barreiro et al. 2011); however, in temperate systems their quantity typically increases in winter months (Ochieng and Erftemeijer 1999, Barreiro et al. 2011) due to the natural senescence of plants and increases in winter storms. These seaweeds act as 'spatial subsidies' (sensu Polis et al. 1997) from the marine environment to the nutrient poor, sandy beaches. The subsidizing role of this beach-cast phytodetritus (hereafter called "wrack") has been reported in the literature (see Colombini and Chelazzi 2003 and references therein). Despite the influence of kelps on macrofaunal assemblages (Inglis 1989, Mews et al. 2006, Olabarria et al. 2010) there have been few studies done on the macrofaunal responses to other macroalgae or changes in macroalgal composition.

Wrack is used both as a habitat and a food source for invertebrates such as amphipods, isopods, and herbivorous and carnivorous beetles (Bustamante et al. 1995, Colombini et al. 2000, McLachlan and Brown 2006, Rodil et al. 2008, Crawley et al. 2009). How wrack impacts beach communities depends on: the amount of seaweed deposited on the beach, the species composition of the wrack, its spatial distribution, and the length of time it has remained on the beaches (Griffiths and Stentondozey 1981, Inglis 1989, Orr et al. 2005, Rodil et al. 2008, Pelletier et al. 2011). The physical complexity (Hicks 1980) and nutritional quality (Pennings et al. 2000) of the various seaweed species can be factors that influence which macrofauna will colonize the seaweeds and these qualities can change over time as seaweeds decay on the beach (Rodil et al. 2008).

With the spread of non-indigenous species around the world, wrack spatial subsidies are being modified. Studies have found that introduced seaweeds in the subtidal range affect the species composition of wrack washing up on nearby beaches (Jiménez et al. 2015a) which could have consequences for macrofaunal communities. Despite the ubiquity of wrack on beaches, little attention has been paid to how non-indigenous algal species are altering these spatial subsidies. Pennings et al. (2000) found that supralittoral invertebrates such as amphipods and isopods showed feeding preferences for various seaweed species which indicate different seaweed species may be colonized differently. To our knowledge, the only study comparing the impact of native and non-indigenous seaweed on supralittoral communities found that S*argassum muticum* hosted a different community of invertebrates than the native algae *Saccorhiza polyschides* (Rodil et al. 2008).

Baynes Sound on the east coast of Vancouver Island is inundated with several tons of wrack during winter months (Kingzett et al. unpublished data). It consists primarily (~90%) of the non-indigenous seaweed *Mazzaella japonica* but also contains large quantities of *Fucus* spp. and the other invasive seaweed *Sargassum muticum* (Chapter 4). These two seaweeds were thought to have been introduced to Vancouver Island as hitchhikers on oysters which were brought from Japan (Scagel 1956, Saunders 2009) and both have established populations in the area. In addition to the disturbance of multiple introduced species, a harvest of *M. japonica* has been occurring on beaches during winter months. Harvesting has been suggested as a potential management strategy for invasive species (Villanueva et al. 2010); however, it is essential to understand if a non-indigenous seaweed has become naturalized and is a functioning member of the system before its removal should be considered. Thus, the need to understand how these seaweeds are colonized by macrofauna is two fold to determine if not having evolved in the ecosystem affects faunal community composition and to provide relevant information for policy makers.

We used artificial wrack patches comprised of either the non-indigenous seaweeds *Mazzaella japonica* and *Sargassum muticum* or the native species *Fucus* spp. to answer the question of whether seaweed species composition impacts macrofaunal colonization over a three-week period. We tested the hypotheses that the 1) species composition of the wrack would impact invertebrate community structure, 2) two sites would be colonized differently, 3) invertebrate abundance and diversity would change over time as the seaweed decayed, 4) dominant semi-terrestrial herbivores would have preference for particular seaweeds, and 5) percent organic content as a metric for seaweed quality would impact the total abundance of colonizing invertebrates. We predicted seaweed species identity would allow for increased colonization of invertebrates (Rodil et al. 2008), and there would be a difference between the two sites due to their differing abiotic characteristics. Additionally, we predicted organisms such as amphipods would colonize wrack quickly and other organisms such as flies and beetles would appear later in succession (Inglis 1989, Dugan et al. 2003, Pelletier et al. 2011) and talitrid amphipods would prefer feeding on the native seaweed (Pennings et al. 2000).

Finally, we predicted organic content would influence total invertebrate abundance (Rodil et al. 2008).

5.2 Methods

5.2.1 Study area

We completed a colonization study at two beach sites approximately 3 km apart: RV Park Beach (49°27’39 N, 124°43’16 W) and Buccaneer Beach (49°26’42 N, 124°40’51 W) in Baynes Sound, Vancouver Island, British Columbia (Fig. 5.1). These sites were chosen as there is a significant wash up of beach-cast wrack (Chapter 4) and *Mazzaella japonica* seaweed harvesting is currently being conducted at both sites. Both sites face North East, but there are some differences; the RV Park Beach is sandy whereas Buccaneer Beach is a mixture of both sand and small cobbles.

5.2.2 Field Experimental Design

Artificial patches of wrack were established in the high intertidal, above the highest tide level predicted for the duration of the experiment and were comprised of single seaweed species treatments to determine colonization preference. The seaweeds used were two non-indigenous seaweeds, *Mazzaella japonica* and *Sargassum muticum,* and a native species, *Fucus* spp. The experiment began on 21 November, 2013 and lasted for 21 days with a total of five sampling periods. Live seaweeds were collected by hand via SCUBA from nearby rocky shores on the morning the experiment began and divided into two-liter patches to create the artificial patches. For the five time periods, three replicate patches at each site (n = 3 per time period per seaweed species for a total of 45 patches per site) were placed along an 80 meter transect in a randomized block design. Patches were placed at least one meter apart and invertebrates were allowed to colonize for 2,4,7,14, and 21 days. On the appropriate collection day each replicate patch of seaweed was sprayed with insecticide to kill any flying invertebrates such as flies and beetles. After allowing patches to sit for five minutes, all seaweed and any visible fauna were placed into a dilute formalin solution to remove invertebrates from the seaweed. Remaining fluid was strained through a 1 mm mesh filter to collect the invertebrates which were then fixed in a 7% formalin solution. One piece of seaweed from each sample was rinsed in freshwater and returned to the lab where it was frozen until it could be processed. During sampling, both air and wrack patch temperatures were taken. All samples were collected except for four samples at RV Park Beach on day 21 which had been washed away due to high tide and storm surge leaving *M. japonica* = 2, *S. muticum* = 2, *Fucus* spp. = 1 for that particular date.

5.2.3 Laboratory Analyses

Invertebrates were sorted, identified to the lowest possible taxonomic level, and counted. Seaweed pieces retained from the field were dried in a 60°C oven until they reached a constant weight after which they were placed in a muffle oven at 500°C for three hours to determine the seaweed organic content.

5.2.4 Feeding Preference Assay

Feeding preference of the dominant semi-terrestrial invertebrates inhabiting the wrack was determined for the three seaweed species included. The day before the feeding assay began, fresh samples of seaweeds were collected as were 100 *Traskorchestia* spp. (Amphipoda: Talitridae). The day the experiment began approximately one gram of each seaweed was spun in a salad spinner for 20 seconds to determine seaweed wet weight. Ten amphipods were placed into each treatment container fitted with mesh windows to allow for airflow. The ten treatment containers were placed outside in a sheltered area to experience natural temperature and light regimes and the amphipods were allowed to feed on the three species of seaweed for one week. Five control containers were treated equivalently to the treatment containers except they did not receive amphipods to determine autogenic change. The amphipod feeding assay was conducted from December 12-19, 2013. At the end of the seven-day feeding period, amphipods were removed from the containers, seaweeds were rinsed of any debris, spun for 20 seconds and then reweighed; control containers were treated the same.

5.2.5 Statistical Analyses

All data were analyzed using the R statistical software (R Core Team 2015). Due to the loss of the four samples from the RV Park site, only four sampling periods were analyzed statistically from that site. Community composition over time was compared using repeated measures permutation analysis of variance (PERMANOVA) for seaweed species, day, site, and all of the potential interactions (Anderson 2001, Anderson and Walsh 2013). To better understand the impacts of the three seaweed species overtime, we conducted individual PERMANOVA analyses for each site and each time period. All PERMANOVA were run using the vegan package (Okansen et al. 2016) with a Bray-Curtis distance matrix and 999 permutations. Data were visualized using non-metric multidimensional scaling (nMDS), a Bray-Curtis distance matrix and 1000 maximum starts. Total animal abundance and species richness were examined for each site using a repeated measures analysis of variance (RM-ANOVA) including seaweed species (3 levels) and time (4 time periods for RV Park and 5 time periods for Buccaneer Beach) as fixed factors and block (3 levels) as a random factor followed by post-hoc comparisons for significant results. Buccaneer Beach total abundance was log transformed but richness was normally distributed following Shapiro-Wilks test for normality. Species richness at the RV Park Beach was also normally distributed but abundance was log transformed to meet normality.

The four dominant members of the invertebrate community were also examined using RM-ANOVA with seaweed species (3 levels) and time (4 time periods for RV Park and 5 time periods for Buccaneer Beach) as fixed factors and block (3 levels) as a random factor for each site separately and appropriate post-hoc tests were done for any significant results. For RV Park Beach talitrid amphipods and anthomyiid fly data were log (x+1) transformed, and oligochaete and mite data were rank transformed (Conover and Iman 1981) to meet assumptions of normality. At Buccaneer Beach, amphipod and fly data were log (x+1) transformed, oligochaete data were rank transformed, and mite data were square root transformed.

Amphipod feeding data were analyzed using Manly's α index of feeding preference (Manly 1974). Average percent change in mass of each seaweed species in control containers was calculated to determine autogenic change during the experiment (Peterson and Renaud 1989). Average percent autogenic change was then multiplied to all initial seaweed masses. Total grams eaten of each seaweed was then calculated using the initial values accounted for autogenic change. To determine amphipod seaweed preference, the feeding preference index was calculated using the following equation (Krebs 2009):

$$\alpha_i = \frac{r_i}{n_i} * \frac{1}{\sum^m (r_j/n_j)}$$

Preference is determined by:

$\alpha_i = 1/m$=no preference for species i

$\alpha_i > 1/m$=preference for species *i*

$\alpha_i < 1/m$=avoidance of species *i*

where m = the number of seaweeds presented to the amphipods and is thus three for this assay. Alpha = 0.333, therefore values calculated above 0.333 indicate preference, below indicate avoidance. 95% confidence intervals were calculated. Where these bars do not overlap, the alpha value indicates statistically significant preferences.

Seaweed organic content data were analyzed using RM-ANOVA for the two sites and separately for each seaweed species. Time (4 levels at RV Park and 5 levels at Buccaneer) was the fixed factor and block (3 levels) was a random factor. Percent organic content was also correlated with total individuals to determine if there was a relationship between organic content and invertebrate colonization.

5.3 Results

A total of 22 101 invertebrates belonging to 29 species were collected and identified from the samples. Anthomyiid flies, halacarid mites, *Traskorchestia* spp., and microdrile oligochaetes were the numerically dominant organisms found in the samples accounting for 24, 23, 21, and 17 percent of the total individuals, respectively (Table 1). These four groups accounted for over 85% of the total abundance of invertebrates. Other colonizers included collembolans, several beetle species, fly pupae, and nematodes. *Sargassum muticum* was colonized by the most individuals (51% of total invertebrates) followed by *Mazzaella japonica* (32%) and lastly *Fucus* spp. (17%) (Table 5.1).

Colonization differed for the three species over time at the two different sites in a three-way interaction (Table 5.2). Further analyses showed that the effect of the different seaweed species was more pronounced at Buccaneer Beach than at the RV Park beach

(Table 5.3) and this was also evidenced in the nMDS visualizations of the data (Fig 5.2). There was approximately an order of magnitude more individuals at the RV Park Beach than at Buccaneer Beach (Fig 5.3). At both sites, numbers of individuals significantly increased over time (Fig 5.3, Table 5.4) and were significantly different between the seaweed species at Buccaneer Beach; *Sargassum muticum* was colonized by significantly more invertebrates than the other two seaweeds ($p>0.03$). Though not statistically significant, the trend was the same at the RV Park Beach. At both sites, there was a two-way interaction for seaweed species and date influencing species richness with a general increase in richness over time (Fig 5.3, Table 5.4).

Seaweed species and time both significantly affected the abundance of amphipods at each site (Fig 5.4a and b, Table 5.5 and 5.6). At RV Park Beach *Fucus* sp. was colonized the quickest but by day 14, *Sargassum muticum* and *Mazzaella japonica* had statistically more species ($p=0.042$). This trend was not the same at Buccaneer Beach. While seaweed species was significant ($p=0.047$, Table 5.6), *S. muticum* was colonized the fastest (Fig 5.4b) and had significantly more invertebrates than *M. japonica* ($p=0.039$) but not *Fucus* spp. ($p=0.34$). There was also a significant effect of time on anthomyiid flies at both sites. There were 200 times the number of flies on all seaweed species on day 14 as compared to day 7 at the RV Park Beach ($p<0.005$ for all comparisons, Fig 5.4c) and 10 times more flies on day 21 as compared to day 14 at Buccaneer Beach ($p<0.03$ for all comparisons, Fig 5.3d). Halacarid mites at the RV Park Beach were not significantly impacted by seaweed species but were significantly higher on day 14 ($p<0.05$ for all comparisons) (Fig 5.4e). At Buccaneer Beach significantly more mites colonized *S. muticum* than ether *M. japonica* ($p=0.002$) and *Fucus* ($p=0.0001$) (Fig 5.4f).

Amphipods significantly preferred feeding on *Sargassum muticum* over the other two species and significantly avoided *Fucus* spp. (Fig 5.5). For all seaweed species, percent organic content decreased over the course of the study (Fig 5.6). There was no obvious effect of percent organic content on total abundance of individuals at either site (Fig 5.7). In all cases the temperature of the wrack patches was the same as the air temperature which was between 1ºC and 3ºC at RV Park Beach and -1ºC and 2ºC at Buccaneer Beach.

5.4 Discussion

Overall, colonization of wrack in Baynes Sound by invertebrates was greater than what similar studies have found in Spain (Olabarria et al. 2007, Rodil et al. 2008), but species richness was similar to other studies in multiple locations (Jedrzejczak 2002, Dugan et al. 2003, Rodil et al. 2008). The dominant species that colonized the wrack patches in this study were anthomyiid flies followed by halacarid mites, and talitrid amphipods which is consistent with other studies (Griffiths and Stentondozey 1981, Inglis 1989, Colombini et al. 2000, Rodil et al. 2012), though other studies have found significantly more tenebrionid and staphlynid beetles (Rodil et al. 2008). This lack of beetles could be due to the level of urban development near the beaches.

The two sites had significantly different community compositions, RV Park Beach had over an order of magnitude more individuals than Buccaneer Beach most likely due to the difference in the habitat between the two sites. RV Park Beach is a fully sand beach but Buccaneer Beach is a is a combination sand and gravel beach; additionally, Buccaneer Beach always experienced lower air temperatures than RV Park and often had frost on the ground during sampling (K. Pawluk, pers obs). Seaweed species also impacted the total number of individuals which was greatest for *Sargassum muticum* patches followed by *Mazzaella japonica* and lastly by *Fucus* spp; the pattern was most evident on days 14 (at RV Park Beach and Buccaneer Beach) and 21 (at Buccaneer Beach) of the study. This is in contrast with Rodil et al. (2008) who found greater colonization on the native seaweed *Saccorhiza polyschides* than the introduced *S. muticum*. The depressed colonization on *Fucus* spp. could be due to the high levels of phenols contained in the seaweed which decrease both its palatability and rate of decay (Targett et al. 1992). Previous studies have shown that beach-dwelling herbivores generally do not prefer *Fucus* spp. when other brown seaweeds are available (Pennings et al. 2000).

The pattern of succession found in this study is different than previously found in the literature where colonization occurred fastest in the first week of colonization (Olabarria et al. 2007, Rodil et al. 2008); however, Ford et al. (1999) found that colonization took longer during winter months than summer months (when most researchers conduct colonization studies). There was a significant increase in species richness colonizing the artificial patches over time; though, seaweed species did not influence this. Again, this result was different from what other studies had found previously where seaweed species impacted colonization rate (Olabarria et al. 2007, Rodil et al. 2008).

All dominant invertebrate abundance increased throughout the course of the experiment except for talitrid amphipods at Buccaneer Beach. Adult flies do not use wrack as a significant food source (Rodil et al. 2008) but as a habitat to lay their eggs (Griffiths and Stentondozey 1981). Though fly abundance increased at both sites during the final sampling periods (day 14 at RV Park Beach and day 21 at Buccaneer Beach) there were few fly larvae found. This could suggest that flies had not yet had time to deposit eggs on the seaweeds. Though time was important for fly colonization, there was no effect of seaweed species which may indicate that anthomyiid flies are a generalist species and lay eggs on any available algal substratum. This conjecture, however, is in opposition to the study by Rodil et al. (2008) who found that flies significantly preferred native seaweeds over invasive seaweeds. While there is no obvious explanation for this difference, it could be due to the nutritional differences between the different seaweed species utilized in the two studies.

Amphipods showed varying colonization of the seaweeds over time. At the RV Park Beach there were significantly more amphipods colonizing the two introduced seaweeds later in time, but at Buccaneer Beach there was no consistent pattern of preference. Other studies have shown that amphipod colonization occurs quickly (Pelletier et al. 2011) with maximum abundance occurring within the first week wrack is available (Inglis 1989, Olabarria et al. 2007). At both sites amphipods showed a preference for colonizing and feeding on *Sargassum muticum.* Amphipod feeding preference for feeding on *S. muticum* is not surprising as these results were supported by a feeding study done in Chapter 4 as well as a stable isotope study which found that *S. muticum* represented a large portion of the isotope signature of talitrid amphipods (Rossi et al. 2010). The combined results of the colonization study and the feeding assay suggest that *S. muticum* is used both as a habitat and a food source by amphipods. Oligochaete and mite abundance increased later in the colonization process, a finding consistent with other studies (Inglis 1989). This is unsurprising as oligochaetes are known to feed on microbes (Giere and Pfannkuche 1982) which increase in abundance on seaweeds as they decay.

Organic content is one metric of seaweed quality and though there was overall a decrease in percent organic content over time it did not correlate with invertebrate abundance. Further examination of the seaweed characteristics is necessary to better predict what factors influence colonization such as seaweed structure (Hicks 1980, Hooper and Davenport 2006), chlorophyll a, protein, and carbohydrate content which have been shown to influence colonization (Rodil et al. 2008). It would also be interesting to compare colonization in the winter months when the majority of the wrack is washing up on beaches as compared to summer months when the subsidy is limited and invertebrate abundance is higher due to increased metabolic rates and decreased generation time.

This study indicates that wrack species identity affects the abundance of colonizing invertebrates supporting previous research which showed invasive and native wrack species are not used equally. Surprisingly, the two non-indigenous seaweeds had the highest rates of colonization; though, *Sargassum muticum* was most preferred as a habitat and as a food source suggesting even though it is not be the most abundant wrack component (Chapter 4) that it is an important nutrient source. Determining the way the invertebrates are impacted is important as these species are prey for large invertebrates and vertebrates, thus, having repercussions on upper trophic levels (Spiller et al. 2010). These results have management implications which would suggest that though both introduced seaweeds act as habitats and food sources, *S. muticum* has a greater impact on beach-dwelling invertebrate communities. Further experiments need to determine if there is a threshold amount of seaweed necessary on beaches to ensure sufficient nutrient flow to microbes and invertebrates and the remained of the ecosystem to ensure that if harvest is to continue, the ecosystem functions as it did prior to the introduction of *S. muticum* and *M. japonica*.

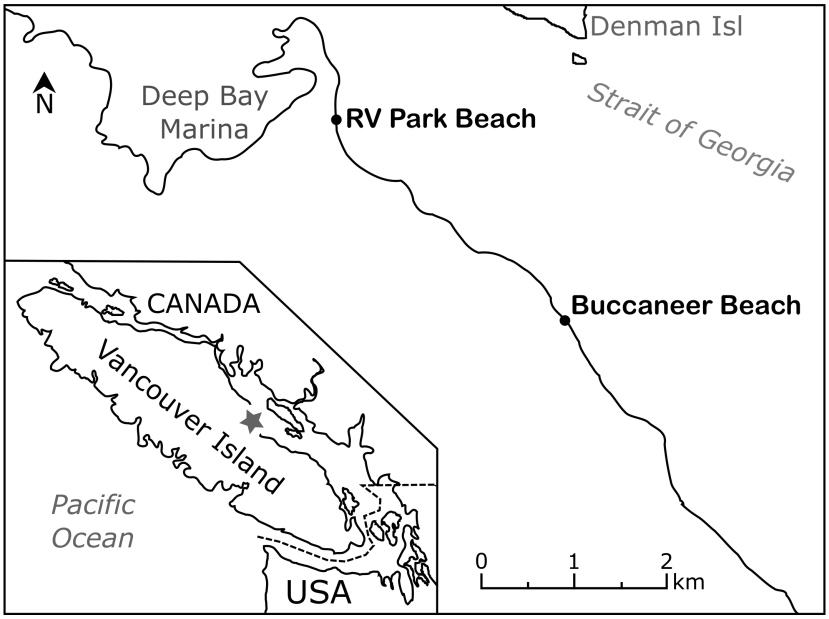

Figure 5.1 Study sites in Baynes Sound, British Columbia. Colonization studies occurred at the beaches indicated.

Table 5.1. Total number of the dominant invertebrates and all other species colonizing the three seaweed species for the two sites combined (n = 29 for *Sargassum muticum* and *Mazzaella japonica*, 28 for *Fucus* spp.).

	Sargassum	***Mazzaella***	***Fucus***	Total
Anthyomyiid sp 1	2334	1801	1171	5306
Halicarid mites	2904	1583	503	4990
***Traskorchestia* spp**	2486	1433	796	4715
Oligochaete	1784	1258	770	3812
All others	1769	928	581	3278
Total	11277	7003	3821	**22101**

Table 5.2 Results of the repeated measures PERMANOVA for the three seaweed species over the five time periods. Bold values indicate statistical significance, n=3 per species per time period.

	df	SS	MS	Pseudo-F	P
Seaweed	2	1.2525	0.6263	4.3336	**0.001**
Day	4	5.6567	1.4142	9.7859	**0.001**
Site	1	2.9018	2.9018	20.0801	**0.001**
Seaweed*Day	8	2.9784	0.3723	2.5763	**0.001**
Seaweed*Site	2	0.6875	0.3474	2.3786	**0.010**
Day*Site	4	3.5187	0.8797	6.0872	**0.001**
Seaweed*Day*Site	8	1.8598	0.2325	1.6087	**0.005**
Residuals	56	8.0927	0.1445		

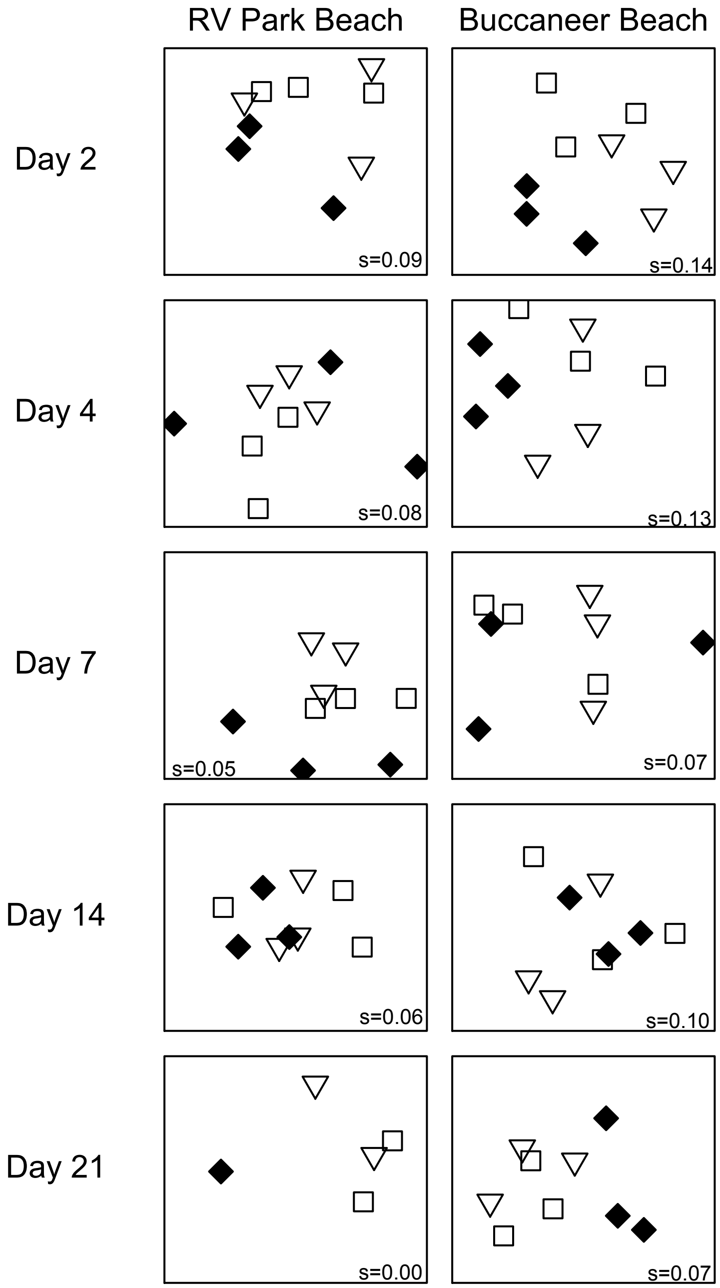

Figure 5.2 Non-metric multidimensional scaling of invertebrate communities at the two sites over the five sampling periods n = 3 per seaweed species (◆ = *Fucus*, ☐ = *Mazzaella,* ▽ = *Sargassum*) per time period except for the RV Park day 21 (*Mazzaella* = 2, *Sargassum* = 2, *Fucus* =1).

Table 5.3 Results of the PERMANOVA for each time period for the RV Park and Buccaneer Beaches. Significant values indicated in bold n = 3 per seaweed species per time period.

	df	SS	MS	Pseudo-F	P
RV Park Day 2					
Seaweed	2	0.5788	0.2894	2.0024	**0.011**
Residuals	6	0.8672	0.1445		
RV Park Day 4					
Seaweed	2	0.6756	0.3378	1.5328	0.084
Residuals	6	1.3222	0.2204		
RV Park Day 7					
Seaweed	2	0.9945	0.4972	3.9077	**0.003**
Residuals	6	0.7634	0.1272		
RV Park Day 14					
Seaweed	2	0.3913	0.1957	1.746	0.139
Residuals	6	0.6724	0.1121		
Buccaneer Day 2					
Seaweed	2	0.6930	0.3465	2.8847	**0.011**
Residuals	6	0.7207	0.1201		
Buccaneer Day 4					
Seaweed	2	0.6602	0.3301	2.2626	**0.028**
Residuals	6	0.8753	0.1459		
Buccaneer Day 7					
Seaweed	2	0.8545	0.4272	2.9095	**0.004**
Residuals	6	0.8810	0.1468		
Buccaneer Day 14					
Seaweed	2	0.8822	0.4411	2.6961	**0.016**
Residuals	6	0.9817	0.1636		
Buccaneer Day 21					
Seaweed	2	0.8330	0.4165	3.0139	**0.011**
Residuals	6	0.8291	0.1382		

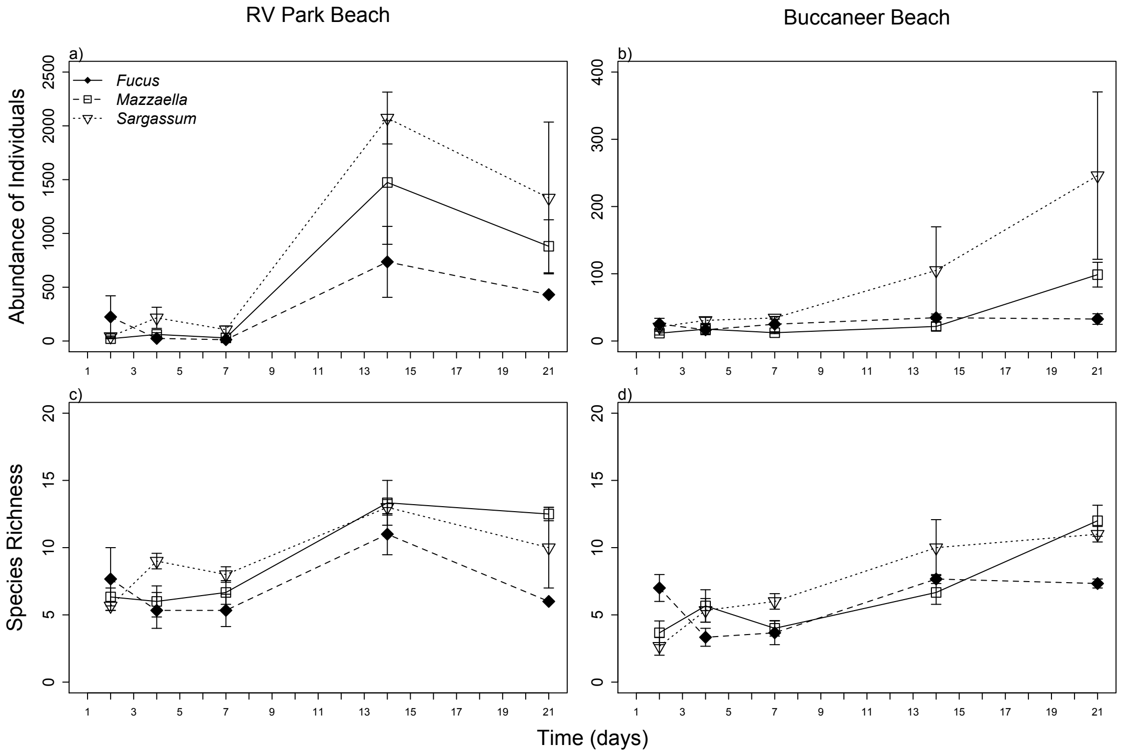

Figure 5.3 Effect of native and introduced seaweed on invertebrate colonization over time for total invertebrates at a) RV Park and b) Buccaneer Beaches and invertebrates species richness at c) RV Park and d) Buccaneer Beaches. Mean (±SE), n=3 per species per time period, except for day 21 at Mailbox Beach (*Mazzaella* = 2, *Sargassum* = 2, *Fucus* =1). Note differences in y-axes.

Table 5.4 Effect of seaweed species *Fucus* spp., *Mazzaella japonica*, and *Sargassum muticum* on invertebrate total abundance and species richness. Results of repeated measures ANOVA, n = 3 per seaweed species per time period for RV Park Beach (4 time periods) and Buccaneer Beach (5 time periods). Bold values indicate statistical significance.

	df	F-value	p
Species Abundance			
RV Park Beach			
Seaweed	2	3.7139	0.0892
Date	3	57.8786	**<0.0001**
Seaweed*Date	6	2.6018	0.0539
Buccaneer Beach			
Seaweed	2	11.1176	**0.0096**
Date	4	12.2637	**<0.0001**
Seaweed*Date	8	1.8819	0.1104
Species Richness			
RV Park Beach			
Seaweed	2	0.6843	0.5399
Date	3	35.002	**<0.0001**
Seaweed*Date	6	2.6595	**0.0501**
Buccaneer Beach			
Seaweed	2	4.2632	0.0705
Date	4	19.8085	**<0.001**
Seaweed*Date	8	3.8527	**0.0048**

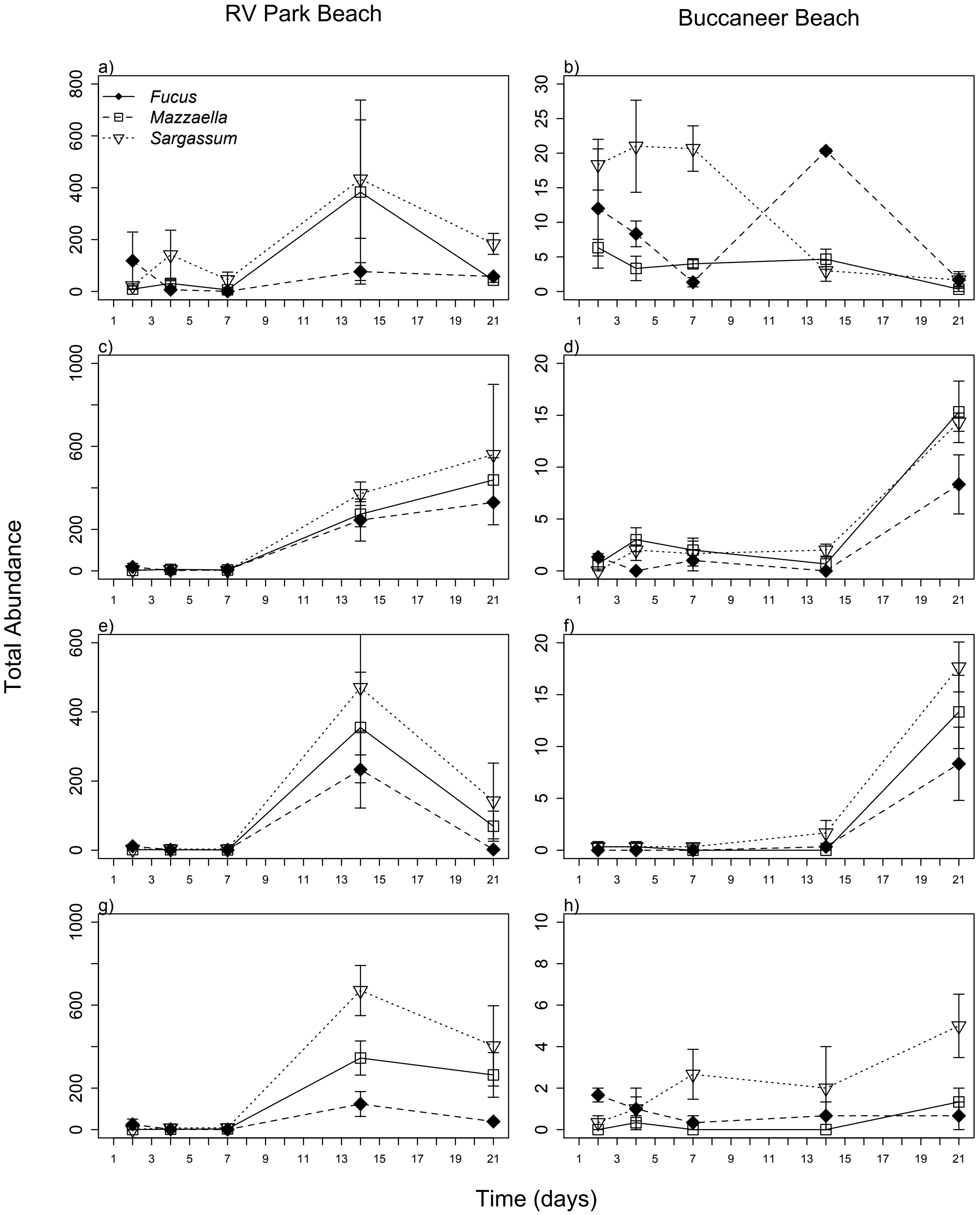

Figure 5.4 Effect of different seaweed species on various invertebrate abundances for the two different sites: RV Park Beach (a, c, e, g) and Buccaneer Beach (b, d, f, h) for *Traskorchestia* spp. (a and b), anthomyiid flies (c and d), oligochaetes (e and f), and mites (g and h). Points represent mean ± SE, n = 3 per seaweed per time except for the RV Park day 21 points (*Mazzaella* = 2, *Sargassum* = 2, *Fucus* =1).

Table 5.5 Results of the repeated measures ANOVA for the dominant invertebrates at the RV Park Beach for the 4 different time periods, n = 3 per species per time period. Bold values indicate statistical significance.

	df	F-value	p
Traskorchestia spp.			
Seaweed	2	5.28043	**0.0476**
Date	3	9.9914	**0.0004**
Seaweed*Date	6	1.5755	0.2113
Anthomyiid sp1			
Seaweed	2	0.0459	0.9555
Date	3	85.9641	**<0.0001**
Seaweed*Date	6	2.21054	0.0898
Oligochaete			
Seaweed	2	2.5757	0.1558
Date	3	18.5609	**<0.0001**
Seaweed*Date	6	1.6139	0.2005
Halacarid mites			
Seaweed	2	3.5666	0.0954
Date	3	32.9521	**<0.0001**
Seaweed*Date	6	2.2537	0.0848

Table 5.6 Results of the repeated measures ANOVA for the dominant invertebrates at the Buccaneer Beach for the 5 different time periods, n = 3 per species per time period. Bold values indicate statistical significance.

	df	F-value	p
Traskorchestia spp.			
Seaweed	2	8.3714	**0.0184**
Date	4	8.2161	**0.0003**
Seaweed*Date	8	4.1240	**0.0033**
Anthomyiid sp 1			
Seaweed	2	5.0207	0.0523
Date	4	42.6280	**<0.0001**
Seaweed*Date	8	1.6809	0.1548
Oligochaete			
Seaweed	2	3.1800	0.114
Date	4	17.2234	**<0.0001**
Seaweed*Date	8	0.5261	0.8251
Halacarid mites			
Seaweed	2	8.4436	**0.0180**
Date	4	1.7162	0.1793
Seaweed*Date	8	1.6373	0.1665

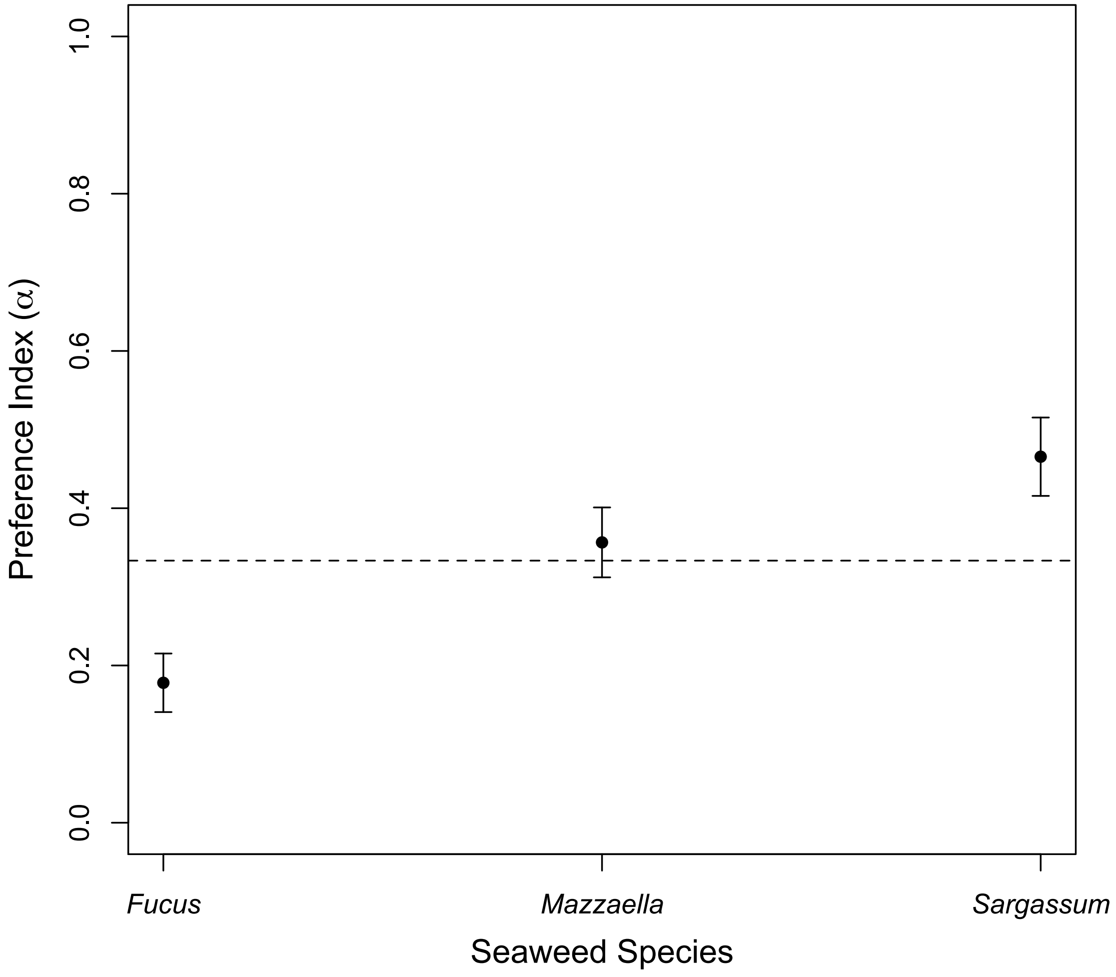

Figure 5.5 Feeding preference of *Traskorchestia* spp. on the three species used for the colonization study. Dashed line denotes value of zero preference (α = 0.33), values above the line denote prey preference, values below the line represent avoidance. Bars represent 95% CI, where CI does not overlap the line preference or avoidance is significant, n = 10.

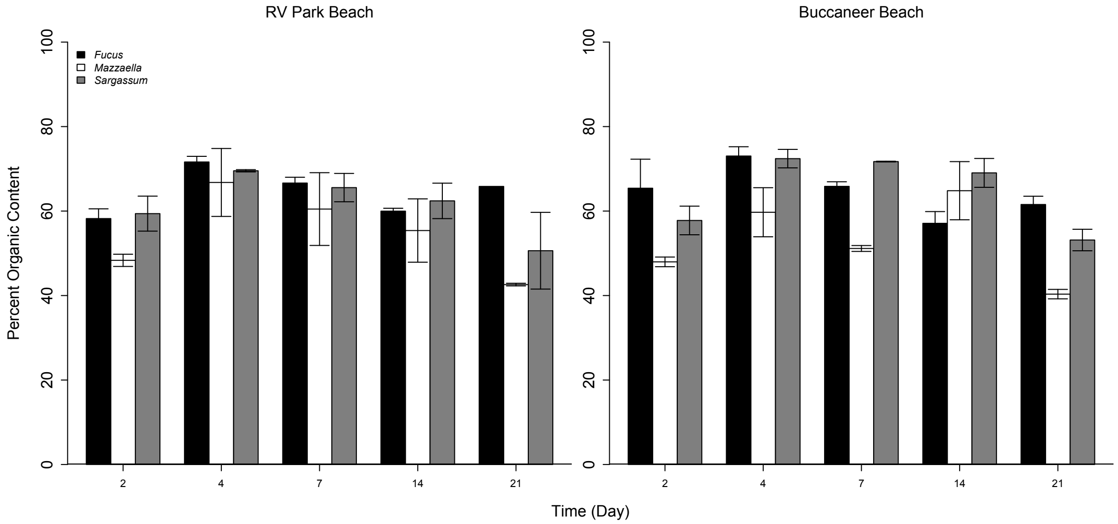

Figure 5.6 Percent organic content for the three seaweeds at the RV Park Beach and Buccaneer Beach over the 21-day study. Bars represent mean ± SE, n = 3 per seaweed per time except for the RV Park day 21 points (*Mazzaella* = 2, *Sargassum* = 2, *Fucus* =1).

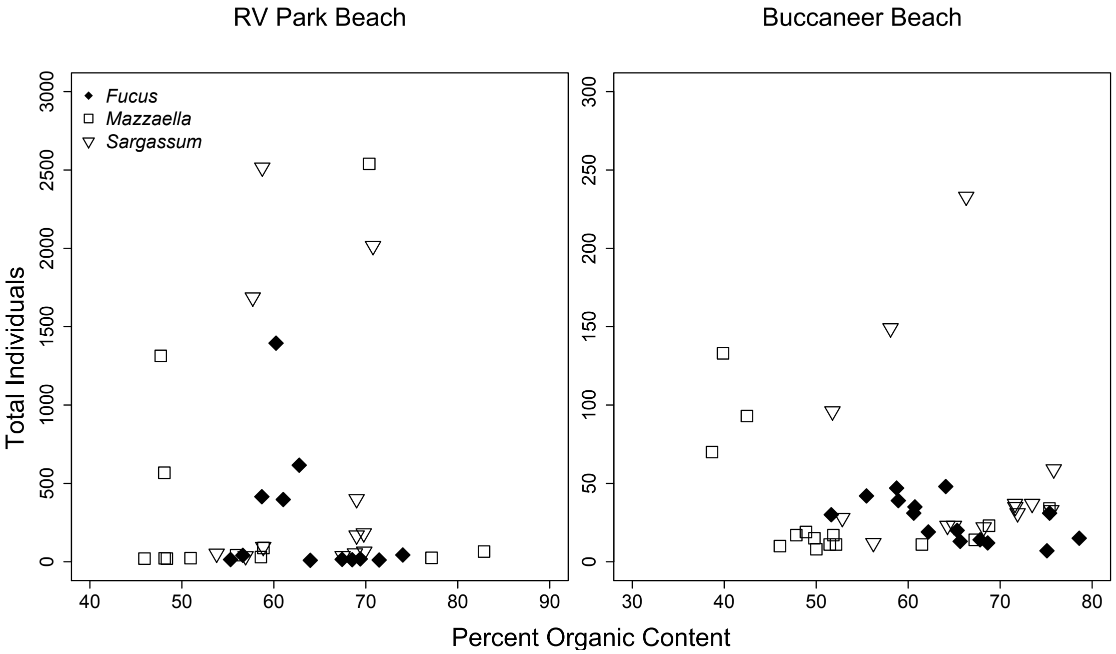

Figure 5.7. Percent organic content as a predictor for total individuals colonizing wrack at the RV Park Beach ($R^2 < 0.001$) and Buccaneer Beach ($R^2 = 0.025$).

Chapter 6 – Conclusions

Non-indigenous seaweeds can have a myriad of impacts on the recipient communities they inhabit. However, these impacts have been poorly studied save for the most prominent invasive seaweeds (Schaffelke and Hewitt 2007, Maggi et al. 2015, Davidson et al. 2015) and prior to this thesis, no research has examined how two non-indigenous seaweeds interact in the same region of the recipient habitat. In addition, many studies of non-indigenous seaweeds have focused predominantly on the growing range of the seaweed but did not consider their impacts as a spatial subsidy in neighbouring beach habitats, an issue which has been recently raised in the literature (Harris et al. 2014). In the previous chapters we demonstrated that a previously unstudied, non-indigenous seaweed, *Mazzaella japonica,* has negative impacts on native species abundance and richness in subtidal habitats (Chapter 2). Furthermore, we showed that *M. japonica* outcompetes *Sargassum muticum*, one of the best-studied invasive seaweeds (Engelen et al. 2015) (Chapter 2&3). We also show that these two non-indigenous seaweeds have the opposite effect on higher trophic levels as they provide a food source (Chapter 3&4) and habitat (Chapter 5) for invertebrates. However, the magnitude of impact of these two non-indigenous seaweeds is not equivalent on either trophic level.

6.1 Competition with Native Seaweeds

Removal of two non-indigenous algae *Mazzaella japonica* (Chapter 2&3) and *Sargassum muticum* (Chapter 3) had positive impacts on native algae, allowing for increases in abundance and diversity, likely due to reduction in competition for space and light (Britton-Simmons 2004, White and Shurin 2011). This demonstrates that when growing together, non-indigenous species have direct negative impacts on native seaweeds. However, removal of *M. japonica* had a greater impact on native seaweeds than *S. muticum.* As well*,* removal of *M. japonica* resulted in seaweed communities more similar to plots from which both non-indigenous seaweeds had been removed whereas removal of *S. muticum* resulted in seaweed communities more similar to control plots (Chapter 3). While this was not the outcome originally predicted, it is perhaps not surprising given the overall greater abundance of *M. japonica* compared to *S. muticum* in the ecosystem.

Both removal studies (Chapter 2&3) showed that removal of invasive species had a greater impact on recovery of native species abundance and richness in the fully subtidal transects (Buccaneer Beach) than on the intertidal transects (Mailbox Beach). Previous work theorized this could be due to the increased density of plants in subtidal areas as compared to intertidal ones thus having greater impact (Sánchez and Fernández 2005, White and Shurin 2011, Jackson et al. 2015). This was not consistent with our results which found in areas where *Mazzaella japonica* grows without *Sargassum muticum*, pre-removal percent cover of *M. japonica* was roughly the same at the two sites (90% cover at Mailbox Beach vs 88% cover at Buccaneer Beach) (Chapter 2). Due to the more consistent abiotic conditions in subtidal habitats which lead to lower physiological stressors, competition is a more important force in structuring species distributions which is supported by the results found in this study.

6.2 Interaction of Multiple Invasive Seaweeds

Mazzaella japonica had subadditive impacts on *Sargassum muticum* as its removal allowed for *S. muticum* colonization in plots where it did not exist at the beginning of the experiment (Chapter 2) and increased percent cover in plots where the two seaweeds co-existed (Chapter 3). The evidence from Chapters 2&3 suggest that *M. japonica* is the dominant competitor in the ecosystem. This is not entirely surprising as *M. japonica* shares many of the characteristics that studies have identified as making *S. muticum* an excellent invader. Although both seaweeds have perennial holdfasts which are able to overwinter, *M. japonica*'s holdfast is larger and much more difficult to remove from the substratum. While *M. japonica* may not be as effective in dispersing throughout its recipient range as *S. muticum*, it is a better competitor for space.

Interestingly, populations of *S. muticum* experienced a greater increase in percent cover in intertidal regions as compared to subtidal regions when *M. japonica* was removed (Chapter 3). This difference strengthens the contention that *M. japonica* is a stronger competitor than *S. muticum* where competition is often the dominant factor influencing subtidal distributions of native seaweeds.

6.3 Beach-Cast Wrack

Marine seaweeds constitute a nutrient sink in subtidal regions where they grow (Hanisak 1993) which can later become a spatial subsidy after plants are dislodged and washed onto nutrient-poor beach communities (Orr et al. 2005, Mews et al. 2006, Ince et al. 2007). Seaweeds can be a subsidy either through decay, or feeding by herbivores, or as a habitat. Both *Mazzaella japonica* and *Sargassum muticum* are washed onto beaches as wrack and are of the five most abundant components of the wrack community; however, *M. japonica* was by far the dominant wrack species (at least 88%) (Chapter 4).

Though *M. japonica* is the dominant seaweed washing up on beaches in Baynes Sound, it does not appear to be as important a spatial subsidy as other seaweeds(Chapter 4&5), specifically *S. muticum* and *Chondracanthus exasperatus*. However, due to its exceptionally high abundance it is still essential for the ecosystem. Decay and decomposition of *M. japonica* was significantly less than both *C. exasperatus* and *S. muticum* but greater than *Fucus* spp. *S. muticum* had the highest rates of decay and decomposition after *C. exasperatus* but these differences were not statistically significant. Additionally, *S. muticum* wrack was colonized by the greatest number of invertebrates on the beaches (Chapter 5). *M. japonica* was colonized by fewer individuals than *S. muticum* but by more than the native seaweed *Fucus spp*. (Chapter 5). These data suggest that though non-indigenous, beach-dwelling invertebrates prefer to use *S. muticum* and *M. japonica* as habitats.

Multiple amphipod feeding studies showed these highly abundant detritivores preferred to feed on *S. muticum* over all other seaweeds (Chapter 4&5) refuting the results of the commonly discussed 'enemy release hypothesis' (Elton 1958) whereby non-indigenous species are released from predation effects due to their novelty. This result has been found for other herbivore which has also preferentially fed on *S. muticum* (Cacabelos et al. 2010, Britton-Simmons 2011)

These data together suggest that *S. muticum* may play a disproportionately significant role as a spatial subsidy as compared to its abundance. In contrast *M. japonica,* which had the greatest biomass on beaches, is not as important for supporting supralittoral invertebrate communities.

6.4 Overall Community Impacts

To date, this is the first *in situ* manipulative study to examine the impacts and interactions of two invasive seaweeds in multiple habitats. This thesis highlights the need to study not just the growing range, but also the regions where dislodged seaweeds wash up and act as spatial subsidies in order to fully understand the impacts that invasive seaweeds can have on their recipient ecosystem. As was previous found (Thomsen et al. 2014), these two non-indigenous seaweeds appear to have greater negative impacts on species in the same trophic levels than those on higher trophic levels. Indeed, both seaweeds appear to have generally positive impacts on higher trophic levels with the exception of large bodied motile marine invertebrates in the growing range of the seaweeds. The impacts that these two seaweeds have are likely to increase as their invasion continues. Unfortunately, the lack of baseline data for much of the BC coast will hamper the ability to determine the full scope of the impacts.

6.5 Management Options for *Mazzaella japonica* and *Sargassum muticum*

There are five potential seaweed management recommendations that have been elucidated through this work:

Option 1: Manage both seaweeds as invasive seaweeds.

This form of management would require a massive eradication effort of both seaweeds ensuring the holdfasts and most (if not all) of the plant fragments are removed from the ecosystem. A potential benefit from a large one-time harvest would be financial gains for the harvesters who extract carrageenan from *Mazzaella japonica*. Also, if successful, this option should allow for an increase in native biodiversity and recruitment of native kelps in the growing region of these two seaweeds. There are, however, some potential drawbacks to this option. The eradication effort may be unsuccessful especially for *S. muticum* as it is well established all around Vancouver Island. Previous attempts to eradicate *S. muticum* have shown to be ineffectual (Critchley 1986). A significant financial cost may not result in an ecological advantage (Meyerson and Reaser 2002). As well, removal of these seaweeds could have possible negative impacts on beach-dwelling invertebrate communities which would lose a potentially important spatial subsidy (Chapter 4&5) forcing managers to determine if marine algae diversity is more important than supralittoral invertebrate abundance. The large disturbance the eradication might have could allow for the establishment of other non-indigenous species given there are propagules in the area (Byers 2002, Valentine and Johnson 2003, Britton-Simmons and Abbott 2008). Additionally, herring (*Culpea pallasii*) spawn on both *M. japonica* and *S. muticum* (K. Pawluk, pers. obs.) and would therefore lose a spawning substratum.

Option 2: Manage only *Mazzaella japonica* as an invasive seaweed.

As *Mazzaella japonica* has the greatest impact on the native seaweed communities (Chapter 3) and has a lesser positive impact on abundance of beach dwelling communities as compared to *Sargassum muticum* (Chapter 4&5), removing just the one seaweed could be another option. Management of just *S. muticum* by eradication would not be a recommended option, as its removal would most likely allow for an increase in *M. japonica* population. If *M. japonica* eradication is successful, native species could recover in the fully subtidal areas (Chapter 2) and *S. muticum* could increase in population (Chapter 3) which, in turn, could have positive impacts on beach-dwelling invertebrates (Chapter 4&5). Again, like the option one, the large disturbance caused from the eradication attempt could allow for the recruitment of other non-indigenous species or allow *S. muticum* to increase its population with unknown consequences to the native communities.

Option 3: Manage *Mazzaella japonica* for a continued sustainable harvest.

Harvest of native seaweed populations, primarily of *Chondrus crispus* (Sharp and Pringle 1990, Rebours et al. 2014), has been occurring on the east coast of Canada since the early 1940s and has been suggested as a potential management strategy for invasive algae (Villanueva et al. 2010). While previous research has shown harvesting or reducing quantities of beach cast-wrack can be detrimental to ecosystems this research has focused solely on the harvest of native seaweeds (Kirkman and Kendrick 1997, Dugan and Hubbard 2010). Currently, *Mazzaella japonica* is being harvested after it has washed up onto beaches in my study sites during winter months. Managing for a sustainable harvest would require the maintenance of stable populations in the growing range to provide the plant densities necessary to wash onto beaches. However, this management strategy would also require a harvesting method that did not damage the supralittoral ecosystem (Reyes-Martinez et al. 2015) while removing the seaweed material and that ensured populations of *M. japonica* did not continue to spread beyond the harvest area to minimize ecosystem impact.

Option 4: Manage a subtidal harvest of *Mazzaella japonica*

Given that *Mazzaella japonica* has the greatest influence on subtidal seaweeds and has a resilient, long-lived holdfast, a subtidal harvest could be one option allowing for economic development while reducing the impact of this seaweed. Though this endeavour would be challenging technologically due to the subtidal nature, it would present many ecological and social benefits. *M. japonica* would be removed from the region where it is in high density (~88 percent cover) and exerts the most negative community impacts. Hand harvesting would increase collection specificity reducing the removal of native seaweeds. Interestingly, the life-history strategy of this species (its long-lasting encrusting holdfast) that I believe makes it such an effective competitor, may also be the factor that could allow for its sustainable harvest. The technique of harvesting the fronds without disturbing holdfasts, shown to be effective for native seaweeds (Seeley and Schlesinger 2012) would allow for continual plant re-growth. In this process, enough material is removed to be economical with minimal disturbance to ecosystems. Additionally, the removal of live plants would reduce the overall reproductive output of the population due to removal of reproductive individuals before propagules are released. Harvesting could occur year-round, except during herring spawning season, providing a more stable industry rather than the current boom and bust scenario of winter harvesting. Large machinery would be unnecessary on beaches reducing the social strain of this harvest and decreasing impact on forage fish spawning habitat.

Option 5: Inaction

Not managing or utilizing these species is another viable option (Thresher and Kuris 2004, Hewitt et al. 2009). If evolutionary origin is not considered, there is currently a thriving seaweed population in the growing range and a large spatial subsidy supporting nutrient poor beach communities. Thus, if biodiversity is not necessary or valued, inaction could be a potential management option. Inaction could also prevent an expensive, likely ineffectual, and highly disruptive eradication program. However, this management strategy is unlikely to be chosen because the harvest has been taking place for three years and the Ministry of Agriculture would need substantial evidence clearly demonstrating removal of seaweed is having significant negative impact in order to revoke or not renew harvest licenses. At this point, there is little evidence to support the notion that harvesting is have significant impact to the ecosystem.

Questions remain to be answered before determining which management strategy should be adopted. These issues span both ecological and social considerations. The central ecological question requiring elucidation is to determine if one dominant seaweed (or two in this case) is functionally equivalent to an ecosystem with high biodiversity. Specifically, assuming native seaweed populations do increase after removal of invaders, one needs to ask if they have the same positive effect on marine invertebrates, beach-cast wrack, and herring spawn that *S. muticum* and *M. japonica* currently have. There are also many social issues regarding the harvest as beaches where the seaweeds wash up are directly adjacent to developed properties. Community member feel strongly about industrialization of their beaches and about removal of natural resources (regardless to whether or not those resources should be there in the first place). Ultimately, the question the Ministry of Agriculture needs to answer is if they are going to manage *Mazzaella japonica* as an invasive species or as a natural resource. Given the results of my research program, I would suggest that if there is no evidence that beach harvesting impacts beach-spawning forage fish this harvest is not detrimental to the beach habitats given that some amount of wrack is left on beach. However, a subtidal harvest, though technically challenging, would have the greatest positive impacts on the Baynes Sound ecosystem.

6.6 Ongoing Research

One major component I was unable to address sufficiently for this thesis was the transfer of nutrients from *Mazzaella japonica* and *Sargassum muticum* through the food chain. Herbivores are often suggested as a natural control for primary producers; however, this is an unreasonable assumption if those herbivores preferentially feed on native seaweeds as many do (Cacabelos et al. 2010). Currently, I am working with a master's student and a directed studies student to trace the incorporation of these two introduced seaweeds and the most abundant native seaweeds through the food chain using stable isotopes.

To expand on our knowledge of the potential for *Strongylocentrotus droebachiensis* control of *Mazzaella japonica* and *Sargassum muticum* Sarah Dudas (Vancouver Island University) and I are co-supervising an honours student who is examining the effect of single species feeding assays on urchin test and gonad growth of *S. droebachiensis* to determine if there is any energetic benefit or disadvantage of feeding on the introduced species.

6.7 Future Research

One goal in community ecology is to determine general "laws" that govern how species interact so scientists can predict how organisms will respond to changes in the biota of an ecosystem (Lawton 1999). A more specific goal in invasion ecology is to predict the impact of introducing a new species to an ecosystem so that management, if necessary, can be put in place early enough to eradicate the invader without disrupting the ecosystem. *Sargassum muticum* and *Mazzaella japonica* impact native seaweed communities via the same mechanisms: they both compete for space with multiyear holdfasts and they alter light regimes by shading. However, one of the main conclusions of my research is that *M. japonica* has the potential to outcompete the globally relevant and well studied *S. muticum,* and that overall, *M. japonica* has a greater impact on native seaweed communities and while it theoretically contributes lots of nutrients to the supralittoral ecosystem it is not the preferred food source by the invertebrate community. This highlights the need to study multiple invasions simultaneously as one might predict the seaweed with the greater invaded range would have greater impacts. Were *S. muticum* to be eradicated from the ecosystem, there is the possibility that *M. japonica* could be released from the little competitive interaction it does have with *S. muticum* and expand its range and impact on the already depressed native seaweed communities.

While there is a much more comprehensive understanding of the invasive capabilities of *Sargassum muticum* with over 650 papers having been published on it (Engelen et al. 2015), there are many areas of research that are still required to fully understand this invasive species. To better understand invasion processes and evolution of both *S. muticum* and *Mazzaella japonica* a more detailed knowledge of the ecology of these two species in their native range is essential (Engelen et al. 2015). Direct comparisons between characteristics in native ranges and recipient ranges can help elucidate generalizations about what allows particular species to be good invaders. Due to circumstantial evidence regarding timing and initial records of the invasion of *S. muticum*, there is a high level of certainty of the invasion vector for both of these species; however, still to be understood is the timeline of the invasions.

Clearly, much less is known about *Mazzaella japonica* leaving a broad range of future studies to be completed. First and foremost, detailed maps of where *M. japonica* grows need to be created to allow for monitoring of its population dynamics to determine if the populations are spreading, at equilibrium, or shrinking. A better understanding of the life history strategies of *Mazzaella japonica* is essential to more fully understand its capacity as an invader. Growth rates, plant and holdfast longevity, and reproductive capacity need be determined to understand why this species is such a successful invader in Baynes Sound. Reproductive plants were found year-round, but I did not have the opportunity to quantify them or determine the fecundity of this species. Another important population factor to determine is the dispersal potential of *M. japonica*. Currently, we have no knowledge of this potential; a key piece of information needed if we are to predict potential population spread. Haploid and diploid ratios of these plants should be determined along with any biophysical differences between these two morphs. Additionally, factors that make Baynes Sound a recipient habitat prone to invasion need to elucidated.

Though we now have a basic understanding of the role that *Mazzaella japonica* and *Sargassum muticum* play as wrack for invertebrates, there are many dynamics still to be understood. *M. japonica* washes up in excessive quantities which could impact physical and abiotic beach dynamics such as: dune formation, shore armouring, and oxygen utilization. The role of oxygen is particularly important. If seaweed piles become too dense and stagnate on beaches, oxygen may be depleted and impact both invertebrates and beach-spawning forage fish such as sand lance and surf smelt.

Much of our knowledge of invasive seaweeds comes from work done on intertidal populations; potentially, leaving a large area where population impacts are not being examined. While there are pockets of research where subtidal work is being conducted (*Codium fragile* in Nova Scotia and *Caulerpa* spp. in the Mediterranean) much of what is known about impact of non-indigenous seaweeds is from work being done at low tide thus capturing a limited component of these populations. Intertidal zones are generally known to be more physiologically limiting and may dampen competitive interactions between species. Replicated studies of introduced seaweeds in their intertidal range as well as in their subtidal range within the same field sites need to be conducted to determine if there are true differences in the impact of NIS between these two areas or if this is a sampling artefact.

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