IMPACTS OF NEWLY ESTABLISHED NON-INDIGENOUS GREEN CRAB (*CARCINUS MAENAS*) ON NATIVE FAUNA IN PLACENTIA BAY, NEWFOUNDLAND

by

© Melanie A. Rossong

A Thesis Submitted to the

School of Graduate Studies

in Partial Fulfillment of the Requirements for the Degree of

Doctor of Philosophy

Department of Biology

Memorial University of Newfoundland

March 2016

St. John’s, Newfoundland and Labrador

ABSTRACT

The recent invasion of the European green crab (*Carcinus maenas*) populations in Placentia Bay, Newfoundland and Labrador (NL) raises great concern about potential impacts on local fisheries and native biodiversity. Green crab are highly adaptable and in both native and invaded areas, green crab are well established predators that can outcompete other similarly sized decapods. The main objectives of this thesis were to: 1) identify the native species that green crab compete with for resources; 2) determine the depths and substrate types in which these interactions likely occur; 3) assess the indirect effects of green crab on native crustaceans and their changes in behavior; 4) assess the impacts of green crab on benthic community structure; 5) compare the NL population with other Atlantic Canadian populations in terms of competitive abilities; and 6) compare morphological features of the NL population with other Atlantic Canadian populations. I found that green crab overlap in space and diet with both rock crab (*Cancer irroratus*) and American lobster (*Homarus americanus*), potentially leading to a shift in habitat. Laboratory studies on naïve juvenile lobster also suggested shifts in behavior related to green crab, in that lobster decreased foraging activity and increased shelter use in the presence of green crab. Benthic community analyses showed fewer species in mud, sand, and eelgrass sites heavily populated by green crab compared to sites without green crab, although results depended on the taxa involved and I could not eliminate environmental differences through a short term caging study. Foraging ability of green crab varied in intraspecific competition experiments, with populations from NL and Prince Edward Island dominating longer-established populations from Nova Scotia

and New Brunswick. Additional studies excluded claw size as a factor driving these results and behavioral differences likely reflected differences in invasion time and population genetics. Overall, green crab in Placentia Bay appear to be altering community structure of benthic invertebrates through predation and they also appear to indirectly impact native crustaceans through competition.

ACKNOWLEDGEMENTS

I would like to thank both my supervisors Pedro Quijón and Paul Snelgrove for their support, patience, and understanding throughout the completion of my thesis. I would also like to thank my family: my husband Tim who offered lots of positive encouragement, field support, statistical guidance and advice, and to my beautiful little girl Eldyn who allowed me to finish edits during nap times. I would also like to acknowledge my supervisory committee, Cynthia McKenzie and Andrea Locke who provided helpful insight and financial support. Comments from my examining committee were greatly appreciated. Thanks to J. Williams, H. Squires, M. Comeau, B. Comeau, D. Giard, R. Leblanc, G. Paulin, T. Wells, R. Bernier, A. Nadeau, C. McCarthy, T. Pickering, L. Poirier, A. Vickerson, K. Matheson, C. Vickers, D. Wilcott, R. Rivkin, P. Sargent, W. Coffey, M. Pittman, B. O'Donnell, W. Petley, A. Harnish, C. Mellish, W. Robbins, T. Wheeler, for assistance with animal collection, field work and lab work. Funding for this research was provided by Natural Sciences and Engineering Research Council of Canada Discovery Grants to PQ and PS, a contract from Fisheries and Oceans Canada as part of the Aquatic Invasive Species Research Program funding to CM and AL, NSERC PGS-D, Newfoundland and Labrador Research Development Council Ocean Industries Research Scholarship and a MUN School of Graduate Studies Fellowship to MR.

Table of Contents

List of Tables

List of Figures

1. A review of the impacts of the non-indigenous European green crab on marine sedimentary habitats

The discovery of non-indigenous European green crab, *Carcinus maenas* L. (1758), in coastal Newfoundland in 2007 created immediate concern among scientists and fishermen that major ecological impacts might ensue, including detrimental effects on lobster and other natural populations (CBC 2007). The basis for this concern was the major changes often reported during some, but not all, marine invasions. While some invasive species contribute to ecological and economic alteration of communities, invasive species may increase native species biodiversity (Bruno et al. 2005) through facilitation. Ecosystems in general may not be fully "saturated" and the addition of invasive species may not reduce native species when available resources are abundant (Bruno et al. 2005). Because fewer past studies have focused on marine invasive species, in contrast to those in terrestrial environments (Bruno et al. 2005), less is known about the consequences of new invaders in marine coastal ecosystems. My review summarizes the impacts of green crab in newly invaded benthic communities, and introduces new research on changes to Newfoundland ecosystems following the onset of this invasion.

1.1. Aquatic Invasive Species

Various factors can shape an ecosystem, including climate change, rising sea level, and presence of keystone predators, but predicting the consequences of introductions of invasive species in newly established areas remains uncertain. For this thesis, I define invasive species as organisms that have been moved from one area to another, outside of their native range, with the potential to rapidly colonize new areas

causing environmental and socioeconomic impacts (Ricciardi and Cohen 2007). Range expansions of invasive species usually occur through natural mechanisms or human-mediated transfer. The transfer of aquatic invaders is typically attributed to larval transport via ship fouling, ballast (solid and liquid), drilling platforms, transport of commercial goods, aquaculture, and release through scientific research (Carlton 1989; Lockwood et al. 2007).

Once introduced into an area, a population may become established. Environmental conditions appropriate for the physiology of the invader along with high propagule pressure (number of individuals released) both increase the probability of establishment in a new area (Lockwood et al. 2007). Carlton (1999) estimated that ballast waters transport more than 10,000 species daily (worldwide), however, only 5 to 20% of all species successfully invade an area (Lockwood et al. 2007). Effective invaders often have wide physiological tolerance, short generation time, and high genetic variability (Lockwood et al. 2007). Once established, invasive species can often reach high densities as a result of low competition, predation, and parasite pressure (Behrens Yamada et al. 2005). High densities often allow invasive species to avoid local extirpation, however, even with eradication of the invasive species, the likelihood of re-introduction remains high if the original transfer vector remains available (Lockwood et al. 2007).

1.1.1. Problems Associated with Invasive Species

Invasive species can compete for resources, alter habitat, spread disease, and prey on native species (Jensen et al. 2002). In some cases, invasive species can greatly diminish native population size and thereby affect the structure of the natural community.

Displaced native species may relocate to other areas where conditions are less favorable, ultimately reducing their productivity or survival (Jensen et al. 2002).

Competitive exclusion suggests that two species cannot share the same ecological niche or role within an ecosystem, without detrimental effects on the less competitive species (e.g. Krohne 2001). Establishment of invasive species in areas where native species share certain aspects of the same niche, limiting resources (e.g. food, shelter) may lead to intense competition. Competition for these resources will either reduce populations of one or both species, or displace one species from the area (Krohne 2001; Lockwood et al. 2007). Large population size and lower predation pressures may give invasive species an advantage over native species for available resources.

Invasive species have altered natural communities for many centuries (Grosholz and Ruiz 1995), often dominating in their new environments. For example, the invasive zebra mussel (*Dreissena polymorpha*) is thought to have arrived in ballast water from Europe in the 1980s, and quickly spread from Lake St. Clair, near Detroit to the Great Lakes causing major ecological changes (Johnson and Carlton 1996). Zebra mussels have displaced populations of native unioniid clams, changing large-scale energy and nutrient flow in the ecosystem (Johnson and Carlton 1996). Similarly, an increase in chlorophyte blooms of *Cladophora glomerata* in the Great Lakes also coincided with greater densities of zebra mussels (Higgins et al. 2008). Algal blooms often occur with nutrient enrichment, however, increased water clarity, and phosphorus recycling provided by the filter feeding mussels allowed for these chlorophyte blooms to establish (Higgins et al.

2008), resulting in large-scale decay of organic material and decreased dissolved oxygen in the ecosystem.

In other freshwater communities, non-native crayfish species threaten native crayfish diversity. In competition experiments, growth of native crayfish *Oronectus virilis* decreased when competing with invasive *O. rusticus* (Hill and Lodge 1999). A decrease in native crayfish growth may indicate increased predation risk and competition for shelter, resulting in decreased fecundity from poorer overall condition (Hill and Lodge 1999).

Marine invasive crustaceans typically arrive during their planktonic larval phase but their presence becomes evident only when they reach adulthood. In 1989, the Japanese crab *Hemigrapsus sanguineus,* was sighted along the New Jersey coast and has since spread from Cape Cod to North Carolina. Although smaller than most intertidal crabs, studies show the Japanese crab to be aggressive and much more likely to instigate an agonistic response in laboratory competition experiments (MacDonald et al. 2007). Invasive crabs numerically dominate the intertidal zone in some areas (Jensen et al. 2002).

In Canada, the Chinese mitten crab, *Eriocheir sinensis* has occurred intermittently in the Great Lakes since the 1950s. Mitten crab have an unusual life history for crustaceans in requiring both freshwater and marine systems to survive. The adults live in freshwater, spawn in marine systems, and release their larvae in estuaries (Anger 1991; Clark et al. 1998). In 2004, the discovery of an adult Chinese mitten crab in the St. Lawrence Estuary's brackish water increased the probability of successful recruitment

and population viability (deLafontaine 2005). Although little is known about direct impacts on native organisms, burrowing behavior of *E. sinensis* decreases vegetation and affects stream bank stability (Dittel and Epifanio 2009).

1.2. European Green Crab

The native range of the European green crab, *Carcinus maenas,* spans the east coast of the Atlantic Ocean from northern Europe to northern Africa (Grozholz and Ruiz 1996; Audet et al. 2003). However, in the past hundred years populations of green crab have spread significantly worldwide in the Western Atlantic, Australia, South America, Japan and the Northeast Pacific (Cohen et al. 1995; Grosholz and Ruiz 1995; McDonald et al. 2001).

Genetic evidence points to two distinct invasions along the east coast of North America (Roman 2006), in the early 1800s and in the late 1980s (Carlton and Cohen 2003). The 1800s populations represented the first wave of invasions, comprised of one to four haplotypes thought to have originated from the southern United Kingdom (Roman 2006; Blakeslee et al. 2010). Both Bay of Fundy (NB) and western North American populations originated from the first invasion. The last invasion of the eastern seaboard, which took place in Nova Scotia, Canada in the 1980s has resulted in a range expansion averaging 63 km annually (Grosholz and Ruiz 1995), encompassing northern and eastern Nova Scotia, Prince Edward Island and Placentia Bay, Newfoundland in 2007 (Blakeslee et al. 2010). The two to three distinct European haplotypes that comprise northern NS and PE populations, likely originated from populations at the northern extent of their European range and represent the second invasion of Atlantic Canada (Roman 2006). The

second population likely originated from a more cold-tolerant source population spreading more quickly by oceanic currents (Roman 2006), mixing the two introduced populations in southern NS (Blakeslee et al. 2010).

Green crab can readily adapt to and survive in a wide range of abiotic conditions. Populations are widely distributed within protected marine and estuarine environments in mud, sand, or rocky substratum (Cohen et al. 1995). In its native range, green crab occupy a variety of habitats from protected low energy systems to semi-exposed rocky shores (Grosholz and Ruiz 1996), resulting in potential for greater expansion within invaded habitats. Although considered intertidal, green crab live to depths of at least 6 m. Some populations remain primarily subtidal, while others move with the tides to feed (Cohen et al. 1995).

In general, green crab can survive large temperature and salinity fluctuations as well as desiccation (Audet et al. 2003; Klassen and Locke 2007). They prefer salinities ranging from 10 to 30 ppt and temperatures between 3 °C and 26 °C, but can tolerate wider ranges (Klassen and Locke 2007). The larvae are less tolerant than adults, and successful reproduction and larval development occur only within a much narrower range of salinities (15 to 26 ppt) and temperatures (9 to 22.5 °C) (Nagaraj 1993; Anger et al. 1998). This ability to resist environmental stressors allows populations to persist in their new environments. Moist green crab can survive outside of water for 60 days (Carlton and Cohen 2003), and can live for about 3 months without feeding (Clay 1965).

Two main factors are thought to regulate green crab populations within its invaded range. Cold temperatures and currents seem to limit the northward spread of green crab

within the United States and Canada from the original 1800s invasion (DeRiviera et al. 2005). During colder than normal winters, green crab numbers decline considerably, however, in warmer surface water temperatures green crab can increase in number and expand poleward (Berrill 1982; Behrens Yamada et al. 2005; McDonald et al. 2006). In general, greater larval sensitivity to colder temperatures limits their ability to survive (Hines et al. 2004). Dispersal models of genetic haplotypes indicate downstream dispersal of green crab larvae from the second invasion, spreading to populations in NB and the eastern United States (Pringle et al. 2011), and attributing population spread to oceanic transport. In the eastern United States the presence of blue crab, *Callinectes sapidus* may determine the southern limit of green crab. Research suggests that blue crab consume green crab, and occupy a niche similar to green crab (DeRivera et al. 2005). In laboratory experiments, blue crab consumed smaller green crab, even with alternate food sources available (DeRivera et al. 2005). However, green crab juveniles were competitively dominant in limited food source experiments with juvenile blue crab (MacDonald et al. 2007).

When initially invading a new environment, *Carcinus maenas*, like other successful invaders, lack natural predators and populations typically reach very large densities. This increase of organisms can dramatically affect the invaded area because resources become limiting and competition pressures increase. Within its native and invaded range, green crab is a successful predator, in some cases, reducing populations of some benthic invertebrates, while outcompeting and displacing other crustaceans during foraging (McDonald et al. 2001). Green crab predation can alter molluscan shell

morphology (e.g. increased thickness; Grozholz and Ruiz 1995), reduce population abundance of soft shell clams *Mya arenaria* (Floyd and Williams 2004), and change natural food webs by causing a local decline in native species abundances (Grozholz et al. 2000; McDonald et al. 2001).

1.2.1. Impacts on Native Species

Green crab's competitive ability may contribute to the decline of various benthic organisms, habitats, and local fisheries. In its native range, green crab regulates the structure of benthic communities through predation, competition, and sediment disturbance (McDonald et al. 2006). As green crab populations in new areas increase, so do potential changes to community structure and ecosystem stability. Changes to community may alter water filtration rates and availability of nutrients, as well as, predator and prey populations (Grosholz and Ruiz 1995).

1.2.1.1. Bivalves

Green crab prey on bivalves more than any local known predator in New England (Lohrer and Whitlatch 2002; MacDonald et al. 2007). Their spread in the 1950s to Maine was one of the major contributing factors in the collapse of the Gulf of Maine soft-shell clam (*Mya arenaria*) population. During this period, harvested landings decreased by 6.6 million kg for an economic loss of $2.3 million (Behrens Yamada et al. 2005). Within three years of arrival in Western North America in the early 1990s, green crab had reduced numbers of native *Nutricola tantilla* and *N. confusa* with densities five times less

than previously observed and no signs of recovery (Grosholz et al. 2000). In California, green crab decreased clam abundance by an order of magnitude (Tanner 2007).

In lab experiments, individual juvenile green crab (<40 mm CW) consumed approximately 150 clams (*Nutricola* spp.) in 12 hour feeding trials (Grosholz et al. 2000). When given a choice, green crab preferred soft-shell clams over blue mussels, *Mytilus edulis* and American oysters, *Crassostrea virginica*. However, green crab consumed less preferred prey (i.e. mussels and oysters) once clams were eliminated (Pickering and Quijón 2011). In a caging experiment, green crab selected smaller clams (<17 mm) over larger ones, perhaps because smaller clams have thinner shells, reduced handling time, or greater availability at the surface compared to deeper, larger clams (Floyd and Williams 2004). Thus, the effects of green crab on benthic invertebrates may only be evident after several generations of failed recruitment by impacted populations, depending on the species.

Blue mussels also commonly occur in green crab stomachs (Elner 1981). Juvenile green crab consumed fewer mussels than rock crab, *Cancer irroratus*, however, adult green crab consumed mussels at a rate two to 20 times greater than juvenile green crab (Breen and Metaxas 2008). In the Wadden Sea, juvenile green crab density can reach 1000 individuals/m^2 in blue mussel patches (Baeta et al. 2005). Under laboratory conditions, blue mussels responded to green crab within 30 days by producing thicker shells, larger adductor muscles, and by increasing byssal thread production (Frandsen and Dolmer 2002). These defensive mechanisms occur more frequently in habitats with low complexity (i.e. mudflats) and fewer refuges to reduce predation pressure. In structurally

more complex habitats (i.e. salt marshes), predation rates by green crab are typically 30% lower than in mudflats (Frandsen and Dolmer 2002).

1.2.1.2. Gastropods

Green crab predation has also driven natural selection in shells of *Nucella lapillus* and other gastropod species (Vermeij 1982). High predation pressure has promoted changes in both the morphology and physiology of multiple species. For example, the shells of *Littorina obtusata* and *Nucella lapillus* have thickened by 50 to 82% and 12%, respectively since the arrival of green crab in the Gulf of Maine (Smith 2004). Greater shell mass characterized *L. obtusata* populations that co-occurred with green crab with larger crusher claws (Edgell and Rochette 2008).

Green crab prey more readily on *L. obtusata*, a species native to Atlantic Canada, than the non-native *Littorina littorea* (which co-occurs with green crab in Europe). Lower anti-predator defences in *Littorina obtusata* may reflect less time to co-evolve with green crab. In feeding experiments, green crab consumed both species when shells were crushed first but fed almost entirely (only 1 out of 150 crabs consumed *L. littorea*) on *L. obtusata* when shells were intact (Edgell and Rochette 2008). In eastern North America, southern populations of green crab (1st invasion) have larger claws and can break larger snail (*L. obtusata*) shells than northern populations (Smith 2004).

1.2.1.3. Crustaceans

Most crustaceans behave according to the predictions of Game Theory (Maynard Smith 1974). This theory suggests organisms reduce the risk of injury by assessing their

opponent before battle, which crustaceans may do by displaying a behavior called a "meral spread". Crabs use this behavior to increase apparent size by elevating their body and clearly displaying their weapons (Glass and Huntingford 1988; Huber and Kravitz 1995; Sneddon et al. 1997). In lobster, if an opponent's claws appear significantly larger, the subordinate animal retreats. If the two opponents are evenly matched, battle may ensue over the resource. Once a winner is established the defeated lobster retreats. In juvenile lobster, defence may be established by an escape response known as a tailflick (Hudon 1987). Thorpe et al. (1994) suggested greater accuracy in recent models of game theory. It is now believed that crustaceans adjust their behavior according to the value they place on the resource. When resources are limited, they may engage in an interaction regardless of the challenger's size.

Green crab can affect native crustaceans beyond direct predation by decreasing invertebrate prey abundance and increasing competition (e.g. Elner 1981). In laboratory experiments, green crab outcompeted west coast of North America native crabs, *Hemigrapsus* spp. and *Cancer magister*, (Grosholz and Ruiz 1995; McDonald et al. 2001; Jensen et al. 2002) for limited food resources. In competition experiments, green crab also spent significantly more time feeding than Dungeness crab, *C. magister*, which could not successfully approach the feeding green crab (McDonald et al. 2001). Another study examined *C. maenas* and two species of *Hemigrapsus* crab, the native *H. oregonensis* and the invasive *H. sanguineus* (Jensen et al. 2002). Green crab were considered the dominant competitor in laboratory interactions with native crabs, with more successful approaches to take over the resource and significantly more time spent with the bait. In the Jensen et

al. 2002 study, the invasive *Hemigrapsus sanguineus* outcompeted green crab. However, after many interactions, green crab learned to avoid direct interaction with the competitively better opponent (Jensen et al. 2002; Roudez et al. 2008).

Although adult blue crabs are thought to limit the southern expansion of green crab, green crab may impact blue crab populations. In laboratory studies, juvenile green crab outcompeted juvenile *Callinectes sapidus* and *H. sanguineus* (MacDonald et al. 2007). Of all the agonistic interactions, green crab outcompeted *C. sapidus* most often. Green crab was the most persistent species and managed to feed regardless of which species occupied the resource (MacDonald et al. 2007).

In eastern Canada, native rock crab, *Cancer irroratus* occur in the intertidal and lower subtidal environments, potentially overlapping spatially with green crab in invaded areas. In behavioural experiments, juvenile rock crab exhibited lower growth rates (Breen and Metaxas 2005) and increased shelter usage (Matheson and Gagnon 2012a) in the presence of green crab. Adult green crab initially found food first in 90% of trials, however, dominance over the resource varied depending on water temperature (Matheson and Gagnon 2012b).

Green crab overlap in space and diet with juvenile American lobster (*Homarus americanus*; Lynch and Rochette 2009; Elner 1981). Thus, newly established populations of green crab in North America may influence lobster as well as native crab. Researchers hypothesized that high densities of green crab may depress carrying capacity of coastal habitats by limiting available space and food for native crustaceans (Elner 1981). In laboratory experiments, adult green crab fed on juvenile lobster (Rossong et al. 2006),

however, adult lobster also feed on adult green crab (Elner 1981; Karnofsky et al. 1989; Lynch and Rochette 2009). These reciprocal impacts may maintain population balances for both organisms.

In laboratory studies of competition for limited food between green crab and various lobster size classes, green crab outcompeted juvenile (28-53 mm carapace length (CL)), and sub-adult (55-70 mm CL) lobster for food resources (Rossong et al. 2006; Williams et al. 2006). In both cases, green crab found the food first and in the majority of experimental trials lobster could not displace green crab. When sub-adult lobster fed first, rather than simultaneous release, lobster defended the resource and green crab were unsuccessful at displacing lobster from the resource (Williams et al. 2006). In a shelter experiment, green crab caught and consumed juvenile lobster. The more time the lobster spent outside of the shelter the greater chance of mortality (Rossong et al. 2006).

1.2.1.4. Other Organisms

Because green crab prefer sheltered areas with access to both freshwater and saltwater inputs, estuaries represent an ideal habitat. Green crab in Placentia Bay, NL have been observed feeding on various fish species in eelgrass habitats used as nursery grounds (C. McKenzie pers. comm.). Similarly, stomach contents of green crab in the Eastern US revealed the presence of winter flounder (*Pseudopleuronectes americanus*) proteins. Although not a significant part of their diet, 4.8% of 313 individuals tested positively for winter flounder in their stomachs (Taylor 2005). The larger the green crab, the greater the likelihood of winter flounder and plaice (*Pleuronectes platessa*) in stomach contents (Taylor 2005).

In some areas of eastern North America the decline of eelgrass (*Zostera marina*) beds correlates with the introduction and establishment of green crab (Garbary et al. 2004; Malyshev and Quijón 2011). Green crab often prey on infauna by uprooting eelgrass shoots, thereby decreasing available shelter for organisms and increasing their vulnerability (Cohen et al. 1995; Davis et al. 1998). Green crab tear and cut the sheath bundle of eelgrass while foraging and burrowing (Davis et al. 1998). In a mesocosm study of transplanted eelgrass, green crab densities of four or more individuals/m^2 destroyed 39% of transplanted shoots (36 shoots per treatment) within one week (Davis et al. 1998). Green crab not only destroy eelgrass indirectly through bioturbation but evidence suggests juveniles also graze directly on eelgrass (Malyshev and Quijón 2011; Garbary et al. 2013).

Changes in community structure can alter the biodiversity of the system and ultimately facilitate colonization by new invasive species (Carlton and Cohen 2003; Stachowicz et al. 2007). Changes in food web structure can affect not only yearly residents of the habitat but may also have large-scale consequences on migrating bird species and their reproductive success (Jamieson et al. 1998). In Bodega Bay, CA an enclosure study of green crab revealed significantly lower densities of bivalves (*Transennella confusa, T. tantilla*), and crustaceans (*Cumella vulgaris, Corophium* sp.) in experimental sites compared to control sites (Grosholz and Ruiz 1995). In the experimental sites, higher numbers of some species of polychaetes (*Lumbrineris zonata*; Grosholz and Ruiz 1995) suggested depression of some species and facilitated population growth in other species. For example, the depletion of the US west coast native clam,

Nutricola spp. by the green crab allowed establishment of the invasive clam, *Gemma gemma* (Lockwood et al. 2007) by reducing competition pressure.

As discussed in this review, green crab has impacted various species in North America since its introduction, and in some cases, facilitated the success of other invaders. A review of these impacts through direct predation or competition pressures by green crab on native species is provided in Table 1-1.

Table 1-1. Summary of predator (P) and competitor (C) relationships between green crab (GC) and various benthic species.

Organism	**P**	**C**	**Impact**
Mya arenaria	X		collapse of populations in New England and Nova Scotia, preferred smaller clams (1,2)
Nutricola tantilla	X		reduced numbers, no signs of recovery (2)
Nutricola confusa	X		reduced numbers, no signs of recovery (2)
Mytilus edulis	X		adult GC consume 2-20 times more mussels than juveniles; defence mechanisms present with GC (3,4)
Nucella lapillus	X		defence mechanisms (5,6,7)
Littorina obtusata	X		defence mechanisms (6,7)
Littorina littorea	X		defence mechanisms; less impacted due to thicker shells (7)
Hemigrapsus oregonensis		X	GC competitively dominant (8)
Cancer magister	X	X	GC competitively dominant, GC preys on juveniles (9)
Hemigrapsus sanguineus		X	GC outcompeted by adults; among juvenile GC competitively dominant (8,10)

Table 1-1. Summary of predator (P) and competitor (C) relationships between green crab (GC) and various benthic species (cont.).

Organism	**P**	**C**	**Impact**
Cancer irroratus		X	juvenile rock crab consume more resources than GC (3)
Dyspanopeus sayi	X	X	GC compete with mud crab for clams; GC consume mud crab (11)
Callinectes sapidus		X	juvenile GC competitively dominant (10)
Homarus americanus	X	X	GC competitively dominant against juveniles and sub-adult lobster; opposite for adults (12,13,14)
Transennella confusa	X		lower densities in enclosure experiment (15)
Transennella tantilla	X		lower densities in enclosure experiment (15)
Cumella vulgaris	X		lower densities in enclosure experiment (15)
Corophium sp.	X		lower densities in enclosure experiment (15)
Gemma gemma			facilitation by green crab, higher densities of organism in enclosure experiment (15)
Lumbrineris zonata			facilitation by green crab, higher densities of organism in enclosure experiment (15)

Table 1-1. Summary of predator (P) and competitor (C) relationships between green crab (GC) and various benthic species (cont.).

Organism	**P**	**C**	**Impact**
Pseudopleuronectes americanus	X		Proteins found in GC stomachs (17)
Pleuronectes platessa	X		Proteins found in GC stomachs (17)

(1) Floyd and Williams 2004; (2) Grosholz et al. 2000; (3) Breen and Metaxas 2008; (4) Frandsen and Dolmer 2002; (5) Vermeij 1982; (6) Smith 2004; (7) Edgell and Rochette 2008; (8) Jensen et al. 2002; (9) McDonald et al. 2001; (10) MacDonald et al. 2007; (11) Quijón et al. unpublished data; (12) Rossong et al. 2006; (13) Williams et al. 2006; (14) Williams et al. 2009; (15) Grosholz and Ruiz 1995; (16) Lockwood et al. 2007; (17) Taylor 2005.

1.3. Current Study

Most competition and predation experiments using green crab and various native benthic invertebrates have been conducted under laboratory conditions and few experiments have looked directly at impacts in the field. Moreover, most previous field studies concentrated on the effects of green crab on individual benthic invertebrate species, and community-level effects examined in my study broaden our understanding of green crab impacts with a specific focus on community structure.

In 2007, green crab were first observed in North Harbour, Newfoundland (NL) and since this initial observation have established high densities. They quickly spread within the next six years from the head of Placentia Bay extending to Lamaline along the west coast, and Placentia on the east coast (Figure 1-1). In addition, isolated populations were discovered in western NL, near Stephenville and Corner Brook. The potential impacts of green crab on habitats and species within Placentia Bay, NL have yet to be determined and are particularly interesting because their arrival marks a northward extension in North America and initial interactions with native species have not been previously documented. The overall goal of this thesis is to determine effects of green crab populations in Placentia Bay on native species through habitat and dietary overlap, agonistic interactions between species, and community level effects on benthic biodiversity. I have organized this thesis into five chapters followed by a summary of my results, where each chapter deals with different aspects of green crab ecology in the hopes of gaining insight into potential impacts of green crab in Placentia Bay.

The exploratory first study (Chapter 2) adds information on population abundances of green crab at two different locations, during two seasons, and over multiple years, to determine the depths at which green crab occur and document any temporal changes in abundance. More importantly, diver transects determine which native species overlap in space with green crab. Once native species overlap with green crab was determined, I examined dietary overlap for common prey items. Specifically, I used stomach contents analyses and stable isotopes to evaluate potential short (days) and long-term (months) dietary overlap, respectively. This experiment provides both baseline data about populations of green crab in the head of Placentia Bay and potential native species impacts through limited habitat and food availability caused by the introduction of green crab.

Chapter 3 examines indirect impacts of shelter and foraging behaviour through laboratory experiments with juvenile lobster. Often invasion ecology focuses on direct impacts; however, alterations in behaviour can ultimately cause detrimental effects that are often overlooked. This chapter uses lobster not previously exposed to green crab, to determine if shelter usage and foraging change with the presence of a caged green crab compared to control trials with no green crab.

Chapter 4 addresses this idea further but in the context of benthic community structure. Through field comparison of habitats with green crab and without green crab, I determined whether green crab change species abundance and overall community structure. I follow this observational experiment with a caging study to determine whether green crab were the direct cause of any alterations in benthic communities.

Chapters 5 and 6 address the green crab invasion throughout Atlantic Canada. As a result of multiple introductions, the genetic structure of green crab populations differ and I therefore examined whether these differences result in any behavioural (Chapter 5) and morphological (Chapter 6) differences among populations. In Chapter 5, I use a limited food source experiment to examine intraspecific competition among green crab from different populations and with different genetic history to determine if behavioural differences exist in foraging ability. Chapter 6 tested whether weapon size influenced foraging ability from the competition experiment. I addressed this question by collecting additional crabs from three provinces and at three sites within each province to examine claw size variability. I then compare the claw morphology results to foraging success to determine whether crabs with bigger claws achieve greater foraging success than individuals with smaller claws.

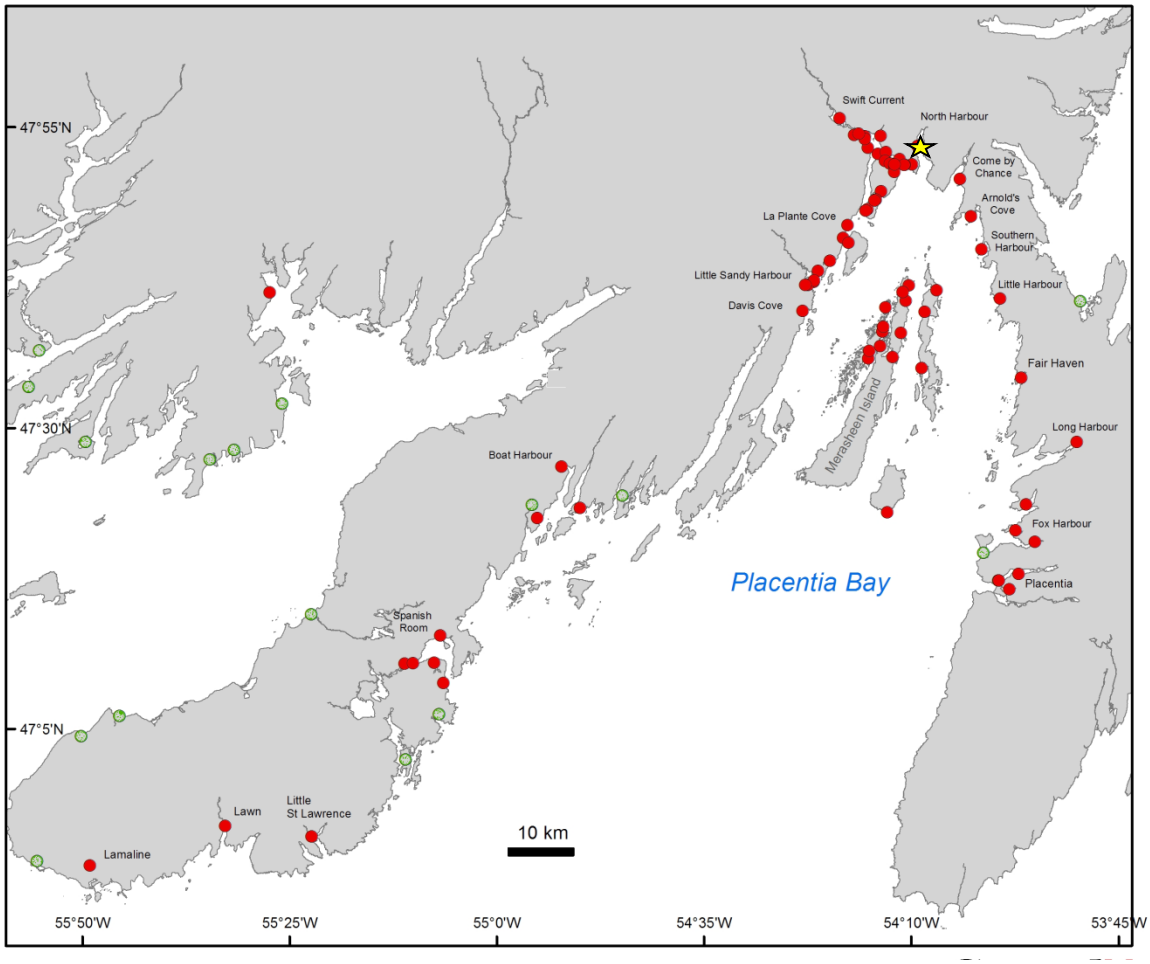


Figure 1-1. The known distribution of green crab in Placentia Bay, NL as of 2013 (solid red circles). (Source C. McKenzie; Department of Fisheries and Oceans Canada). The yellow star denotes the assumed point of origin and the highest population density. Green circles denote sampled areas where no green crab were detected.

1.4. References

Anger K (1991) Effects of temperature and salinity on the larval development of the Chinese mitten crab *Eriocheir sinensis* (Decapoda: Grapsidae) Mar. Ecol. Prog. Ser. 72: 103-110.

Anger K, Spivak E, Luppi T (1995) Effects of reduced salinities on development and bioenergetics of early larval shore crab, *Carcinus maenas*. J. Exp. Mar. Biol. Ecol. 220: 287-304.

Audet D, Davis DS, Miron G, Moriyasu M, Benhalima K, Campbell R (2003) Geographic expansion of a nonindigenous crab, *Carcinus maenas* (L.), along the Nova Scotian shore into the southeastern Gulf of St. Lawrence, Canada. J. Shellfish Res. 22: 255-262.

Baeta A, Cabral HN, Neto JM, Marques JC, Pardal MA (2005) Biology, population dynamics and secondary production of the green crab *Carcinus maenas* (L.) in a temperate estuary. Estuar. Coast. Shelf S. 65: 43-52.

Behrens Yamada S, Dumbauld BR, Kalin A, Hunt CE, Figlar-Barnes R, Randall A (2005) Growth and persistence of a recent invader *Carcinus maenas* in estuaries of the northeastern Pacific. Biol. Invasions 7: 309-321.

Berrill M (1982) The life cycle of the green crab *Carcinus maenas* at the Northern end of its range. J. Crustacean Biol. 2: 31-39.

Blakeslee AMH, McKenzie CH, Darling JA, Byers JE, Pringle JM, Roman J (2010) A hitchhiker's guide to the Maritimes: anthropogenic transport facilitates long-distance dispersal of an invasive marine crab to Newfoundland. Diversity Distrib. 16: 879-891.

Breen E, Metaxas A (2005) Effects of juvenile non-indigenous *Carcinus maenas* on the growth and condition of juvenile *Cancer irroratus*. J. Exp. Mar. Biol. Ecol. 377: 12-19.

Breen E, Metaxas A (2008) A comparison of predation rates by non-indigenous and indigenous crabs (juvenile *Carcinus maenas*, juvenile *Cancer irroratus*, and adult *Dyspanopeus sayi*) in laboratory and field experiments. Estuaries Coasts 31: 728-737.

Bruno JF, Fridley JD, Bromberg KD, Bertness MD (2005) Insights into biotic interactions from studies of species invasions. In: Species Invasions Insights into Ecology, Evolution and

Biogeography, ed. DF Sax, JJ Stachowicz, SD Gaines pp. 13-40, Sinauer Associates, Sunderland Mass.

Carlton JT (1989) Man's role in changing the face of the ocean: Biological invasions and implications for conservation of near-shore environments. Conserv. Biol. 3: 265-273.

Carlton JT (1999) The scale and ecological consequences of biological invasions in the World's oceans. In: Invasive Species and Biodiversity Management, ed. OT Sandlund, PJ Schei, A Viken, pp. 195-212, Dordrecht, The Netherlands: Kluwer Academic.

Carlton JT, Cohen AN (2003) Episodic global dispersal in shallow water marine organisms: the case history of the European shore crabs *Carcinus maenas* and *C. aestuarii*. J. Biogeogr. 30: 1809-1820.

Casey P (2005) Insights into ecology. In: Species Invasions Insights into Ecology, Evolution and Biogeography, ed. DF Sax, JJ Stachowicz, SD Gaines pp. 9-13. Sinauer Associates, Sunderland Mass.

CBC news (2007) Invasive Crabs found in N.L. waters. http://www.cbc.ca/news/canada/ newfoundland-labrador/invasive-crab-found-in-n-l-waters-1.669993

Chapman JW, Carlton JT (1994) Predicted discoveries of the introduced isopod *Synidotea laevidorsalis* (Miers, 1881). J. Crustacean Biol. 700-714.

Clark PF, Rainbow PS, Robbins RS, Smith B, Yeomans WE, Thomas M, Dobson G (1998) The alien Chinese mitten crab *Eriocheir sinensis* (Crustacea: Decapoda: Brachyura), in the Thames catchment. J. Mar. Biol. Assoc. UK 78: 1215-1221.

Clay E (1965) Literature survey of the common fauna of estuaries. 16. *Carcinus maenas* L., Imperial Chemical Industries Ltd., Brixham Laboratory, UK.

Cohen AN, Carlton JT, Fountain MC (1995) Introduction, dispersal and potential impacts of the green crab *Carcinus maenas* in San Francisco Bay, California. Mar. Biol. 122: 225-237.

Davis RC, Short FT, Burdick DM (1998) Quantifying the effects of green crab damage to

eelgrass transplants. Restor. Ecol. 6: 297-302.

de Lafontaine Y (2005) First record of the Chinese mitten crab (*Eriocheir sinensis*) in the St. Lawrence River, Canada. J. Great Lakes Res. 31: 367-370.

DeRivera CA, Ruiz GM, Hines AH, Jivoff P (2005) Biotic resistance to invasion: native predator limits abundance and distribution of an introduced crab. Ecology 86: 3364-3376.

Dittel AI, Epifanio CE (2009) Invasion biology of the Chinese mitten crab *Eriocheir sinensis*: A brief review. J. Exp. Mar. Biol. Ecol. 374: 79-92.

Edgell TC, Rochette R (2008) Differential snail predation by an exotic crab and the geography of shell claw covariance in the Northwest Atlantic. Evolution 62: 1216-1228.

Elner R (1981) Diet of green crab *Carcinus maenas* (L.) from Port Herbert, southwestern Nova Scotia. J. Shellfish Res. 1: 89-94.

Floyd TA, Williams PJ (2004) Impact of green crab (*Carcinus maenas* L.) predation on a population of soft-shell clams (*Mya arenaria* L.) in the southern Gulf of St. Lawrence. J. Shellfish Res. 23: 457-462.

Frandsen RP, Dolmer P (2002) Effects of substrate type on growth and mortality of blue mussels (*Mytilus edulis*) exposed to the predator *Carcinus maenas*. Mar. Biol. 141: 253-262.

Garbary DJ, Miller AG, Seymour N, Williams J (2004) Destruction of eelgrass beds in Nova Scotia by the invasive green crab. In: Status and Conservation of Eelgrass (*Zostera marina*) in Eastern Canada, pp. 13-14. Ed. by A. R. Hanson. Canadian Wildlife Service Technical Report, 412. 40 pp.

Garbary DJ, Miller AG, Williams J, Seymour NR (2013) Drastic decline of an extensive eelgrass bed in Nova Scotia due to the activity of the invasive green crab (*Carcinus maenas*). Mar. Biol. 161: 1-13.

Glass CW, Huntingford FA (1988) Initiation and resolution of fights between swimming crabs (*Liocarcinus depurator*). Ethology 77: 237-249.

Grosholz ED, Ruiz GM (1995) Spread and potential impact of the recently introduced European green crab, *Carcinus maenas*, in central California. Mar. Biol. 122: 239-247.

Grosholz ED, Ruiz GM (1996) Predicting the impact of introduced marine species: Lessons from the multiple invasions of the European green crab *Carcinus maenas*. Biol. Conserv. 78: 59-66.

Grozholz ED, Ruiz GM, Dean CA, Shirley KA, Maron JL, Connors PG (2000) The impacts of a nonindigenous marine predator in a California Bay. Ecology 81: 1206-1224.

Higgins SN, Malkin SY, Howell ET, Guildford SJ, Campbell L, Hiriart-Baer V, Hecky RE (2008) An ecological review of *Cladophora glomerata* (Chlorophyta) in the Laurentian Great Lakes. J, Phycol. 44:839-854.

Hill AM, Lodge DM (1999) Replacement of resident crayfishes by an exotic crayfish: The roles of competition and predation. Ecol. Appl. 92: 678-690.

Hines A, Ruiz G, Hitchcock N, de Rivera C (2004) Projecting range expansion of invasive European Green crabs (*Carcinus maenas*) to Alaska: Temperature and Salinity tolerance of larvae. Environmental Science and Management pp 24.

Huber R, Kravitz EA (1995) A quantitative analysis of agonistic behavior in juvenile American lobster (*Homarus americanus* L.). Brain Behav. Evol. 46: 72-83.

Hudon C (1987) Ecology and growth of postlarval and juvenile lobster, *Homarus americanus*, off Iles de la Madeleine (Quebec). Can. J. Fish. Aquat. Sci. 44: 1855-1869.

Jamieson GS, Grosholz ED, Armstrong DA, Elner RW (1998) Potential ecological implications from the introduction of the European green crab, *Carcinus maenas* (Linneaus), to British Columbia, Canada, and Washington, USA. J. Nat. Hist. 32: 1587-1598.

Jensen GC, McDonald PS, Armstrong DA (2002) East meets west: competitive interactions between green crab *Carcinus maenas*, and native and introduced shore crab *Hemigrapsus spp.* Mar. Ecol. Prog. Ser. 225: 251-262.

Johnson LE, Carlton JT (1996) Post establishment spread in large scale invasions: Dispersal mechanisms of the zebra mussel *Dreissena polymorpha*. Ecology 77: 1686-1690.

Karnofsky EB, Atema J, Elgin RH (1989) Field observations of social behaviour, shelter use, and foraging in the lobster, *Homarus americanus*. Biol. Bull. 176: 239-246.

Klassen G, Locke A (2007) A biological synopsis of the European green crab, *Carcinus maenas*. Can. Manuscr. Rep. Fish. Aquat. Sci. no. 2818: vii+75 pp.

Krohne DT (2001) General Ecology. Wadsworth, Belmont, California.

Lockwood JL, Hoopes MF, Marchetti MP (2007) Invasion Ecology. Blackwell Publishing, UK.

Lodge DM (1993) Biological invasions: Lessons for ecology. Trends Ecol. Evol. 8: 133-137.

Lohrer AM, Whitlatch RB (2002) Relative impacts of two exotic brachyuran species on blue mussel populations in Long Island sound. Mar. Ecol. Prog. Ser. 227: 135-144

Lynch BR, Rochette R (2009) Spatial overlap and interspecific interactions between subadult American lobster and green crab. J. Exp. Mar. Biol. Ecol. 369: 127-135.

MacDonald JA, Roudez R, Glover T, Weis JS (2007) The invasive green crab and Japanese shore crab: Behavioural interactions with a native crab species, the blue crab. Biol. Invasions 9: 837-848.

Malyshev A, Quijón PA (2011) Disruption of essential habitat by a coastal invader: new evidence of green crab effects on eelgrass beds. ICES J. Mar. Sci. 68: 1852-1856.

Matheson K, Gagnon P (2012a) Temperature mediates non-competitive foraging in indigenous rock (*Cancer irroratus* Say) and recently introduced green (*Carcinus maenas* L.) crabs from Newfoundland and Labrador. J. Exp. Mar. Biol. Ecol. 414-415: 6-18.

Matheson K, Gagnon P (2012b). Effects of temperature, body size and chela loss on competition for limited resource between indigenous rock crab (*Cancer irroratus* Say) and recently introduced green crab (*Carcinus maenas* L.). J. Exp. Mar. Biol. Ecol. 428: 49-56.

Maynard Smith J (1974) The theory of games and the evolution of animal conflicts. J. Theor. Biol. 47: 209-221.

McDonald PS, Jensen GC, Armstrong DA (2001) The competitive and predatory impacts of the nonindigenous crab *Carcinus maenas* (L.) on early benthic phase Dungeness crab *Cancer magister* Dana. J. Exp. Mar. Biol. Ecol. 258: 39-54.

McDonald PS, Holsman KK, Beauchamp DA, Dumbauld BR, Armstrong DA (2006) Bioenergetics modeling to investigate habitat use by the nonindigenous crab, *Carcinus maenas*, in Willapa Bay, Washington. Estuaries Coasts 29: 1132-1149.

Nagaraj M (1993) Combined effects of temperature and salinity on the zoeal development of the green crab, *Carcinus maenas* (Linnaeus, 1758) (Decapoda: Portunidae). Sci. Mar. 57: 1-8.

Pickering T, Quijón PA (2011) Potential effects of a non-indigenous predator in its expanded range: assessing green crab, *Carcinus maenas*, prey preference in a productive coastal area of Atlantic Canada. Mar. Biol. 158: 2065-2078.

Pringle JM, Blakeslee AMH,Byers JE, Roman J (2011) Asymetric dispersal allows an upstream region to control population structure throughout a species' range. PNAS 108:15288-15293.

Ricciardi A, Cohen J (2007) The invasiveness of an introduced species does not predict its impact. Biol. Invasions 9: 309-315.

Roman J (2006) Diluting the founder effect: cryptic invasions expand a marine invader's home range. P. Roy. Soc. B-Biol. Sci. 273: 1-7.

Rossong MA, Williams PJ, Comeau M, Mitchell S, Apaloo J (2006) Agonistic interactions between invasive green crabs *Carcinus maenas* (Linnaeus) and juvenile American lobster *Homarus americanus* (Milne Edwards). J. Exp. Mar. Biol. Ecol. 329: 281-288.

Roudez RJ, Glover T, Weis JS (2008) Learning in an invasive and a native predatory crab. Biol. Invasions 10: 1191-1196.

Simberloff D, Von Holle B (1999) Positive interactions of nonindigenous species: Invasional meltdown? Bio. Invasions 1: 21-32.

Smith LD (2004) Biogeographic differences in claw size and performance in an introduced crab predator *Carcinus maenas*. Mar. Ecol. Progr. Ser. 276: 209-222.

Sneddon L, Huntingford FA, Taylor AC (1997) Weapon size versus body size as a predictor of winning in fights between shore crabs, *Carcinus maenas* (L.). Behav. Ecol. Sociobiol. 41: 237-242.

Stachowicz JJ, Bruno JF, Duffy JE (2007) Understanding the effects of marine biodiversity on communities and ecosystems. Annu. Rev. Ecol. Evol. Syst. 38: 739-766.

Tanner JE (2007) The influence of introduced European green crabs (*Carcinus maenas*) on habitat selection by juvenile native blue crabs (*Portunus pelagicus*). Estuaries Coasts 30: 601-606.

Taylor DL (2005) Predatory impact of the green crab (*Carcinus maenas* Linnaeus) on post-settlement winter flounder (*Pseudopleuronectes americanus* Walbaum) as revealed by immunological dietary analysis. J. Exp. Mar. Biol. Ecol. 324: 112-126.

Thorpe KE, Huntingford FA, Taylor AC (1994) Relative size and agonistic behaviour in the female velvet swimming crab, *Necora puber* (L.) (Brachyura, Portunidae). Behav. Process 32: 235-246.

Vermeij GJ (1982) Phenotypic evolution in a poorly dispersing snail after arrival of a predator. Nature 299: 149-150.

Williams PJ, Floyd TA, Rossong MA (2006) Agonistic interactions between invasive green crabs, *Carcinus maenas* (Linnaeus) and sub-adult American lobster, *Homarus americanus* (Milne Edwards). J. Exp. Mar. Biol. Ecol. 329: 66-74.

Williams PJ, MacSween C, Rossong MA (2009) Competition between invasive green crab (*Carcinus maenas*) and American lobster (*Homarus americanus*). New Zeal. J. Mar. Fresh. Res. 43: 29-34.

Co-Authorship Statement

Chapter 2: M. Rossong designed both the spatial and dietary experiments, collected and analyzed data, and prepared the manuscript. P. Quijón and P. Snelgrove contributed ideas, and advised on data analyses. C. McKenzie contributed ideas, particularly in methodology, and assisted with data collection. A. Locke and the rest of the co-authors contributed to the editing of the manuscript.

Chapter 3: M. Rossong designed and set-up the foraging experiments, collected and analyzed data, and prepared the manuscript. P.J. Williams contributed ideas, and assisted with experimental set-up. P. Quijón, P. Snelgrove and the rest of the co-authors contributed to the editing of the manuscript.

Rossong MA, Quijón PA, Williams PJ, Snelgrove PVR (2011) Foraging and shelter behavior of juvenile American lobster (*Homarus americanus*): the influence of a non-indigenous crab. J. Exp. Mar. Biol. Ecol. 403: 75-80.

Chapter 4: M. Rossong designed both the benthic and caging experiment, collected and analyzed data, and prepared the manuscript. P. Quijón and P. Snelgrove contributed ideas, helped with study design, and advised on data analyses. C. McKenzie contributed ideas, and assisted with data collection. A. Locke and the rest of the co-authors contributed to the editing of the manuscript.

Chapter 5: M. Rossong designed intraspecific competition experiments, collected and analyzed data, and prepared the manuscript. T. Barrett assisted with animal collection, and advised on data analyses. C. McKenzie and A. Locke assisted with animal collection.

P. Quijón and P. Snelgrove contributed ideas, and all of the co-authors contributed to the editing of the manuscript.

Rossong MA, Quijón PA, Snelgrove PVR, Barrett TJ, McKenzie CH, Locke A (2012) Regional differences in foraging behaviour of invasive green crab (*Carcinus maenas*) populations in Atlantic Canada. Biol. Invasions 14: 659-669.

Chapter 6: M. Rossong designed claw variability study, collected and analyzed data, and prepared the manuscript. T. Barrett assisted with animal collection, data collection, and advised on data analyses. C. McKenzie and A. Locke assisted with animal collection. P. Quijón and P. Snelgrove contributed ideas, and all of the co-authors contributed to the editing of the manuscript.

2. Spatial and dietary overlap of invasive green crab and native decapods in Placentia Bay, Newfoundland

2.1. Abstract

Invasive European green crab (*Carcinus maenas*) were first observed in Placentia Bay, NL in 2007 and the population quickly increased and spread throughout the bay. The impacts of this invasion on native species and habitats are unknown. This study examines spatial and dietary overlap between green crab and other native decapods in Placentia Bay to determine which species may be affected by this invasion. Surveys conducted by divers indicated habitat overlap among green crab, rock crab (*Cancer irroratus*) and lobster (*Homarus americanus*) at a 0 to 9 m depth range. Although abundances varied with year and season, the data suggest that native species may have shifted to deeper water. We used stomach content and stable isotope analyses to examine short- and long-term dietary similarities, respectively, and showed that all three species shared common prey items. Rock crab and green crab stomachs had 13 of 21 prey items in common, although diet preference varied. Polychaetes and bivalves dominated items in green crab stomachs whereas shrimp, hermit crab, and algae dominated rock crab stomachs. The long-term diet of green crab depended on body size. Small green crab (≤ 50 mm carapace width (CW)) appeared to feed at a similar trophic level as larger green crab but they likely consumed different prey items. The results show moderate overlap in habitat and prey preference between green crab and rock crab. Negative effects potentially associated with this spatial and dietary overlap should be carefully assessed to determine community-level effects.

2.2. Introduction

Habitat plays an important role in the survival of an organism, by providing food resources and protection against predators and environmental disturbances. However, some aspects of habitat may become limiting, especially with the introduction of invasive species with overlapping requirements. Invasive species often prey upon, or compete with, native species for resources such as food and shelter (Cohen et al. 1995). Consequently, if resources are limited, invasive species can ultimately cause declines in populations, niche alteration, behavioural changes, and even local extirpation of native species (Lockwood et al. 2007).

European green crab were first observed and confirmed in Placentia Bay, Newfoundland in 2007 (Klassen and Locke 2007). The population increased rapidly and the green crab invasion raised concerns with local fishermen regarding potential impacts on native species, particularly Atlantic rock crab and American lobster. Rock crab occur in several habitat types but typically occupy sand and mud habitats from the upper intertidal to lower subtidal regions. In Newfoundland, rock crab commonly occupy depths of 5 to 20 m (Robichaud et al. 2000). Lobster are more limited in terms of habitat, relying on cobble and rocky substrate to build burrows. Newly settled (shelter-restricted) lobster depend on burrow building (<20 mm carapace length (CL); Lawton and Lavalli 1995), but as lobster size increases, lobster actively forage farther outside their shelters as energy demands outweigh predation risk (Lawton and Lavalli 1995). Relative to rock crab and lobster, green crab are highly adaptable, have fewer habitat requirements, and a greater tolerance to environmental variability, establishing populations in a wide array of habitats

(e.g. mud, sand, rocky intertidal, and saltmarshes; Klassen and Locke 2008), with varying salinities (4 to 52 ppt; Cohen et al. 1995), and temperatures (0 to 35 ºC; Hidalgo et al. 2005). Unlike crustaceans that require structures to create shelters, green crab can readily bury in most substrates.

Green crab and lobster migrate with depth during tidal cycles (Hunter and Naylor 1993; Jones and Schulman 2008), but green crab, rock crab and the juvenile stage of lobster (<40 mm CL) overlap spatially in the subtidal zone (1.2 m below chart datum; Lynch and Rochette 2009). In winter months, estuarine green crab are assumed to migrate offshore and overwinter in deeper water much like lobster, creating additional opportunity for interaction (Broekhuysen 1936), although the overwintering of green crab in Placentia Bay is still unclear and under investigation (McKenzie pers.comm.). Moreover, in the Bay of Fundy, New Brunswick adult green crab overlap with a range of lobster sizes, including juveniles and adults, and particularly smaller-sized lobster in the lower subtidal zone (Lynch and Rochette 2009). Thus, if species overlap in space, dietary overlap likely occurs for similar-sized crustaceans.

The crab digestive system allows bulk consumption, with a large cardiac stomach for storage and a smaller pyloric stomach that subsequently processes food items (Griffen and Mosblack 2011). This system supports brief bouts of foraging or foraging under ideal conditions with an extended rest period. Short-term diet is often inferred from stomach content analyses, however, many factors may affect diet including habitat, season, sex, moult stage, interspecific interactions and food availability (Baeta et al. 2006; Antonio et al. 2011; Griffen and Mosblack 2011; Watts et al. 2011). Moreover, stomach content

analysis biases heavily towards hard, calcified prey structures (Carmichael et al. 2004; Watts et al. 2011). Stable isotope analysis is often used in conjunction with stomach contents, assuming that levels of light and heavy isotopes of elements in an organism's tissues reflect typical prey consumption (Watts et al. 2011). Similarly, during biological assimilation selective extraction of lighter isotopes leaves heavier isotopes within the tissue for a longer time (Bodin et al. 2007). This time frame depends on tissue type with longer turnover rates in muscle (months) than organ tissues (days or weeks) (Freire et al. 2009).

Carbon-13 (^{13}C) and nitrogen-15 (^{15}N) isotopes have been used as indicators of an organism's food sources and trophic level (Peterson and Fry 1987). The value of $\delta^{15}N$ in marine ecosystems increases with trophic level, and traditionally, a difference of 3.4‰ between organisms indicates feeding at a new trophic level (Watts et al. 2011). The $\delta^{13}C$ is often used to differentiate prey items from benthic versus pelagic sources in marine ecosystems, with less negative values associated with benthic prey (Watts et al. 2011). Although more useful than stomach contents in inferring long-term diet, stable isotope signatures cannot quantify specific prey items and thus provide only a general indicator of similarity in diet. A combination of both methods provides a more complete understanding of diet (Antonio et al. 2011).

This study examines spatial and dietary overlap among green crab, rock crab, and lobster to evaluate potential competition for shelter and food resources in Placentia Bay, Newfoundland. Gut content analyses suggest that in the field, lobster and green crab readily consume similar food resources (Karnofsky 1989; Elner 1981); however, little is

known about green crab diet in Newfoundland. This study documents short-term (stomach contents) and long-term (stable isotopes) food uptake as complementary means to evaluate dietary overlap. The objective of this chapter was to determine 1) whether rock crab and lobster overlap with green crab in space and diet.

2.3. Methods

2.3.1. Spatial Overlap

Surveys were conducted by divers along transects in June and September 2009 (June 24-25th; September 16-17th) and 2011 (June 21st; September 21-22nd), and June 2010 (June 16-17th) at two sites within Placentia Bay (Table 2-1). Multiple hurricanes in succession precluded fall sampling in 2010. All sampling took place late morning to early afternoon. The first site was located at a headland of the small fishing community of North Harbour (47° 50' 49.4" N, 54° 05' 01.5" W) near the head of Placentia Bay where green crab populations were first identified. The second sampling site, Baker's Cove (47° 49' 55.7" N, 54° 07' 15.1" W), was also located at the head of Placentia Bay. Both sites were sheltered, with mixed cobble substrate and algal covered boulders. The June 2009 survey was an exploratory study to examine green crab and juvenile lobster, in collaboration with the Department of Fisheries and Oceans (DFO). No juvenile lobster were found at the North Harbour site in 2009 so my study initially focused only on green crab; however, I expanded sampling to include both rock crab and adult lobster at Baker's Cove when no green crab were found at this site. By the next sampling period in September 2009, green crab were present at both sampling sites, so I retained both sites for the study.

At each site a single 100 m transect (lead line with floats on either end) was placed running parallel to the shore at 1, 3, 6 and 9 m depths (n = 4 transects per site). The North Harbour transects ran parallel to the shore, however, the curved shoreline at the Baker's Cove site required a curved transect line to align with the correct depth. The lead line was deployed from the boat and aligned on the ocean bottom by scuba divers. Water depth was determined with an echosounder on the boat. In some cases the depth varied slightly along the transect (±1 m) depending on the terrain of the substrate. Surface water temperatures were taken at each site and mini logger data was later obtained from Department of Fisheries and Oceans from an area near the North Harbour site.

Divers collected all green crab, rock crab, and lobster observed within 1 m on either side of the transect line. The species, sex, and size (carapace width (CW) for crab and carapace length (CL) for lobster) were recorded, as well as sediment type and the presence of any other species. Species counts were estimated in some cases when organisms escaped from the divers. Fukui traps (63 cm x 46 cm x 23 cm, 1.6 cm mesh opening; see Gillespie et al. 2015) were placed along or near the transect area overnight to determine if additional species were present within the area (some species could have remained hidden with the presence of the divers). Both the sampling methods (i.e. diver transects and Fukui traps) and the depths chosen may have biased species size distributions towards larger adults, potentially missing juvenile stages of all species.

2.3.2. Dietary Overlap

2.3.2.1. Stomach Contents

Green crab (n = 55), rock crab (n = 43), and lobster (n = 2) collected in the September 2009 survey were returned to the laboratory for stomach content analysis. These organisms were frozen and stored at DFO, St. John's, NL until analysis. An independent consultant specializing in stomach content analysis, Dr. Hubert Squires (Fisheries and Oceans, retired, since deceased, pers. comm.), analyzed the stomach contents of all organisms in March 2010. Stomachs were removed and dissected and organisms were identified to species level in most cases. Prey items were quantified when possible, or simply categorized as present or absent. If a species was present but the number of individuals could not be quantified then one individual for that species was used in the analyses. Overall, unidentified material comprised approximately 2%.

2.3.2.2. Stable Isotopes

During the 2011 transect surveys, divers collected green crab and rock crab from North Harbour, along with various potential prey items, recording date, site, depth, and time. Lobster could not be included in the stable isotope analysis because no lobster were collected during this sampling period. Potential prey items were selected based on preliminary information from the stomach content analyses conducted the previous year, but also included other taxa found along the transects. Sediment samples were taken from each site to collect infaunal organisms to include as potential prey items. All samples were frozen prior to sample preparation. In the laboratory, muscle tissue was removed

from the crusher claw of green and rock crab. The entire organism was used for prey items. Potential prey items were dissected to extract tissues from shells where necessary (e.g. bivalves, crustaceans, gastropods). All tissue samples were placed in labeled glass vials and dried in an oven for 48 hours at 60°C. Dried samples were then crushed into a fine powder using mortar and pestle and weighed to 0.4 mg in a tin capsule. These samples were then labeled and transported in a well plate for carbon (C) and nitrogen (N) stable isotope analysis at the Canadian Rivers Institute's Stable Isotopes in Nature Laboratory in Fredericton, NB. Masses of each isotope were determined using a mass spectrometer using standard methodology (Jardine et al. 2003). Results are reported as the ratio of the heavy to light isotope relative to the ratio of a standard material using the delta notation (δX) and are expressed in units of parts per thousand (‰):

$$\delta X = [(R_{sample}/R_{standard}) - 1] \times 1000$$

where X is the heavy isotope (^{13}C or ^{15}N) and R denotes the ratio of the heavy to light isotope (^{13}C /^{12}C or ^{15}N /^{14}N) of the sample or standard material (Jardine et al. 2003). The laboratory provided values for $\delta^{13}C$, $\delta^{15}N$, % C, % N and the C:N ratio for each sample. The mean $\delta^{13}C$ and $\delta^{15}N$ and standard error of the mean were calculated for each species and graphed as a scatterplot.

2.3.3. Statistical Analyses

2.3.3.1. Spatial Overlap

Spatial overlap in body size between green crab and rock crab was assessed by comparing carapace width using a three-way analysis of variance (ANOVA) with complete interactions using depth (0, 3, and 6 m), species (green crab and rock crab), and month (June and September) as factors. Crab at 9 m depth were removed from the analysis because of low sample size. Data were pooled across years and sites because insufficient degrees of freedom precluded inclusion of these factors in the ANOVA model. When a significant three-way interaction was detected, an interaction plot was used to identify the levels of the factors that strongly contributed to the interaction. Two-way ANOVAs were then conducted separately for the factor that strongly contributed to the interaction. For the two-way ANOVAs, effects for each factor were interpreted when the interaction term was not significant and pairwise comparisons were conducted to assess differences among levels. When the interaction term was significant, pairwise comparisons were conducted for the crossed levels for the two factors.

Abundances of green crab and rock crab were also assessed using a three-way ANOVA with complete interactions using depth (0, 3, and 6 m), species (green crab and rock crab), and month (June and September) as factors. Only the main effect of species was significant; therefore, abundances were compared separately by species using two-way ANOVAs with site and year as factors.

Statistical analyses were conducted using Minitab 15 software (State College, PA) using a significance level of $\alpha = 0.05$. Tukey's honestly significant difference method was

used for all pairwise comparisons using a family-wise error rate of 0.05. The residuals of the ANOVA models were assessed for normality and homogeneity of variances using Anderson-Darling and Levene's tests, respectively. Abundances were $\log_{10}$-transformed to meet these assumptions.

2.3.3.2. Dietary Overlap

Stomach contents were examined using multi-dimensional scaling (MDS) plots and analysis of similarities (ANOSIM) in PRIMER (version 5.2.4; Clarke 1993) to explore similarity in prey species for green crab and rock crab. Differences in prey species composition were assessed using one way ANOSIMs (4^{th} root transformation) with the following factors: 1) species (rock crab or green crab); 2) carapace width (CW; >50 or <50 mm CW); 3) depth (1, 3, 6, or 9 m); and 4) sampling location (Baker's Cove or North Harbour). The $\delta^{13}C$ and $\delta^{15}N$ signatures were compared using two-sample t-tests to assess differences between species and between body size categories.

2.4. Results

2.4.1. Spatial Overlap

2.4.1.1. Size Distribution

A three-way ANOVA on carapace width detected a significant interaction ($F_{2,676}$ = 6.72; p < 0.001) among depth, species, and month (Table 2-1). An interaction plot identified that differences in carapace width at 6 m depth were dependent on month (Figure 2-2). Crab were larger in June only at 6 m depth compared to September. Two-

way ANOVAs were therefore conducted separately by month (Table 2-2), and indicated a significant interaction ($F_{2,294}$ = 6.19; p = 0.002) between depth and species in June. Pairwise comparisons showed that rock crab at 6 m depth were significantly larger than all other combinations of species and depths, whereas green crab at 1 m depth were significantly smaller than all other combinations of species and depths (except rock crab at 3 m). In September, the interaction between depth and species was not significant ($F_{2,382}$ = 0.82; p = 0.442). Rock crab were significantly larger than green crab ($F_{1,382}$ = 28.70; $p < 0.001$) and carapace width differed significantly among depths ($F_{2,382}$ = 5.27; p = 0.006). Pairwise comparisons revealed significantly smaller crab collected at 1 m than at 3 m.

2.4.1.2. Crab Abundances

The interaction terms in the three-way ANOVA on abundance with factors depth, species, and month were not significant (Table 2-3). Only the main effect of species was significant ($F_{1,41}$ = 4.58; p = 0.041). Abundances of green crab were significantly greater ($F_{1,16}$ = 6.03; p = 0.026) at North Harbour relative to Baker's Cove and abundances did not differ significantly ($F_{2,16}$ = 2.12; p = 0.153) among years (Table 2-4; Figure 2-3A). For rock crab, the interaction between site and year was significant ($F_{2,14}$ = 4.32; p = 0.035; Table 2-4). Pairwise comparisons revealed that rock crab abundance at Baker's Cove in 2009 was significantly greater than in 2010 and 2011; differences were not significant between Baker's Cove and North Harbour or within years at North Harbor (Figure 2-3B; Figure 2-4; Figure 2-5).

2.4.1.3. Overlap Between Crab and Lobster

Despite generally low numbers, lobster were nonetheless present. Juvenile lobster in low abundances are notoriously difficult to collect because of their cryptic nature (Wahle and Steneck 1991), but given their ecological and economic importance in the region I chose to include them, noting the need for cautious interpretation with such small sample size.

Adult lobsters occurred in low abundances in Baker's Cove in June and September 2009 and June 2011. We found lobster in June 2009 at both 1 and 3 m depths, only at 3 m depth in September 2009, and at a depth of 6 m in June 2011(Figure 2-5). No lobsters were present in North Harbour transects. Lobsters occurred at the same depths as rock crab, however, overlap in all species occurred in fall 2009.

2.4.2. Dietary Overlap

2.4.2.1. Stomach Contents

Sixty-nine percent of the sampled green crab stomachs (n = 38) contained prey representing 62 prey organisms. Empty stomachs were found in 27% of green crab from Baker's Cove (n = 15) and only 4% from North Harbour (n = 2). The most common food items were polychaetes (dominated by the Genus *Nereis*, in 42% of stomachs) and soft shell clams (*Mya arenaria*; in 29% of stomachs). Other taxa present in lower abundances included crustaceans (*Crangon septemspinosa*, *Eualus pusiolus, Pagurus* sp., and *Homarus americanus*), algae (filamentous green and fucoids), bivalves of the genus *Mytilus*, gastropods of the genera *Thais* and *Littorina*, and sponges.

Rock crab stomachs contained a total of 49 organisms. Of the 43 rock crab stomachs sampled, 81% of stomachs contained prey items. Empty stomachs were found in 12% of rock crab from Baker's Cove (n = 5) and 7% from North Harbour (n = 3). Crustaceans (*Crangon septemspinosa* and *Eualus pusiolus* in 31% of stomachs), plants/algae (*Zostera marina* and unknown filamentous algae in 23% of stomachs), polychaetes (*Nereis* sp. in 17% of stomachs) and sponges (in 14% of stomachs) dominated prey items. Other prey included crustaceans (*Carcinus maenas*, *Pagurus* sp., *Homarus americanus*), bivalves (*Mya arenaria*), gastropods (*Thais* sp., *Littorina* spp., *Acmae* sp., *Euspira heros*) and fucoids.

Only two lobster were examined for stomach contents. The most abundant prey item for lobster was *Thais* sp. with a total of 11 opercula in two stomachs. Other prey items included plants (*Zostera marina*), fish (*Gadus* sp.), echinoderms (*Stronglyocentrotus droebachiensis*), gastropods (*Littorina* sp.) and crustaceans (*Pagurus* sp.; *Eualus pusiolus*).

Green crab, rock crab, and lobster shared five prey species with the greatest overlap being in gastropod prey (*Thais* sp., *Acme* sp., and *Littorina* spp.) as well as green algae and hermit crab (*Pagarus* sp.) (Table 2-5). Rock crab and green crab diet overlapped most, sharing 13 of 21 possible prey items that included algae, polychaetes, bivalves and sponges in addition to the taxa listed above. The potential overlap in prey species differed between sites (Figure 2-6). At Baker's Cove, stomach contents of both crab species consisted primarily of bivalves and crustaceans with almost 15% more bivalves in green crab stomachs and 5% more crustaceans in rock crab stomachs. In

contrast, stomach contents differed at North Harbour with polychaetes (43%) dominating most green crab stomachs and crustaceans (42%) dominating rock crab stomachs (Figure 2-6).

Green crab diet varied with size. Smaller green crab (≤50 mm; Table 2-5) fed on gastropods and sponges, which were absent from stomachs of larger green crab (>50 mm). The stomach contents of rock crab and lobster shared similar items compared to small and large green crab; however, smaller green crab shared more prey items with lobster. This relationship was assessed further with an analysis of similarity, however, low global R values associated with low sample sizes precluded detecting any significant relationships among species and size ranges.

2.4.2.2. Stable Isotope Analysis

The $\delta^{13}C$ values for green crab (n = 20) ranged from -14.62 to -18.22 ($\bar{x}$ = -16.59) and the $\delta^{15}N$ values ranged from 10.61 to 12.73 ($\bar{x}$ = 11.55; Figure 2-10). The $\delta^{13}C$ values for rock crab (n = 9) ranged from -15.21 to -18.10 ($\bar{x}$ = -16.88) and 11.51 to 12.77 for $\delta^{15}N$ ($\bar{x}$ = 11.88; Figure 2-10). A two-sample t-test showed no significant difference in $\delta^{13}C$ (t_{27} = 0.90, p = 0.375) and $\delta^{15}N$ (t_{27} = 1.83, p = 0.079) signatures between rock and green crab. Linear regressions of $\delta^{13}C$ and $\delta^{15}N$ on CW were significant ($F_{1,18}$ = 8.74, p = 0.008 and $F_{1,18}$ = 8.40, p = 0.010, respectively) for green crab, suggesting that CW (i.e. body size) was a significant predictor of isotopic signature. Examination of the scatterplot of the isotopic signatures versus CW revealed two groupings of crab (separated at a CW of approximately 50 mm; Figure 2-8 and Figure 2-9). The linear regression of $\delta^{13}C$ on CW for rock crab was not significant ($F_{1,7}$ = 1.15, p = 0.319), in

contrast to a significant linear regression of $\delta^{15}N$ on CW for rock crab ($F_{1,7}$ = 34.48, p = 0.001).

Mean isotope signatures were compared after separating green crab into two size categories. “Small” green crab were defined to be ≤50 mm CW and “large” green crab > 50 mm CW. The mean $\delta^{13}C$ signature for small green crab (n = 8) was -17.40 and large green crab (n = 12) was -15.85; these means differed significantly (t_{18} = 3.82, p = 0.001) Figure 2-10). The $\delta^{15}N$ signature for small (mean = 11.5, n = 8) and large (mean = 11.82, n = 12) green crab differed significantly (t_{18} = 3.63, p = 0.002).

2.5. Discussion

2.5.1. Crab Abundances

Densities of green crab did not statistically differ among years despite harvesting strategies by the Fish, Food and Allied Workers Union (FFAW) and Department of Fisheries and Oceans (DFO) to suppress green crab in North Harbour from 2009 to 2011, but overall green crab were significantly more abundant at North Harbour than at Baker’s Cove. Green crab were first observed in North Harbour, and populations have increased over subsequent generations.

In Baker’s Cove, green crab were absent in June of 2009 but present in low numbers in September transects later that year. This change may have resulted from green crab overwintering behavior or from spread of green crab within Placentia Bay. Since the initial surveys in 2009, green crab have spread to most locations along the western portion of Placentia Bay and as far south as the town of Placentia on the eastern side (C McKenzie pers. comm.). No differences in annual abundance in North Harbour suggest

larval rather than adult movement to the Baker's Cove location, however, additional research would be required to determine the extent of both stages of movement.

2.5.2. Spatial Overlap

Green crab overlapped with rock crab spatially at both sites. At Baker's Cove, rock crab abundance declined after 2009, and rock crab and lobster distributions apparently shifted to deeper water, although seasonal variability was high. It is unclear from the results of this study whether the presence of green crab from September 2009 onward caused the local decline in rock crab within the transects sampled (up to 9 m) or the change in depths observed. Green crab typically occupy shallower depths than rock crab, suggesting a greater likelihood that rock crab shift to deeper water to minimize interactions. Although other studies in the southern Gulf of St. Lawrence suggest coexistence between green crab and rock crab in invaded areas, Bélair and Miron (2009) suggest greater abundance of green crab in warmer months and higher rock crab activity when temperatures begin to decrease after September. Although rock crab and lobster utilize similar rocky habitats, some studies suggest that these species may co-occur because differences in resource use allow co-existence with minimal competition (Hudon and Lamarche 1989; Bélair and Miron 2009).

Larger rock crab (>68 mm CW) show no differences in foraging when exposed to chemical cues from green crab or other conspecifics (Matheson and Gagnon 2012a) suggesting that, at least temporarily upon first encounters, green crab presence has no effect on rock crab feeding. In contrast, green crab appear to affect juvenile rock crab (< 19 mm CW) in the Bras D'Or Lakes, NS area. In the presence of green crab, juvenile rock

crab intermoult periods were longer compared to individuals held only with conspecifics, however, longer intermoult periods stopped when rock crab exceeded green crab in size (Breen and Metaxas 2009). Similarly, in Newfoundland, small rock crab (<50 mm CW) exhibited five times higher rates of sheltering behaviour (burying in sediments) than green crab (Matheson and Gagnon 2012a; Matheson and Gagnon 2012b) suggesting that high densities of green crab may limit rock crab initial growth and their vulnerability to predation.

Green crab overlapped with lobster only at Baker's Cove. North Harbour was selected as one of the study areas precisely because lobster were reportedly fished near transect locations. It is possible that the fishery or an unknown seasonal shift in distribution may have displaced substantial numbers of these lobster. In addition, even though my initial intention was to examine overlap with juvenile lobster the absence of juveniles compelled me to focus on adult lobster, which occupy much shallower water in Placentia Bay than previously thought. Juvenile lobster may indeed have been absent, or they may have been camouflaged within the algal covered substrate, thus making identification by divers difficult. Recurrent visual observations suggest that adult lobster occurred in greater numbers than reported on transects, however, divers were unable to catch them.

In laboratory studies, green crab negatively impacted juvenile lobster (25-55 mm CL). In a shelter experiment (Rossong et al. 2006), juvenile lobster that remained within an artificial shelter had higher survival; green crab often captured and consumed those that spent less time within their shelter. Similarly, juvenile lobster with no previous

exposure to green crab spent less time foraging and more time in shelter in the presence of a caged green crab (Rossong et al. 2011; see Chapter 3).

2.5.3. Dietary Overlap

Stomach contents and stable isotope analyses both indicated dietary overlap between green crab and rock crab. Rock crab and green crab diets were comprised mainly of polychaetes, bivalves, and crustaceans. These stomach contents were consistent with findings for rock crab in New York and the southern Gulf of St. Lawrence (Hudon and Lamarche 1989; Stehlik 1993), and green crab in the US, Canada, and Europe (Ropes 1968; Elner 1981; Chaves et al. 2010), with some differences in dominant prey items. Diets differed somewhat between large rock crab (>50 mm) and green crab although further examination of stomach contents indicated no clear distinctions. This lack of difference may reflect variability in diet and low sample size that resulted in some prey items occurring in only one stomach, limiting our ability to detect any dietary differences.

The results of the present study suggest that diet may shift with size in green crab. Ontogenetic shifts in diet have been reported for blue crab, *Callinectes sapidus* (Douglass et al. 2011), where diet of crab <20 mm consisted of sediment, macrophytes, amphipods, and polychaetes, and switched to crabs and barnacles in individuals >20 mm. In my study, stomachs of smaller green crab contained a greater variety of food items than large individuals, suggesting overlap with both rock crab and lobster during a limited period of green crab ontogeny.

Comparison of isotope signatures across size classes mirrored those changes, with lower carbon and nitrogen in small green crab compared with large green crab. The

similarity in $\delta^{15}N$ signatures suggests similar trophic positions, however, the discrepancy in $\delta^{13}C$ values indicates consumption of different prey items (Fantle et al. 1999). The $\delta^{13}C$ values in large green crab were consistent with primarily benthic feeding, whereas more negative $\delta^{13}C$ values suggest prey items of both benthic and pelagic origin (Watts et al. 2011). For example, smaller individuals of spider crab (*Maja brachydactyla*) had lower carbon and nitrogen signatures than their larger counterparts (Bodin et al. 2007). These authors attributed differences in carbon values to a shift towards prey items that typically feed on benthic algae or organic matter. This interpretation is consistent with the stomach contents of smaller green crab in my study (i.e. rich in gastropods and sponges; Bodin et al. 2007).

With regard to lobster populations, the low sample size of lobster precludes any conclusive evaluation of dietary overlap. However, the stomach contents from a single lobster suggests consumption of prey similar to that of green crab, such as gastropods and hermit crab. Lobster was also identified as a potential food item in two green crab stomachs. Identification was based on pieces of exoskeleton within the stomachs so it is possible that it may have been misidentified, the crabs could have scavenged on a dead lobster or a recent molt, or that they indeed feed on smaller sized lobster. Therefore interpretation of stomach contents analyses based on just a few individuals requires great caution.

Unfortunately, divers were unable to obtain additional lobster for stable isotope analysis from my two study sites. In a preliminary study, isotope signatures suggest overlap in long-term diet with both green and rock crab, however, future sampling is

necessary with a larger sample size. Previous studies from locations in western NL, Placentia Bay, NL, Northumberland Strait, and the Gulf of St. Lawrence show that lobster stomachs contain food items similar to those found in green crab stomachs: polychaetes, periwinkles, rock crab, mussels, and echinoderms, suggesting dietary overlap with a preference for energy rich items (Squires 1970; Scarratt and Lowe 1972; Carter and Steele 1982; Hudon and Lamarche 1989).

Green crab exhibit competitive advantages under laboratory settings when interacting with species from a similar resource guild. With a limited food source, green crab typically located food first and dominated foraging time over Dungeness crab (*Cancer magister*) and blue crab (*Callinectes sapidus*) as well as juvenile and sub-adult lobster (McDonald et al. 2001; Rossong et al. 2006; MacDonald et al. 2007; Williams et al. 2009). In interspecific encounters between NL green crab and "naïve" (not previously exposed to green crab) rock crab, green crab encountered the food source first in over 90% of trials when matched with various size ranges of rock crab. However, dominance changed with both size and temperature. Interestingly, larger rock crab (>90 mm CW) had greater foraging success than green crab in lower water temperatures (4°C) than in higher water temperatures (>12°C; Matheson and Gagnon 2012b). This difference suggests that green crab could potentially out-compete rock crab regardless of size during warmer months (June- September), when resources are most abundant and needed most for reproduction. The low temperatures reported in that study (<4 °C) occur at my study site only from December until June. In Denmark, foraging rates of green crab are thought to decrease 15 to 20 times during this period, and green crab often bury within sediments

until temperatures increase above 8°C (Aagaard et al. 1995). Thus, even with the spatial and diet overlap reported here, interactions may be temporally limited to periods of warm conditions when both crab species forage actively.

2.6. Conclusions

This study suggests that green crab overlap habitats occupied by rock crab and lobster, and green crab share prey with both species. My study did not specifically address prey preference and dominant prey species, and thus could not fully evaluate the extent of overlap. Future research with increased sample sizes, and from various locations with complementary prey preference experiments, would provide more detailed information on dietary impacts of rock crab and lobster with green crab. With densities as high as those observed in Placentia Bay, green crab likely encounter native species frequently during time periods of active foraging. If competitive dominance reported in previous studies proves correct, lower availability of both food and shelter for native species may occur. Determining the extent of this habitat overlap, and how the recent invasion may influence predation rates or potential competition pressures, will require further laboratory and field experiments.

2.7. References

Aagaard A, Warman CG, Depledge, MH (1995) Tidal and seasonal changes in the temporal and spatial distribution of foraging *Carcinus maenas* in the weakly tidal littoral zone of Kerteminde Fjord, Denmark. Mar. Ecol. Prog. Ser. 122: 165-172.

Antonio ES, Kasai A, Ueno M, Ishihi Y, Yokoyama H, Yamashita Y (2011) Diet shift in the sand shrimp *Crangon urital* along the estuary-marine gradient. J. Crustacean Biol. 31: 635-646.

Baeta A, Cabral HN, Pardal MA (2006) Ecology of the green crab, *Carcinus maenas* (L, 1758) in a temperate estuary, Portugal. Crustaceana 79: 1181-1193.

Bélair M, Miron G (2009) Predation behaviour of *Cancer irroratus* and *Carcinus maenas* during conspecific and heterospecific challenges. Aquat. Biol. 6: 41-49

Bodin N, LeLoc F, Hily C, Caisey X, Latrouite D, Le Guellec A (2007) Variability of stable isotope signatures (δ13C and δ15N) in two spider crab populations (*Maja brachydactyla*) in Western Europe. J. Exp. Mar. Biol. Ecol. 343: 149-157.

Broekhuysen GL (1936) On development, growth, and distribution of *Carcinus maenas* (L.) Archs. Neer. Zool. 2: 257-399.

Breen E, Metaxas A (2009) Effects of juvenile non-indigenous *Carcinus maenas* on the growth and condition of juvenile *Cancer irroratus*. J. Exp. Mar. Biol. Ecol 377: 12-19.

Carmichael RH, Rutecki D, Annett B, Gaines E, Valiela I (2004) Position of horseshoe crab in estuarine food webs: N and C stable isotopic study of foraging ranges and diet composition. J. Exp. Mar. Biol. Ecol 299: 231-253.

Carter JA, Steele DH (1982) Stomach contents of immature lobster (*Homarus americanus*) from Placentia Bay, Newfoundland. Can. J. Zool. 60: 337-347.

Chaves ML, Horta MS, Chainho P, Costa MJ, Costa LJ (2010) New additions to the feeding ecology of *Carcinus maenas* (L. 1758) in a south-western Europe estuary (Portugal). Cahiers de Biologie Marine 51: 229-238.

Clarke KR (1993) Non-parametric multivariate analyses of changes in community structure. Aust. J. Ecol. 18: 117-143.

Cohen AN, Carlton JT, Fountain MC (1995) Introduction, dispersal and potential impacts of the green crab *Carcinus maenas* in San Francisco Bay, California. Mar. Biol. 122: 225-237.

Douglass JG, Duffy JE, Canuel EA (2011) Food web structure in a Chesapeake Bay eelgrass bed as determined through gut contents and 13C and 15N isotope analysis. Estuaries Coasts 34: 701-711.

Elner RW (1981) Diet of green crab *Carcinus maenas* (L.) from Port Hebert, southwestern Nova Scotia. J. Shellfish Res. 1: 89-94.

Fantle MS, Dittel AI, Schwalm SM, Epifanio CE, Fogel ML (1999) A food web analysis of the juvenile blue crab, *Callinectes sapidus*, using stable isotopes in whole animals and individual amino acids. Oecologia 120: 416-426.

Freire J, Carabel S, Verísimo P, Bernárdez C, Fernández L (2009) Patterns of juvenile habitat use by the spider crab *Maja brachydactyla* as revealed by stable isotope analyses. Sci. Mar. 73: 39-49.

Gillespie GE, Norgard TC, Anderson ED, Haggarty DR, Phillips AC (2015) Distribution and biological characteristics of European green crab, *Carcinus maenas*, in British Columbia, 2006-2013. Canadian Technical Report of Fisheries and Aquatic Sciences 3120.

Griffen BD, Mosblack H (2011) Predicting diet and consumption rate differences between and within species using gut ecomorphology. J. Anim. Ecol. 80: 854-863.

Hidalgo FJ, Barón PJ, Orensanz JML (2005). A prediction come true: the green crab invades the Patagonian coast. Biol. Inv. 7: 547-552.

Hudon C, Lamarche G (1989) Niche segregation between American lobster *Homarus americanus* and rock crab *Cancer irroratus*. Mar. Ecol. Prog. Ser. 52: 155-168.

Hunter E, Naylor E (1993) Intertidal migration by the shore crab *Carcinus maenas*. Mar. Ecol. Prog. Ser 101: 131-138.

Jardine, TD, McGeachy, SA, Paton, M, Savoie, M, Cunjak, RA (2003) Stable isotopes in aquatic systems: sample preparation, analysis, amd interpretation. Can. Manuscr. Rep. Fish. Aquat. Sci. No. 2656: 39p.

Jones PL, Schulman MJ (2008) Subtidal-intertidal trophic links: American lobsters [*Homarus americanus* (Milne-Edwards)] forage in the intertidal zone on nocturnal high tides. J. Exp. Mar. Biol. Ecol. 361: 98-103.

Karnofsky EB, Atema J, Elgin RH (1989) Field observations of social behaviour, shelter use, and foraging in the lobster, *Homarus americanus*. Biol. Bull. 176: 239-246.

Klassen G, Locke A (2007) A biological synopsis of the European green crab, *Carcinus maenas*. Can. Manuscr. Rep. Fish. Aquat. Sci. no. 2818.

Lawton P, Lavalli KL (1995) Postlarval, juvenile, adolescent and adult ecology. In: Factor, J.R. (Ed.), Biology of the Lobster, *Homarus americanus*. Academic Press Inc, San Diego, pp. 47-88.

Lockwood JL, Hoopes MF, Marchetti MP (2007) Invasion Ecology. Blackwell Publishing, UK.

Lynch BR, Rochette R (2009) Spatial overlap and biotic interactions between sub-adult American lobsters, *Homarus americanus*, and the invasive European green crab *Carcinus maenas*. J. Exp. Mar. Biol. Ecol. 369: 127-135.

MacDonald JA, Roudez R, Glover T, Weis JS (2007) The invasive green crab and Japanese shore crab: behavioral interactions with a native crab species, the blue crab. Biol. Invasions 9: 837-848.

Matheson K, Gagnon P (2012a) Effects of temperature, body size and chela loss on competition for limited resource between indigenous rock crab (*Cancer irroratus* Say) and recently introduced green crab (*Carcinus maenas* L.). J. Exp. Mar. Biol. Ecol. 428: 49-56.

Matheson K, Gagnon P (2012b) Temperature mediates non-competitive foraging in indigenous rock (*Cancer irroratus* Say) and recently introduced green (*Carcinus maenas* L.) crab from Newfoundland and Labrador. J. Exp. Mar. Biol. Ecol. 414-415: 6-18.

McDonald PS, Jensen GC, Armstrong DA (2001) The competitive and predatory impacts of the nonindigenous crab *Carcinus maenas* (L.) on early benthic phase Dungeness crab *Cancer magister* Dana. J. Exp. Mar. Biol. Ecol. 258: 39-54.

Peterson BJ, Fry B (1987) Stable isotopes in ecosystem studies. Annu. Rev. Ecol. Syst. 18: 293-320.

Robichaud DA, Lawton P, Strong MB (2000) Exploratory fisheries for rock crab, *Cancer irroratus*, and Jonah crab, *Cancer borealis*, in Canadian Lobster Fishing Areas 34, 35, 36 and 38. DFO Can. Sci. Advis. Sec. Res. Doc. 2000/051: 27 pp.

Rossong MA, Quijón PA, Williams PJ, Snelgrove PVR (2011) Foraging and shelter behaviour of juvenile American lobster (*Homarus americanus*): the influence of a non-indigenous crab. J. Exp. Mar. Biol. Ecol. 403: 75-80.

Rossong MA, Williams PJ, Comeau M, Mitchell S, Apaloo J (2006) Agonistic interactions between invasive green crab *Carcinus maenas* (Linnaeus) and juvenile American lobsters *Homarus americanus* (Milne Edwards). J. Exp. Mar. Biol. Ecol. 329: 281-288.

Ropes JW (1968) The feeding habits of the green crab *Carcinus maenas* (L.). Fish Wildl. Sew. Fish. Bull. 67: 183-203.

Scarratt D J, Lowe R (1972) Biology of the rock crab (*Cancer irroratus*) in Northumberland Strait. J. Fish. Res. Bd. Can. 29: 161-166.

Squires HJ (1970) Lobster (*Homarus americanus*) fishery and ecology in Port au Port Bay, Newfoundland, 1960-65. Proc. Natl. Shellfish. Ass. 60: 22-39.

Stehlik LL (1993) Diets of the brachyuran crabs *Cancer irroratus, C. borealis* and *Ovalipes ocellatus* in the New York Bight. J. Crust. Biol. 13: 723-735.

Wahle RA, Steneck RS (1991) Recruitment habitats and nursery grounds of the American lobster Homarus americanus: A demographic bottleneck. Mar. Ecol. Prog. Ser. 69: 231-243.

Watts AJR, Mccafferty DJ, Newton J, Bailey DM (2011) Does seabird carrion contribute to the diet of the shore crab *Carcinus maenas* on the Isle of May, Scotland? An isotopic perspective. J. Mar. Biol. Assoc. UK 91: 1459-1464.

Welch WR (1968) Changes in abundance of the green crab, *Carcinus maenas* (L.) in relation to the recent temperature changes. Fish. Bull. 67: 337-345.

Williams PJ, MacSween C, Rossong MA (2009) Competition between invasive green crab (*Carcinus maenas*) and American lobster (*Homarus americanus*). New Zeal. J. Mar. 43: 29-33.

Table 2-1. Analysis of variance table for the three-way analysis of variance on carapace width with factors depth, species, and month.

Source	**Degrees of Freedom**	***F*-statistic**	***p*-value**
Depth	2	18.41	<0.001
Species	1	59.65	<0.001
Month	1	1.98	0.160
Depth*Species	2	2.52	0.081
Depth *Month	2	12.45	<0.001
Species*Month	1	1.54	0.206
Depth*Species*Month	2	6.72	0.001
Error	676		

Table 2-2. Analysis of variance table for the two-way analysis of variance on carapace width conducted separately by month with factors depth and species.

Month	**Source**	**Degrees of Freedom**	***F*-statistic**	***p*-value**
June	Depth	2	19.29	<0.001
	Species	1	30.70	<0.001
	Depth*Species	2	6.19	0.002
	Error	294		
September	Depth	2	5.27	0.006
	Species	1	28.70	<0.001
	Depth*Species	2	0.82	0.442
	Error	382		

Table 2-3. Analysis of variance table for the three-way analysis of variance on abundance (log_{10}-transformed) with factors depth, species, and month.

Source	**Degrees of Freedom**	***F*-statistic**	***p*-value**
Depth	2	1.46	0.249
Species	1	4.58	0.041
Month	1	0.49	0.488
Depth*Species	2	0.11	0.898
Depth *Month	2	1.57	0.226
Species*Month	1	1.02	0.320
Depth*Species*Month	30	1.90	0.168
Error	41		

Table 2-4. Analysis of variance table for the two-way analysis of variance on abundance (log_{10}-transformed) conducted separately by species with factors site and year.

Month	**Source**	**Degrees of Freedom**	***F*-statistic**	***p*-value**
Green crab	Site	1	6.03	0.026
	Year	2	2.12	0.153
	Site*Year	2	0.25	0.785
	Error	16		
Rock crab	Site	1	1.40	0.256
	Year	2	5.18	0.021
	Site*Year	2	4.32	0.035
	Error	14		

Table 2-5. Prey items within the stomachs of invasive green crab (*n* = 38), native rock crab (*n* = 43), and lobster (*n* = 2) from two sites within Placentia Bay, NL.

Organism	Green Crab (≤ 50 mm)	Green Crab (> 50 mm)	Rock Crab	Lobster
Gastropods				
Thais sp.	X_a		X_a	X_b
Whelk (unknown)	X_b		X_b	X_b
Littorina sp.	X_b		X_b	X_b
Acmae sp.			X_b	
Plants and Algae				
Zostera marina			X_{ab}	
Green algae	X_b	X_{ab}	X_{ab}	X_{ab}
Brown algae	X_{ab}	X_a	X_{ab}	
Bivalves				
Mya arenaria	X_{ab}	X_{ab}	X_{ab}	
Mytilus sp.	X_b	X_{ab}		
Crustaceans				
Amphipod (unknown)			X_a	
Carcinus maenas			X_{ab}	
Pagurus sp.	X_b	X_b	X_a	X_b
Homarus americanus		X_{a*}	X_{a*}	
Shrimp (unknown)	X_{ab}	X_{ab}	X_{ab}	

Table 2-5. Prey items within the stomachs of invasive green crab (*n* = 38), native rock crab (*n* = 43), and lobster (*n* = 2) from two sites within Placentia Bay, NL (cont.).

Organism	**Green Crab (≤ 50 mm)**	**Green Crab (> 50 mm)**	**Rock Crab**	**Lobster**
Sponges	X_{ab}		X_{ab}	
Polychaetes				
Nereis sp.	X_{ab}	X_{a}	X_{ab}	
Fish				
Gadus sp.				X_{b}
Echinoderms				
S. droebachiensis				X_{b}

"X" represents the presence of the organism. The letters represent the site of collection where a = North Harbour, NL and b = Baker's Cove, NL; * indicates uncertainty (may be juvenile lobster or rock crab).

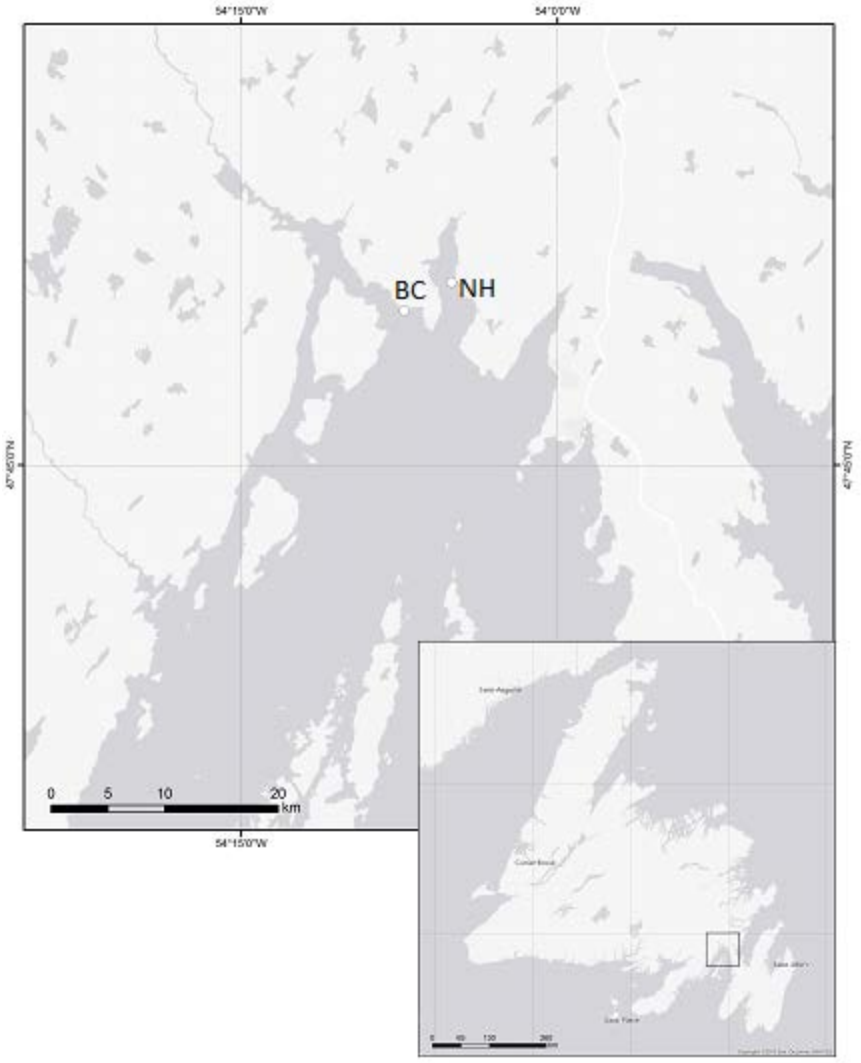


Figure 2-1. Transect locations for two sites (NH = North Harbour and BC = Baker's Cove) within Placentia Bay, NL.

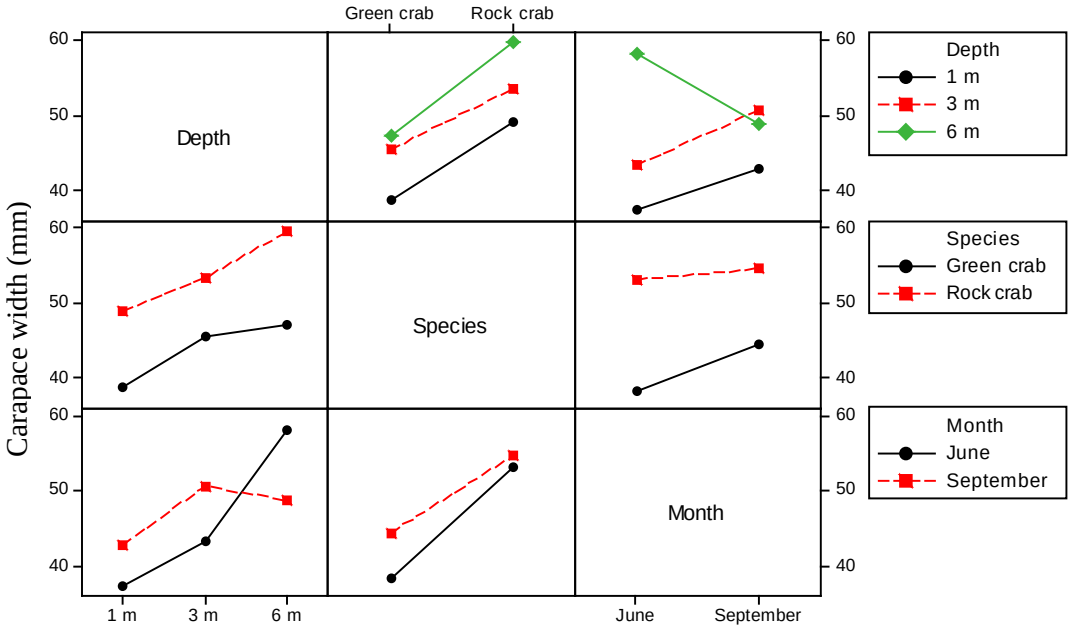
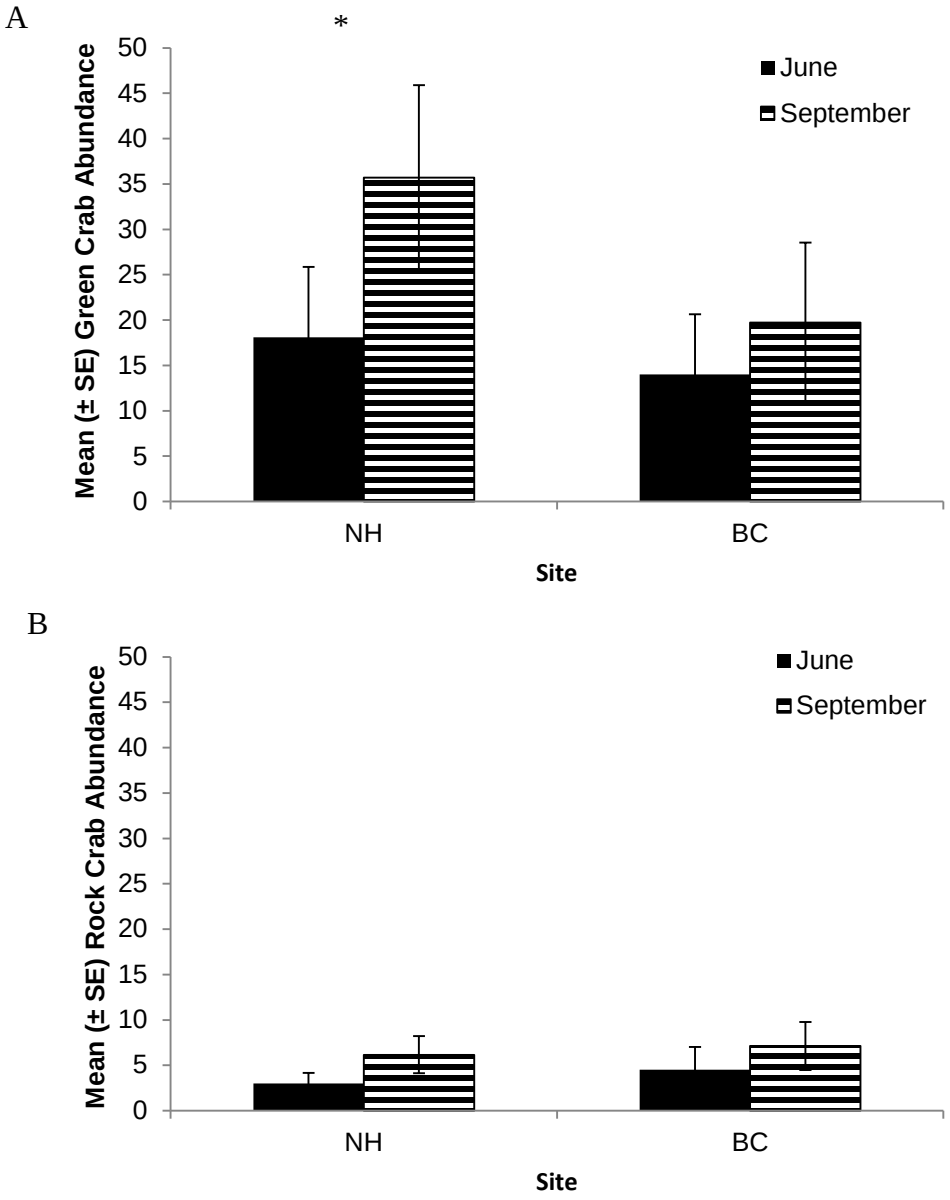


Figure 2-2. An interaction plot for mean carapace width of crab collected from transects in 2009, 2010 and 2011 in North Harbour and Baker's Cove, NL relative to depth (1, 3 and 6 m), species (rock and green crab) and sampling month (June or September).



* indicates a significant difference between sites.

Figure 2-3. Mean abundances of green crab (A) and rock crab (B) observed by divers along 100m transects at four depths (1, 3, 6, and 9 m) from 2009 to 2011 at North Harbour, NL (NH) and Baker's Cove, NL (BC) in June and September. A survey was not conducted in September 2010 due to poor weather conditions.

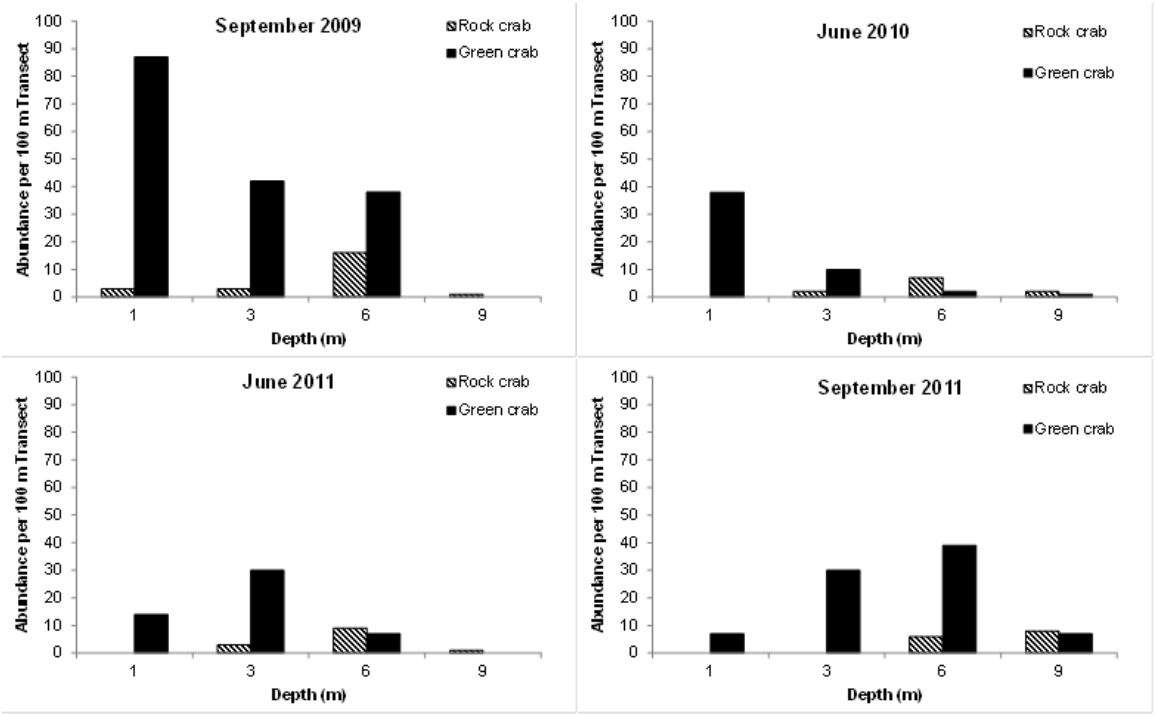


Figure 2-4. Abundances of rock crab (hatched) and green crab (solid black) observed by divers along a single 100 m transects at four depths (1, 3, 6, and 9 m) in North Harbour, NL in September 2009 and 2011 and in June 2010 and 2011.

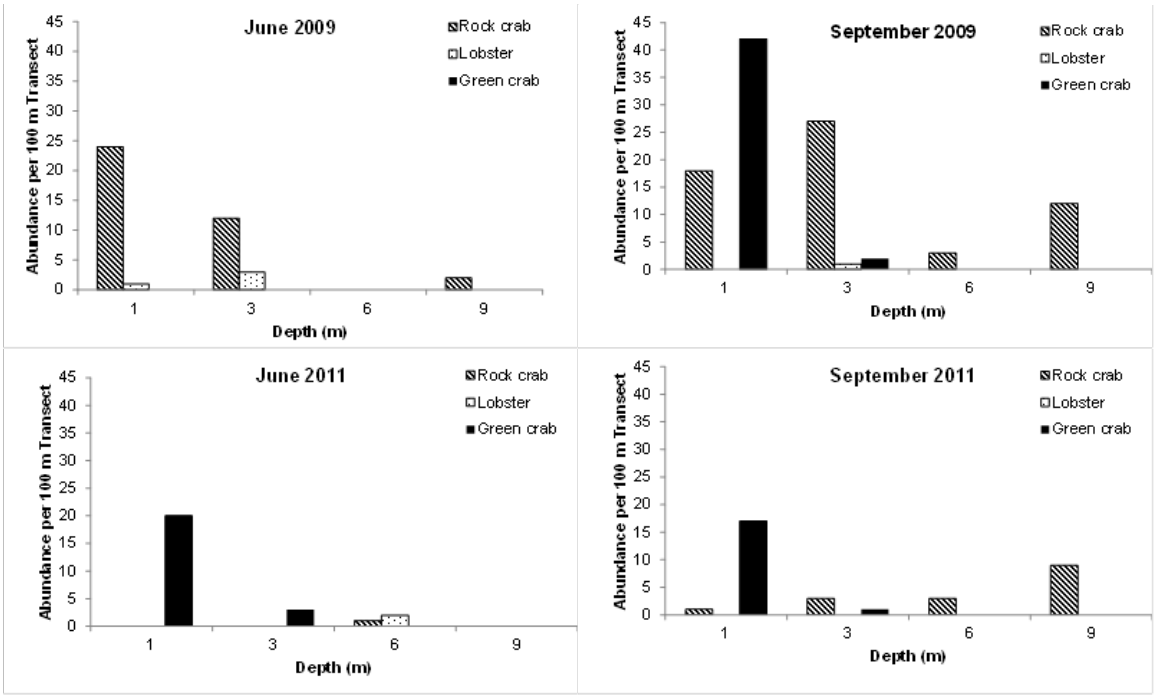
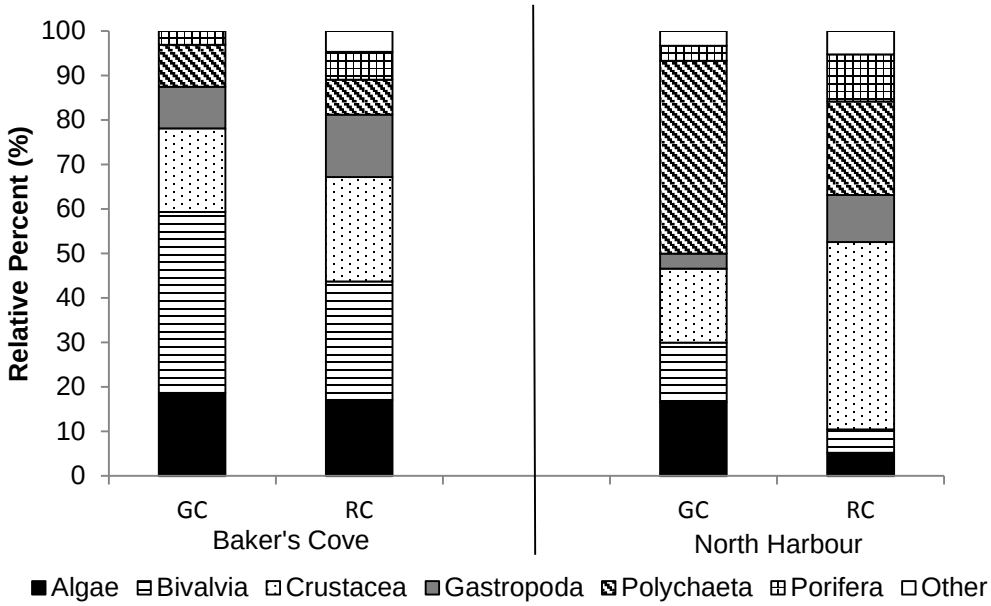


Figure 2-5. Abundances of rock crab (hatched), lobster (stippled) and green crab (solid black) observed by divers along a single 100 m transects at four depths (1, 3, 6, and 9 m) in Baker's Cove, NL in September 2009 and 2011 and in June 2009 and 2011.



The “other” component contained fish, echinoderms and eelgrass.

Figure 2-6. Percentage of various prey items in stomach contents found in green crab (GC; *n* = 15 Baker’s Cove; *n* = 22 North Harbour), and rock crab (RC; *n* = 21 Baker’s Cove; *n* = 13 North Harbour) from Baker’s Cove and North Harbour, NL.

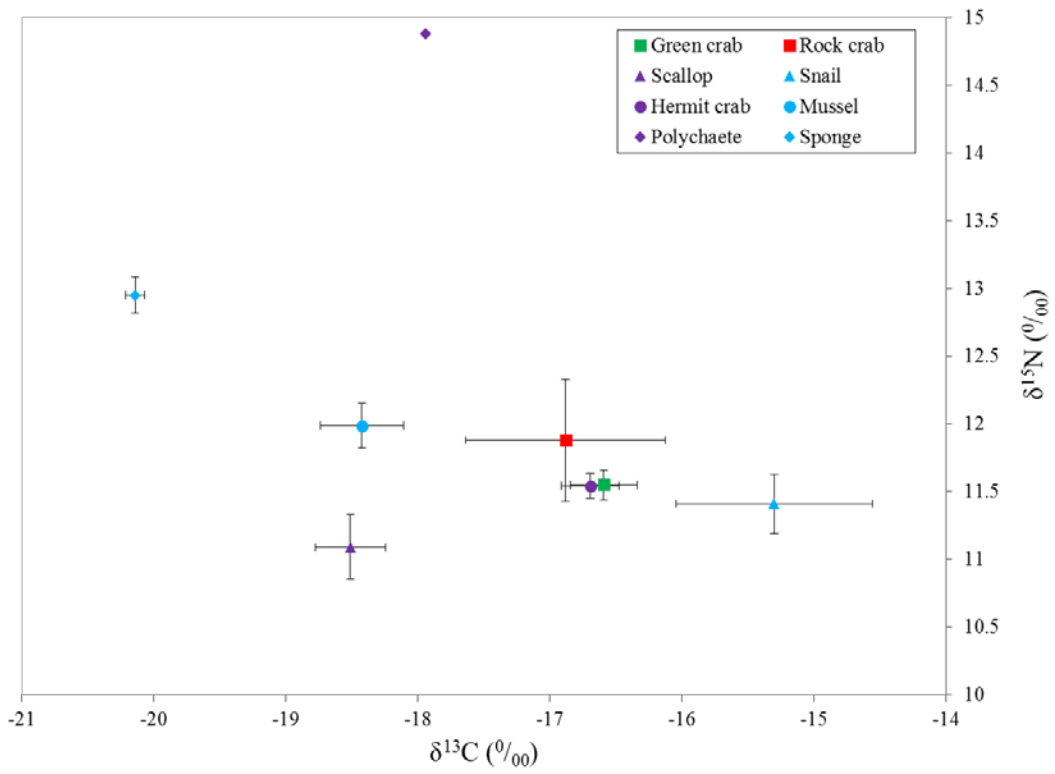


Figure 2-7. Plot of $\delta^{15}N$ and $\delta^{13}C$ stable isotope signatures from biota collected in North Harbour, NL in September 2011. The predators in this study were rock crab (red square) and green crab (green square); all other items were considered potential prey items.

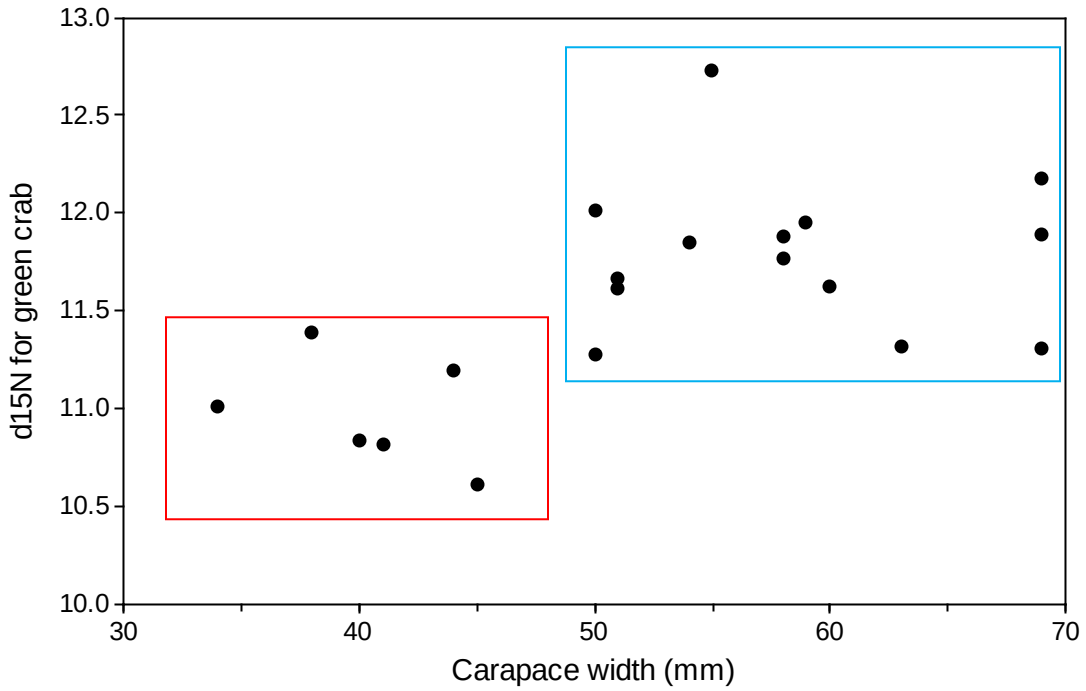


Figure 2-8. The relationship between $\delta^{15}N$ values and carapace width (CW mm) from green crab in North Harbour, NL, 2011. Red and blue boxes show different groupings in δ15N, separated at approximately 50 mm CW.

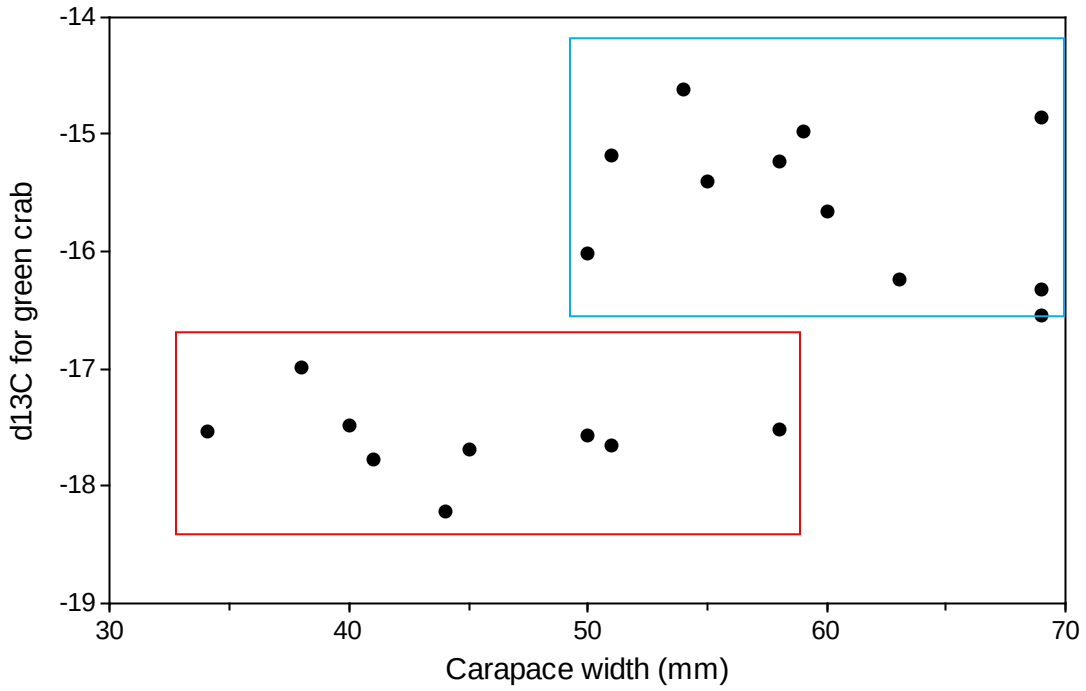


Figure 2-9. The relationship between carapace width (CW mm) and $\delta^{13}C$ values obtained from stable isotope analysis for green crab in North Harbour, NL, 2011. Red and blue boxes show different groupings in $\delta^{13}C$.

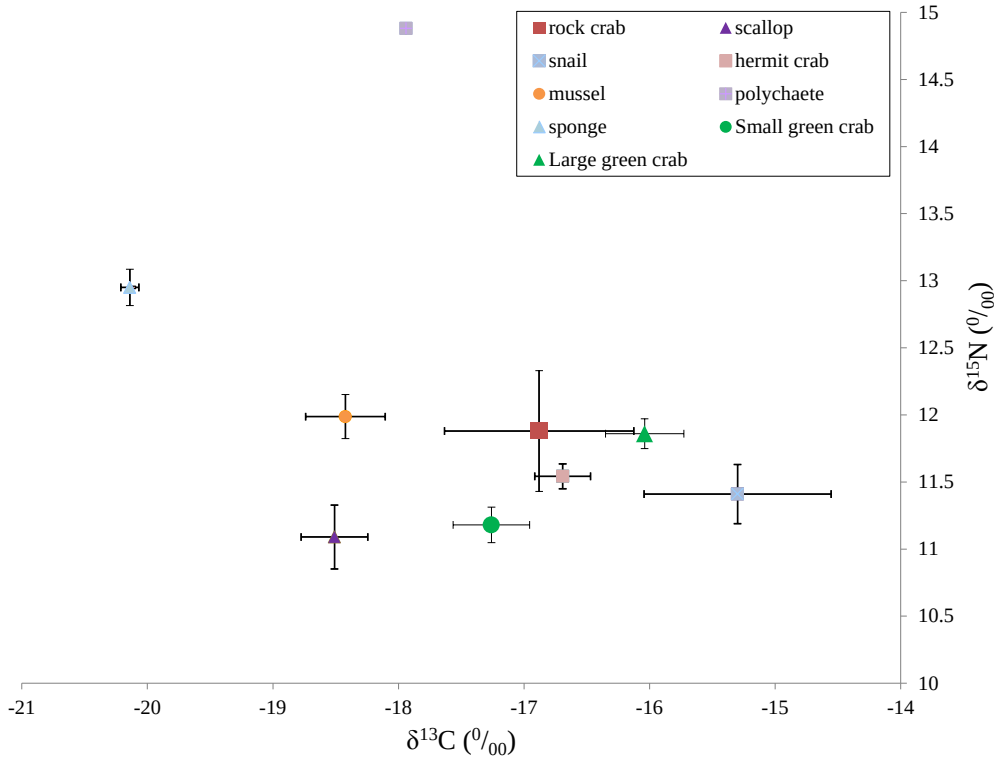


Figure 2-10. Plot of $\delta^{15}N$ and $\delta^{13}C$ stable isotope signatures from biota collected in North Harbour, NL in September 2011. The predators in this study were rock crab (red square) and two sizes of green crab (small ≤ 50 mm; green circle; large >50 mm; green triangle); all other items were considered prey items.

3. Feeding and shelter behaviour of juvenile American lobster (*Homarus americanus*): the influence of a non-indigenous crab

3.1. Abstract

American lobster (*Homarus americanus*) is currently the most important commercial fishery in Atlantic Canada. The recent arrival and establishment of invasive European green crab (*Carcinus maenas*) in this region may pose a threat to this industry because of likely interactions between these species. Adult green crab are dominant predators that rapidly increase in population size in newly invaded areas and potentially compete with juvenile lobster for limited resources. Previous studies suggest that juvenile lobster utilize shelter to avoid predation but shelter dependence decreases as they mature and develop predator defence mechanisms. Smaller lobster must therefore trade-off energetic needs with predation risk. In laboratory experiments the effect of the presence of adult green crab on feeding and shelter behaviour of juvenile lobster (25-51 mm carapace length) was examined by offering juvenile lobster protective shelter and an adjacent food patch in the presence or absence of green crab. Lobster behaviour was monitored in each trial over a period of eight hours. Smaller juvenile lobster (<35 mm carapace length) spent significantly less time feeding, spent more time within the shelter, and spent more time locating the food source in the presence of a green crab than in their absence. Green crab can therefore influence feeding and shelter usage of small juvenile lobster, though results show this influence decreases in lobster >36 mm carapace length that are less shelter-dependent and more frequent foragers.

3.2. Introduction

Optimal foraging theory predicts that organisms forage in a way to maximize caloric intake and fitness with the least amount of energy expended (McArthur and Pianka 1966; Emlen 1966); thus more energy is gained by reduced search time and less handling of the food item. Foraging decisions include tradeoffs in terms of the quality and density of food, distances between food patches, and prey size (e.g. Alcock 2009; Pyke 1984). However, for crustaceans and other prey species that utilize shelters, the risk of predation may ultimately alter foraging behaviour.

Shelter availability and use is thought to play a critical role in the recruitment dynamics of lobster. Smaller lobster are able to bury themselves in the substrate (Cobb 1971; Berrill and Stewart 1973), but exhibit a strong preference for natural shelters created by rocks and crevices (Hudon and Lamarche 1989; Barshaw et al. 1994). All size ranges of lobster utilize rocks and crevices, but dependence is greater in early benthic phases and juvenile lobster (Cobb 1971). Access to shelters is thought to limit lobster recruitment (Lawton and Lavalli 1995) because shelters protect juvenile lobster from predators and thus enhance survival (Lawton and Lavalli 1995; Hudon 1987). Numerous observations suggest that juvenile lobster prefer to spend the majority of their time in shallow and wide, opaque shelters (Cobb 1971), with limited foraging excursions. As lobster increase in size, they spend more time foraging outside of shelters (Lawton and Lavalli 1995). Predator avoidance is therefore size specific and may result in a trade-off between safety and reduced foraging rate (Abrahams and Dill 1989; Wahle 1992). For example, in the presence of a caged sculpin (*Myoxocephalus aeneus*), smaller lobster

(< 30 mm CL) spent less time foraging than larger lobster (30 to 38 mm CL) (Wahle 1992). Similarly, in the presence of caged tautog (*Tautoga onitis*), juvenile lobster consumed fewer mussels and often brought mussels back to the safety of the shelter before consuming them compared to trials with no caged tautog (Spanier et el. 1998).

Similar feeding responses may occur in the presence of other, potentially threatening predators such as the non-indigenous green crab, (*Carcinus maenas*) (Klassen and Locke 2007). This invasive species has spread since the 1800s (Grosholz and Ruiz 1995), and reached the east coast of North America in the 1850s. Over the subsequent 100 years, it expanded from New Jersey to southern Nova Scotia (Grosholz and Ruiz 1995; Audet et al. 2003; Roman 2006) and increasingly overlapped the geographic range of lobster. A second introduction of genetically distinct green crab in the 1980s has expedited broader expansion within eastern Nova Scotia, Prince Edward Island and, most recently, in Newfoundland (Roman 2006; Blakeslee et al. 2010). Green crab consume similar prey to lobster, and likely compete for food with lobster and other predators at this latitude. Green crab prey on bivalves (Palacios and Ferraro 2003; Floyd and Williams 2004; Klassen and Locke 2008), juvenile fish (Taylor 2005), other crab species (Grosholz et al. 2000; McDonald et al. 2001) and juvenile lobster in laboratory settings (aquaria 0.9 m diameter; carapace length (CL) <57 mm; Rossong et al. 2006). The literature on laboratory experiments also reports that green crab outcompete *Hemigrapsus spp.*, *Cancer magister* crab (Jensen et al. 2002; McDonald et al. 2001; Grosholz and Ruiz 1995), and juvenile American lobster (Rossong et al. 2006; Williams et al. 2006) for limited food resources. However, studies by Bélair and Miron (2009a,b) have also shown

co-existence without apparent interference between green crab and rock crab (*Cancer irroratus*).

The current establishment and growth in green crab populations within lobster grounds of eastern North American shores seem harmless in some regions but increases the likelihood of interactions between these two species in others. Recent invasions of areas with juvenile lobster habitats, in particular, raise concerns for the potential detrimental effects of adult green crab on lobster recruitment. Field studies within the Bay of Fundy, New Brunswick, have confirmed the spatial overlap of adult green crab and juvenile lobster (<40 mm CL) with the highest overlap in the shallow subtidal zone (<1.2 m chart datum; Lynch and Rochette 2009). Although direct interactions between green crab and juvenile lobster have been demonstrated already, indirect effects remain largely unexplored.

A laboratory setting was used to determine whether the presence of green crab influences the behaviour of juvenile lobster. Although green crab predation on juvenile lobster is an obvious concern, the potential alteration of lobster behaviour in the presence of increasing numbers of green crab is expected to expand to broader scales, and potentially alter the behaviour of lobster. The feeding behaviour of several size ranges of juvenile lobster exposed to the presence of a caged green crab was examined. The objectives of this study were to: 1) document whether lobster spend more time within a shelter in the presence of green crab; 2) document whether lobster consume prey items (mussels) in the presence of green crab; and 3) assess whether behaviour is related to size of juvenile lobster. Given that the lobster in this study were collected in areas not yet

invaded by green crab, and are therefore naïve to the influence of this new predator, two null hypotheses were tested: i) juvenile lobster feeding and shelter seeking behaviour are not altered in the presence of green crab, and ii) shelter and feeding behaviour in the presence or absence of green crab are not related to the size of the juvenile lobster.

3.3. Methods

3.3.1. Collection and Care of Lobster and Crab

Scuba divers collected juvenile lobster (n = 17 males, n = 13 females) within a size range of 25 to 52 mm CL (mean CL ± SD for males = 37 ± 8 mm, 18.1-94.6 g; females = 38 ± 5 mm, 21.4 -82.7 g) on September 2, 2009 in North Rustico, Prince Edward Island, Canada. Juvenile lobsters were collected from an area on PEI where green crab have not been observed during frequent dives in the area (Michel Comeau, Fisheries and Oceans Canada). Male green crab ranging in size from 65 to 76 mm carapace width (CW) (n = 3; CW = 70.4 ± 4.4 mm SD; mass = 106.6 ± 27.0 g SD) were collected from baited traps in Pomquet Harbour, Nova Scotia on September 4, 2009. Both species were transported in coolers with ice packs and kelp to the animal care facility at St. Francis Xavier University, Antigonish, Nova Scotia. Lobster and crab were maintained in separate holding tanks in a temperature (10°C) and light (12 hour light/dark) controlled room. Each animal was placed in a rectangular container (25 x 45 cm) on shelves within a larger tank. Ultraviolet and bio-filtered water (salinity 31 ppt) was pumped into a storage container above the shelf, where it trickled down tubing into each container and supplied fresh, oxygenated water. Lobster and green crab were held in the laboratory for one week prior to experiments and fed a daily diet of mussels.

3.3.2. Experimental Set-up and Video-taping

Experiments were conducted in a single 90-cm diameter, cylindrical plastic tank filled with seawater typically found in juvenile lobster habitat (10°C, 31 ppt), to a depth of approximately 45 cm. The bottom of the tank was uniformly covered in coarse sand to a depth of 5 cm. A shelter (PVC pipe cut in half along its long axis, 15 cm length, 6 cm height) and an empty wire mesh cage (25 cm x 12 cm x 10 cm) to hold a green crab were added to the tank. All variables (e.g. salinity, temperature, cage, shelter) remained constant for the control and reference trials. Juvenile lobster ranging in size from 25 to 52 mm CL, were randomly assigned to each treatment to ensure no confounding size biases. Experiments were videotaped with two CCD, low light cameras (Panasonic WV-BP334) mounted over the tank 1 m above the sediment. Two infrared illuminators (Extreme CCTV Moonlight-IR) were used to minimize behavioural alterations associated with bright light (Weissburg and Zimmer-Faust 1994). Novex (NOVEX2000 V. 3.01) software transmitted signals from the cameras to a recorder located outside the room.

3.3.3. Feeding and Shelter Behaviour

Juvenile lobster were starved for 64 hours prior to experimental trials to standardize hunger levels (48 hours before the trial and during the 16 hour acclimation period; Mascaro and Seed 2001). Prior to the experiment, lobsters were removed from their individual housing unit within a few feet of where the experimental tank was located, and they were immediately placed in the water of the experimental tank. Individuals were acclimated in the tank for 16 hours. The long acclimation period prior to

the experiment was because of handling and to allow the lobster to exhibit sheltering behaviors.

After the acclimation period, a covered (crushed) mussel was introduced (mean weight ± SD = 15.2 ± 5.8 g) as a food patch. The mussel was secured to a piece of plastic with a cable tie and was placed in the middle of the tank for every trial. There were two treatments; a control treatment (n = 15 trials) with an empty wire cage and a green crab treatment (n = 15 trials) where an adult green crab was added to the wire cage at the end of the acclimation period. An airstone was used for 15 minutes to disperse the crab scent throughout the tank for the green crab treatment and for consistency for the control treatment. After this 15 minute period, the crushed mussel was uncovered and recording commenced for each trial for approximately 8 hours (mean time ± SD = 474 ± 50 minutes; the 15 minute acclimation of the green crab and set-up was included in this time period resulting in slight differences in trial length). The trial was considered complete after the 8 hours, regardless of when the entire mussel was consumed since it was impossible to determine from the video.

After completion of the experiments, each lobster was banded, tagged, and observed in a separate holding tank for two weeks to confirm that each individual was not an aggressive pre-moult lobster (Tamm and Cobb 1978). Green crab were fed and returned to the holding containers for use in subsequent trials. Each type of trial alternated in series of three trials (i.e. 3 control trials followed by 3 trials using green crabs) to minimize tank changes between taping and reduce the number of crab to be subsequently euthanized. All lobster were used only once in the experiment and water changes were

completed after each trial. After the trials and the two-week monitoring period, green crab and lobster were euthanized to comply with animal care protocols for animals held in laboratory facilities.

3.3.4. Video and Statistical Analysis

The time spent in the shelter, time spent feeding on the mussel, the frequency and location (near food patch or in shelter) of lobster feeding, and any other distinctive behaviours were recorded and analyzed. For the purpose of this study, foraging behavior was defined as the time feeding on the mussel and does not include search time. From the video analyses handling of the food item and directly consuming the mussel was considered feeding or foraging. Shelter behavior included the lobster within or partly in the shelter. Often the lobster moved sediment out of the shelter but in these cases the lobster left the shelter for less than 5 seconds and this was still considered “sheltering behaviour”. The proportion of time spent on different activities was used as a response variable due to slight differences in the length of the trials.

Data did not meet the assumption of normality with data transformations so treatment differences were tested with Mann-Whitney U tests. A two-sample t-test was used to verify that the random allocation of lobster to treatment groups resulted in similar sized lobster between treatments. The relationship between each response variable (the proportion of time in the shelter and with the mussel, the number of times feeding, and the time taken by the lobster to first encounter the mussel) and juvenile lobster size was assessed using linear regression. The result of the experiment showed that lobster either

spent almost no time (<1% time) in the shelter or a significant portion of time (>65% of time) in the shelter.

The proportion of time in shelter was therefore analyzed using a logistic binary regression with levels of 0 = no shelter usage (i.e. lobster spent less than 1 % of the trial in the shelter) and 1 = significant shelter usage (i.e. lobster spent greater than 65% of the trial in the shelter). None of the lobster spent between 1 and 65% of the time in the shelter. The 65% cutoff was selected based on the distribution of the proportion of time spent in the shelter. A Pearson chi-square goodness of fit test was used to assess the fit of the binary logistic regression model. The data was analyzed using a binary logistic regression because overlap in carapace size precluded use of a step function. A binary logistic regression assigns a predicted probability of using the shelter for the range of carapace lengths where there is overlap in the response.

All statistical analyses were conducted using Minitab 15 software (State College, PA) using a significance level of 0.05.

3.4. Results

3.4.1. Feeding Behaviour

The proportion of time that the lobster fed in the absence of a green crab ranged from 0 to 0.27 (mean ± SE = 0.073 ± 0.014) and for lobster in the presence of a green crab ranged from 0.0008 to 0.31 (mean ± SE = 0.091 ± 0.028) (Figure 3-1A). The difference between the treatments was not statistically significant (Mann-Whitney U = 234; p = 0.968; n = 15 per treatment). The linear regression of the proportion of time spent feeding on juvenile lobster carapace length (Figure 3-1B) was not significant in the absence of a

green crab ($F_{1,13}$ = 0.01; p = 0.935; r^2 = 0.010); however, the linear regression was significant in the presence of a green crab ($F_{1,13}$ = 5.95; p = 0.030; r^2 = 0.314) with a positive slope indicating that larger sized lobster spent significantly more time feeding.

The time required for juvenile lobster to initially locate the mussel did not differ significantly between treatments (Mann-Whitney U = 208.5, p = 0.329; n = 15 per treatment). Lobster size was not a significant predictor of time to the mussel in the absence of a green crab ($F_{1,13}$ = 0.01; p = 0.945; r^2 < 0.001) or in the presence of a green crab ($F_{1,13}$ = 4.54; p = 0.053; r^2 = 0.259) (Figure 3-2A). The number of times the lobster fed during the trials was not significantly different between treatments (Mann-Whitney U = 241; p = 0.739). The linear regression of the number of times the lobster fed during the trial (Figure 3-2B) was not significant in the absence of a green crab ($F_{1,13}$ = 0.42; p = 0.529; r^2 = 0.031) or in the presence of a green crab ($F_{1,13}$ = 3.97; p = 0.234; r^2 = 0.234).

3.4.2. Shelter Behaviour

During the 16-hour acclimation period before the beginning of the trial, lobster exhibited three main behaviours: 1) they excavated sediment from below the shelter, creating a depression, 2) they collected coarser sediments near the shelter to create a barrier at one end of the shelter, or 3) they moved around the tank and did not enter the shelter. Lobster that utilized the shelter during the trials often left the shelter to collect more sediment and then continued their sheltering behavior.

The proportion of time that the lobster used the shelter in the absence of a green crab ranged from 0 to 0.84 (mean ± SE = 0.33 ± 0.086) and for lobster in the presence of a green crab ranged from for 0 to 0.93 (mean ± SE = 0.34 ± 0.11) (Figure 3-3). The

difference between the treatments was not statistically significant (Mann-Whitney U = 223.5; p = 0.719; n = 14 for caged treatment; n = 15 for control treatment). In one green crab trial, a lobster carried the mussel to the shelter prior to consumption. This particular trial was removed from the data analysis of shelter use because feeding time could not be distinguished from time in shelter.

A binary logistic regression with the response variable of shelter use (0 = no shelter usage and 1 = shelter usage) versus lobster CL was significant (G = 9.004; p = 0.003) (Figure 3-4). The 1% and 65% cut-offs were selected based on the distribution of the proportion of time spent in the shelter (Figure 3-3B). The Pearson chi-square goodness of fit test revealed that there was no evidence that the model did not fit the data (χ^2 = 10.35; p = 0.586) and CL was a significant predictor of shelter usage (Z = -2.03; p = 0.042). The regression equation was:

$$\pi(x) = \frac{\exp(\alpha + \beta x)}{1 + \exp(\alpha + \beta x)}$$

where $\pi(x)$ is the probability that a lobster with CL = x will use the shelter, α = 12.71, and β = -0.3517.

Although these data cannot be extrapolated to the full range of lobster sizes, the model can be used to predict the carapace length of a lobster such that the probability that the lobster will use the shelter is 50%. This carapace length can be calculated to be 36 mm.

3.5. Discussion

3.5.1. Feeding Behaviour

As suggested by previous studies (e.g. Lawton and Lavalli 1995) the present study found that during the juvenile phase, lobster behaviour may change substantially with a very modest change in size. Smaller lobster (<36 mm CL) spent significantly less time feeding and took more time to find a food source in the presence of a caged green crab than in its absence. This response is consistent with other laboratory studies of Spanier et al. (1998; 35-57 mm CL) and Wahle (1992; 9-38 mm CL) who documented a reduction in the quantity of food consumed by juvenile lobster in the presence of two predatory fish species. In the Spanier et al. (1998) study, individual lobster exposed to a predator spent less time feeding, consumed less food, and frequently transported mussels to their shelter for consumption. The results of the present study reinforce the trade-off between risk reduction and energetic demands (Lima and Dill 1990; Wahle 1992), where perceived predation risk alters behavioural decisions of foraging animals (Abrahams and Dill 1989).

Smaller sized juvenile lobster (<36 mm CL) spent not only less time feeding but spent significantly more time in the shelter in the presence of green crab. Lobster are known to leave the protection of their shelter to forage and then transport food to their shelter for consumption, repeating the behaviour once the food has been consumed (Lawton and Lavalli 1995). This behaviour was expected to change during development because lobster defence mechanisms change with size (Hudon 1987) and the risk of predation decreases. Smaller lobster can only react to predators by rapidly flexing their abdomen, whereas larger lobster have much bigger, well-developed claws that can be

used for protection (Lang et al. 1977; Hudon 1987). In the absence of green crab, juvenile lobster of all sizes pursued the food source within 70 minutes of the beginning of the trials. However, in the presence of green crab, smaller lobster took more time to begin feeding. This increase in time to begin feeding was primarily related to the increased time spent in the shelter away from elevated predation risk. Irrespective of size, lobster in the control trials often encountered but did not immediately feed on the food resource. A similar delay was not apparent in the green crab trials, possibly because the lobster wanted to reach the food patch before the green crab. Lobster and green crab food competition experiments have shown that the first organism to obtain a food resource is more likely to defend it and is less likely to be displaced by competitors (Rossong et al. 2006; Williams et al. 2006; Williams et al. 2009).

The frequency with which lobster fed was also dependent on size but only in the trials where green crab were present. Smaller lobster typically fed less than 6 times during the 8 hour trial, and in the majority of trials the lobster moved to the mussel only once or twice, remaining there until the mussel was completely consumed. Larger sized juvenile lobster visited the food patch much more frequently, in some cases more than 15 times, even when green crab were present. For these larger juvenile lobster, no evidence of agonism against the caged green crab was detected in the video for any of the trials. Foraging blue crab (*Callinectes sapidus*) exhibit similar behaviour when conspecifics were within a 5-m radius of food patch (Clark et al. 1999). Juvenile American lobster visit food patches more frequently when conspecifics are nearby (Spanier et al. 1998), because short-duration trips may reduce the risk of agonistic encounters when

competitors are present (Clark et al. 1999). Whereas small juveniles remain in the shelter to avoid predators, larger lobster may forage more frequently to limit competition.

3.5.2. Shelter Behaviour

In nature, lobster within the size range of 35-80 mm carapace length are less shelter dependent than smaller lobster (Lawton and Lavalli 1995; Hudon 1987). In the present study, small lobster (<36 mm) spent significantly more time in the shelter when a green crab was present than in the control group. In the presence of green crab, juvenile lobster spent either the majority of the trial (>65 %) or practically no time (<1%) in the shelter, which was significantly related to body size. In a shelter competition experiment between American lobster and green crab, lobster that spent more time in shelter were less likely to suffer mortality by green crab than lobster that spent more than 10% of the trial away from the shelter (Rossong et al. 2005). Field (Richards and Cobb 1986) and laboratory studies (Spanier et al. 1998) have demonstrated that shelter reduces predation loss for juvenile lobster. The size range of lobster in the present study was narrow (26 mm), however, in the presence of a green crab resulted in a significant negative relationship between shelter utilization and lobster size.

Sheltering behaviour was consistent with other observations reported in the literature. Cobb (1971) observed similar “bull-dozing” activity in his experiments and noted that lobster preferred shelters with a single opening over multiple openings. Field observations suggest that similar sheltering behaviour (barricading one or both openings) takes place during pre-moulting periods (Karnofsky et al. 1989). Although lobster exhibited this type of behaviour, no evidence of any moulting related signs that could

alter the results of the present study were detected during or after the two-week period of the experiment.

Juvenile lobster (Richards and Cobb 1986; Wahle 1992; Spanier et al. 1998), and crayfish (*Astacus astacus*; Appleberg et al. 1993) increase shelter use in the presence of a predator or predator odour. When exposed to sculpin or sculpin odour, juvenile lobster remained in their shelter an average of 68% of the time compared to controls (Wahle 1992). The proximity and abundance of a food source and the encounter rates with predators can both greatly influence shelter use in juvenile lobster (Lawton 1987). As lobster become increasingly hungry, they also increase foraging behaviour and thus predation risk (Lawton 1987). Similarly, when lobster are food limited they "accept" increased predation risk by increasing their foraging area (Lawton 1987). Increased shelter use, as seen by juvenile lobster, may be particularly adaptive against predators that use movement to locate their prey. Vulnerable juveniles are generally more likely than adults to modify their behaviour in response to predation (Garvey et al. 1994).

3.5.3. Conclusions

The present study provides insight into indirect behavioural impacts of the presence of invasive green crab. Lynch and Rochette (2009) encountered adult green crab within one meter of juvenile lobster along transects within the lower subtidal zone. The present study showed that smaller juvenile lobster (<36 mm) reduced feeding and increased shelter use when a green crab was in close proximity, however, caution is required in extrapolating these findings to larger temporal and spatial scales because this study was completed under small-scale laboratory conditions. For example, is the reduced

feeding and increased shelter usage by small juvenile lobster a short-term initial response to a new predator or does this behaviour continue over time? Nonetheless, considered in tandem with previous work on these species (e.g. Rossong et al. 2006; Williams et al. 2006; Lynch and Rochette 2009), this study suggests that the presence of green crab can affect small juvenile lobster behaviour when in close proximity. Field experiments would be useful for testing the hypotheses in the present study, but because of the challenges and limited feasibility of such field studies, this study was necessary for testing specific hypotheses regarding size-related behavioural changes. Given that green crab populations continue to grow and expand in Atlantic Canada, their presence may not only modify the resources available for lobster populations (positively or negatively, depending on size), but may also alter lobster behavioural patterns. What remains unclear is the relative weight of positive effects (prey provision to large lobster) and detrimental effects (lowered feeding in small juvenile lobster due to behavioural response to green crab). Until we have a better understanding the interactions between invasive green crab and native American lobster, the establishment and ongoing increase in green crab populations remains a potential concern for the lobster fishery.

3.6. References

Abrahams MV, Dill LM (1989) A determination of the energetic equivalence of the risk of predation. Ecology 70: 999-1007.

Alcock J (2009) Animal Behavior: An evolutionary approach. 9th edition, Sinauer Associates Inc.

Appelberg M, Söderbäck B, Odelström T (1993) Predator detection and perception of predation risk in the crayfish *Astacus astacus*. L. Nordic. J. Freshw. Res. 68: 55-62.

Audet D, Davis DS, Miron G, Moriyasu M, Benhalima K, Campbell R (2003) Geographic expansion of a nonindigenous crab, *Carcinus maenas* (L.), along the Nova Scotian shore into the southeastern Gulf of St. Lawrence, Canada. J. Shellfish Res. 22: 255-262.

Barshaw DE, Able KW, Heck KL (1994) Salt marsh peat reefs as protection for postlarval lobsters *Homarus americanus* from fish and crab predators: comparisons with other substrates. Mar. Ecol. Prog. Ser. 106: 203-206.

Bélair M, Miron G (2009a) Predation behaviour of *Cancer irroratus* and *Carcinus maenas* during conspecific and heterospecific challenges. Aquat. Biol. 6: 41-49.

Bélair M, Miron G (2009b) Time budget of *Cancer irroratus* (Say) and *Carcinus maenas* (L.) under various temperature and prey density conditions during conspecific and heterospecific challenges. J. Shellfish Res. 28: 923-930.

Berrill M, Stewart R (1973) Tunnel digging in mud by newly settled American lobsters, *Homarus americanus*. J. Fish. Res. Board Can. 30: 285-287.

Blakeslee AMH, McKenzie CH, Darling JA, Byers JE, Pringle JM, Roman J (2010) A hitchhiker's guide to the Maritimes: anthropogenic transport facilitates long-distance dispersal of an invasive marine crab to Newfoundland. Diversity Distrib. 16: 879-891.

Clark ME, Wolcott TG, Wolcott DL, Hines AH (1999) Interspecific interference among foraging blue crabs *Callinectes sapidus*: interactive effects of predator density and prey patch distribution. Mar. Ecol. Prog. Ser. 178: 69-78.

Cobb JS (1971) The shelter-related behavior of the lobster, *Homarus americanus*. Ecology 52: 108-115.

Elner RW, Campbell A (1987) Natural diets of lobster *Homarus americanus* from barren ground and macroalgal habitats off southwestern Nova Scotia, Canada. Mar. Ecol. Prog. Ser. 37: 131-140.

Emlen JM (1966) The role of time and energy in food preference. Am. Nat. 100: 611-617.

Floyd T, Williams J (2004). Impact of green crab (*Carcinus maenas* L.) predation on a population of soft-shell clams (*Mya arenaria* L.) in the southern Gulf of St. Lawrence. J. Shellfish Res. 23: 457-462.

Garvey JE, Stein RA, Thomas HM (1994) Assessing how fish predation and interspecific prey competition influence a crayfish assemblage. Ecology 75: 532-547.

Grosholz ED, Ruiz GM, Dean CA, Shirley KA, Maron JL, Connors PG (2000) The impacts of a nonindigenous marine predator in a California Bay. Ecology 81: 1206-1224.

Grosholz ED, Ruiz GM (1995) Spread and potential impact of the recently introduced European green crab, *Carcinus maenas*, in central California. Mar. Biol. 122: 239-247.

Hudon C (1987) Ecology and growth of postlarval and juvenile lobster, *Homarus americanus*, off iles de la Madeleine (Quebec). Can. J. Fish. Aquat. Sci. 44: 1855-1869.

Hudon C, Lamarche G (1989) Niche segregation between American lobster *Homarus americanus* and rock crab *Cancer irroratus*. Mar. Ecol. Prog. Ser. 52: 155-168.

Jones PL, Schulman MJ (2008) Subtidal-intertidal trophic links: American lobsters [*Homarus americanus* (Milne-Edwards)] forage in the intertidal zone on nocturnal high tides. J. Exp. Mar. Biol. 361: 98-103.

Jensen GC, McDonald PS, Armstrong DA (2002) East meets west: competitive interactions between green crab *Carcinus maenas*, and native and introduced shore crab *Hemigrapsus spp*. Mar. Ecol. Prog. Ser. 225: 251-262.

Karnofsky EB, Atema J, Elgin RH (1989) Field observations of social behaviour, shelter use, and foraging in the lobster, *Homarus americanus*. Biol. Bull. 176: 239-246.

Klassen G, Locke A (2007) A biological synopsis of the European green crab,

Carcinus maenas. Can. Manuscr. Rep. Fish Aquat. Sci. no 2818, vii + 75 p.

Lang F, Govind CK, Costello WJ, Greene SI (1977) Developmental neuroethology: changes in escape and defensive behaviour during growth of the lobster. Science 197: 682-685.

Lawton P (1987) Diel activity and foraging behavior of juvenile American lobsters, *Homarus americanus*. Can. J. Fish. Aquat. Sci. 44: 1195-1205.

Lawton P, Lavalli K (1995) Postlarval, juvenile, adolescent and adult ecology. In: Factor JR (ed.) Biology of the lobster, *Homarus americanus*. Academic Press Inc, San Diego, pp. 47-88.

League-Pike PE, Schulman MJ (2009) Intraguild predators: Behavioral changes and mortality of the green crab (*Carcinus maenas*) during interactions with the American lobster

(*Homarus americanus*) and jonah crab (*Cancer borealis*). J. Crustacean Biol. 29: 350-355.

Lima SL, Dill LM (1990) Behavioral decisions made under the risk of predation: a review and prospectus. Can. J. Zool. 68: 619-640.

Lynch BR, Rochette R (2009) Spatial overlap and biotic interactions between sub-adult American lobsters, *Homarus americanus*, and the invasive European green crab *Carcinus maenas*. J. Exp. Mar. Biol. 369: 127-135.

MacArthur RH, Pianka ER (1966) On optimal use of a patchy environment. Am. Nat. 100: 603-609.

Mascaro M, Seed R (2001) Foraging behavior of juvenile *Carcinus maenas* (L.) and *Cancer pagurus*. L. Mar Biol 139: 1135-1145.

McDonald PS, Jensen GC, Armstrong DA (2001) The competitive and predatory impacts of the nonindigenous crab *Carcinus maenas* (L.) on early benthic phase Dungeness crab *Cancer magister* Dana. J. Exp. Mar. Biol. Ecol. 258: 39-54.

Palacios KC, Ferraro SP (2003) Green crab (*Carcinus maenas* L.) consumption rates on and prey preferences among four bivalve prey species. J. Shellfish Res. 22(3): 865-871.

Pyke GH (1984) Optimal foraging theory: A critical review. Ann. Rev. Ecol. Syst. 15: 523-575.

Richards RA, Cobb JS (1986) Competition for shelter between lobsters (*Homarus americanus*) and Jonah crabs (*Cancer borealis*): Effects of relative size. Can. J. Fish. Aquat. Sci. 43: 2250–2255.

Roman J (2006) Diluting the founder effect: cryptic invasions expand a marine invader's home range. Proc. R. Soc. Lond. B. Biol. Sci. 273: 1-7.

Rossong MA, Williams PJ, Comeau M, Mitchell S, Apaloo J (2006) Agonistic interactions between invasive green crabs *Carcinus maenas* (Linnaeus) and juvenile American lobsters *Homarus americanus* (Milne Edwards). J. Exp. Mar. Biol. Ecol. 329: 281-288.

Silva IC, Alves MJ, Paula J, Hawkins SJ (2010) Population differentiation of the shore crab *Carcinus maenas* (Brachyura: Portunidae) on the southwest English coast based on genetic and morphometric analyses. Sci. Mar. 74: 435-444.

Spanier E, McKenzie TP, Cobb JS, Clancy M (1998) Behavior of juvenile American lobsters, *Homarus americanus*, under predation risk. Mar. Biol. 130: 397-406.

Tamm GR, Cobb JS (1978) Behavior and the crustacean molt cycle: changes in aggression of *Homarus americanus*. Science 200: 78-81.

Taylor DL (2005) Predatory impact of the green crab (*Carcinus maenas* Linnaeus) on post-settlement winter flounder (*Pseudopleuronectes americanus* Walbaum) as revealed by immunological dietary analysis. J. Exp. Mar. Biol. Ecol. 324: 112-126.

Wahle RA (1992) Body size dependent anti-predator mechanisms of the American lobster. Oikos 65: 52-60.

Weissburg MJ, Zimmer-Faust RK (1994) Odor plumes and how blue crabs use them in finding prey. J. Exp. Biol. 197: 349-375.

Williams PJ, Floyd TA, Rossong MA (2006) Agonistic interactions between invasive green crabs, *Carcinus maenas* (Linnaeus) and sub-adult American lobsters, *Homarus americanus* (Milne Edwards). J. Exp. Mar. Biol. Ecol. 329: 66-74.

Williams PJ, MacSween C, Rossong MA (2009) Competition between invasive green crab (*Carcinus maenas*) and American lobster (*Homarus americanus*). N. Z. J. Mar. Freshw. Res. 43: 29-34.

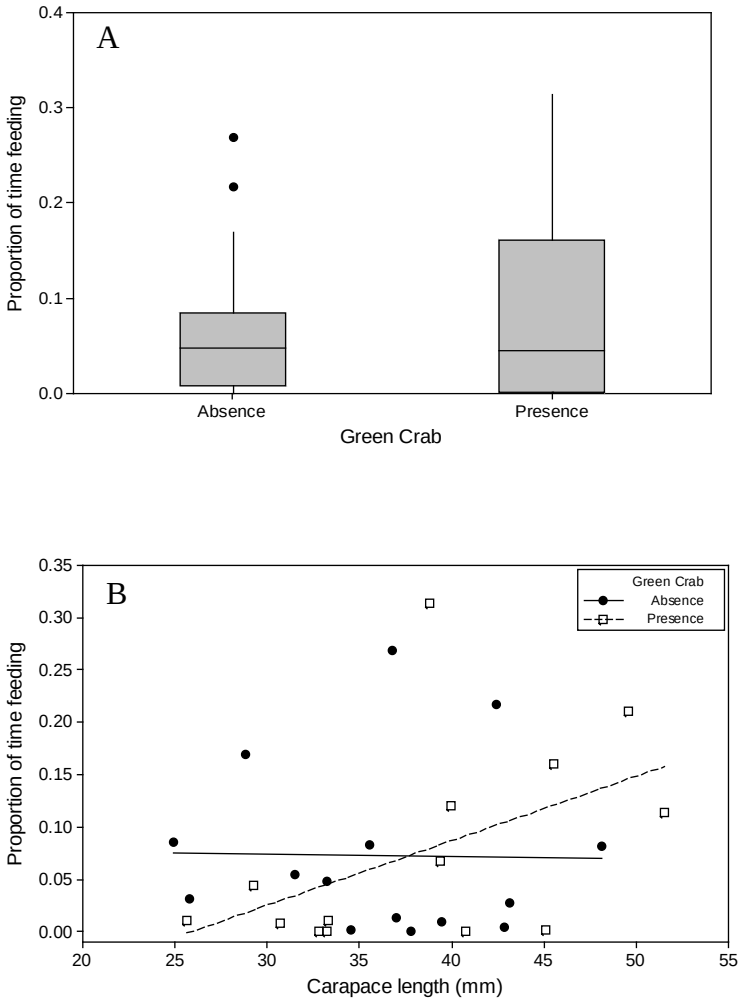


Figure 3-1. A) Boxplot of the proportion of time that the lobster spent feeding on the mussel (*Mytilus edulis*) in the presence of a caged green crab (n = 15) and absence of a caged green crab (control; n = 15). Box represents the first quartile, the median, and the third quartile. Observations that are 1.5 times the interquartile range beyond the box are plotted as individual points. No significant difference between treatments (p = 0.968). B) Scatterplot of the proportion of time spent feeding versus lobster carapace length in the presence of a caged green crab (significant regression; p = 0.030) and absence of a caged green crab (control; regression not significant; p = 0.935).

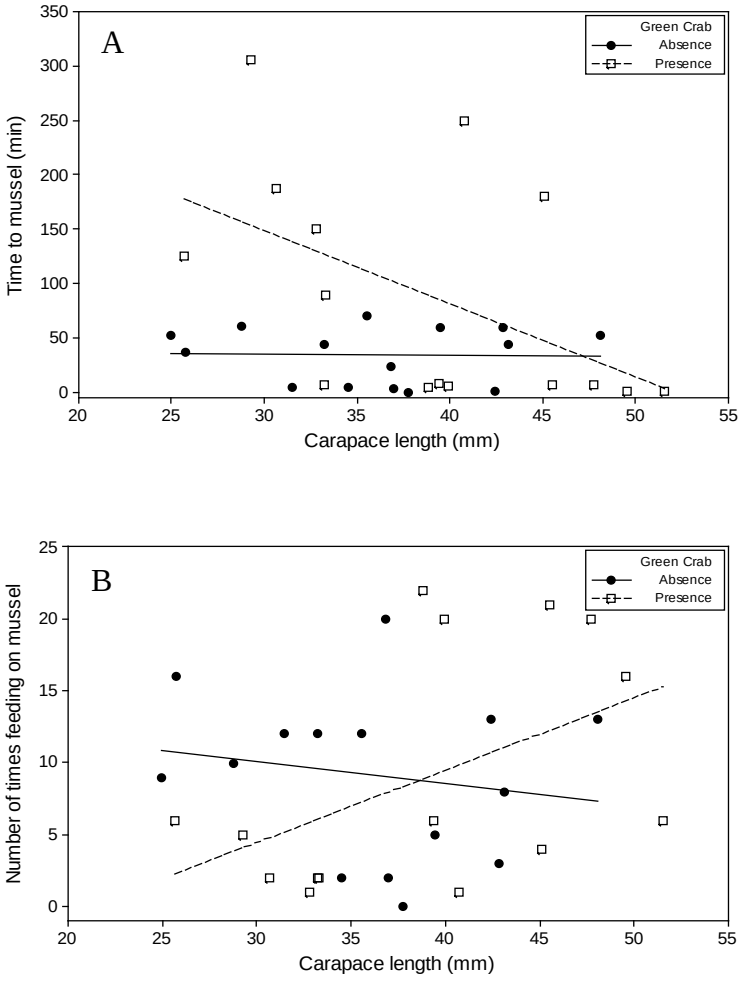


Figure 3-2. A) Scatterplot of the time elapsed before the lobster begins to feed on a mussel versus the carapace length of the lobster in the presence of a caged green crab (regression not significant; $p = 0.053$) and absence of a caged green crab (regression not significant; $p = 0.945$). B) Scatterplot of the number of times the lobster was observed feeding on a mussel versus the carapace length of the lobster in the presence of a caged green crab (regression not significant; $p = 0.234$) and absence of a caged green crab (regression not significant; $p = 0.529$).

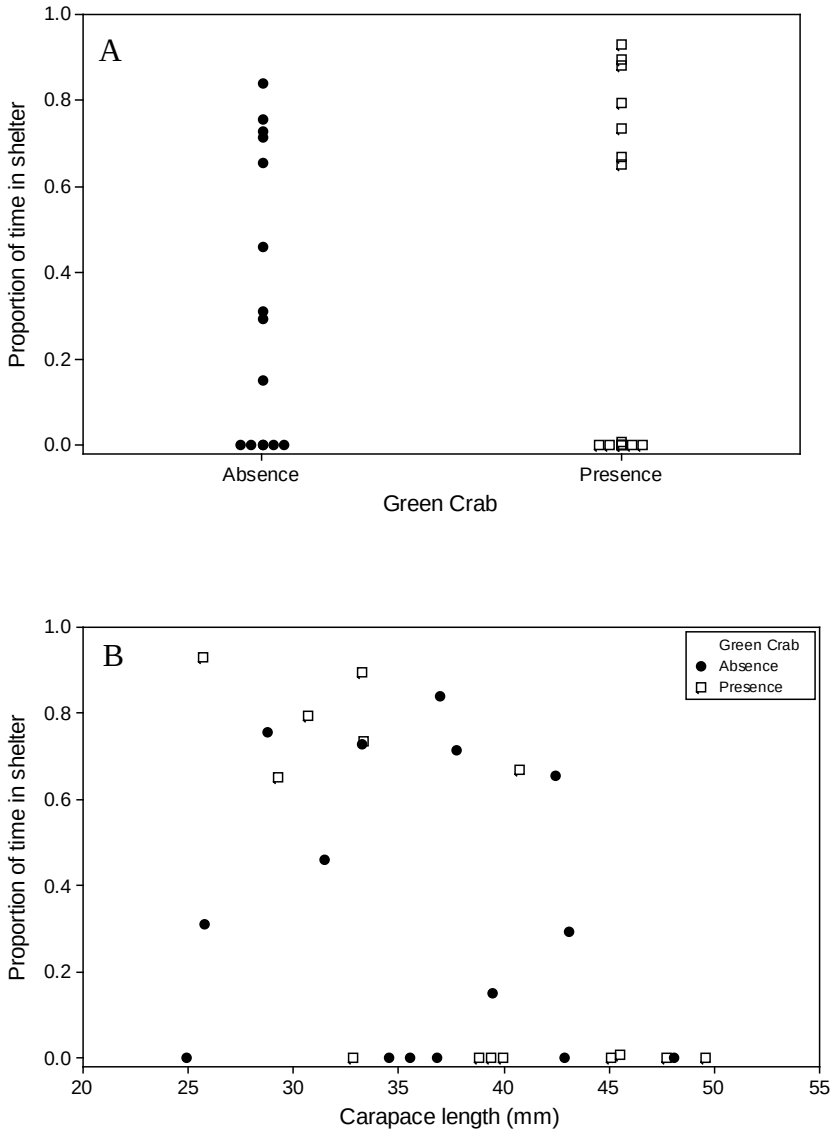


Figure 3-3. A) Individual value plot of the proportion of time that the lobster spent in the shelter in the presence of a caged green crab (n = 14) and absence of a caged green crab (n = 15). B) Scatterplot of the proportion of time the lobster spent in the shelter versus the carapace length of the lobster in the presence of a caged green crab and absence of a caged green crab.

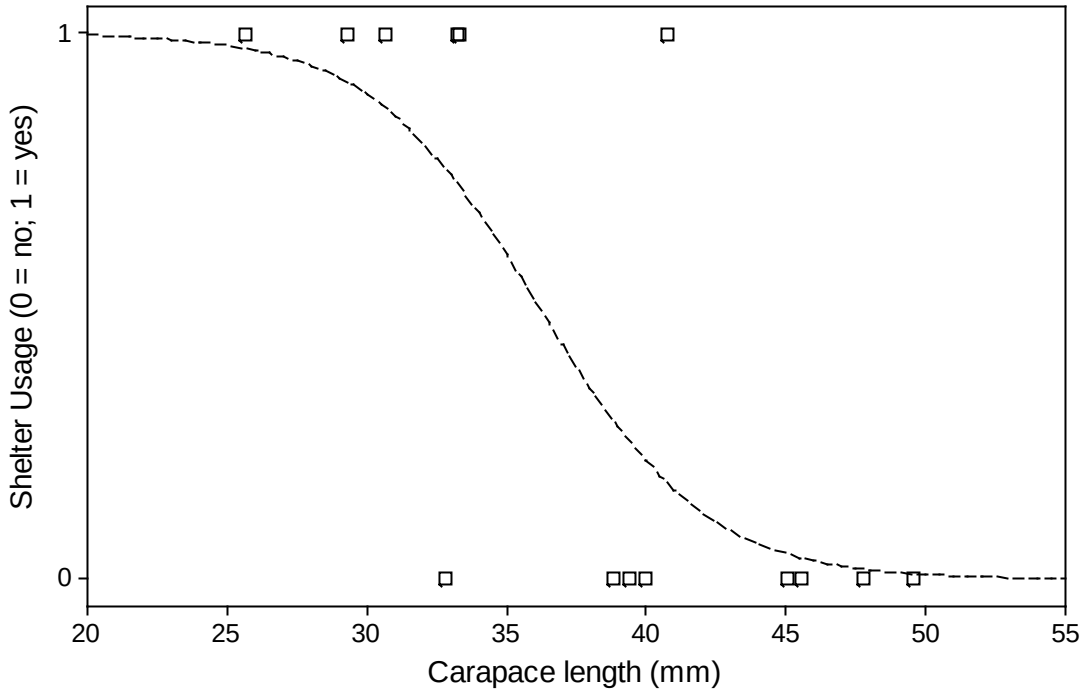


Figure 3-4. Scatterplot of shelter usage (0 = no; 1 = yes) versus carapace length of lobster in the presence of a caged green crab with a fitted binary logistic regression curve. Shelter usage was defined as no = lobster that spent less than 1% of the trial in the shelter and yes = lobster that spent more than 65% of the trial in the shelter. Carapace length was a significant predictor (p = 0.042) in the logistic regression and the model fit the data (Pearson chi-square goodness of fit p = 0.586). No lobster spent between 1 and 65% of the time in the shelter (see Figure 3-3B).

4. Potential influence of non-indigenous green crab on benthic community structure in contrasting habitats of Placentia Bay, Newfoundland

4.1. Abstract

In its native distribution range, the European green crab (*Carcinus maenas*) can alter benthic community structure through consumption of prey and habitat alteration. Similar effects in community structure are expected to occur in newly invaded areas, where the arrival of this species threatens benthic invertebrates. In this study, infaunal and epifaunal communities (family level taxonomy) were examined in three habitats (mud, sand, and eelgrass) in study areas that were invaded by green crab and not invaded by green crab, to determine whether spatial differences in benthic communities were related to the recent arrival of green crab. A short-term caging study was conducted to assess potential effects of green crab on the infaunal community. Spatial comparisons showed that taxonomic composition and density differed between invaded and control areas and among habitats. Total epifaunal density and the density of all dominant (greater than 5% of all samples) taxa were lower in invaded sites, regardless of habitat type. Epifaunal taxa richness was significantly lower in invaded mud and eelgrass habitats. Infaunal densities varied across habitats, with lowest densities in invaded mud sites. Infaunal taxa richness was greater in invaded sand habitats and lower in invaded mud and eelgrass habitats. Lower diversity for epifauna and infauna occurred in all invaded habitats. Despite differences between invaded and control sites, the lack of differences among treatments in the caging experiment precluded conclusively attributing the changes in benthic composition and density to green crab impacts.

4.2. Introduction

Benthic environments are often defined through differences in particle size, hydrodynamic regime, and the presence of plants or animals that add complexity (e.g. mussel and oyster beds; Orth et al. 1984; Wilson 1991). Biological disturbance through predation or physical disruption may further influence benthic communities by indirectly modifying structural characteristics of the habitat, particularly in intertidal and shallow water communities (Woodin 1999).

Foraging by epibenthic predators can influence community structure by altering water content and particle composition (Botto and Iribane 1999). Although these activities typically reduce macrofaunal diversity, some species can flourish under high disturbance conditions and locally reach high abundances (Sheehan et al. 2010). The impact of predators on benthic communities often depends on prey composition. If predation is important in a sedimentary habitat, it primarily impacts suspension feeders, low mobility species, and tube building infauna living closer to the surface, with little effect on highly mobile deep-burrowing species (Orth et al. 1984; Wilson 1991; Quijón and Snelgrove 2008). For example, sediment disruption can reduce larval settlement, growth in some species of bivalves (Rhoads and Young 1970), and abundances of tube building spionids through suffocation by sediments (Wilson 1981).

Invasive species often represent a novel threat to native species and community structure (Dunston and Johnson 2004; Lockwood et al. 2007), particularly when those invasive species are also predators or bioturbators. Invasive species, by definition, can spread into new areas relatively quickly, and once established potentially can alter

populations and existing community structure (Grosholz and Ruiz 1995; Grosholz et al. 2000; Beisner et al 2006). For example, the arrival and population increase of European green crab in New England has been linked to the rapid decline of soft-shell clams (*Mya arenaria*), which resulted in ecological change as well as economic loss (Behrens Yamada et al. 2005). Similar declines in other shellfish species have been recorded along the east and west coasts of North America (Grosholz et al. 2000; Breen and Metaxas 2008; Floyd and Williams 2004). Green crab can also structure communities in their native range through both predation and sediment disturbance (McDonald et al. 2006). Therefore, the invasion of this species into novel habitats is expected to impact individual prey species and associated benthic communities.

Green crab were first observed in Newfoundland coastal waters in 2007 where they now occur in the intertidal and subtidal zones of a wide range of habitats (Klassen and Locke 2007). Examination of community structure in contrasting nearshore habitats offers a unique opportunity to evaluate their initial biological effects in Placentia Bay, NL. This information is particularly valuable given that most studies on green crab impacts focus on individual species only (e.g. Floyd and Williams 2004; McDonald et al. 2001; Rossong et al. 2006), whereas few have focused on community-level effects (but see Grosholz et al. 2000). This study compares benthic community structure in three distinctive sedimentary habitat types from green crab invaded and control sites. The objectives of this study were to: 1) determine whether habitat type influences how the presence and activity of green crab drive community differences, and 2) assess the

influence of green crab on sedimentary epifauna and infauna using a short-term caging manipulative experiment.

4.3. Methods

4.3.1. Benthic Biodiversity Sampling

Intertidal sampling was conducted in two control areas (where green crab have not been reported) and two invaded areas (where green crab have been reported) in three habitat types: muddy sediments, sandy sediments and sediments associated with eelgrass beds (n = 12 sites). The sites were distributed in Placentia Bay and St. Mary's Bay, Newfoundland (Figure 4-1). Control sites were not all sampled in Placentia Bay because of habitat differences in southern Placentia Bay compared to northern invaded areas, uncertainty of green crab densities along the western side of Placentia Bay, and logistic constraints (e.g. time to get to the sites, field preservation). The sites chosen in St. Mary's Bay were similar in habitat composition and freshwater inputs, and were located at the head of the bay similar to the invaded sites in Placentia Bay.

At each site, three 25-m transects were placed parallel to the shore and spaced 5 m apart to characterize low, mid, and high beach tide exposure communities. The shallowest transect was placed at the low tide mark and the highest transect was placed near the high tide level, varying primarily in the duration of tidal exposure. Quadrats (0.5 x 0.5 m (0.25 m^2)) were sampled every 5 m along the 25 m transect (n = 5 per transect; n = 15 per site). Within each quadrat, all epifauna were enumerated. Infauna were sampled with a push corer (7 cm diameter) to a depth of 10 cm (n = 15 per site). The core was generally taken in the middle of the quadrat, however, if the core could not be pushed through the

substrate (e.g. large rocks or dense algae), the core was taken as close to the middle of the quadrat as possible. All samples were labeled and transported to the Ocean Sciences Centre at Memorial University where they were processed over a 0.5 mm sieve, fixed in a 10% formalin and seawater solution for a 60 hour period, and then rinsed with fresh water prior to storage in a 70% ethanol solution with Rose Bengal (stain) to facilitate identification.

Infaunal samples (n = 180) were examined under a dissecting microscope to separate all macrofaunal organisms, which were then identified to the family level. This level of taxonomic resolution was based on the time to identify organisms and previous work in coastal Newfoundland (Quijón and Snelgrove 2006) and elsewhere (Carney 2007) that suggests family level taxonomic resolution can differentiate communities reasonably well in environments with modest numbers of species per family and when considerable differences between treatments or groups are expected to occur.

4.3.2. Green Crab Inclusion Cage Study

To experimentally assess if potential differences in benthic community structure at invaded and control sites were driven by green crab predation or disturbance, an inclusion caging study was conducted in Ship Harbour, NL (47° 21' 37.89" N, 53° 54' 4.23" W) in June 2011. Ship Harbour is located in eastern Placentia Bay in similar habitat to the other sites sampled in this study. This site was picked because at the time of the study this site had low densities of green crab. The caging study could not be replicated in St. Mary's Bay because green crab had not yet invaded this bay and the risk of crab escaping from the enclosure was too great.

Open bottom cages (length x width x height = 1 x 1 x 0.3 m for mud and sand sites; 1 x 1 x 0.5 m for eelgrass sites) were constructed of plastic coated wire with a mesh opening of 1.0 cm^2. Cages were placed in the lower intertidal zone approximately 5 m apart with sides pushed ~5 to 10 cm into the sediment to avoid crab escape and anchored with a few large rocks placed on top of the cage. Cages were deployed simultaneously in all three habitats [mud, eelgrass (composed mostly of mud substrate) and sand] at low tide for a one-week period in areas of low green crab density. A one-week deployment was based on a similar caging study (Gregory and Quijón 2011) where green crab impacts were detected in a short time period.

Treatments were randomly assigned to one of: inclusion cages (one crab placed inside each cage; n = 6 replicate cages per substrate), partial cages (half of the full cage; to assess potential effects of caging; n = 6 replicate cages per substrate), and control (no cage, areas marked with stakes; n = 6 replicate cages per substrate). Core samples (7 cm diameter pushed to 10 cm depth; processed as described above) were taken prior to the placement of each cage and at the end of the experiment (7 days) to compare infaunal densities. All the cages were inspected after three days to remove any debris or stranded algae and to check for potential crab escape. No evidence of crab escape, sediment accumulation or cage malfunction were detected. However, a storm damaged and swept many cages onto the high beach at the sand site. The remaining cages were re-secured, but included only two green crab inclusions and no partial cages (see below). Because green crab were found in higher abundances in both eelgrass and mud habitats than sand habitats in Placentia Bay, and because of time constraints, the study was not repeated.

4.3.3. Data Analysis

4.3.3.1. Benthic Biodiversity Sampling

Raw abundance data were converted into densities as number of organisms per square metre. Abundance data were divided by the area or volume of the sampling equipment for both epifauna (0.25 m^2) and infauna (0.0038 m^2). Density data were used to determine the following benthic invertebrate summary variables:

- Total Density
- Densities of common taxa (making up more than 5% of the samples)
- Taxa richness
- Shannon-Wiener Diversity index (base e)
- Evenness
- Presence and absence of taxa by site
- Benthic community analyses based on Bray-Curtis similarity matrices and multi-dimensional scaling plots.

Rare taxa (composing less than 5% of the samples) were excluded only to reduce the number of figures in this chapter and the number of statistical comparisons, but all taxa present has been included in Table 4-6 and Table 4-7.

A three-way ANOVA was used to assess differences associated with habitat type (mud, eelgrass, sand), area (invaded or control), and beach tidal zone (low, mid, high). The ANOVA for epifaunal and infaunal densities showed significant interactions between treatment and habitat and no significant effect of beach tidal zone (Table 4-1; Table 4-2); data were therefore analyzed separately by habitat and pooled across beach tidal zones. If

assumptions of normality (Anderson-Darling test for normality) and equal variances (Levene's test) were met, a one-way ANOVA was used to test for differences between invaded and control sites. For comparisons that violated assumptions of normality, a non-parametric Kruskal-Wallis test was used.

Benthic community structure was examined using Bray Curtis similarity matrices and multi-dimensional scaling plots (MDS; 4^{th} root transformed, not standardized) using PRIMER (Clarke 1993). Analysis of similarity (ANOSIM) was used to assess similarities in benthic community among habitats and between invaded and control areas (Clarke 1993).

4.3.3.2. Green Crab Inclusion Cage Study

For the short-term caging experiment, a four-way ANOVA was used to assess total infaunal densities using habitat type (mud, eelgrass, sand), area (invaded or control), beach tidal zone (low, mid, high), and time (before and after the inclusion experiment) as factors. Densities were 4^{th} root transformed based on a Box-Cox transformation to meet the assumption of normality and equality of variances of residuals. The main effect of time (before and after the inclusion experiment) for total density was not significant, therefore only the data from the after period were used to assess differences in community structure and densities of the dominant taxa. The three caging treatments were compared using two-way ANOVAs with substrate and cage type as factors based on log transformed data. MDS plots and ANOSIM were used to present results and to assess differences in the similarity of community structure among levels of each factor.

4.4. Results

4.4.1. Benthic Biodiversity Sampling

All samples were analyzed separately for epifaunal and infaunal sampling given the marked differences in sampling protocol and surface area and volume sampled.

4.4.1.1. Epifauna

Gastropods, bivalves, and crustaceans dominated the epifauna, particularly periwinkles (*Littorina* spp.) and mussels (*Mytilus* spp.). The families Littorinidae, Mytilidae, and Gammaridae dominated sand habitats whereas eelgrass and mud habitats supported high densities of soft-shell clams (*Mya* sp.). ANOSIM revealed significant differences among habitats (*Global R* = 0.138; $p = 0.001$) and between treatments (*Global R* = 0.237; $p = 0.001$) but no differences among beach tidal heights (*Global R* = -0.008; $p = 0.681$). MDS plots of epifauna by habitat separated invaded and control areas for all three habitat types (*Global R* = 0.957; $p = 0.001$ for sand; *Global R* = 0.7; $p = 0.001$ for mud; *Global R* = 0.309; $p = 0.001$ for eelgrass; Figure 4-2).

Univariate density comparisons showed significantly lower total densities (Figure 4-3) in invaded mud and eelgrass sites (Table 4-3). In all habitats, Mytilidae densities were significantly lower at invaded sites (Table 4-3); similarly, Littorinidae densities at eelgrass and mud sites were significantly lower in invaded sites compared to controls (Table 4-3; Figure 4-3).

Most epifaunal taxa occurred at both control and invaded sites. The dogwhelk (*Nucella* sp.) was present in low abundance but only at invaded sand and eelgrass sites

(Table 4-6). Amphipods were only observed in quadrats at control sand and mud sites. No epifauna were recorded in Gooseberry Cove.

4.4.1.2. Infauna

Infaunal organisms included 24 families comprised of polychaetes (11), bivalves (2), amphipods (5); isopods (1), gastropods (2) as well as 3 other miscellaneous groups. The numerically dominant families included the polychaetes Capitellidae, Spionidae and Phyllodocidae, the periwinkles, Littorinidae, and amphipods and isopods. Analyses of similarity (ANOSIM) revealed significant differences between control and invaded sites (*Global R* = 0.169; $p = 0.001$) but no differences among beach tidal levels (*Global R* = -0.001; $p = 0.528$). MDS plots for each habitat type are summarized in Figure 4-4. Communities associated with mud (*Global R* = 0.707; $p < 0.001$; Figure 4-4A), sand (*Global R* = 0.123; $p = 0.037$; Figure 4-4B) and eelgrass (*Global R* = 0.311; $p < 0.001$; Figure 4-4C) all differed significantly between control and invaded sites. The low R value for sand suggests similar benthic communities at invaded and control sites.

In general, total infaunal densities were higher in mud compared to both sand and eelgrass substrates, and in control mud sites compared to invaded sites for 4 of the 6 dominant taxa (Table 4-4; Figure 4-5). In sand sites, phyllodocid polychaete, isopod, and amphipod densities were significantly higher in invaded sites (Table 4-4; Figure 4-5) than in control sites. Results were mixed in eelgrass beds; total densities were significantly lower in invaded sites and lower densities also characterized invaded sites for Littorinidae ($F = 36.45$; $p < 0.001$) and Capitellidae ($F = 17.8$; $p < 0.001$). The

opposite pattern was seen for Isopoda (F = 10.34; p = 0.001) and Gammaridae (F = 11.15; p = 0.001; Figure 4-5) which both increased at invaded sites.

Most infaunal taxa occurred at both control and invaded sites and their presence was largely dependent on habitat (Table 4-7). *Mytilus* species were present at all control sites but were observed only in invaded sand sites. Ostracods were observed only at two control sites. Reference mud sites and one eelgrass site had the greatest total richness (15 taxa).

4.4.1.3. Community Structure Comparisons

For both epifauna and infauna, the number of families per sample varied with substrate and between treatments. Fewer families of epifauna characterized invaded sites than control sites but the difference was significant only for mud and eelgrass sites (Table 4-3; Figure 4-6). Infaunal mud and eelgrass at invaded sites had significantly fewer taxa than control sites (Table 4-4), but significantly more taxa at the sand sites. Infaunal evenness was significantly higher in invaded mud sites compared to controls (Table 4-4; Figure 4-6). Shannon-Wiener diversity index for epifauna was significantly lower in invaded sites for all 3 substrates (Figure 4-6; $p < 0.003$ for all). In contrast, Shannon-Wiener infaunal diversity was significantly higher for invaded sand (F = 8.39; p = 0.006) sites but lower at mud sites (F = 10.78; p = 0.001) compared to controls. No differences were observed for eelgrass sites.

4.4.2. Green Crab Inclusion Cage Study

Because of the reduced number of samples in sandy sediments (see Methods), I was only able to compare total density of infaunal organisms in mud and eelgrass beds. Across substrates, core samples encompassed 30 different families spanning polychaetes (16), crustaceans (7), gastropods (4), and bivalves (3). MDS plots separated mud and eelgrass benthic communities (ANOSIM *Global R* = 0.204, $p = 0.001$) but the caging factor was not significant (Figure 4-7; *Global R* = -0.068, $p = 0.756$ for mud; *Global R* = 0.08, $p = 0.226$ for eelgrass).

The absence of significant interaction terms allowed direct comparison of main level effects in the four-way ANOVA comparing total density (Table 4-5). Total density did not differ significantly between time period ($F_{1,36} = 2.96$; $p = 0.094$), among cage types ($F_{2,36} = 0.48$; $p = 0.624$), or tidal beach height ($F_{2,36} = 2.59$; $p = 0.089$) but differed between mud and eelgrass habitats ($F_{1,36} = 46.78$; $p < 0.001$). Analyses showed no statistically significant differences for densities of the common taxa or for benthic community variables (Figure 4-8; Figure 4-9).

4.5. Discussion

4.5.1. Benthic Biodiversity Sampling

My results reflected expected differences in species composition and density in response to habitat type (Snelgrove 1998) but provide mixed evidence on the effects of presence or absence of green crab on benthic biodiversity. Community structure differed significantly among mud, eelgrass, and sand sampling sites, suggesting a strong influence

of habitat. Community structure and individual taxa densities differed between control sites and those currently exposed to green crab populations, however, because the caging study was inconclusive, I could not determine a causal relationship. High variability in community structure among widely spaced sites also limited definitive conclusions. Therefore comparisons for each habitat are discussed separately.

4.5.1.1. Epifauna

Epifaunal composition in eelgrass and mud were generally similar but less similar than infaunal organisms. Total densities were significantly lower across all substrates in invaded areas compared with control areas. In all cases except Myidae in mud sites, bivalve and gastropod densities were significantly lower in areas with green crab in comparison to control sites. This pattern was consistent with results of other experimental studies assessing green crab impacts (e.g. Grosholz and Ruiz 1995; Grosholz et al. 2000). Bivalves and gastropods are preferred prey species for green crab in both their introduced and native ranges (Klassen and Locke 2007; Pickering and Quijón 2011). Hence, lower density and, in some cases, absence of these prey items were expected in areas now densely populated by green crab. Similarly, studies have linked bivalve declines to increased abundance of other non-native species (e.g. red king crab, *Paralithodes camtschaticus* in the Barents Sea (Britayev et al. 2010)).

4.5.1.2. Infauna

Spatial differences in benthic community among control and invaded sites varied with habitat type and appeared more pronounced in muddy sediments. Green crab can

manipulate prey and forage in mud substrates much more readily than in eelgrass or sandy sediments. Densities of all major families (including bivalves, polychaetes, amphipods, isopods, and gastropods) were lower in green crab invaded mud sites than in control sites. The only exceptions were phyllodocid and spionid polychaetes. Phyllodocid worms occur in relatively low densities and live deep in sediments below the upper few centimeters where green crab typically prefer to feed (see Floyd and Williams 2004). In contrast, surface-feeding spionids would be available in the preferred sediment depth of green crab, but may reduce their vulnerability by burrowing in the sediment (Cheverie 2012). Typically, crab foraging disturbance decreases polychaete abundances (Botto and Iribarne 1999; Fernandes et al. 1999; Gregory and Quijón 2011), as observed with Capitellidae in my study. In addition, significantly higher taxonomic richness and diversity at control sites indicates that green crab may reduce infaunal diversity, at least at small spatial scales.

In sandy sediments, isopods, amphipods and Phyllodocidae densities were highest in invaded areas. These results may relate to bioturbation, a process that can redistribute organic matter and ultimately affect both vertical distribution and community structure (Dauwe et al. 1998). The reworking of sandy sediments by green crab may allow a greater number of early colonizing organisms to flourish (e.g. Isopoda) while negatively impacting other organisms. In terms of community structure, the average number of taxa and Shannon-Weiner diversity were significantly higher at invaded sandy sites. Sand substrates offer interstitial spaces and rebuff foraging more effectively than mud, potentially decreasing predation rates and supporting higher numbers of infaunal taxa.

Total density was lower in invaded eelgrass sites. This observation contradicts previous studies that show that complex structures such as rhizomes and other plant material may reduce the effects of epibenthic predation (Orth et al. 1984; Summerson and Peterson 1984). In my study, lower densities of suspension feeders, gastropods, and some polychaetes characterized invaded sites. In contrast only mobile amphipods and isopods occurred in higher abundances in invaded areas, likely because both taxa quickly colonize disturbed ecosystems (Lenihan and Oliver 1995). In another study, predation rates by epibenthic predators on blue mussels were 70% lower in seagrass beds compared to adjacent mudflats (Frandsen and Dolmer 2002), explaining the absence of a predation effect on densities of a wide variety of infaunal species in eelgrass beds (cf. Summerson and Peterson 1984).

The effects of green crab on the eelgrass plant itself may partly explain lower infaunal and epifaunal abundances in my green crab impacted eelgrass sites. In other studies, green crab have reduced eelgrass biomass through digging of pits, destruction of plant shoots (Garbary et al. 2013), and grazing by juveniles (Malyshev and Quijón 2011). Despite increases in eelgrass cover elsewhere in Newfoundland (e.g. Warren et al. 2010), a similar decline in eelgrass cover has occurred in Placentia Bay (C McKenzie, pers. comm.) where my study took place, suggesting that green crab may be reducing habitat complexity relative to control sites, possibly leading to long-term changes in infaunal organisms.

4.5.2. Green Crab Inclusion Cage Study

Although community structure differed among sites depending on green crab presence or absence, my sites also displayed considerable natural variability. Both epifauna and infauna varied appreciably between locations and among samples within locations. Differences in fauna over small spatial scales may arise from variability in environmental parameters, slight variations in habitat (beach slope, presence of algae etc.), other predators (e.g. shorebirds) and currents. In this study I explicitly controlled for habitat (i.e. I compared similar habitats across sites). However, slight changes in conditions can nonetheless affect species composition and abundance above and beyond obvious among-habitat differences. I therefore cautiously conclude that green crab may be one of several major drivers of spatial community structure in these habitats (see below).

Epibenthic predators and their effects on community structure have been widely studied (Wilson 1991). Most studies use exclusion or inclusion of predators with cages to reduce predation intensity or to examine the impacts of one particular predator by confining it to a cage for a specified time period (Berge and Alvarez-Valderhaug 1983; Wilson 1991). Most predator exclusion experiments demonstrate increased abundance and biomass of infauna when predators are removed, whereas species diversity either increases or remains unchanged (see Wilson 1991; Quijón and Snelgrove 2005). However, cage studies are often criticized because the structures themselves can alter hydrodynamics and thus sedimentation rates, larval transport, and food supply which can ultimately change benthic composition (Hulberg and Oliver 1980).

My caging study assessed experimentally whether green crab presence was causally related to spatial differences in infauna and epifauna in Placentia Bay. I detected several changes in infaunal species diversity and abundance within 7 days in partial and full cages whereas reference sites remained relatively unchanged. Despite the fact that reference sediment types did not significantly differ from partial cages, I cannot fully discard a potential artifact effect. Green crab or a combination of green crab and some caging effect that could not visually be identified likely caused the modest number of observed differences.

In similar caging experiments conducted on Prince Edward Island, infaunal organisms in inclusion cages containing low and high densities of green crab declined by 50% compared to cages without green crab over a similar time period (Gregory and Quijón 2011). Similarly, green crab significantly reduced the abundance of soft-shell clams (*Mya arenaria*), small gastropods, and polychaetes in caging studies conducted in Nova Scotia (Floyd and Williams 2004). In my eelgrass site, as in Gregory and Quijón (2011), polychaetes declined most in full cages whereas bivalves such as Myidae and Mytilidae remained largely unchanged for all cage treatments. It is noteworthy that bivalve abundances declined over the 7 days of the experiment in both substrates whereas polychaete densities remained relatively similar across treatments. Previous literature suggests that a single green crab can reduce infaunal densities within a limited time frame over small spatial scales (e.g. Gregory and Quijón 2011, Floyd and Williams 2004). However, my results indicate that these effects may change among habitats and may be easily confounded by other variables, particularly for infaunal organisms. Unfortunately,

the location selected for my field experiment lacked a prominent epifaunal component, which were the group of organisms that appeared to change most in previous spatial comparisons (Lohrer and Whitlatch 2002; Baeta et al. 2005). The limited ability to draw conclusions on the causal effects of green crab on these communities requires further experiments in these and other habitats while demonstrably minimizing potential caging artifacts. Experiments conducted over a longer time period (e.g. a month) or with variable green crab densities would likely add further insight, however, green crab caging studies conducted over a longer time period and with higher densities in Nova Scotia also produced inconclusive results (Thompson 2007; Cheverie 2012).

4.5.3. Conclusions

This study shows clear differences in epifaunal and infaunal community composition and density among sand, mud and eelgrass habitats. Locations exposed to green crab and locations not yet invaded clearly differed, however, the caging study did not confirm that these changes were the result of green crab. Although additional factors likely contributed to spatial variability, the high densities of this new invader likely contribute to the spatial differences I report. Because green crab are generalist predators, their overall impact exceeds that of specialized feeders because they adapt their feeding behaviour based on food availability (Cohen et al. 1995; Enderlein and Wahl 2004). Green crab can potentially decrease the density and diversity of native benthic communities, and their recent arrival in Placentia Bay offered a unique opportunity to study the initial impact of this species in three distinctive habitats. Unfortunately, the rapid spread of the species severely limited the number of reference sites available for

direct comparisons within a local area. High variability in community structure among widely spaced sites limited stronger conclusions regarding the specific effects of green crab and their contribution to spatial differences. Thus, assessing the impacts of this invasive species as it continues to spread across the region requires further study.

4.6. References

Behrens Yamada S, Dumbauld BR, Kalin A, Hunt CE, Figlar-Barnes R, Randall A (2005) Growth and persistence of a recent invader *Carcinus maenas* in estuaries of the northeastern Pacific. Biol. Invasions 7: 309-321.

Beisner BE, Hovius J, Hayward A, Kolasa J, Romanuk TN (2006) Environmental productivity and biodiversity effects on invertebrate community invasibility. Biol. Invasions 8: 655-664.

Berge JA, Valderhaug VA (1983) Effect of epibenthic macropredators on community structure in subtidal organically enriched sediments in the inner Oslofjord. Mar. Ecol. Prog. Ser. 11: 15-22.

Botto F, Iribarne O (1999) Effect of the burrowing crab *Chasmagnathus granulata* (Dana) on the benthic community of a SW Atlantic coastal lagoon. J. Exp. Mar. Biol. Ecol. 241: 263-284.

Breen E, Metaxas A (2008) A comparison of predation rates by non-indigenous and indigenous crab (juvenile *Carcinus maenas*, juvenile *Cancer irroratus*, and adult *Dyspanopeus sayi*) in laboratory and field experiments. Estuaries Coasts 31: 728-737.

Britayev TA, Rzhavsky AV, Pavlova LV, Dvoretskij AG (2010) Studies on impact of the alien red king crab (*Paralithodes camtschaticus*) on the shallow water benthic communities of the Barents Sea. J. Appl. Ichthyol. 26: 66-73.

Carney RS (2007) Use of diversity estimations in the study of sedimentary benthic communities. Oceanogr. Mar. Biol. Annual Rev. 45: 139-172.

Cheverie A (2012) Predator effects of the invasive green crab (*Carcinus maenas*) and the native rock crab (*Cancer irroratus*) on soft-sediment macrofauna. MSc thesis, Dalhousie University, Halifax, NS.

Clarke KR (1993) Non-parametric multivariate analyses of changes in community structure. Aust. J. Ecol. 18: 117-143.

Cohen AN, Carlton JT, Fountain MC (1995) Introduction, dispersal and potential impacts of the green crab *Carcinus maenas* in San Francisco Bay, California. Mar. Biol. 122: 225-237.

Dauwe B, Herman PMJ, Heip CHR (1998). Community structure and bioturbation potential of macrofauna at four North Sea stations with contrasting food supply. Mar. Ecol. Prog. Ser. 173: 67-83.

Dunstan PK, Johnson CR (2004). Invasion rates increase with species richness in a marine epibenthic community by two mechanisms. Oecologia 138: 285-292.

Enderlein P, Wahl M (2004). Dominance of blue mussels versus consumer-mediated enhancement of benthic diversity. J. Sea Res. 51: 145-155.

Floyd TA, Williams PJ (2004) Impact of green crab (*Carcinus maenas* L.) predation on a population of soft-shell clams (*Mya arenaria* L.) in the southern Gulf of St. Lawrence. J. Shellfish Res. 23(2): 457-462.

Frandsen RP, Dolmer P (2002) Effects of substrate type on growth and mortality of blue mussels (*Mytilus edulis*) exposed to the predator *Carcinus maenas*. Mar. Biol. 141: 253-262.

Garbary DJ, Miller AG, Williams J, Seymour NR (2013) Drastic decline of an extensive eelgrass bed in Nova Scotia due to the activity of the invasive green crab (*Carcinus maenas*). Mar. Biol. 161: 1-13.

Gregory GJ, Quijón PA (2011) The impact of a coastal invasive predator on infaunal communities: Assessing the roles of density and a native counterpart. J. Sea Res. 66: 181-186.

Grosholz ED, Ruiz GM (1995). Spread and potential impact of the recently introduced European green crab, *Carcinus maenas*, in central California. Mar. Biol. 122: 239-247.

Grozholz ED, Ruiz GM, Dean CA, Shirley KA, Maron JL, Connors PG (2000) The impacts of a nonindigenous marine predator in a California Bay. Ecology 81: 1206-1224.

Hulberg LW, Oliver JS (1980) Caging manipulations in marine soft-bottom communities: importance of animal interactions or sedimentary habitat modifications. Can. J. Fish. Aquat. Sci. 37: 1130–1139.

Klassen G, Locke A (2007) A biological synopsis of the European green crab, *Carcinus maenas*. Can. Manuscr. Rep. Fish. Aquat. Sci. no. 2818.

Lenihan HS, Oliver JS (1995) Anthropogenic and natural disturbances to marine benthic communities in Antarctica. Ecol. Appl. 5: 311-326.

Lockwood JL, Hoopes MF, Marchetti MP (2007) Invasion Ecology. Blackwell Publishing , UK.

Lohrer AM, Whitlatch RB (2002) Relative impacts of two exotic brachyuran species on blue mussel populations in Long Island sound. Mar. Ecol. Prog. Ser. 227: 135-144

Malyshev A, Quijón PA (2011) Disruption of essential habitat by a coastal invader: new evidence of the effects of green crabs on eelgrass beds. ICES J. Mar. Sci. 68: 1852-1856.

McDonald PS, Holsman KK, Beauchamp DA, Dumbauld BR, Armstrong DA (2006) Bioenergetics modeling to investigate habitat use by the nonindigenous crab, *Carcinus maenas*, in Willapa Bay, Washington. Estuaries Coasts 29: 1132-1149.

McDonald PS, Jensen GC, Armstrong DA (2001) The competitive and predatory impacts of the nonindigenous crab *Carcinus maenas* (L.) on early benthic phase Dungeness crab *Cancer magister* Dana. J. Exp. Mar. Biol. Ecol. 258: 39-54.

Orth RJ, Heck Jr KL, van Montfrans J (1984) Faunal communities in seagrass beds: A review of the influence of plant structure and prey characteristics on predator: prey relationships. Estuaries 7: 339-350.

Pickering T, Quijón PA (2011) Potential effects of a non-indigenous predator in its expanded range: assessing green crab, *Carcinus maenas*, prey preference in a productive coastal area of Atlantic Canada. Mar. Biol. 158: 2065-2078.

Quijón PA, Snelgrove PVR (2005) Differential regulatory roles of crustacean predators in sub-arctic, soft-sediment system. Mar. Ecol. Prog. Ser. 285: 137-149.

Quijón PA, Snelgrove PV (2006). The use of coarser taxonomic resolution in studies of predation on marine sedimentary fauna. J. Exp. Mar. Biol. Ecol. 330: 159-168.

Quijón PA, Snelgrove PVR (2008) Trophic complexity in marine sediments: new evidence from the Gulf of St. Lawrence. Mar. Ecol. Prog. Ser. 371: 85-89.

Rhoads DC, Young DK (1970). The influence of deposit-feeding organisms on sediment stability and community trophic structure. J. Mar. Res. 28: 150-178.

Rossong MA, Williams PJ, Comeau M, Mitchell S, Apaloo J (2006) Agonistic interactions between invasive green crab *Carcinus maenas* (Linnaeus) and juvenile American lobsters *Homarus americanus* (Milne Edwards). J. Exp. Mar. Biol. Ecol. 329: 281-288.

Seitz RD (2011) Gradient effects on structuring of soft-bottom benthic infauna: *Macoma balthica* and predation, recruitment, and food availability. J. Exp. Mar. Biol. Ecol. 409: 114-122.

Sheehan EV, Coleman RA, Thompson RC, Attrill MJ (2010) Crab-tiling reduces the diversity of estuarine infauna. Mar. Ecol. Prog. Ser. 411: 137-148.

Snelgrove PVR (1998) The biodiversity of macrofaunal organisms in marine sediments. Biodivers. Conserv. 7: 1123-1132.

Summerson HC, Peterson CH (1984) Role of predation in organizing benthic communities of a temperate-zone seagrass bed. Mar. Ecol. Prog. Ser. 15: 63-77.

Thompson J (2007) Population-level effects of the European green crab (*Carcinus maenas* L.) in an eelgrass community of the southern Gulf of St. Lawrence. MSc thesis, University of New Brunswick, Saint John, NB.

Warren MA, Gregory RS, Laurel BJ, Snelgrove PVR (2010). Increasing density of juvenile Atlantic (*Gadus morhua*) and Greenland cod (*G.ogac*) in association with spatial expansion and recovery of eelgrass (*Zostera marina*) in a coastal nursery habitat. J. Exp. Mar. Biol. Ecol 394: 154-160.

Wilson WH Jr (1981) Sediment mediated interactions in a denscly populated infaunal assemblage: the effects of the polychaete *Abarenicola pacifica*. J. Mar. Res. 39: 735-48.

Wilson WH (1991) Competition and predation in marine soft-sediment communities. Annu. Rev. Ecol. Syst. 21: 221-241.

Woodin SA (1999) Shallow water benthic ecology: A North American perspective of sedimentary habitats. Austral Ecol. 24: 291-301.

Table 4-1. Analysis of variance table for the three-way analysis of variance on total density for epifauna with factors, area (invaded, control), habitat, and tidal beach height.

Source	**Degrees of Freedom**	***F*- Statistic**	***p*-value**
Area	1	44.4	<0.001
Habitat	2	1.67	0.192
Beach	2	0.57	0.564
Area*Habitat	2	6.63	0.002
Area*Beach	2	0.87	0.423
Habitat*Beach	4	<0.01	1.000
Area*Habitat*Beach	4	0.46	0.767
Error	162		
Total	179		

Table 4-2. Analysis of variance table for the three-way analysis of variance on total density for infauna with factors, area (invaded, control), habitat, and tidal beach height.

Source	**Degrees of Freedom**	***F*- statistic**	***p*-value**
Area	1	0.25	0.618
Habitat	2	16.61	<0.001
Beach	2	1.04	0.355
Area*Habitat	2	18.53	<0.001
Area*Beach	2	0.10	0.908
Habitat*Beach	4	0.92	0.454
Area*Habitat*Beach	4	0.87	0.482
Error	162		
Total	179		

Table 4-3. A comparison of control and invaded areas for benthic invertebrate endpoints for the epifaunal component of the benthic study for sand, mud, and eelgrass habitats.

Habitat	**Endpoint**	**Test**	**Test Statistic**	**df (N)**	**df (D)**	***p*-value**
Sand	SDI	K-W	24.98	1		<0.001
	Evenness	K-W	16.09	1		<0.001
	Richness	K-W	0.20	1		0.657
	Mytilidae	K-W	19.14	1		<0.001
	Littorinidae	K-W	0.83	1		0.361
	Total Density	K-W	0.83	1		0.361
Mud	SDI	K-W	8.91	1		0.003
	Evenness	K-W	9.69	1		0.002
	Richness	K-W	29.27	1		<0.001
	Mytilidae	K-W	12.71	1		<0.001
	Littorinidae	K-W	47.76	1		<0.001
	Myidae	K-W	0.02	1		0.879
	Total Density	ANOVA	113.73	1	58	<0.001
Eelgrass	SDI	ANOVA	19.96	1	52	<0.001
	Evenness	K-W	6.19	1		0.013
	Richness	K-W	15.31	1		<0.001
	Mytilidae	K-W	21.38	1		<0.001
	Littorinidae	K-W	7.18	1		0.007
	Myidae	K-W	16.01	1		<0.001
	Total Density	ANOVA	28.53	1	58	<0.001

df = degrees of freedom; N = numerator (if applicable); D = denominator; K-W = Kruskal-Wallis; ANOVA = Analysis of Variance; SDI = Shannon Diversity Index

Table 4-4. A comparison of control and invaded areas for benthic invertebrate endpoints for the infaunal component of the benthic study for sand, mud, and eelgrass habitats.

Habitat	Endpoint	Test	Test Statistic	df (N)	df (D)	*p*-value
Sand	SDI	ANOVA	8.39	1	50	0.006
	Evenness	ANOVA	0.17	1	50	0.685
	Richness	ANOVA	13.66	1	58	<0.001
	Gammaridae	K-W	9.35	1		0.002
	Littorinidae	K-W	0.48	1		0.488
	Isopoda	K-W	9.28	1		0.002
	Capitellidae	K-W	0.22	1		0.637
	Spionidae	K-W	<0.01	1		0.979
	Phyllodocidae	K-W	32.05	1		<0.001
	Total Density	K-W	2.76	1		0.097
Mud	SDI	K-W	10.78	1		0.001
	Evenness	ANOVA	15.28	1	57	<0.001
	Richness	ANOVA	144.07	1	58	<0.001
	Gammaridae	K-W	11.45	1		0.001
	Littorinidae	K-W	36.45	1		<0.001
	Isopoda	K-W	10.34	1		0.001
	Capitellidae	K-W	17.8	1		<0.001
	Spionidae	K-W	3.38	1		0.066
	Phyllodocidae	K-W	2.98	1		0.084
	Total Density	K-W	28.58	1		<0.001

Continued on next page

Table 4-4. A comparison of control and invaded areas for benthic invertebrate endpoints for the infaunal component of the benthic study for sand, mud, and eelgrass habitats (cont.).

Habitat	Endpoint	Test	Test Statistic	df (N)	df (D)	*p*-value
Eelgrass	SDI	K-W	0.39	1		0.530
	Evenness	ANOVA	4.74	1	58	0.033
	Richness	ANOVA	144.07	1	58	<0.001
	Gammaridae	K-W	11.15	1		0.001
	Littorinidae	K-W	36.45	1		<0.001
	Isopoda	K-W	10.34	1		0.001
	Capitellidae	K-W	17.8	1		<0.001
	Spionidae	K-W	3.38	1		0.066
	Phyllodocidae	K-W	2.98	1		0.084
	Total Density	K-W	28.58	1		<0.001

df = degrees of freedom; N = numerator (if applicable); D = denominator; K-W = Kruskal-Wallis; ANOVA = Analysis of Variance; SDI = Shannon diversity index

Table 4-5. Analysis of variance table for the four-way analysis of variance on total density for the caging study with factors, habitat, cage type, beach, and time.

Source	**Degrees of Freedom**	***F*-statistic**	***p*-value**
Beach	2	2.59	0.089
Habitat	1	46.78	<0.001
Cage	2	0.48	0.624
Time	1	2.96	0.094
Beach*Habitat	2	0.05	0.952
Beach*Cage	4	1.42	0.248
Beach*Time	2	1.51	0.235
Habitat*Cage	2	0.39	0.680
Habitat*Time	1	0.46	0.500
Cage*Time	2	0.54	0.585
Beach*Habitat*Cage	4	1.24	0.310
Beach*Habitat*Time	2	1.38	0.264
Beach*Cage*Time	4	0.15	0.962
Habitat*Cage*Time	2	2.21	0.124
Beach*Habitat*Cage*Time	4	0.69	0.603
Error	36		

Table 4-6. Presence and absence of epifaunal taxa in green crab (invaded) and no green crab (control) sites from benthic quadrat sampling in sand, mud and eelgrass habitats from sites located in Placentia and St. Mary's Bay, NL.

Major Group	Treatment	Control		Invaded		Control		Invaded		Control		Invaded	
	Habitat	Sand				Mud				Eelgrass			
	Site	Point Verde	Gooseberry Cove	Goose Cove	North Hbr	Harricott	North Hbr	North Hbr	North Hbr	North Hbr East	North Hbr East	North Hbr	North Hbr
Amphipoda	-					X							
	Talitridae	X											
Bivalvia	Baltic macoma							X					
	Mytilidae	X		X	X	X	X	X	X	X	X	X	X
	Myidae					X	X	X		X	X		
Decapoda	Carcinus maenas	X		X	X	X	X	X	X	X	X	X	X
Gastropoda	unidentified snails	X		X	X	X	X	X	X	X	X	X	X
	Littorina sp.	X											
	Littorina littorea			X	X	X			X				
	Littorina saxatillus					X							
	moonsnail								X				
	Tectura testudinalis												
	Nucella lapillus			X	X							X	
Isopoda	-					X							
	Total	**5**	**0**	**5**	**5**	**8**	**4**	**5**	**5**	**4**	**4**	**4**	**3**

Note: X= presence of the taxa; '-' = species unidentified to family level.

Table 4-7. Presence and absence of infaunal taxa in green crab (invaded) and no green crab (control) sites from benthic core sampling in sand, mud and eelgrass habitats sites located in Placentia and St. Mary's Bay, NL.

Major Group	Treatment	Control		Invaded		Control		Invaded		Control		Invaded	
	Habitat	Sand				Mud				Eelgrass			
	Site	Point Verde	Gooseberry Cove	Goose Cove	North Hbr	Harricott	North Hbr East	North Hbr	North Hbr	North Hbr East	North Hbr East	North Hbr	North Hbr
	Family												
Amphipoda	-		X	X								X	X
	Calliopidae					X				X		X	X
	Corophidae									X	X	X	
	Gammaridae	X	X	X	X	X	X		X	X	X	X	X
	Hausteridae		X										
	Stenothoidae												X
Bivalvia	-		X										
	Mya arenaria			X		X	X			X	X	X	X
	Mytilus edulis	X	X	X	X	X	X			X	X		
Copepoda	-										X		
Decapoda	-	X		X						X			
Gastropoda	-						X						
	Littorinidae	X	X	X	X	X	X	X		X	X	X	
	Buccinidae			X		X	X	X		X		X	
Isopoda	-	X			X	X	X		X	X	X	X	X
Ostracoda	-	X				X							
Polychaetes	-	X		X	X	X	X	X	X	X	X		X
	Capitellidae	X	X	X	X	X	X	X	X	X	X	X	X
	Gonodidae									X			
	Lumbrineridae					X	X						X
	Maldonidae						X						
	Onuphidae			X		X	X	X		X	X		X
	Paraonidae					X							
	Pectinaria						X						
	Phyllodocidae			X	X	X	X	X	X	X	X	X	X
	Spionidae	X		X	X	X	X	X	X	X	X	X	X
	Serpulidae	X											
	Trichobranchidae	X											
Total		**11**	**7**	**12**	**8**	**15**	**15**	**7**	**6**	**15**	**12**	**11**	**12**

X = presence of the taxa; '-' = species unidentified to family level.

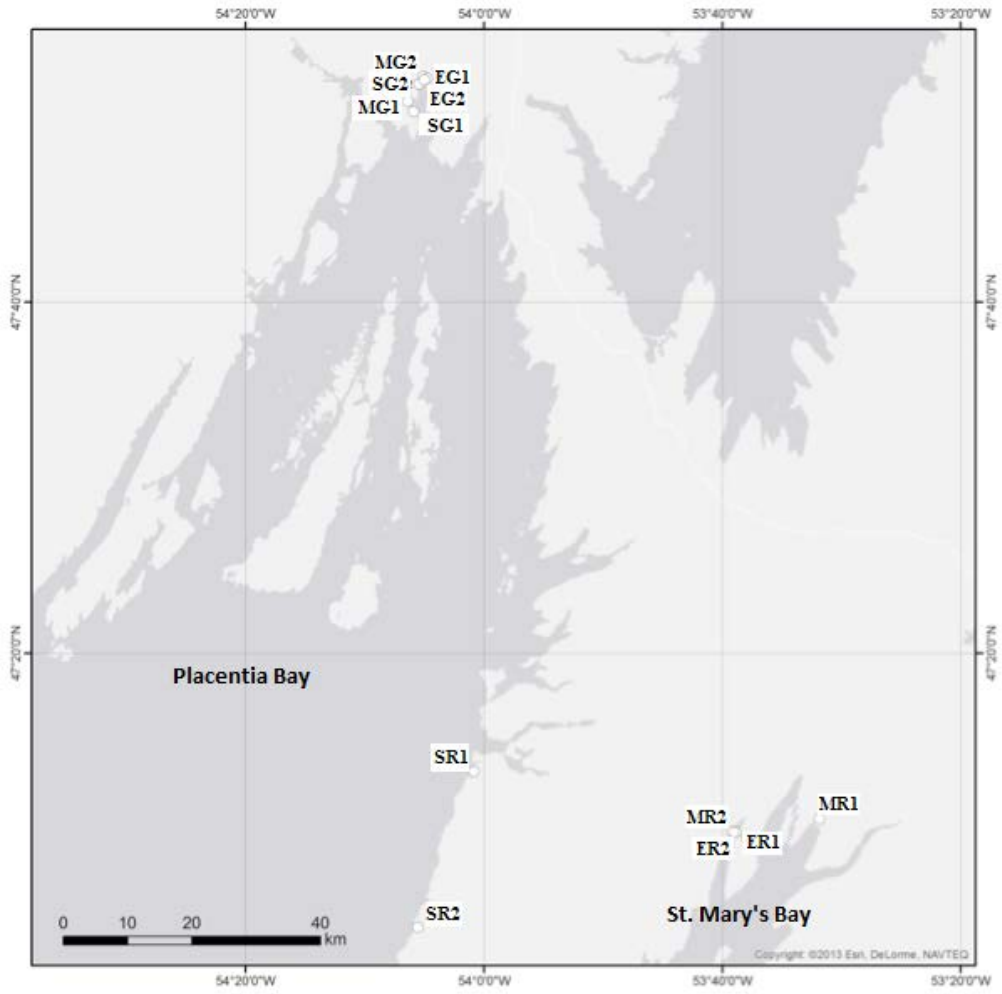
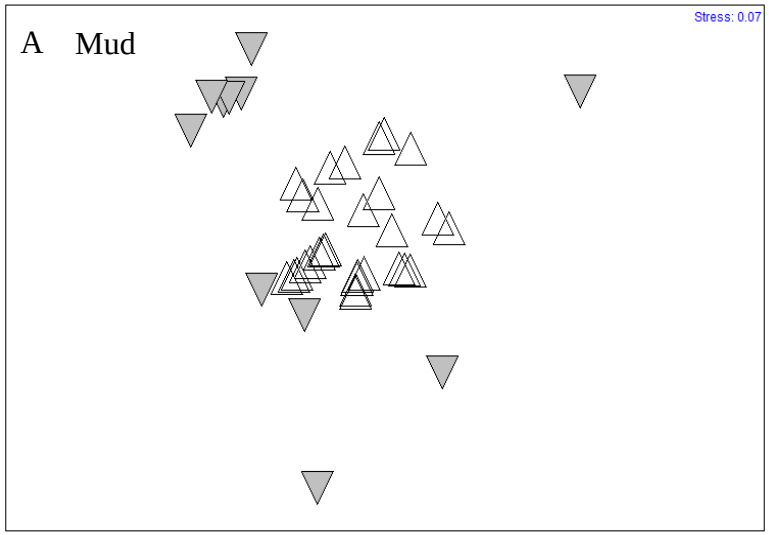
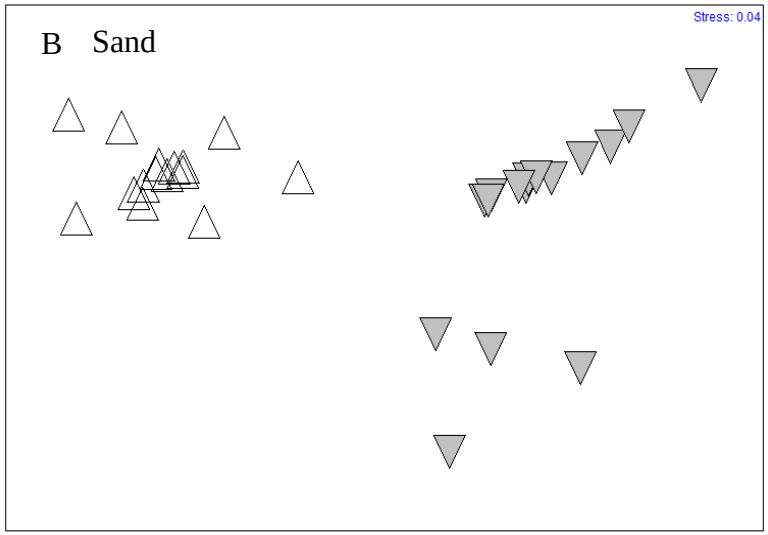


Figure 4-1. Map of sampling sites for mud (M), eelgrass (E) and sand (S) substrates for sites without green crab (R) and sites with green crab (G) in Placentia Bay (SR1 = Point Verde; SR2 = Gooseberry Cove; ME1 and SE1 = North Harbour; ME2, ER1, ER2 and SE2 = Goose Cove) and St. Mary's Bay, NL (MR1= Harricott; MR2, ER1 and ER2 = North Harbour East). Two sites were sampled for each treatment (crab, no crab) for all substrates.





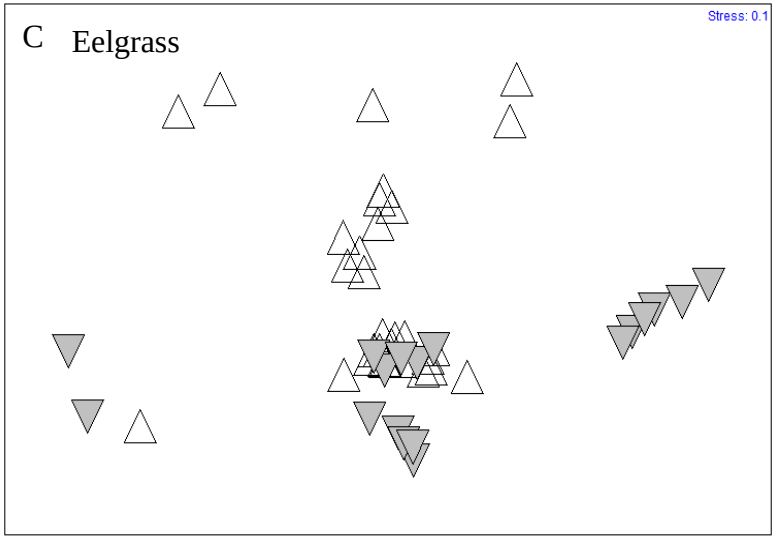
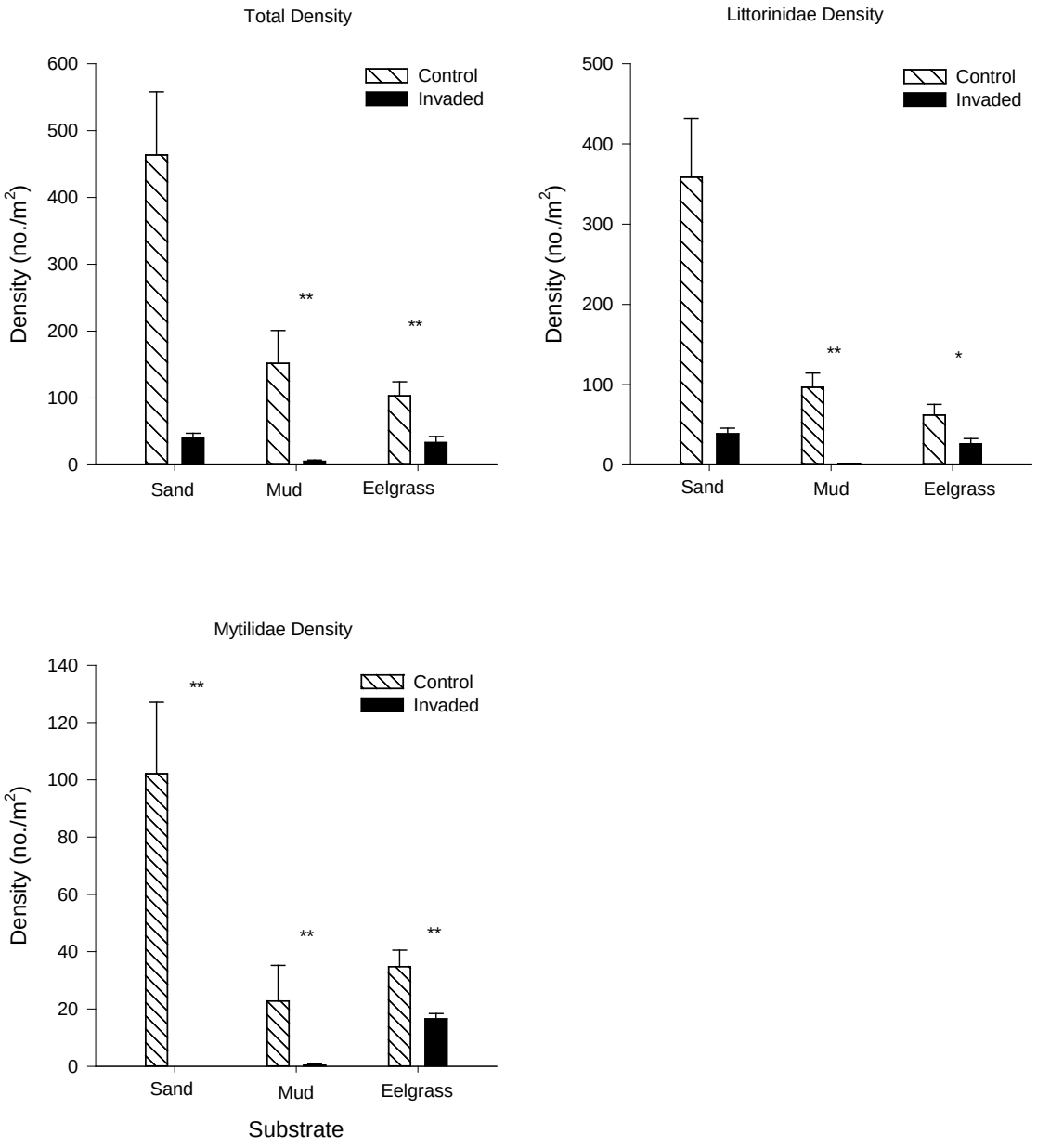


Figure 4-2. Multi-dimensional scaling plot of epifauna for sites without green crab (control; open triangles) and with green crab (invaded; solid triangles) for mud (A; n = 15; *Global* R = 0.7; p = 0.001), sand (B; n = 22; *Global* R = 0.957; p = 0.001), and eelgrass (C; n = 24; *Global* R = 0.309; p = 0.001).



Lines above bars denote 1 standard error and symbols above the bars show statistically significant differences where * < 0.05 and ** < 0.001.

Figure 4-3. Mean density of total epifauna, and epifaunal Littorinidae and Mytilidae densities as a function of control (no green crab) and invaded (with green crab) sites and habitat type (n = 15 per habitat).

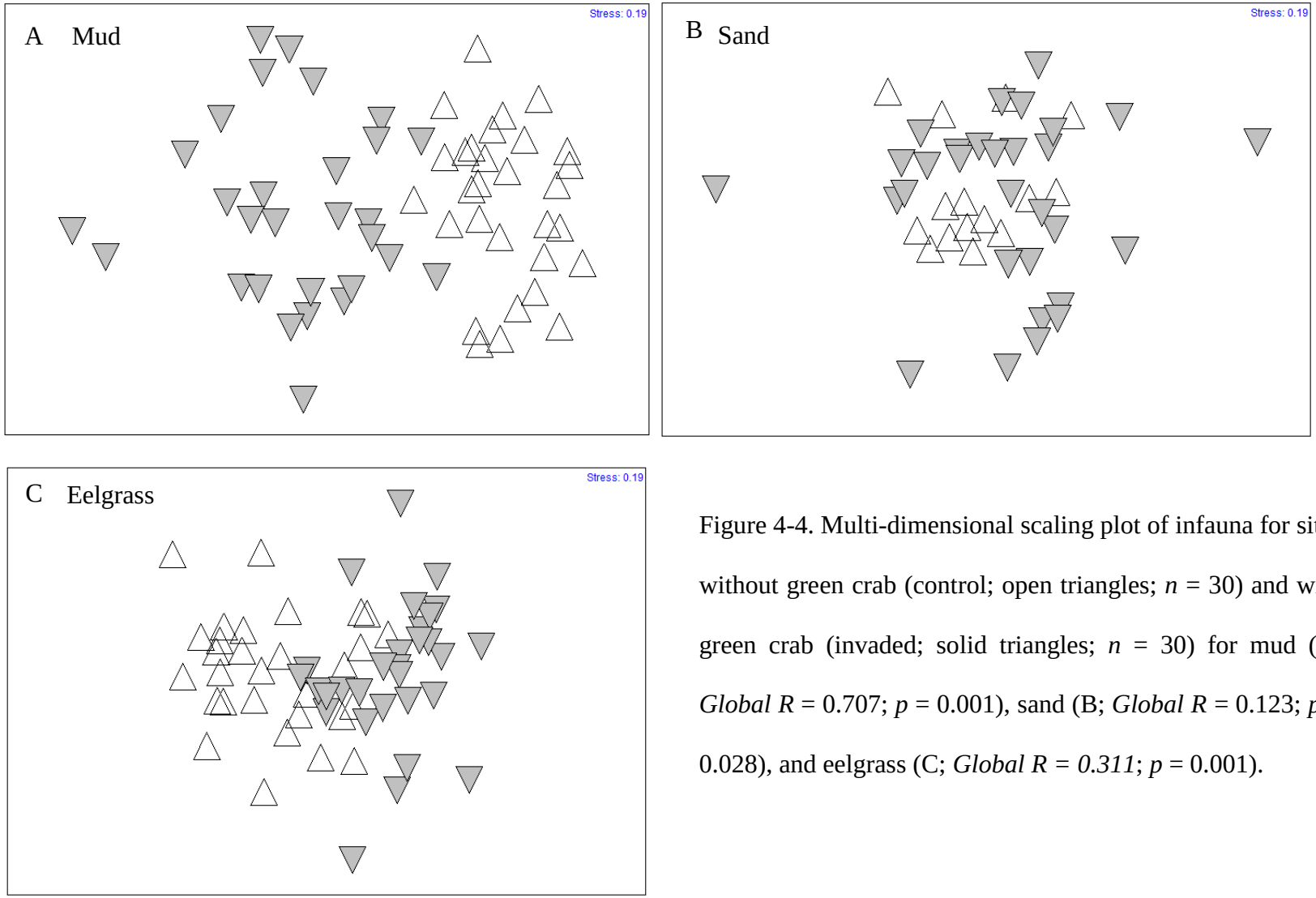
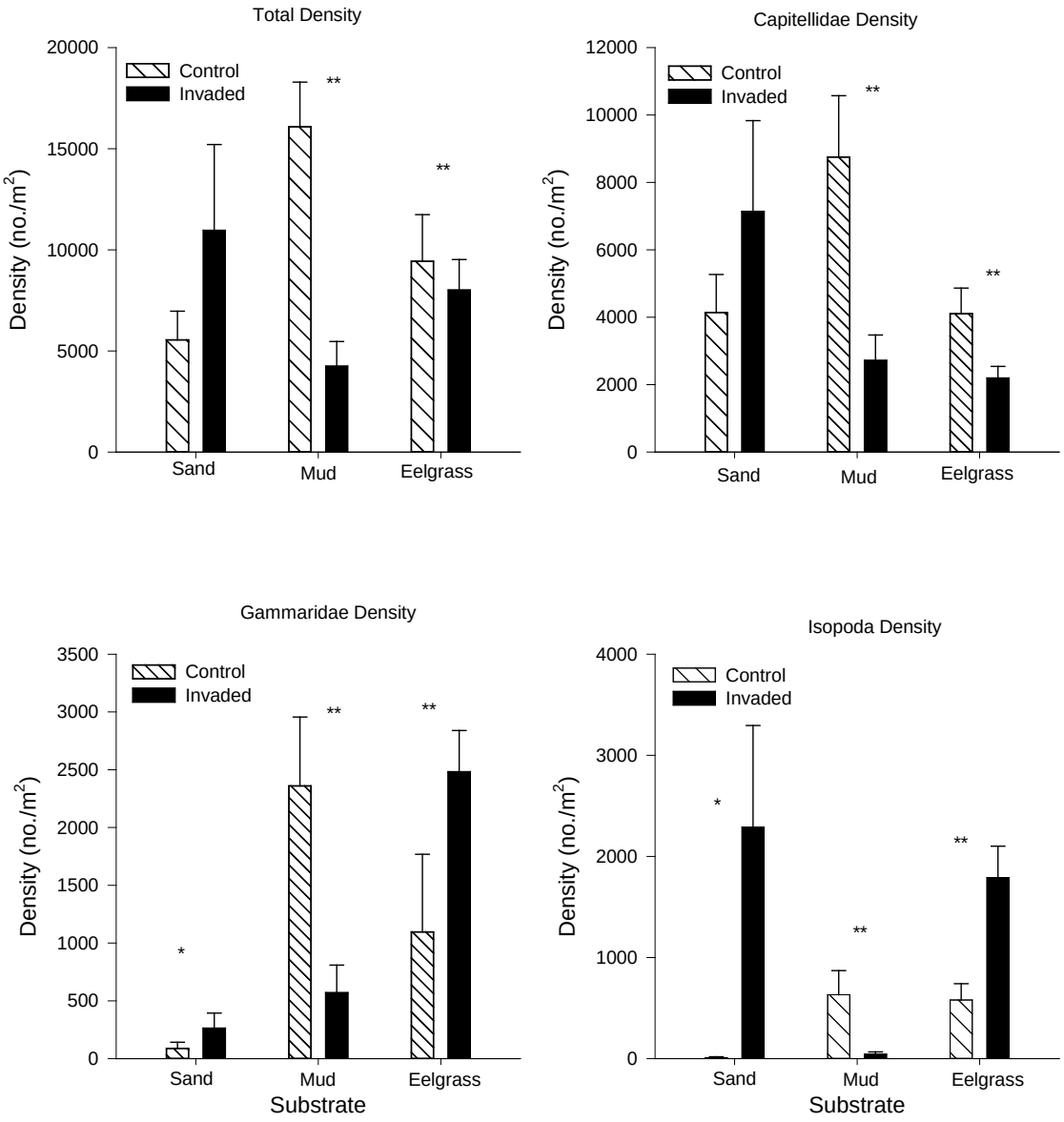
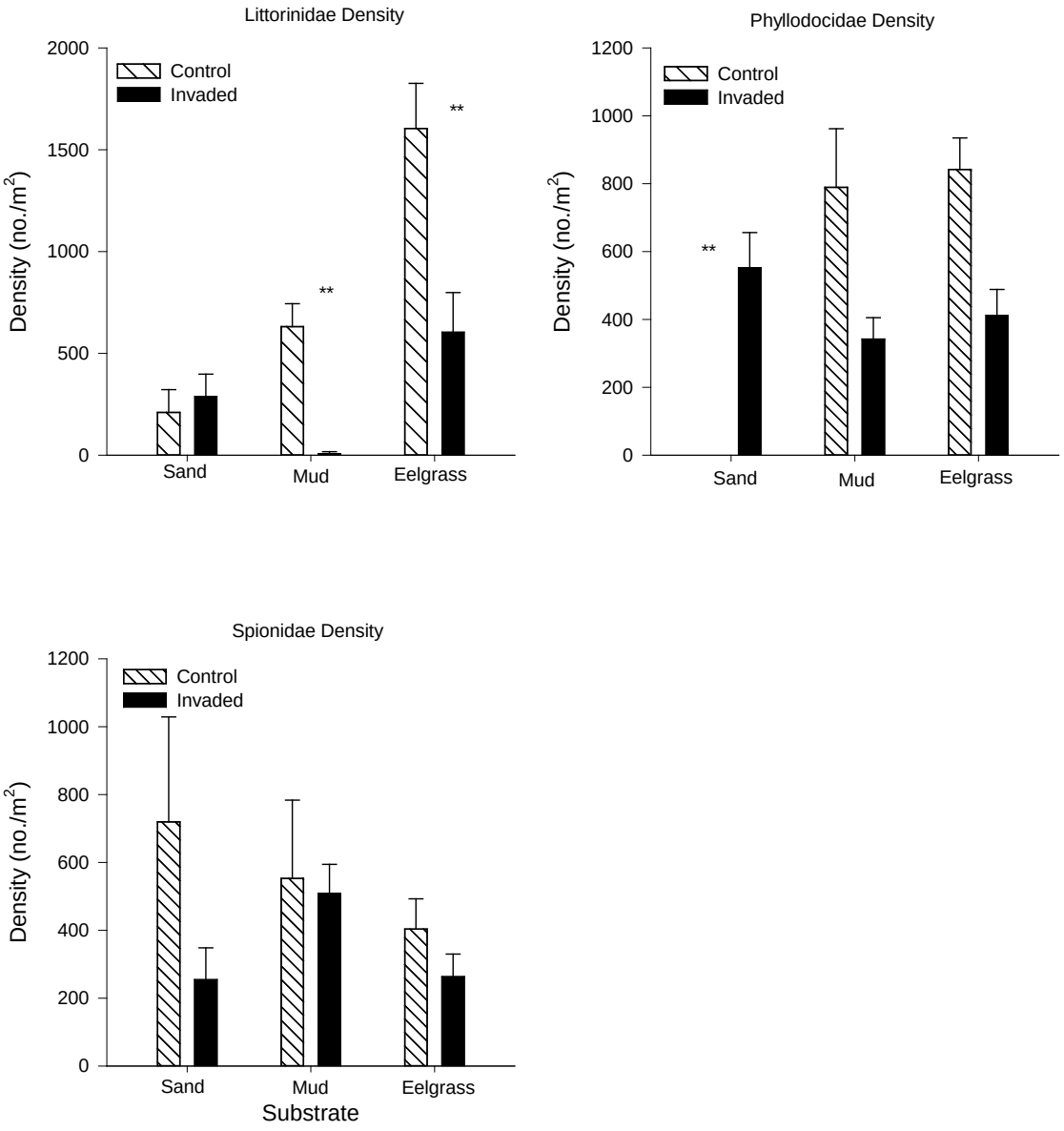


Figure 4-4. Multi-dimensional scaling plot of infauna for sites without green crab (control; open triangles; *n* = 30) and with green crab (invaded; solid triangles; *n* = 30) for mud (A; *Global R* = 0.707; *p* = 0.001), sand (B; *Global R* = 0.123; *p* = 0.028), and eelgrass (C; *Global R = 0.311*; *p* = 0.001).



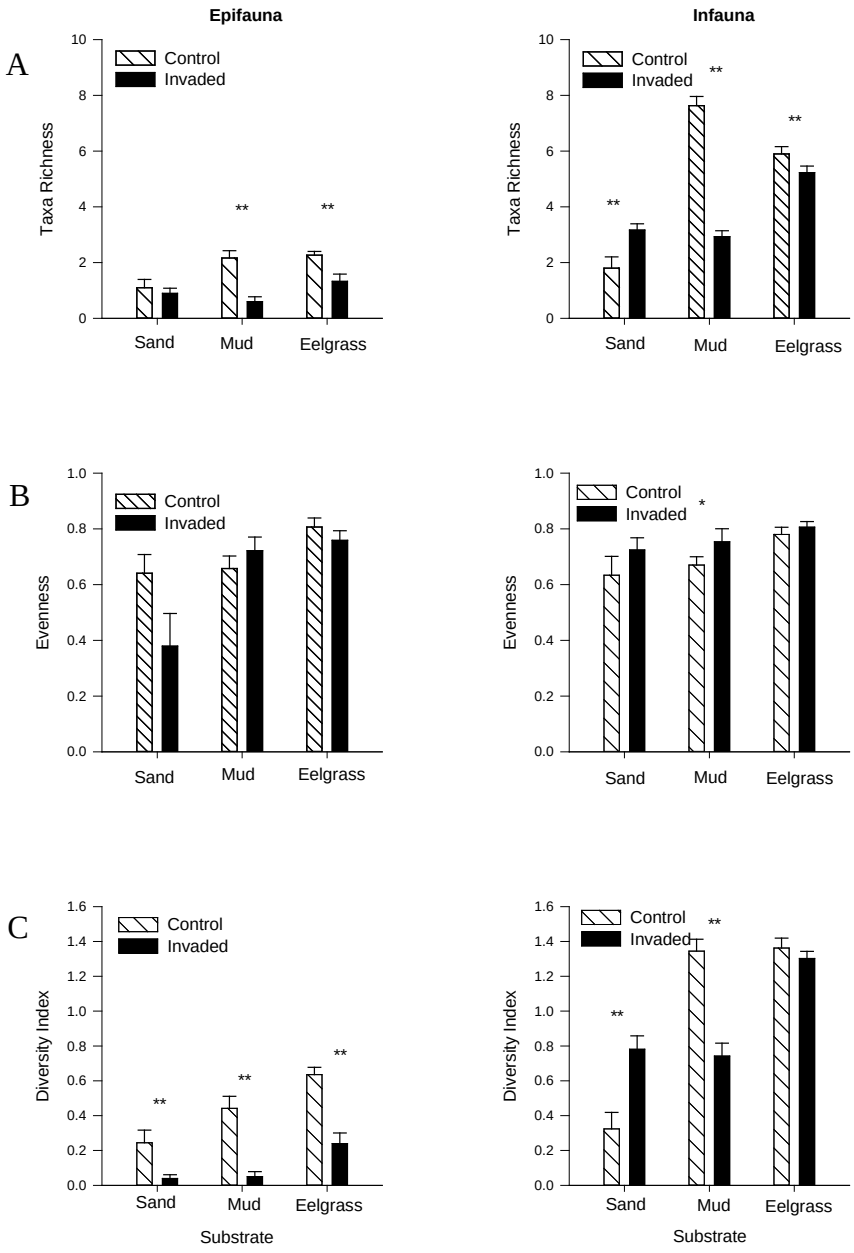
Lines above bars denote 1 standard error and symbols above the bars show statistically significant differences where * < 0.05 and ** < 0.001.

Figure 4-5A. Mean density of common infaunal taxa as a function of green crab presence (invaded) and absence (control) and habitat type (n = 15 per habitat).



Lines above bars denote 1 standard error and symbols above the bars show statistically significant differences where * < 0.05 and ** < 0.001.

Figure 4-5B. Mean density of common infaunal taxa as a function of green crab presence (invaded) and absence (control) and habitat type (n = 15 per habitat).



Lines above bars denote 1 standard error and symbols above the bars show if statistically significant * < 0.05 and ** < 0.001.

Figure 4-6. Effects of green crab presence (invaded) and absence (control) and habitat on community structure. Taxa richness of epifauna and infauna (A). Evenness of epifauna and infauna (B). Shannon diversity of epifauna and infauna (C).

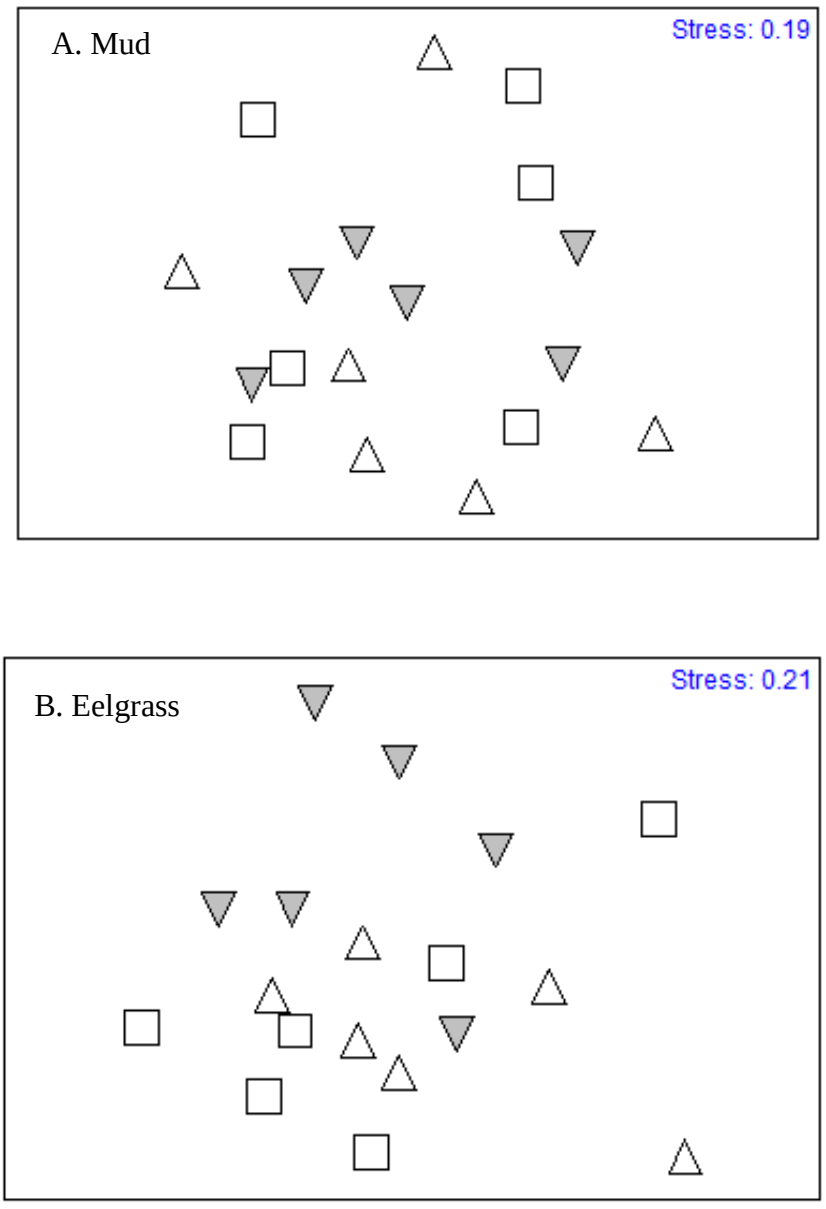
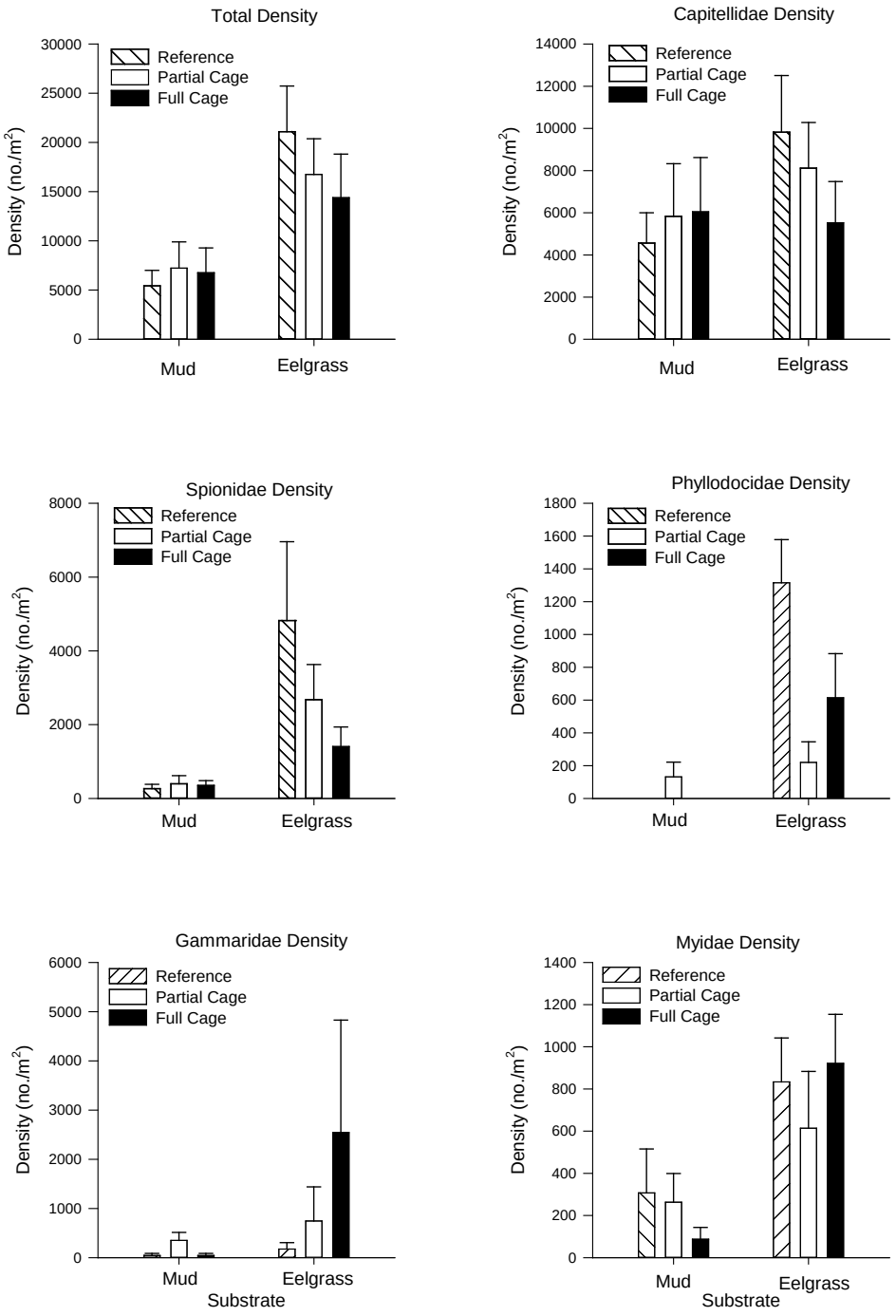
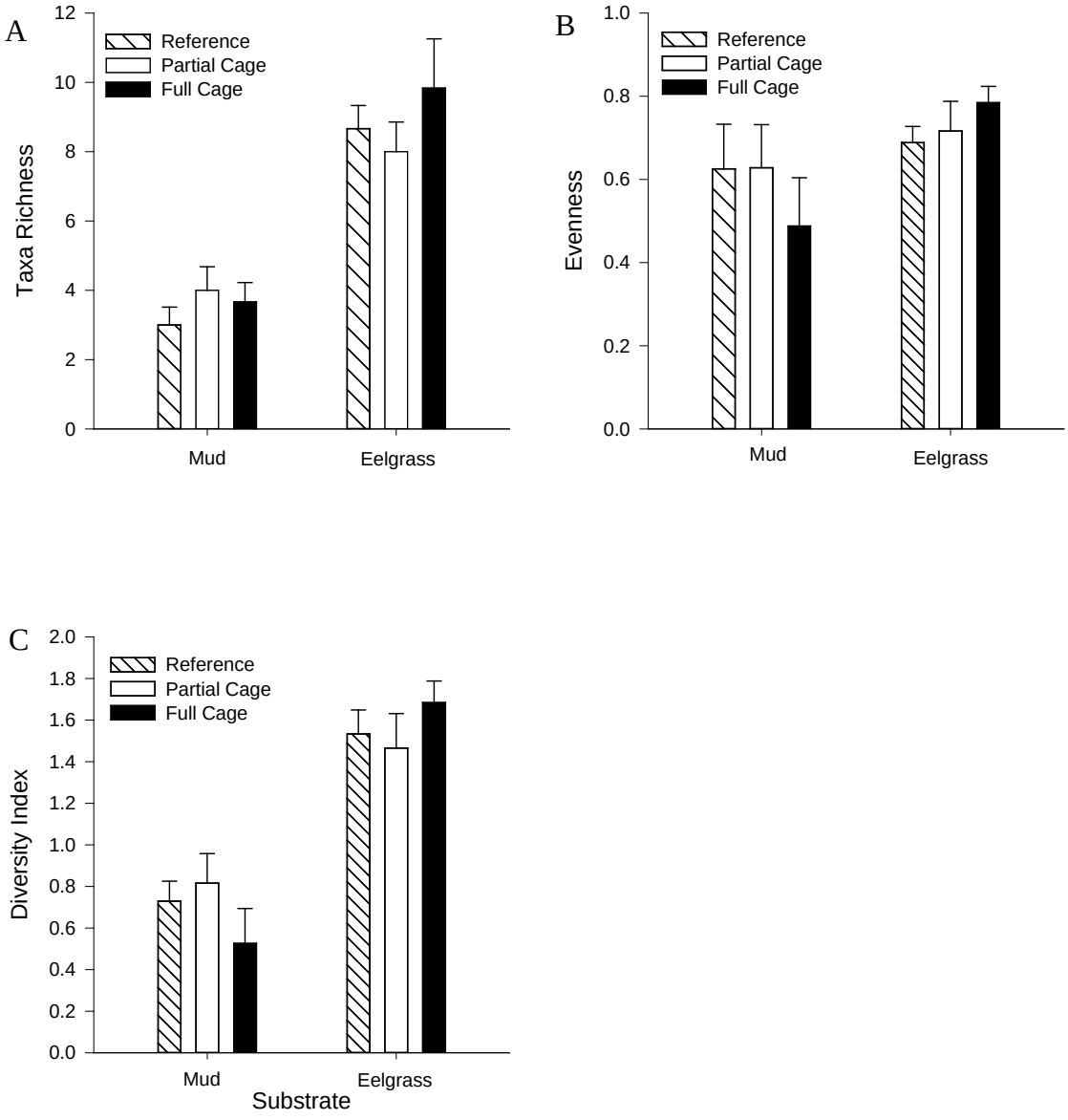


Figure 4-7. Multi-dimensional scaling plot of infauna for different caging treatments for the mud (A) and eelgrass site (B). The treatments included a full cage with green crab (solid triangles), partial cage (open squares) and no cage (reference; open triangles; ANOSIM *Global R* = -0.068, *p* = 0.756; *Global R* = 0.080, *p* = 0.226, respectively).



Lines above bars denote 1 standard error. None of the comparisons were statistically different.

Figure 4-8. Mean density of infauna as a function of cage and sediment type (n = 6 per cage type in each substrate).



Lines above bars denote 1 standard error. None of the comparisons were statistically different.

Figure 4-9. Effects of cage type and substrate on community structure. Taxa richness of infauna (A). Evenness of infauna (B). Shannon diversity of infauna (C).

5. Regional differences in foraging behaviour of invasive green crab (*Carcinus maenas*) populations in Atlantic Canada

5.1. Abstract

Invasive green crab populations initially established in Canada in the Bay of Fundy, New Brunswick in the 1950s and were present in all five Atlantic provinces by 2007. Genetic evidence suggests that the Atlantic Canadian populations originated from at least two separate introductions with differences in time of establishment among regions and possible population-level behavioural differences. This study examines intraspecific foraging behaviour among green crab from different populations, and interspecific foraging competition between genetically similar crab and juvenile lobster. Both foraging experiments involved competition for a limited food source over a one-hour period. In intraspecific match-ups, recent invaders from Newfoundland (NL) were significantly better foragers than long established invaders from Nova Scotia (NS) and New Brunswick (NB) populations; however, no differences between NL and Prince Edward Island (PE) invaders were found. Interspecific competition experiments indicated that the feeding behaviour of recent invaders (NL) and genetically similar but long-established invaders (NS) differed in the presence of juvenile lobster. This study documents behavioural differences among populations of green crab from a small geographic region, which may reflect a combination of both genetic differences and time since population establishment. These differences may result in varying impacts of green crab on newly invaded habitats.

5.2. Introduction

Recent estimates suggest that ballast water may transport more than 10,000 species per day globally (Carlton 1999), but only 5 to 20% of all species successfully establish in a new area (Lockwood et al. 2007). Successful invaders often exhibit wide physiological tolerance, short generation time, and high genetic variability (Lockwood et al. 2007). Consequently, populations of an invader in a new region are unlikely to be genetically uniform, especially those species that span a wide range of environmental conditions in their native range. Once one or more populations of an invasive species establish in a new area, populations typically grow rapidly, often in response to reduced competition, predation, and parasitism pressures (Behrens Yamada et al. 2005).

Indigenous to the northeast Atlantic, the European green crab inhabits the east coast of the Atlantic Ocean from Scandinavia to northern Africa (Grosholz and Ruiz 1996; Audet et al. 2003). Over the past two hundred years, populations of green crab have established worldwide in the northwestern Atlantic, Australia, South America, Japan, and the northeastern Pacific (Cohen et al. 1995; Grosholz and Ruiz 1995). On the east coast of North America alone, genetic evidence suggests that extant populations represent multiple successful invasions (Roman 2006). Green crab first arrived on the east coast of North America in the 1800s but spread to Canada in the 1950s (Carlton and Cohen 2003; Blakeslee et al. 2010). Local and regional larval transport likely facilitated the first Canadian invasion that spread throughout the Bay of Fundy and Atlantic coast of southern Nova Scotia (NS) but appeared to stall near the Halifax area by the 1970s (Carlton and Cohen 2003). A second wave of invaders established in southeastern NS in

the 1980s, subsequently invading coasts around the Gulf of St. Lawrence (northwestern NS, eastern New Brunswick (NB), Prince Edward Island (PE), Magdalen Islands of Quebec (QC)), and most recently Newfoundland (NL) (Klassen and Locke 2007; Blakeslee et al. 2010).

The role of behaviour in invasion success has been under-represented in the literature (Holway and Suarez 1999), even though behaviour likely plays an important role in facilitating successful colonization, establishment, and dispersal. Furthermore, individual variation in behaviours may play a role in population level processes including species distribution (Duckworth and Badyaev 2007). Thus far, two studies have documented the behavior and interactions between a common native species (juvenile American lobster, *Homarus americanus*), and green crab from northern NS and southern NB, respectively (Rossong et al. 2006; Lynch and Rochette 2009). These studies found contrasting results in terms of green crab dominance, a difference that may be related, among other factors, to genetic differences between green crab populations.

Newly arrived invasive species are often perceived as a genetic diversity bottleneck. Individuals within their new range are genetically similar to each other when populations remain small or establish very slowly (Suarez et al. 2008). Green crab within Atlantic Canada show a contrasting pattern and exhibit population diversity levels similar to their native ranges, suggesting multiple invasions or source populations (Roman 2006; Darling 2011). Genetic diversity alone does not necessarily indicate invasion success since populations with a range of levels of genetic diversity have successfully established

(Darling 2011). Instead, genetic diversity may result in variability in the level of potential ecological impacts.

Genetic studies of green crab populations within Atlantic Canada identify spatial and temporal components associated with multiple introductions (Roman 2006). Bay of Fundy populations represented the first wave of invasions, comprised of one to four haplotypes thought to have originated from the southern United Kingdom (Roman 2006; Blakeslee et al. 2010) and that more closely resemble the eastern US population. The two to three distinct European haplotypes that comprise northern NS and PE populations, likely originated from populations at the northern extent of their European range and represent the second invasion of Atlantic Canada (Roman 2006). Mixed haplotypes from the first and second Canadian invasions comprise the NL and southern NS populations (Blakeslee et al. 2010).

This study compares the competitive ability of green crab from the Bay of Fundy (NB; first invasion), Prince Edward Island (PE; second invasion composed of different haplotypes), and Nova Scotia and Newfoundland (NS and NL, genetically mixed populations). The significance of these population differences on green crab ecology and the resulting relative impacts of local invasions on native ecosystems remain largely unexplored. The competitive foraging behaviour of individuals from four Atlantic Canadian provinces was assessed under laboratory conditions using a limited food source. Given that genetic differences may influence competitive ability, aggression, and phenotypic plasticity, it was expected that experimental outcomes would reflect genetic

differences among populations and offer predictive insights into the potential ecological impacts of green crab in these and other regions.

The question of foraging behavioural differences can also be relevant to genetically similar populations with different time of establishment in a given area. Unlike well-established populations of invaders, population sizes of early invaders grow quickly, potentially intensifying interactions with conspecifics and native species by quickly limiting preferred resources (Pintor et al. 2009; Simberloff and Gibbons 2004). This study compared competitive behaviour of green crab from NL (early invaders) and genetically similar crab from southern NS (long-established invaders) and their behaviour in the presence of native competitors, namely juvenile lobster. For these experiments, it was expected that NL green crab would dominate foraging and feed more frequently than NS crab.

5.3. Methods

5.3.1. Field Collections

Green crab (n = 50) were collected using Fukui traps (63 cm x 46 cm x 23 cm, 1.6 cm mesh opening; see Gillespie et al. 2015) in St. Andrews, NB (45° 04’34.57” N, 67° 03’09.55” W), Chance Harbour, NB (45° 07’18.84” N, 66° 21’04.28” W), Port Mouton, NS (43° 52’09.63” N, 64° 49’04.44” W), Souris, PE (46° 21’15.22” N, 62° 52’ 02.62” W) and North Harbour, NL (47° 09’20.90” N, 53° 38’24.82” W) in July 2010 (Figure 5-1). Green crab populations established in these areas by 1951 (both sites in NB), 1960, 1998, and 2007, respectively (Klassen and Locke 2007). All collections occurred in relatively uncontaminated areas (i.e. distant (>30 km) from major urban centers and

industrial facilities to eliminate complications in interpretation associated with other agents of disturbance) with similar mixed mud and rock habitat and wave exposure. Environmental parameters were not measured at the field collection sites. Traps were baited with a standard amount of mackerel or herring and soaked for one to two hours. The brief soaking time may have biased collection towards more aggressive crab (Vasquez Archdale et al. 2003), but was consistent for all populations. Only intact males (49-75 mm carapace width (CW)) were selected and transported to the Atlantic Veterinary College aquatic facility at the University of Prince Edward Island in Charlottetown, PE for use in experimental trials. Crabs were transported in coolers with algae to reduce injury and maintain moisture. The time out of water varied from 1 hour for PE crabs to a maximum of 8 hours for NL crabs that were collected from Placentia Bay, transported by truck to St. John's, NL, and flown to Charlottetown. Divers collected lobster (24-49 mm carapace length (CL); n = 30, both sexes) in North Rustico, PE [46° 27' 29.84" N, 63° 18' 47.12" W; an area without green crab (M. Comeau and A. Locke, Fisheries and Oceans, pers. comms.)]; in August 2010 for transport to the same facility.

5.3.2. Housing and Experimental Tanks

Green crab were separated by collection location and housed in opaque plastic storage containers (108 x 54 x 46 cm) within large round tanks (150 cm diameter x 86.5 cm height; two plastic containers per tank). The bottom of each plastic container was covered with a 2-cm layer of pea gravel, the container was 75% filled with 30 ppt seawater, an airstone was added, and a mesh lid was placed over the container to prevent crab escape. Mixing of water between storage containers was avoided in order to maintain

isolation among populations prior to experimental manipulations. In each large tank, a re-circulating system maintained the crab storage containers at 10.5°C which was similar to field temperatures during collection. Prior to experimental trials, green crab were fed a diet of mussels (one a day for each crab) and the water in the tanks was changed regularly (every other day) to maintain water quality. Green crab were acclimated for a one-week period in the tanks prior to use in the experiment and individual green crab were starved for 48 hours before the experiments to standardize hunger levels (Mascaro and Seed 2001; Rossong et al. 2011). Lobster were banded (both claws), placed individually in housing tanks (30 ppt, 10°C), and fed shrimp every second day during their one-week acclimation period.

The experiments took place in a separate tank. A 1.5 m-diameter tank was filled to a depth of 0.3 m with seawater similar in temperature and salinity to that described above, and the tank bottom was covered with a thin layer of pea gravel. One camera (Speco Technologies Weatherproof DSP VL-66 with infrared) was suspended over the middle of this tank ~1 m from the substrate and a second camera was secured on the side of the tank ~50 cm above the sediment. Both cameras were connected to a 4 channel recorder (Samsung SHR-5042) located in a separate room.

5.3.3. Intraspecific Competition of Green Crab

The first set of experiments examined foraging competition for a limited resource between pairs of individual crabs from different populations (four provinces) shown to have different haplotype probabilities based on studies by Roman (2006) and Blakeslee et al. (2010). The probability of haplotype overlap between NB and PE crabs was very low,

however, the overlap of haplotypes between NL and NS precluded determination of whether genetic haplotypes were more similar to NB or PE. Sampling by Blakeslee et al. (2010) suggests an equal probability of a NL crab sharing the same haplotypes as a crab from NB or PE.

For each trial, green crab in 1 of 6 combinations (n = 15 trials per combination: NB vs NL; NB vs PE; NB vs NS; NL vs PE; NL vs NS; and NS vs PE) were matched together. Green crab in a given trial differed less than 5 mm in size (CW). Before each trial, green crab were measured (± 1 mm), weighed (± 0.01 g), and labeled (waterproof labels glued to the carapace) to facilitate identification during subsequent video analyses. For each trial, both green crab were placed in the tank for a 10-minute acclimation period with the food source covered, then the food was exposed for a 60-minute trial period, similar to other experiments (Jensen et al. 2002; Rossong et al. 2006; Williams et al. 2006). The acclimation period reduced behavioural modifications associated with handling prior to the experiment. A hole was drilled through the shell of a live mussel (*Mytilus edulis*) to facilitate anchoring with a cable tie in the center of the tank, thereby ensuring that interactions took place in the camera's field of view, as in previous experiments (cf. Jensen et al. 2002; Rossong et al. 2006).

All crabs used in experiments were held in holding tanks for two weeks after the experiment prior to euthanization to confirm they did not moult (moult cycle may affect behaviour). In the PE vs NB experiments only 11 of the 15 planned trials were completed due to water quality issues detected in the PE crab tank. All PE green crab were subsequently euthanized and a new group of crabs were collected for the remaining trials

at a later date. Three trials were excluded from the data analysis because green crab detached the mussel, making it difficult to determine interactions.

5.3.4. Feeding Behaviour of Green Crab in the Presence of Juvenile Lobster

The second set of experiments examined the feeding behaviour of two genetically similar green crab populations in the presence of a juvenile lobster. Studies in both southern NS (Elner 1981) and in NL (Chapter 2) suggest that green crab from both locations were previously exposed to lobster, thus reducing any biases on green crab behaviour. The lobsters in this study were not previously exposed to green crab but in similar studies by Rossong et al. (2006), lobster behaviour appeared unaffected by past exposure. The set-up was identical to the intraspecific experiment (see section 5.3.3) except that lobster were paired with green crab from a recently established population (NL; less than 5 yrs) and, a long-established population (NS; more than 45 yrs; n = 25 trials per combination). After the experiment, crab and lobster were labeled, measured, sexed, and held for two weeks prior to euthanizing to confirm that individuals were not aggressive pre-moults (Tamm and Cobb 1978).

5.3.5. Video and Statistical Analysis for Competition Experiments

5.3.5.1. Intraspecific Competition of Green Crab

Upon completion of trials, video footage was analyzed to determine the time taken to find the food source, which individual reached the mussel first, and the total time spent feeding. All video analyses were recorded to the nearest second and all summary statistics are reported with the same precision. The following behaviors were quantified: 1) the

number of approaches by the non-feeding crab on the feeding crab, 2) the frequency at which one crab displaced the other crab from the resource, 3) the duration of interactions, and 4) the intensity of interactions based on a scale from 1 to 3 (1 indicated no physical contact; 2 minimal physical contact, such as when one crab made short duration contact, one push or pinch then retreats; 3 aggressive pinching/pushing by one or both crab).

The amount of time each green crab spent feeding was assessed using paired t-tests, with a Bonferroni adjustment of significance levels ($\alpha = 0.05/n = 0.0083$ for $n = 6$ combinations; Minitab 16, 2010). Differences in body size were assessed using paired t-tests on carapace width (CW) and body weight. A binomial test was used to determine whether crab in each treatment were equally likely to arrive first at the bait.

5.3.5.2. Feeding Behaviour of Green Crab in the Presence of Juvenile Lobster

Video footage from the green crab and lobster experiments was analyzed similar to the intraspecific experiments, however, the lobster did not interact with the green crab or attempt to feed on the mussel. Therefore only time to find the mussel and the amount of time spent feeding were quantified for the green crab. Two-sample t-tests were used to evaluate genetically similar green crab behaviour in the presence of lobster, with treatment groups of NL green crab (from newly-established populations) and NS green crab (from long-established populations). A regression of “time feeding” on “difference in body size” (crab CW – lobster CL) and a regression of “time to locate the mussel” on “difference in body size” were used to assess the influence of body size on both variables.

5.4. Results

5.4.1. Intraspecific Competition of Green Crab

The pairing of crab for each trial resulted in no significant differences in body size (carapace width and body weight) among paired crab for all match-ups (all p-values > 0.150). NL crab were first to the mussel in more trials than NS crab (12 of 15, Figure 5-2) and NB crab (10 of 13, Figure 5-3; Table 5-1) but a binomial sign test revealed ratios not significantly different from 1:1 at the Bonferroni-corrected significance level of 0.0083 ($p = 0.035$ and $p = 0.092$ respectively). PE crab were significantly faster at finding the bait compared to NL crab (14 of 15 trials, $p = 0.001$, Figure 5-4), but not significantly faster compared to NS or NB crab (10 of 15 trials, $p = 0.302$, Figure 5-5; 6 of 11 trials, $p = 1.000$, Figure 5-6, respectively; Table 5-1). NB green crab were faster at finding the bait compared to NS crab in 10 of 14 trials but the ratio was not significantly different from 1:1 ($p = 0.180$; Figure 5-7; Table 5-1).

Green crab from NL spent significantly more time with the food source than green crab from either NB ($t_{12} = 5.48$, $p < 0.001$, Figure 5-3) or NS ($t_{14} = 3.37$, $p = 0.005$, Figure 5-2) populations; however, there was no difference between NL and PE crab ($t_{14} = -0.49$, $p = 0.629$, Figure 5-4). PE crab did not spend more time feeding than NS crab ($t_{14} = -3.01$, $p = 0.009$, Figure 5-5) and NB crab ($t_{10} = -2.08$, $p = 0.064$, Figure 5-6) at the Bonferroni-corrected significance level of 0.0083. No foraging dominance was detected between NB and NS crab ($t_{13} = 0.60$, $p = 0.556$, Figure 5-7).

No significant differences between match-ups were detected in the number of intraspecific interactions and total interaction time ($F_{5,77} = 2.09$, $p = 0.076$; $F_{5,77} = 1.24$, p

= 0.300, respectively; see Table 5-1 for summary). The longest interaction times occurred in the PE vs NB match-ups, followed closely by the match-ups with NL crabs. Intraspecific interaction intensity varied in all match-ups. In some cases, intensity increased as the trial continued, but in other cases more passive encounters followed a few intense battles. In 22% of the trials, the first crab to the mussel was never displaced from the resource, whereas the other crab took over for some portion of the trial. However, in most cases, ownership of the mussel reverted to the initial feeder.

5.4.2. Feeding Behaviour of Green Crab in the Presence of Juvenile Lobster

Lobster spent most of the duration of the trial moving around the tank. They often approached the feeding crab but made no physical contact with the crab nor tried to take over the resource. In one trial only, the lobster initiated feeding when the green crab had abandoned the mussel.

During 25 trials, green crab from NL required 476 ± 95 (mean ± SE) seconds to locate the mussel, which they then fed on for 2100 ± 156 seconds. Green crab from NS initially located the mussel in 678 ± 136 seconds and fed for an average of 1561 ± 182 seconds. Although the time to locate the mussels did not differ significantly among populations (t_{42} = -1.22, p = 0.230), NL crabs spent significantly more time (34% longer) on the prey than NS crabs (t_{46} = 2.29, p = 0.027, Figure 5-8).

Regressions of time feeding on “difference in body size were not significant for the NL or NS green crab/lobster trials ($F_{1,23}$ = 3.58, p = 0.071 and $F_{1,21}$ = 0.01, p = 0.933, respectively) and regressions of time to locate the mussel on difference in body size were not significant for the NL or NS green crab/lobster trials ($F_{1,23}$ = 1.84, p = 0.188 and $F_{1,21}$

= 2.31, p = 0.143, respectively), indicating that differences in body size between paired organisms (green crab and lobster) did not influence the responses.

5.5. Discussion

5.5.1. Intraspecific Competition of Green Crab

In this study, NL green crab out-competed individuals from NB and NS populations in foraging experiments. NL crab were first to the mussel in the majority of trials and spent more time feeding on the mussel; however, no significant differences were observed in trials against green crab from PE. PE crab were the first to the mussel in all of their food trials (including NL) and spent more time feeding than NS and NB crab. Although I cannot establish cause and effects unambiguously from these data, these results clearly support the general predictions that; a) green crab populations in Atlantic Canada differ in competitive ability, an aspect of their ecology that genetic differences can at least partly explain; and b) differences in competitive ability between populations of similar genetic makeup may be related to the timing (recent versus historic) of their invasion. Acknowledging the many factors that may influence crab performance and the constraints imposed by a laboratory setting, the behavior observed in these trials is representative of the intensity of these crab interactions.

5.5.2. Genetic Differences

Genetic evidence provides insight into the source of an invasion, transmission routes, and modes of introductions (Mikheyev and Mueller 2007; Darling 2011), but genetic differences may also influence behaviour. Two distinct source populations

comprise eastern Canadian populations (Roman 2006; Darling 2011). One population (NB) shared a similar genetic makeup with the majority of North American populations (Darling et al. 2008), whereas a second population (PE) derives from a completely different source. The lack of environmental barriers allows ready mixing of these populations, creating combinations of haplotypes similar to those in its native range (NS and NL; Roman 2006; Blakeslee et al. 2010). Most studies on green crab impacts thus far (e.g Grosholz et al. 2000; Klassen and Locke 2007) have focused on North American populations exhibiting low genetic diversity (Darling et al. 2008). As a consequence, these populations may differ in competitive abilities in previous studies on green crab behaviour in North America. For instance, interspecific hybridization in plants can produce more competitive invaders (Ellstrand and Schierenbeck 2000). The occurrence of similar genetic mixing among green crab populations could ultimately alter competitive ability with subsequent impacts on native organisms and habitats.

Two previous laboratory experiments on foraging competition between green crab and juvenile lobster (Rossong et al. 2006; Lynch and Rochette 2009) found different levels of competitive dominance in green crab with lobster. The only major difference between the two studies was the origin of the green crab. Rossong et al. (2006) used green crab from the Northumberland Strait, NS (genetically similar to PE populations). These crab were significantly better competitors than lobster, locating and dominating the food source in all trials. Moreover, in 6 of 11, 8 h trials of a shelter experiment, lobster (28-57 mm CL) were consumed by green crab (53-76 mm CW). In contrast, the study with green crab from southern NB (population established in 1950) found that green crab (33-70 mm

CW) were more passive, and that lobster (16-48 mm CL) feeding and mortality remained unaffected by green crab (Lynch and Rochette 2009). The results from the present study are consistent with those two studies and explain their contrasting results. In the PE and NB match-up of the present study, PE crab spent more than twice as much time feeding compared to NB crab. The average of the paired differences was not significantly different from zero (p = 0.064), but the magnitude of the difference was biologically substantial (~150% relative to the mean time feeding for PE). Such differences in competitive ability explain why one green crab population (PE) was able to dominate interspecific trials whereas a second population with a different genetic makeup (NB) did not.

As in the present study, invasions by yellow crazy ants, (*Anoplolepis gracilipes*) on a South Pacific island, and paper wasps (*Polistes dominulus*) in the USA represent multiple invasions with genetic variation among populations (Liebert et al. 2006; Abbott et al. 2007). Intraspecific competition experiments showed different levels of competitiveness between genetically distinct populations of ants (Abbott et al. 2007). The study also found that separate invasions lead to two behaviourally and genetically distinct populations, where only one ant population became highly abundant. The results on green crab competitive ability suggest a similar phenomenon. In practical terms, it is impossible to predict the precise outcome of a green crab invasion into a new locale. However, the results of the present study suggest that the genetic makeup of an invading population could be related to the severity and intensity of potential impacts.

5.5.3. Time Since Establishment

Although genetic differences may explain differences in foraging abilities of green crab populations, other factors (such as time since establishment) likely play a role as well. Genetic evidence suggests that NL green crab originated directly from southern NS (Blakeslee et al. 2010). However, despite their similar genetic makeup, behavioural differences were detected between these two populations in both intraspecific and interspecific competition experiments. In direct match-ups, NL crab spent over three times as much time feeding as NS crab. In trials with a potential competitor (juvenile lobster), as in the intraspecific trials, individual crab from NL spent significantly more time feeding on the mussel than NS crab.

In the interspecific competition trials, the lobster did not physically interact with the green crab, and although a potential effect of the lobster on green crab behaviour cannot be completely ruled out, no evidence of an effect was observed in any of the video recordings. All green crab were housed in the same settings, fed on a schedule of 48 hours starvation prior to use in the experiment to regulate hunger levels, and acclimated for a one-week period prior to experiments. Therefore, other explanations for population differences are crab size differences, food preferences, and contrasting behaviours among populations. Smaller crab may feed less than larger crab, but all crab in intraspecific trials were matched based on a CW size difference of 5 mm or less. Moreover, paired differences in body size were not significant within each match-up. In addition, differences in body size between green crab and lobster did not influence the responses based on the non-significant regressions. With respect to diet differences, green crab from

both southern NS (Elner 1981) and NL (Chapter 2) show that bivalves, and mussels in particular, comprise the largest portion of both diets, so prey preference is unlikely a contributing factor to my results. Behavioural differences among populations are therefore the most plausible explanation and I hypothesize that these differences in foraging ability may be related to local invasion times.

During initial establishment at a new location, densities of green crab increase rapidly, a phenomenon often associated with higher agonistic interactions in similar crab species (Clark et al. 2000; Reichmuth et al. 2011). Green crab are generalist predators (Ropes 1968; Grosholz and Ruiz 1996; Klassen and Locke 2007), capable of depleting food resources in an area before moving on to a new location. In order to survive, green crab must be strong competitors but as populations decline, competition for limited resources among conspecifics presumably decreases (Simberloff and Gibbons 2004). Therefore recent invaders need to be more active foragers in order to become established.

Behaviour is often a good determinant of invasion success (Weis 2010). In the short term, behaviours associated with strong competitive ability allow invaders to maintain high foraging and growth rates, thus increasing the likelihood of successful establishment. In the long term, invader populations may face a boom and bust cycle driven by limited resources or variation in potential dispersal (Williamson and Fitter 1996; Simberloff and Gibbons 2004; Pintor et al. 2009). For example, intraspecific competition in the invasive crayfish (*Pacifastacus leniusculus*) is unusually high when resources are limited, and these heightened levels of aggression limit population growth (Pintor et al. 2008). Similarly, a study on funnel web spiders (*Agelenopsis aperta*) showed

that when resources are depleted (which often occurs in newly invaded areas) spider populations became more competitive than similar populations with an abundant food source (Hendrick and Riechert 1989).

Invaders often prevail over native species in aggressiveness and boldness (Rehage and Sih 2004; Pintor et al. 2008). In newly invaded areas with dietary and spatial overlap with native species, green crab, like other invasive organisms (e.g. crayfish; Pintor et al. 2008), may be more aggressive than in areas where competition is less intense. Aggressive individuals may be one of the drivers of range expansion of an invader. For example, in western bluebirds (*Sialia mexicana*), only aggressive males disperse and colonize new areas (Duckworth and Badyaev 2007) whereas non-aggressive individuals remain well within their natural distribution limits. Once the population has established itself in a new locale and outcompete native species, aggression levels decrease again within several generations (Duckworth and Badyaev 2007). Although the evidence presented in this study is limited in terms of number of sites and populations, its scope is appropriate to the spatial scale of the region and what is known about the history of its invasion. My results suggest decreased foraging intensity with increased time since invasion.

Although foraging success and aggression may be correlated (Reichmuth et al. 2011) it is clear that they are not necessarily equivalent. For example, in a study on juvenile crab foraging, green crab were first to the bait in competition experiments (therefore considered more successful foragers) but were less aggressive than blue crab (*Callinectes sapidus*; MacDonald et al. 2007). Further studies on the relationship between

foraging ability and green crab intraspecific aggression are necessary in order to distinguish the two phenomena. It is clear, however, that aggression, high foraging rate, and superior competitive dominance all play a role in the success of invaders (Pintor and Sih 2010).

5.5.4. Conclusions

This study suggests that genetic make-up and time since establishment both affect the behaviour of an invasive species. Early invaders may initially destroy new habitats but with time their impacts may lessen with changes in behaviour associated with lower population density and decreased competitive pressures. Green crab from PE, and other locations along the Northumberland Strait differ genetically from the rest of Atlantic Canada as well as the eastern US and west coast of North America (Roman 2006). The behaviours of these crab were similar to newly established populations from NL, suggesting they may represent a genetically more aggressive strain or that they have not been established long enough to lose their competitive dominance. Green crab within Atlantic Canada and worldwide have negatively affected native organisms and habitats (Grosholz and Ruiz 1995; Cohen et al. 1995; Klassen and Locke 2007). Although previous studies examined behavior of green crab, population-level differences in behaviour such as those assessed here were previously unexplored. The differences documented here in Atlantic Canadian populations suggest that foraging competition, behaviour, and overall impacts on a native habitat may differ, depending on both genetic makeup and invasion history. Future research using genetic analyses to determine haplotypes of individual crab, baseline studies in native source populations to determine

behavioural differences associated with different haplotypes, increased sample collection from areas with varying invasion times, and further examination of behavioural differences associated with specific environmental parameters, would provide greater insight into the success of invaders.

5.6. References

Abbott KL, Greaves SNJ, Ritchie PA, Lester PJ (2007) Behaviourally and genetically distinct populations of an invasive ant provide insight into invasion history and impacts on a tropical ant community. Biol. Invasions 9: 453-463.

Audet D, Davis DS, Miron G, Moriyasu M, Benhalima K, Campbell R (2003) Geographic expansion of a nonindigenous crab, *Carcinus maenas* (L.), along the Nova Scotian shore into the southeastern Gulf of St Lawrence, Canada. J. Shellfish Res. 22: 255-262.

Behrens Yamada S, Dumbauld BR, Kalin A, Hunt CE, Figlar-Barnes R, Randall A (2005) Growth and persistence of a recent invader *Carcinus maenas* in estuaries of the northeastern Pacific. Biol. Invasions 7: 309-321.

Blakeslee AMH, McKenzie CH, Darling JA, Byers JE, Pringle JM, Roman J (2010) A hitchhiker's guide to the Maritimes: anthropogenic transport facilitates long-distance dispersal of an invasive marine crab to Newfoundland. Divers. Distrib. 16: 879-891.

Carlton JT (1999) The scale and ecological consequences of biological invasions in the World's oceans. In: Sandlund OT, Schei PJ, Viken A (eds) Invasive Species and Biodiversity Management, pp. 195-212, Dordrecht, The Netherlands: Kluwer Academic.

Carlton JT, Cohen AN (2003) Episodic global dispersal in shallow water marine organisms: the case history of the European shore crabs *Carcinus maenas* and *C. aestuarii*. J. Biogeogr. 30: 1809-1820.

Clark MC, Wolcott TG, Wolcott DL, Hines AH (2000) Foraging behavior of an estuarine predator, the blue crab *Callinectes sapidus* in a patchy environment. Ecography 23: 21-31.

Cohen AN, Carlton JT, Fountain MC (1995) Introduction, dispersal and potential impacts of the green crab *Carcinus maenas* in San Francisco Bay, California. Mar. Biol. 122: 225-237.

Darling JA (2011) More than one way to invade: Lessons from genetic studies of *Carcinus* shore crabs. In: Galil BS, Clark PF, Carlton JT (eds) The wrong place- alien marine crustaceans: Distribution, biology and impacts, Springer series in invasion ecology, 1st edition, pp 661-687.

Darling JA, Bagley MJ, Roman J, Tepolt CK, Geller JB (2008) Genetic patterns across multiple introductions of the globally invasive crab genus *Carcinus*. Mol. Ecol. 17: 4992-5007.

Duckworth RA, Badyaev AV (2007) Coupling of dispersal and aggression facilitates the rapid range expansion of a passerine bird. PNAS 104: 15017-15022.

Ellstrand NC, Schierenbeck KA (2000) Hybridization as a stimulus for the evolution of invasiveness in plants? PNAS 97: 7043-7050.

Elner RW (1981) Diet of green crab *Carcinus maenas* from Port Hebert, southwestern Nova Scotia. J. Shellfish Res. 1: 89-94.

Gillespie GE, Norgard TC, Anderson ED, Haggarty DR, Phillips AC (2015) Distribution and biological characteristics of European green crab, *Carcinus maenas*, in British Columbia, 2006-2013. Canadian Technical Report of Fisheries and Aquatic Sciences 3120.

Grosholz ED, Ruiz GM (1995) Spread and potential impact of the recently introduced European green crab, *Carcinus maenas*, in central California. Mar. Biol. 122: 239-247.

Grosholz ED, Ruiz GM (1996) Predicting the impact of introduced marine species: Lessons from the multiple invasions of the European green crab *Carcinus maenas*. Biol. Conserv. 78: 59-66.

Grosholz ED, Ruiz GM, Dean CA, Shirley KA, Maron JL, Connors PG (2000) The impacts of a nonindigenous marine predator in a California bay. Ecology 81: 1206-1224.

Hendrick AV, Riechert SE (1989) Genetically-based variation between two spider populations in foraging behavior. Oecologia 80: 533-539.

Holway DA, Suarez AV (1999) Animal behavior: an essential component of invasion biology. Trends Ecol. Evol. 14: 328-330.

Jensen GC, McDonald PS, Armstrong DA (2002) East meets west: competitive interactions between green crab *Carcinus maenas*, and native and introduced shore crab *Hemigrapsus spp*. Mar. Ecol. Prog. Ser. 225: 251-262.

Klassen G, Locke A (2007) A biological synopsis of the European green crab, *Carcinus maenas*. Can. Manuscr. Rep. Fish Aquat. Sci. no 2818, vii + 75 p.

Liebert AE, Gamboa GJ, Stamp NE, Curtis KM, Turillazzi S, Starks PT (2006) Genetics, behavior and ecology of a paper wasp invasion: *Polistes dominulus* in North America. Ann. Zool. Fennici 43: 595-624.

Lockwood JL, Hoopes MF, Marchetti MP (2007) Invasion Ecology. Blackwell Publishing , UK.

Lynch BR, Rochette R (2009) Spatial overlap and biotic interactions between sub-adult American lobsters, *Homarus americanus*, and the invasive European green crab *Carcinus maenas*. J. Exp. Mar. Biol. Ecol. 369: 127-135.

MacDonald JA, Roudez R, Glover T, Weis JS (2007) The invasive green crab and Japanese shore crab: behavioral interactions with a native crab species, the blue crab. Biol. Invasions 9: 837-848.

Mascaro M, Seed R (2001) Foraging behavior of juvenile *Carcinus maenas* (L.) and *Cancer pagurus*. L. Mar. Biol. 139: 1135-1145.

Mikheyev AS, Mueller UG (2007) Genetic relationships between native and introduced populations of the little fire ant *Wasmannia auropuncata*. Divers. Distrib 13: 573-579.

Pintor LM, Sih A, Bauer ML (2008) Differences in aggression, activity and boldness between native and introduced populations of an invasive crayfish. Oikos 117: 1629-1636.

Pintor LM, Sih A (2010) Differences in growth and foraging behaviour of native and introduced populations of an invasive crayfish. Biol. Invasions 11: 1895-1902.

Pintor LM, Sih A, Kerby JL (2009) Behavioral correlations provide a mechanism for explaining high invader densities and increased impacts on native prey. Ecology 90: 581-587.

Rehage JS, Sih A (2004) Dispersal behavior, boldness and the link to invasiveness: A comparison of four *Gambusia* species. Biol. Invasions 6: 379-391.

Reichmuth JM, MacDonald J, Ramirez J, Weis JS (2011) Fight or flight: an investigation of aggressive behavior and predator avoidance in two populations of blue crabs (*Callinectes sapidus* Rathbun) in New Jersey. Hydrobiologia 658: 173-182.

Roman J (2006) Diluting the founder effect: cryptic invasions expand a marine invader's home range. Proc. R. Soc. Lond. B. Biol. Sci. 273: 1-7.

Ropes JW (1968) The feeding habits of the green crab *Carcinus maenas* (L.). Fish Bull. 67: 183-203.

Rossong MA, Quijón PA, Williams PJ, Snelgrove PVR (2011) Foraging and shelter behavior of juvenile American lobster (*Homarus americanus*): the influence of a non-indigenous crab. J. Exp. Mar. Biol. Ecol. 403: 75-80.

Rossong MA, Williams PJ, Comeau M, Mitchell S, Apaloo J (2006) Agonistic interactions between invasive green crabs *Carcinus maenas* (Linnaeus) and juvenile American lobsters *Homarus americanus* (Milne Edwards). J. Exp. Mar. Biol. Ecol. 329: 281-288.

Simberloff D, Gibbons L (2004). Now you see them, now you don't!- population crashes of established introduced species. Biol. Invasions 6: 161-172.

Suarez AV, Holway DA, Tsutsui ND (2008). Genetics and behavior of a colonizing species: The invasive Argentine ants. Am. Nat. 172: S72-S84.

Tamm GR, Cobb JS (1978) Behavior and the crustacean molt cycle: changes in aggression of *Homarus americanus*. Science 200: 78-81.

Vásquez Archdale M, Anraku K, Yamamoto T, Higashitani N (2003) Behavior of the Japanese rock crab 'Ishigani' *Charybdis japonica* towards two collapsible baited pots: Evaluation of capture effectiveness. Fisheries Sci. 69: 785-791.

Weis JS (2010) The role of behaviour in the success of invasive crustaceans. Mar. Freshw. Behav. Phy. 43: 83-98.

Williams PJ, Floyd TA, Rossong MA (2006) Agonistic interactions between invasive green crabs, *Carcinus maenas* (Linnaeus) and sub-adult American lobster, *Homarus americanus* (Milne Edwards). J. Exp. Mar. Biol. Ecol. 329: 66-74.

Williamson MH, Fitter A (1996) The characters of successful invaders. Biol. Conserv. 78: 163-170.

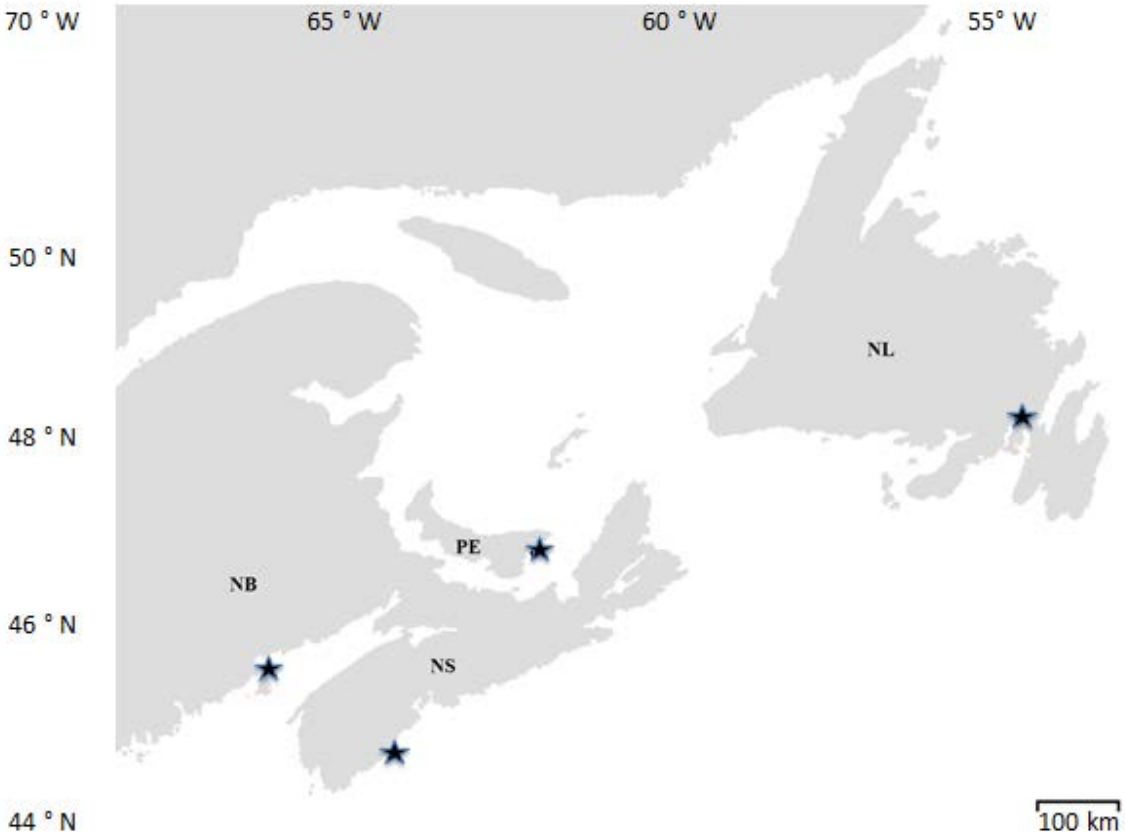


Figure 5-1. Map of the Atlantic Canadian provinces and locations of the sites (represented by a star) from where green crab were collected.

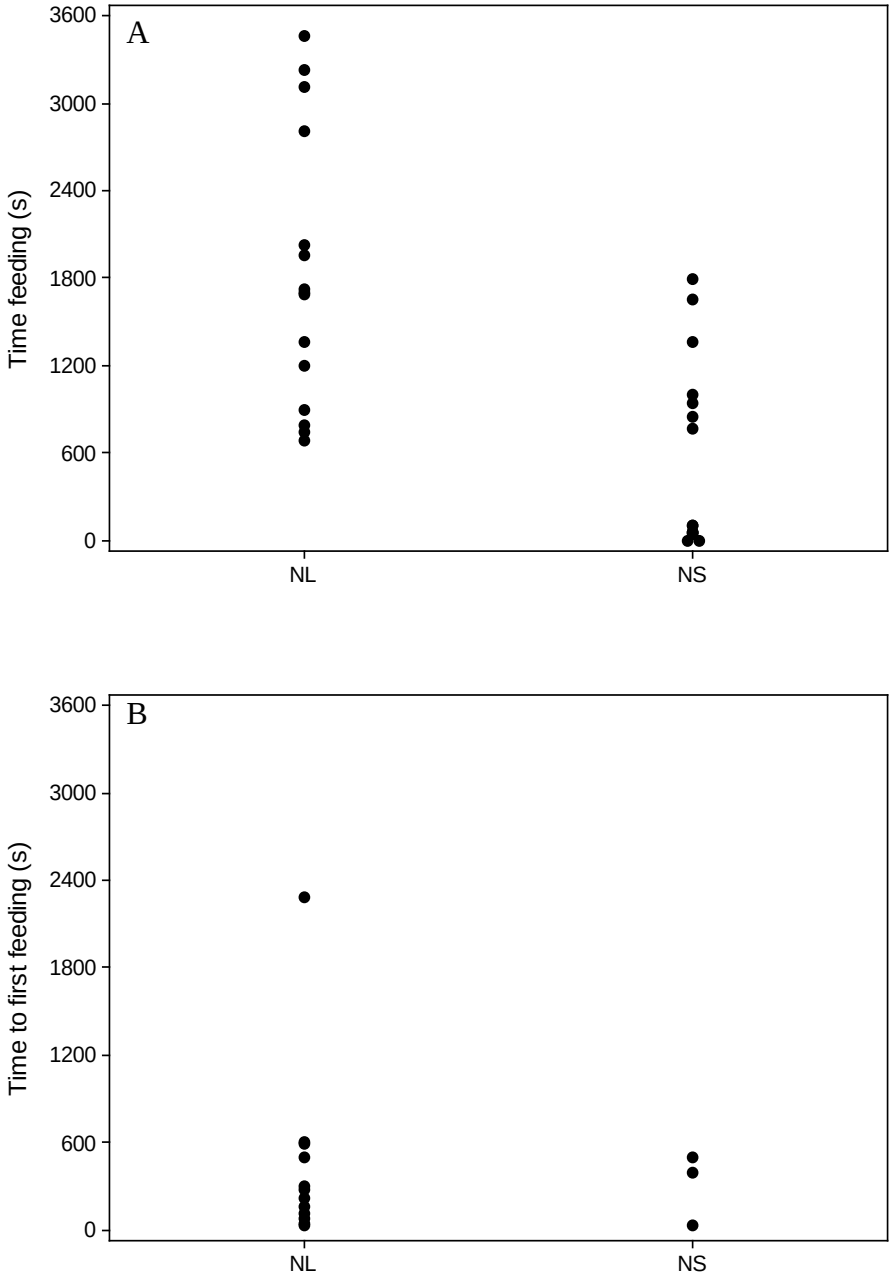


Figure 5-2. Individual value plot of A) time feeding on the mussel by green crab from NL and NS (n = 15; p = 0.005) and B) time to first feeding (NL was first to feed in 12 of 15 trials; p = 0.035).

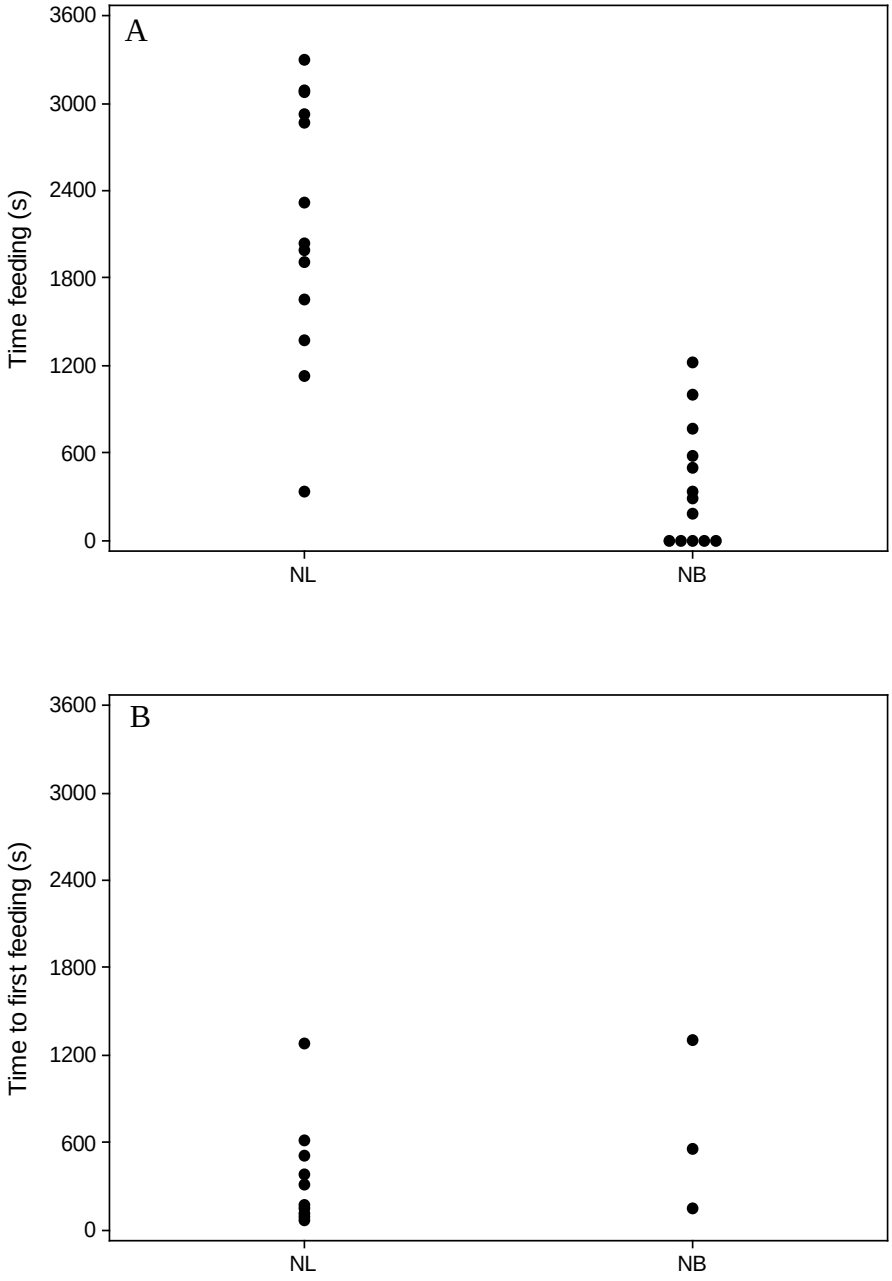


Figure 5-3. Individual value plot of A) time feeding on the mussel by green crab from NL and NB (n = 13; $p < 0.001$) and B) time to first feeding (NL was first to feed in 10 of 13 trials; $p = 0.092$).

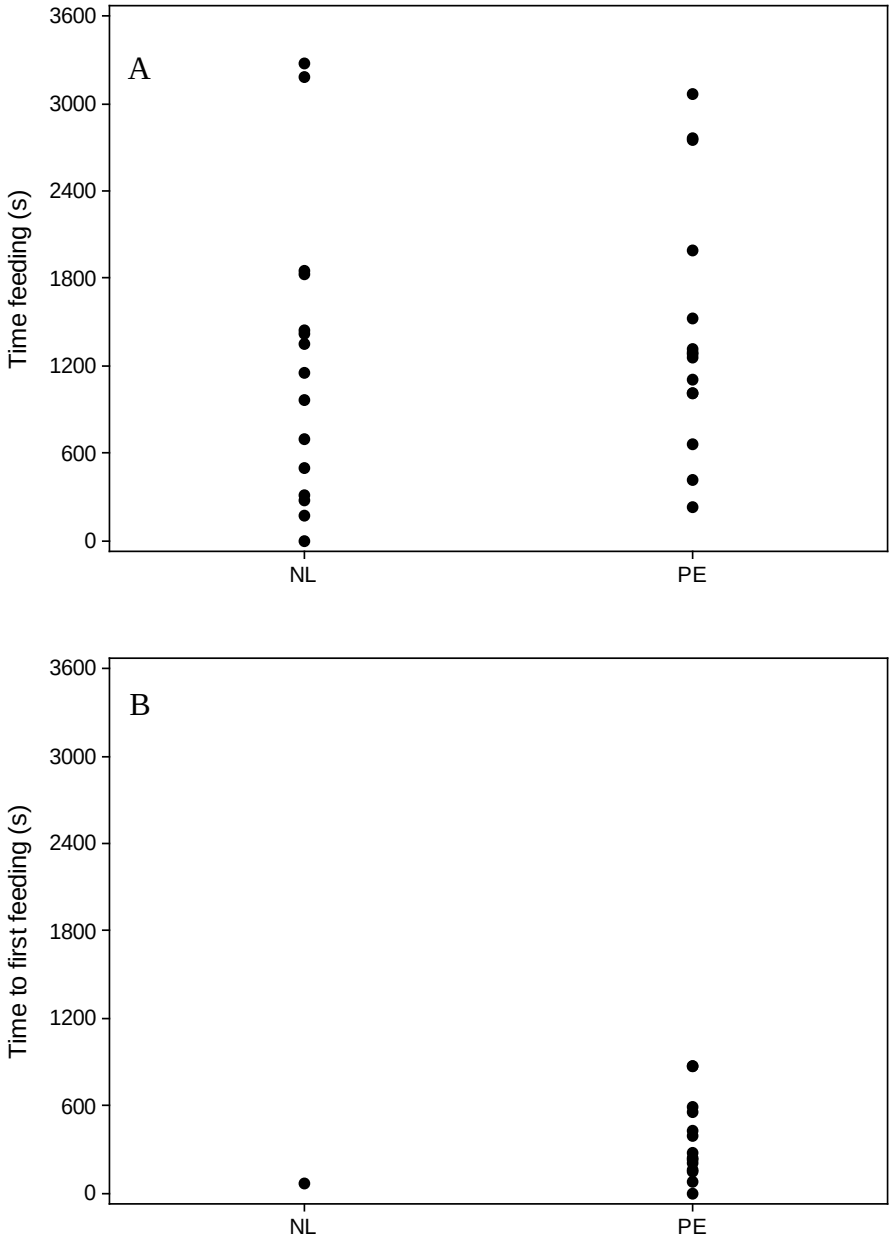


Figure 5-4. Individual value plot of A) time feeding on the mussel by green crab from NL and PE (n = 15; p = 0.629) and B) time to first feeding (PE was first to feed in 14 of 15 trials; p = 0.001).

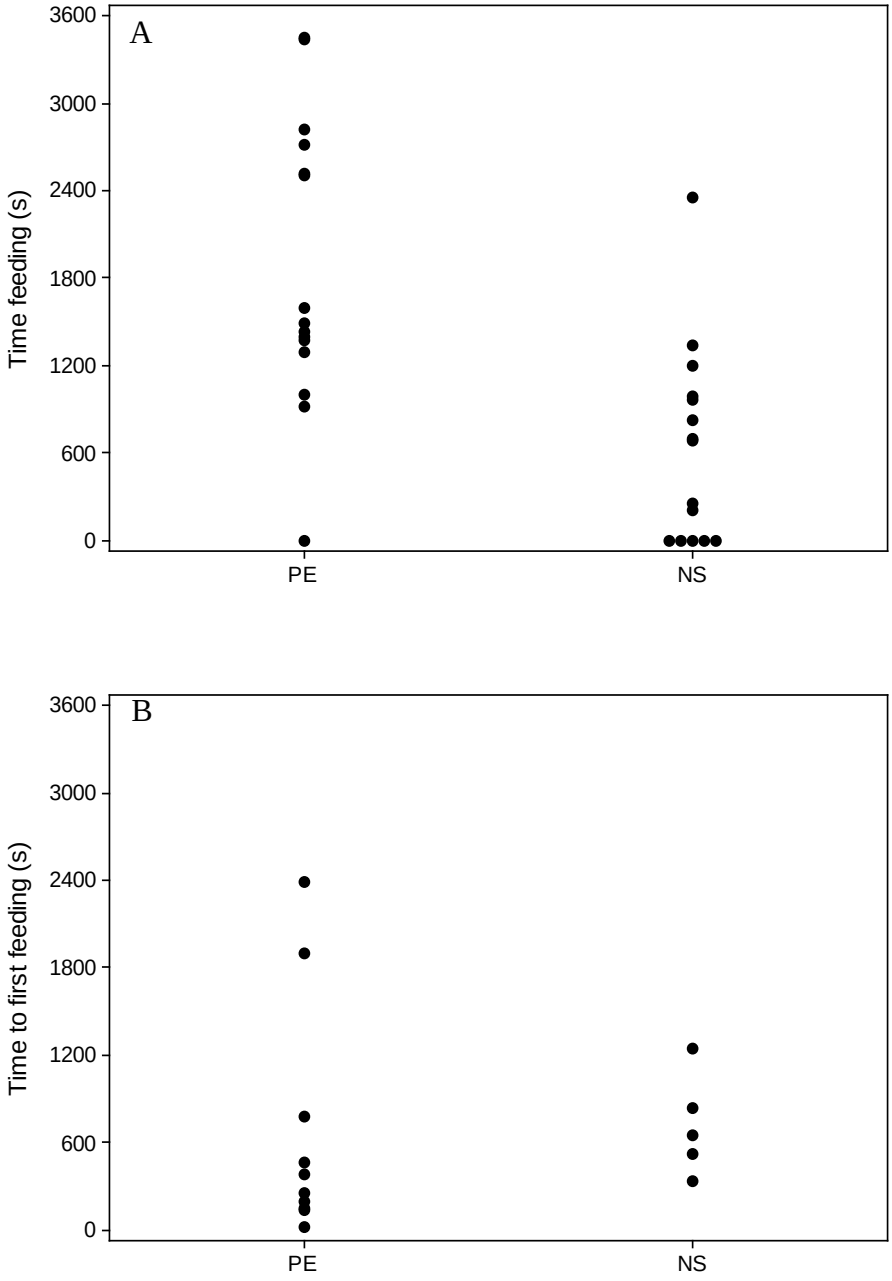


Figure 5-5. Individual value plot of A) time feeding on the mussel by green crab from PE and NS (n = 15; p = 0.009) and B) time to first feeding (PE was first to feed in 10 of 15 trials; p = 0.302).

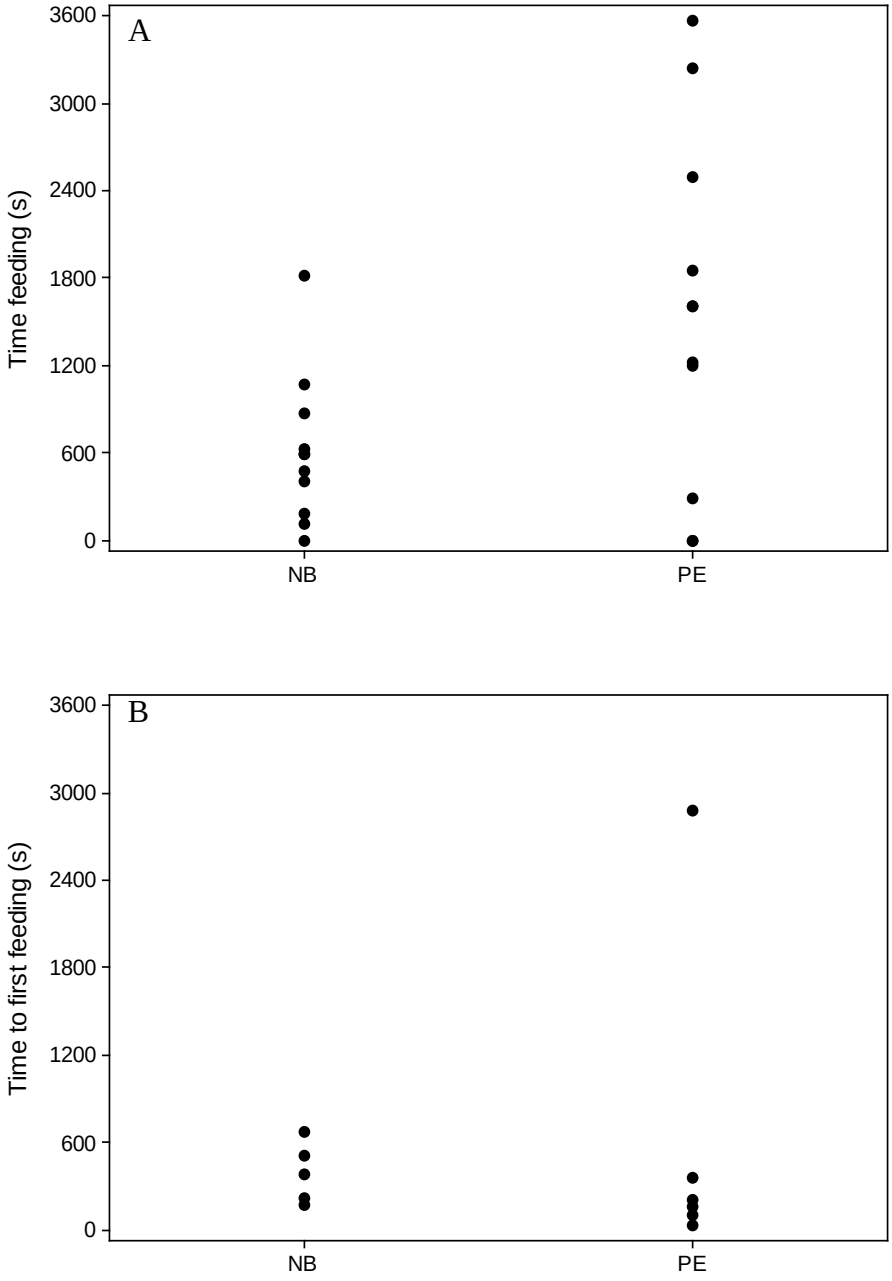


Figure 5-6. Individual value plot of A) time feeding on the mussel by green crab from NB and PE (n = 11; p = 0.064) and B) time to first feeding (PE was first to feed in 6 of 11 trials; p = 1.000).

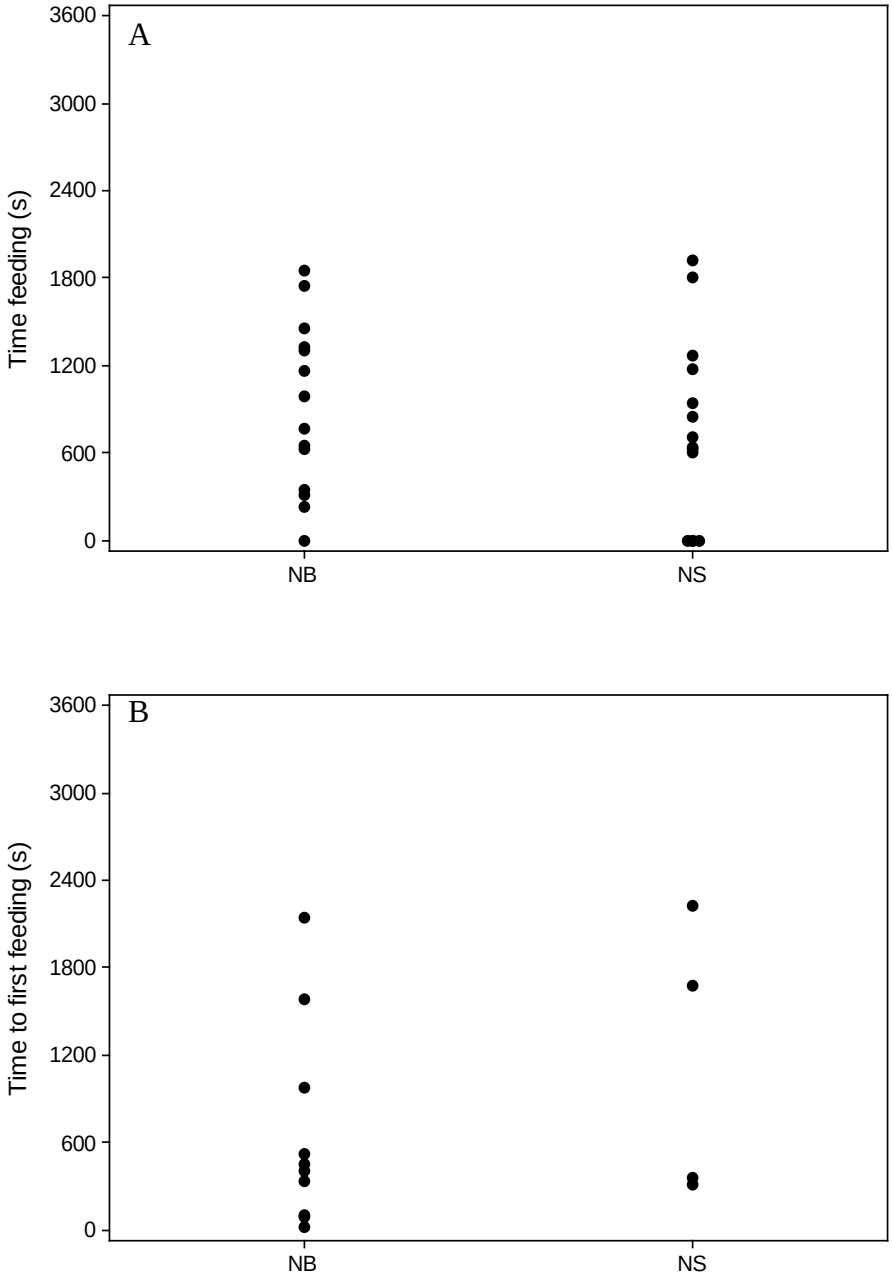
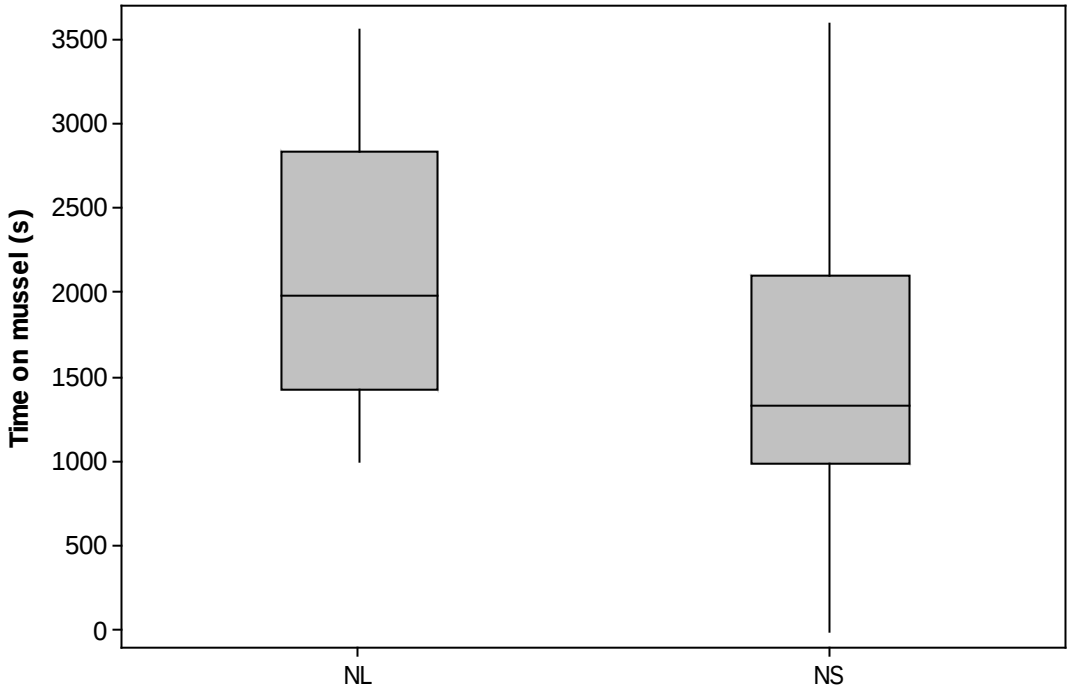


Figure 5-7. Individual value plot of A) time feeding on the mussel by green crab from NB and NS (n = 14; p = 0.556) and B) time to first feeding (NB was first to feed in 10 of 14 trials; p = 0.180).



The horizontal line within the box represents the median value.

Figure 5-8. Boxplot of time spent feeding on the mussel by green crab from NL and NS (n = 25 per treatment; p = 0.027) during one hour trials in the presence of juvenile lobster.

Table 5-1. A summary of the first crab to reach the mussel, the time it took to find the mussel, the amount of time spent feeding on the mussel by green crab 1 and green crab 2 and total interaction time for each match-up.

GC1	GC2	First to Mussel (for the majority of the N trials)	Time to Mussel (s) (mean±SE)	GC1 Time on Mussel (s) (mean±SE)	GC2 Time on Mussel (s) (mean±SE)	Total Interaction Time (s) (mean±SE)	Number of Interactions (mean±SE)	N
NL	PE	PE *	359 ± 67	1229 ± 259	1447 ± 219	314 ± 68	9.1 ± 1.7	15
NL	NB	NL	441 ± 117	2154 ± 246 *	374 ± 115	307 ± 71	7.9 ± 1.3	13
NL	NS	NL	410 ± 144	1825 ± 242 *	589 ± 167	363 ± 96	7.2 ± 1.4	15
NB	PE	PE	519 ± 243	617 ± 154	1554 ± 364	397 ± 69	8.8 ± 1.7	11
NB	NS	NB	803 ± 208	914 ± 156	754 ± 170	166 ± 40	4.1 ± 0.5	14
PE	NS	PE	685 ± 175	1864 ± 256 *	635 ± 175	222 ± 49	5.9 ± 0.9	15

The * indicates a significant result when comparing the two crab treatments. GC1 = the first green crab and its location and GC2 = the second green crab and its location; N = sample size.

6. Claw morphology variation of non-indigenous European green crab populations in Atlantic Canada

6.1. Abstract

Invasive European green crab (*Carcinus maenas*) populations initially established in Canada in the Bay of Fundy, New Brunswick in the 1950s and were present in all five Atlantic Canadian Provinces by 2007. Genetic evidence suggests these Atlantic Canadian green crab populations originated from two separate introductions with differences among regions leading to possible population level morphological differences, particularly in claw size. Given that crab depend heavily on claws for feeding, differences among populations may affect foraging success and ultimately the underlying impacts on benthic communities. This study examined claw morphology of crab from the initial introduction (New Brunswick; NB), the second introduction (Prince Edward Island; PE), and a genetically mixed population (Newfoundland; NL) to evaluate among site differences, and whether claw size accurately predicts the competitive dominance documented in a previous study. Less aggressive NB crab had larger claws than the more aggressive PE populations. Because the largest differences in claw size coincided with the most distinct genetic populations (NB and PE), genetic makeup likely plays a role in the variation observed. Given that claw size can often change in an evolutionary "arms race" between predator and prey, diet must also be considered. With variability in claw size, green crab may be able to feed on a wider array of organisms.

6.2. Introduction

Body size and weapon size are often important for assessing opponents during crustacean agonistic interactions. Game theory predicts avoidance of physical contact if one organism is larger than the other during a given encounter (Huber and Kravitz 1995); however, if similarly matched in size, direct interactions are more likely to occur (Thorpe et al. 1994). Body or weapon size also reflect geographical variation, where genetic makeup and habitat may influence growth. This variation applies particularly well to invasive species such as green crab, in which body size in invaded areas typically exceeds that of individuals in their native range (Pintor et al. 2009). Resources, lower risk of parasites and diseases, and lower competitive and predation pressures in new environments likely drive these differences (Behrens Yamada et al. 2005).

Native populations of green crab also exhibit phenotypic differences in morphology within restricted geographical areas in the United Kingdom (Brian et al. 2006). However, these differences reflect primarily environmental conditions rather than genetic makeup (Brian et al. 2006). Unlike most other crustaceans and contrary to predictions of game theory, green crab often engage in physical contact regardless of size differences between opponents. Hence, at least in this type of intraspecific agonistic encounters, chelae length rather than body size may best predict the outcome of an encounter (Sneddon et al. 1997).

Atlantic Canada offers a unique opportunity to study population morphological variation in green crab. This variation resulted from two geographically separated introductions from different European source populations, which created areas of mixed

genetic variability where populations meet (Blakeslee et al. 2010). Interestingly, recent laboratory behavioral experiments on green crab intraspecific interactions show differences in foraging ability among populations from the region (Rossong et al. 2012; Chapter 5). For instance, crab from the second wave of introductions (Prince Edward Island; PE) were better foragers for a limited resource when matched against crab from the first invasion (New Brunswick; NB). Moreover, the most recent group of invaders (Newfoundland; NL) was superior in terms of foraging to genetically similar populations from Nova Scotia (NS) and NB (Rossong et al. 2012; Chapter 5). This previous work suggested that both genetic variation and time of population establishment may influence foraging ability. Given that both factors apparently influence aggressive behaviour in green crab, morphological differences (e.g. relative claw size) may also contribute to aggressiveness or competitive ability.

Green crab morphology in well-established non-native populations consistently appear to relate to diet and an "arms race" between prey and predators. For example, on the North American east coast, gastropod and bivalve species increase shell thickness in response to green crab; green crab, in turn, increase claw size in response to shell thickness (Vermeijj 1982; Trussell and Smith 2000). Claw size often predicts diet and diet may vary, depending on location. Though generalist predators, green crab may become more specialized based on available resources over time and under increased competitive pressures.

The present study examines the role of green crab morphology with regard to behaviour and geographical variation. As a follow up from a previous study on green crab feeding (Chapter 5; Rossong et al. 2012), this study assessed: a) whether claw size affects

feeding success (i.e. do crabs with larger claw size dominate food resources); b) if so, does population history, habitat, diet or a combination of these factors drive those differences. In addition, a new green crab population was discovered in Stephenville in western NL that was not genetically analyzed at the time of green crab collection. Using the claw morphology of various provinces, including the new Seal Cove population, I also assess: c) whether claw morphology can be used to predict the geographic origin of a new green crab invasion.

6.3. Methods

6.3.1. Field Collection

Preliminary analyses (M. Rossong, unpublished data) suggested geographical differences in green crab claw morphology in the Atlantic region. Green crab were therefore collected from three sites in NB and PE, three sites in eastern NL, and one site in western NL in order to further examine these potential differences (see Figure 6-1 for specific collection sites). Previous work demonstrated that green crab populations from Chance Harbour, St. Andrews, and Dipper Harbour, New Brunswick (NB) were derived from the initial (1950s) introduction of green crab to Canada (Roman 2006). Prince Edward Island (PE) populations from Souris, Georgetown, and Annandale were sourced from a genetically distinct second invasion in 1997. Collections of green crab from the mixed genetic group were obtained from populations in Placentia Bay, Newfoundland and Labrador (NL; North Harbour, Swift Current, Arnold's Cove). An additional green crab population from Seal Cove, St. George's Bay, in western NL had unknown genetic makeup at the time of the study. All crab were collected from Sept 11th to 13th, 2011 using

Fukui traps baited with fish and soaked for 1 to 12 hours depending on location and catch rate. Soak time was consistent with methods in Chapter 5, biasing towards more aggressive male crab in order to assess differences between the two studies. Female crab were observed more often in NB where soak times were longer to obtain a sufficient sample size, but were removed from analysis due to low numbers. In most cases, traps were deployed from government wharves for convenience. Crab were placed on ice, transported to facilities at UPEI (NB and PE crab) or Northwest Atlantic Fisheries Centre (NL crab) and euthanized.

6.3.2. Analysis of Body Measurements

To assess differences in morphology among locations, 13 to 30 male crab from each site were measured for carapace width (CW; ± 1 mm), body weight (± 0.01 g), claw length, claw width (height), and dactyl length (± 1 mm) (Figure 6-2). The majority of crab were intact males, however, in areas where sample sizes were low, crab missing one claw were included in the analyses (fewer than 10% for all sites combined). Analyses excluded crab with regenerated claws.

Analysis of covariance (ANCOVA) was used to assess differences in measures of crusher claw size (claw length, claw width, and dactyl length) among sampling locations, with CW as a covariate to control for differences in body size. Regression slopes were considered parallel when the interaction term in the general linear model was not significant ($\alpha = 0.05$) or when the difference in the coefficient of determination (R^2) between the interaction model and the parallel slope model was less than 2% (Barrett et al. 2010). Assumptions of normality and homogeneity of variances were assessed by

inspection of residual plots, and outliers were assessed by calculating the probability of observing a value as extreme as the value in question, based on Bonferroni adjusted p-values from the Studentized residuals with a significance level of 0.05 (Dohoo et el. 2009). This analysis indicated that a Studentized residual of magnitude 3.76 represented an outlier.

When a significant difference in location was observed in the ANCOVA, multiple comparison tests were conducted to assess two different hypotheses. The first hypothesis addressed whether claw size differed among the 9 study sites. Although we tested this hypothesis with low statistical power (36 total multiple comparisons), differences among the individual sampling sites were also of interest. Tukey's honestly significant differences tests (family error rate = 0.05) were used to assess this hypothesis.

The second hypothesis was whether claw size differed among the three provinces (NB, NL, and PE) and was tested using *a priori* contrasts among provinces (Oehlert 2000). This approach resulted in three contrasts:

$$H_0: \mu_{NB1} + \mu_{NB2} + \mu_{NB3} = \mu_{NL1} + \mu_{NL2} + \mu_{NL3}$$

$$H_0: \mu_{NB1} + \mu_{NB2} + \mu_{NB3} = \mu_{PE1} + \mu_{PE2} + \mu_{PE3}$$

$$H_0: \mu_{NL1} + \mu_{NL2} + \mu_{NL3} = \mu_{PE1} + \mu_{PE2} + \mu_{PE3}$$

where μ_{Xi} represents the mean claw measurement from the i^{th} (i = 1 to 3) site in province X. A Bonferroni adjustment to the significance level was used to control for multiple contrasts (3 comparisons; $\alpha = 0.05/3 = 0.0167$).

6.4. Results

Carapace width ranged from 36 to 79 mm (56.6 ± 10.4; mean ± SD) for NB, 47 to 78 mm (65.2 ± 6.5) for PE, and 35 to 72 mm (56.4 ± 9.1) for NL. Five smaller individuals from NL (CW < 35mm) were removed prior to analysis because these individuals had a strong influence on the regression coefficient. The overlap in CW values was therefore similar across all sampling locations. This removal reduced the combined sample size for all locations to 237 crab.

6.4.1. Differences Among Sites

Green crab were examined for differences in claw measurements relative to overall size (CW; Table 6-1). The interaction term for the ANCOVA of crusher claw length relative to CW was not significant ($F_{8,218} = 1.18$; $p = 0.314$). The interaction term for the ANCOVA of crusher claw width relative to CW was significant ($F_{8,217} = 2.58$; $p = 0.010$). The R^2 of the interaction ANCOVA model was 0.858 and the R^2 of the parallel slope ANCOVA model was 0.844, suggesting that the parallel slope model explained almost as much variability as the interaction model and represented a good fit. One outlier crab from Chance Harbour with a small crusher claw width (Studentized residual = -5.21) was removed. The relationships of crusher claw length and width to CW differed significantly among sample locations ($F_{8,226} = 2.97$; $p = 0.004$, Table 6-1; Figure 6-3A; $F_{8,225} = 21.0$; $p < 0.001$,Table 6-1; Figure 6-3B, respectively). Tukey pair-wise comparisons indicated no significant differences in crusher claw length and width among all sites in NB and all sites in PE. For crusher claw length only Swift Current (NL) differed significantly from North Harbour (NL) and St. Andrews (NB) (Figure 6-5).

Crusher claw width was much more variable among provinces (Figure 6-6) with NL sites overlapping with populations from both PE and NL.

The interaction term for the ANCOVA of crusher claw dactyl length was not significant ($F_{8,208}$ = 0.55; p = 0.817). Crusher claw dactyl length differed significantly among locations ($F_{8,216}$ = 2.21; p = 0.028; Figure 6-3C; Table 6-1). One outlier crab from Georgetown (PE) was removed (Studentized residual = 4.69). Pair-wise comparisons revealed significantly longer dactyls in St. Andrews (NB) and Chance Harbour (NB) crab compared to Swift Current (NL) (Figure 6-7).

The interaction term for the ANCOVA of pincer claw width was significant ($F_{8,204}$ = 2.20; p = 0.029) but coefficients of determination of the interaction ANCOVA model and the parallel slope model were similar (R^2= 0.932 and 0.926 respectively). Three outliers were removed from the analysis (Table 6-1). Pincer claw width differed significantly among locations ($F_{8,212}$= 11.0; p < 0.001; Table 6-1; Figure 6-4B); pair-wise comparisons indicated differences between PE sites and all of the NB sites and overlap with the NL sites (Figure 6-8). No among-site differences were detected for pincer claw length ($F_{8,215}$= 1.19; p = 0.303; Figure 6-4A) or dactyl length ($F_{8,212}$ = 0.883; p = 0.331; Figure 6-4C).

6.4.2. Differences Among Provinces

Contrasts of means among populations showed similar differences in crusher claw as the pair-wise comparisons among locations (Table 6-2). Crusher claw length for NB and PE differed significantly ($F_{1,226,}$= 7.67, p = 0.006) whereas Placentia Bay, NL crab

crusher claw length did not differ significantly from other provinces ($F_{1,226,}$=1.77, p = 0.185 and $F_{1,226,}$= 2.23, p = 0.137 for NB and PE, respectively; Figure 6-5).

Crusher claw width differed significantly among all provinces (p < 0.001 for each contrast; Table 6-2; Figure 6-6). Crusher claw dactyl length did not differ among provinces (Figure 6-7). Pincer claw width differed between NB and PE ($F_{1,212}$ = 75.1, p < 0.001), and NB and NL ($F_{1,212}$ = 51.6, p < 0.001; Figure 6-8) but PE and NL did not differ ($F_{1,212}$ = 2.76, p = 0.098). No significant differences were detected in pincer claw length or dactyls.

6.4.3. Seal Cove

The genetic makeup of green crab from Seal Cove, NL, was unknown at the time of collection but pair-wise comparisons of claw morphologies among sites may indicate whether green crab arrived in Newfoundland through two separate introductions on the west and east coast of NL, or if a single introduction into Placentia Bay contributed to the isolated population in Seal Cove. Seal Cove crab crusher claw widths were most similar to those in Swift Current (Placentia Bay, NL) but differed significantly only from Chance Harbour and St. Andrews (both in NB; Figure 6-6). Pincer claw widths differed significantly between crab from Seal Cove and all sites in NB (Figure 6-8). Crusher and pincer claw lengths and dactyl length of crab from Seal Cove did not differ from any other sites (Figure 6-5; Figure 6-7).

Seal Cove crab had similar crusher claw lengths to NB ($F_{1,255}$ = 0.073, p = 0.787), NL ($F_{1,255}$ = 1.83, p = 0.177) and PE ($F_{1,255}$ = 5.34, p = 0.022) populations (Table 6-3; Figure 6-5). The crusher claw widths at other NL sites were similar to Seal Cove

populations ($F_{1,254}$ = 0.536, p = 0.465) but Seal Cove populations differed significantly from NB ($F_{1,254}$ = 20.7, p < 0.001) and PE ($F_{1,254}$ = 15.0, p < 0.001) populations (Figure 6-6). No differences were detected among contrasts for crusher claw dactyl length (Table 6-3; Figure 6-7). Pincer claw widths differed significantly from the NB population and were similar to Placentia Bay, NL and PE populations. These contrasts suggest that Seal Cove claw sizes were most similar to Placentia Bay populations.

6.5. Discussion

Green crab crusher claws (adjusted for carapace width) were significantly longer and wider in sites associated with the first invasion of Atlantic Canada (NB) and smallest at sites from the second invasion (PE). These findings contradicted my prediction of larger claws in more aggressive or competitively dominant crab found in the most recently invaded areas (cf. Rossong et al. 2012; Chapter 5). In fact, NB crab were the least dominant in competitive experiments, despite significantly larger crusher claws. These results also contrast previous studies where weapon size (not carapace size) primarily determined the outcome of agonistic interactions (Lee and Seed 1992; Sneddon et al. 1997). Whereas most crustaceans appear to assess opponents by displaying their claws in order to reduce injury risk and unnecessary energy expenditure, green crab in invaded areas apparently do not exhibit this conservative behaviour. Video analyses of agonistic encounters support this assertion, in that green crab often forego assessment of their opponent as they enter aggressive combat.

In the present study, despite apparent differences in claw morphology of green crab in Atlantic Canada, I am unable to attribute those differences to specific drivers and

therefore focus this discussion on two potential factors that often shape crustacean morphology. A conclusive explanation for claw differences requires direct studies on the roles of genetics and feeding ecology.

6.5.1. Population Variability and Morphology

The largest claw size difference was observed between the two most genetically distinct populations of green crab (NB and PE), suggesting that differences in claw morphology may be at least partially related to genetic differences. A previous study on native populations of green crab related morphological similarity to genetic population structure (Brian et al. 2006). However, that relationship accounted for only 22% of the variability between genetically similar populations and the authors concluded that environment probably determined phenotypic expression.

The role of genetic haplotypes was examined further by comparing the data of the present study to those obtained from genetically similar crab from another area. Mitchell et al. (2003) collected crabs to examine claw morphology in Antigonish, northern NS, which are genetically similar to the PE crab in my study (Roman 2006). They calculated a "relative claw size" (RCS), by dividing chela width (in their case "chela height") by carapace width, and obtained a mean crusher RCS for males of 0.275 ± 0.046 (mean ± standard deviation; n = 26) and pincer RCS of 0.232 ± 0.026 (n = 28). For the PE crab in the present study, values were 0.271 ± 0.025 (n = 90) and 0.225 ± 0.013 (n = 85), respectively for crusher RCS and pincer RCS. These values for PE and northern NS do not differ significantly based on a two-sample t-test (p = 0.666 for crusher and p = 0.181 for pincer). These results are also consistent with RCS ratios obtained from native

populations in North Wales for crusher claws (0.285 ± 0.032; Lee and Seed 1992), which are genetically similar to PE and northern NS populations. When the RCS of the crusher claw was compared among locations from the data collected in this study and northern NS (Mitchell et al. 2003) significant differences were found between NB and NS (p = 0.008) and between pincer claws for NL and NB (p = 0.006), and for NL and NS (p = 0.019). These results support a strong genetic basis for geographic differences.

Similarly, preliminary analyses of genetic haplotypes (n = 12) suggest that the Seal Cove, NL population is most similar to PE populations (Blakeslee pers. comm.). This result differs from the results of the contrasts in this study, which suggested greatest similarity in overall claw size to Placentia Bay. Confirmation of the origin of the Seal Cove population requires a larger sample size, but available evidence suggests that, although genetics may play a role in overall morphology, other factors also influence these relationships.

6.5.2. The Role of Feeding Ecology on Morphology

Crusher size serves as a template for diet (Elner 1978) where claw width (or height) can be used as a surrogate of crushing strength (Smith 2004; Behrens Yamada et al. 2010). Crab with larger claws should therefore have a selective advantage with a wider range of prey and a lower risk of claw damage associated with feeding (Smith 2004). A trade-off nonetheless exists given that large claws often come at a metabolic cost. Considerable energy contributes to larger claws leaving fewer resources for reproduction and ultimately survival. This consideration is especially important for green crab where larger weapons are not necessarily important for agonistic encounters (Sneddon et al.

1997). This interpretation does not apply to the pincer claw, where we detected no significant differences in pincer claw measurements among populations.

Several studies suggest co-evolution between green crab and native prey (Vermeijj 1982; Trussell and Smith 2000; Freeman and Byers 2006; Rochette et al. 2007). However, in most cases no change in prey defences occurred (Seeley 1986). For example, the snail, *Littorina obtusata* and the blue mussel, *Mytilus edulis* developed thicker shells to reduce green crab predation pressure, and green crab, in turn, evolved larger crusher claws. For instance, Edgell and Rochette (2009) fed NB and Maine green crab thicker shelled snails (*Littorina obtusata*) over an extended period of time (2 moult cycles) and demonstrated that crab developed significantly greater crusher claw volume than those fed a consistent diet of thinner shelled snails. As in the present study, they found no differences in the smaller pincer claw.

The larger crusher claws in NB crab in this study likely reflect thicker shelled or larger prey in that area. This difference may relate to the fact that prey in the Bay of Fundy had more generations to develop adaptations to discourage green crab predation; or that green crab eliminated smaller prey, leaving only less-preferred items, which are harder to open and increase handling time. The smallest claws that characterized the PE populations may reflect food availability within the sedimentary substrates in that region or a time lag in prey defences (Freeman and Byers 2006). A prey preference experiment in PE (Pickering and Quijón 2011) revealed that, when given a choice of bivalve species, green crab of all size ranges prefer soft-shell clams (*Mya arenaria*) over thicker shelled mussels (*Mytilus edulis*) and oysters (*Crassostrea virginica*). In addition, in their field caging experiments, gastropod mortality was evident only when oysters were the only

other food source available (T. Pickering pers. comm.). If green crab prefer thinner-shelled prey items and those prey are readily available then stronger claws may be unnecessary (Behrens Yamada et al. 2010). Optimal foraging theory predicts that it is advantageous to consume medium sized prey, so the most energy is gained at the lowest cost. For small and large prey consumption, search and handling time increases resulting in a higher risk of predation. Thus the benefit of larger claws may depend on diet. Consistently longer claws may benefit crab that prefer soft-shell clams, worms and crustaceans, whereas strong claws (larger claw width) may help in specializing on hard prey (Behrens Yamada et al. 2010). Most clam predation by green crab occurs in the upper layers of the substrate (Floyd and Williams 2004), so soft-shelled clams may alter behaviour in the presence of predators by digging deeper in the sediments and investing more energy in siphon growth (Whitlow 2010).

Interestingly, NL green crab have the second largest claws in my study, which is inconsistent with the predator-prey co-evolution hypothesis presented above. Larger claws may simply adapt to allow a more general diet and the exploitation of a wider range of prey species (Elner 1981). Indeed, stomach content analyses conducted on NL green crab suggest a broad variety of prey, including gastropods, bivalves, crustaceans, and polychaetes (Chapter 2).

Other factors to consider while studying claw morphological variation include sex differences, temperature, and latitude. For instance, when comparing sexes among populations, males often exhibit significantly wider and stronger claws than the more uniform females (Lee and Seed 1992; Brian et al. 2006). This difference was associated with dominance in intraspecific encounters as well as mating behaviour. In my study, I

removed all females from the analysis and tested only males to control for these potential differences. Temperature and location during development can ultimately influence the overall size, moult stage, and growth of an organism (Taylor et al. 2009; Kelly et al. 2011). However, each of these factors may change in ways not fully understood. The influence of water temperature, for example, offers contradictory evidence. One study showed larger overall body size (CW) in northern populations (i.e. colder water temperatures) along the west coast of North America (CW; Kelly et al. 2011). Meanwhile, a second study showed lower claw size and thus lower claw strength in the northernmost sites of the east coast of the US (Taylor et al. 2009). Based on the latter study, one would predict significantly smaller claws in NL based on latitude alone. However, PE crab had the smallest crusher claws despite warmer summer water temperatures within PE's relatively shallow Northumberland Strait. The crabs studied by Taylor et al. (2009) were also from the initial invasion in the 1950s, potentially explaining some of the differences between the two studies. To complicate this argument, study locations on the east (Taylor et al. 2009) and west coast (Kelly et al. 2011) of North America encompassed completely different habitats, and temperature may therefore not drive differences in morphology and may instead reflect differences in community structure and green crab diet.

6.5.3. Conclusions

Published studies indicate that both genetic makeup and geographic variations in diet may play a role in claw morphological differences in green crab within Atlantic Canada. If genetics or diet were the only driving forces, claw variability among sites

within the same province would be minimal. In some cases, where sites were geographically close together, differences were observed in claw size in multiple pair-wise comparisons. Acknowledging expected natural variation in morphology among green crab, pooled samples by provinces with higher sample sizes likely reflect actual population differences more accurately.

With the passage of evolutionary time and with dietary differences among the study locations, differences in morphology of claws may occur as well as in the types of prey consumed. Claw strength can ultimately determine prey availability for consumption. The capacity of invasive crab to develop a broad range of claw sizes within their new habitats, regardless of population origin, may well facilitate successful invasions of less suitable habitats.

6.6. References

Barrett TJ, Tingley MA, Munkittrick KR, Lowell RB (2010) Dealing with heterogeneous regression slopes in analysis of covariance: new methodology applied to environmental effects monitoring fish survey data. Environ. Monit. Assess. 166: 279-291.

Behrens Yamada S, Davidson TM, Fisher S (2010) Claw morphology and feeding rates of introduced European green crabs (*Carcinus maenas* L, 1758) and native Dungeness crabs (*Cancer magister* Dana, 1852). J. Shellfish Res. 29: 471-477.

Behrens Yamada S, Dumbauld BR, Kalin A, Hunt CE, Figlar-Barnes R, Randall A (2005) Growth and persistence of a recent invader *Carcinus maenas* in estuaries of the northeastern Pacific. Biol. Invasions 7: 309-321.

Blakeslee AMH, McKenzie CH, Darling JA, Byers JE, Pringle JM, Roman J (2010) A hitchiker's guide to the Maritimes: anthropogenic transport facilitates long-distance dispersal of a marine crab to Newfoundland. Divers. Distrib. 16: 879-891.

Brian JV, Fernades T, Ladle RJ, Todd PA (2006) Morphological and genetic variability in UK populations of the shore crab, *Carcinus maenas* Linnaeus, 1758 (Crustacea: Decapoda: Brachyura). J. Exp. Mar. Biol. Ecol. 329: 47-94.

Dohoo IR, Martin W, Stryhn H (2009) Veterinary Epidemiologic Research 2nd Edition. VER Inc., Charlottetown, PE.

Edgell TC, Rochette R (2009) Prey-induced changes to a predator's behaviour and morphology: Implications for shell–claw covariance in the northwest Atlantic. J. Exp. Mar. Biol. Ecol. 382: 1-7.

Elner RW (1978) The mechanics of predation by the shore crab *Carcinus maenas* (L.) on the edible mussel, *Mytilus edulis* L. Oecologia 36: 333-344.

Elner RW (1981) Diet of green crab *Carcinus maenas* from Port Hebert, southwestern Nova Scotia. J. Shellfish Res. 1: 89-94.

Floyd T, Williams PJ (2004) Impact of green crab (*Carcinus maenas* L.) predation on a population of soft-shell clams (*Mya arenaria* L.) in the southern Gulf of St. Lawrence. J. Shellfish Res. 23: 457-462.

Freeman AS, Byers JE (2006) Divergent induced responses to an invasive predator in marine mussel populations. Science 313: 831-833.

Huber R, Kravitz, EA (1995) A quantitative analysis of agonistic behavior in juvenile American lobster (*Homarus americanus* L.). Brain Behav. Evolut. 46: 72-83.

Kelley AL, de Rivera CE, Buckley BA (2011) Intraspecific variation in thermotolerance and morphology of the invasive European green crab, *Carcinus maenas*, on the west coast of North America. J. Exp. Mar. Biol. Ecol. 409: 70-78.

Lee SY, Seed R (1992) Ecological implications of cheliped size in crabs: some data from *Carcinus maenas* and *Liocarcinus holsatus*. Mar. Ecol. Prog. Ser. 84: 151-160.

Mitchell SC, Kennedy SM, Williams PJ, Demont ME (2003) Morphometrics and estimates of force generation by the chelae of a North American population of the invasive green crab, *Carcinus maenas* (L.) Can. J. Zool. 81: 203-215.

Oehlert, G.W. 2000. A first course in design and analysis of experiments. W.H. Freeman and Co. New York.

Pickering T, Quijón PA (2011) Potential effects of a non-indigenous predator in its expanded range: assessing green crab, *Carcinus maenas*, prey preference in a productive coastal area of Atlantic Canada. Mar. Biol. 158: 2065-2078.

Pintor LM, Sih A, Kerby JL (2009) Behavioral correlations provide a mechanism for explaining high invader densities and increased impacts on native prey. Ecology 90: 581-587.

Rochette R, Doyle SP, Edgell TC (2007) Interaction between an invasive decapod and a native gastropod: predator foraging tactics and prey architectural defenses. Mar. Ecol. Progr. Ser. 330: 179-188.

Roman J (2006) Diluting the founder effect:cryptic invasions expand a marine invader's home range. Proc. R. Soc. B. 273: 1-7.

Rossong MA, Quijón PA, Snelgrove PVR, Barrett TJ, McKenzie CH, Locke A (2012) Regional differences in foraging behaviour of invasive green crab (*Carcinus maenas*) populations in Atlantic Canada. Biol. Invasions 14: 659-669.

Seeley RH (1986) Intense natural selection caused a rapid morphological transition in a living marine snail. Proc. Natl. Acad. Sci. USA 83: 6897–6901.

Smith LD (2004) Biogeographic differences in claw size and performance in an introduced crab predator *Carcinus maenas*. Mar. Ecol. Prog. Ser. 276: 209-222.

Sneddon L, Huntingford FA, Taylor AC (1997) Weapon size versus body size as a predictor of winning in fights between shore crabs, *Carcinus maenas* (L.). Behav. Ecol. Sociobiol. 41: 237-242.

Taylor GM, Keyghobadi N, Schmidt PS (2009) The geography of crushing: Variation in claw performance of the invasive crab *Carcinus maenas*. J. Exp. Mar. Biol. Ecol. 377: 48-53.

Thorpe KE, Huntingford FA, Taylor AC (1994) Relative size and agonistic behaviour in the female velvet swimming crab, *Necora puber* (L.) (Brachyura, Portunidae). Behav. Processes 32: 235-246.

Trussell GC, Smith LD (2000) Induced defenses in response to an invading crab predator: an explanation of historical and geographic phenotypic change. Proc. Natl. Acad. Sci. USA. 97: 2123-2127.

Vermeijj GJ (1982) Phenotypic evolution in a poorly dispersing snail after arrival of a predator. Nature 299: 149-150.

Whitlow WL (2010) Changes in survivorship, behavior, and morphology in native soft-shell clams induced by invasive green crab predators. Mar. Ecol. 31: 418-430.

Table 6-1. Test statistics, coefficients of determination, and p-values for analyses of covariance of claw size versus carapace width by site.

Response variable	**Interaction *F*-Statistic**	**Interaction Model R^2**	**Interaction P-value**	**Location *F*-Statistic**	**Parallel Slope Model R^2**	**Location P-value**	**Outliers Removed from Analysis**
Crusher claw length	$F_{8,218}$ = 1.18	0.901	0.314	$F_{8,226}$ = 2.97	0.897	0.004	
Crusher claw width	$F_{8,217}$ = 2.58	0.858	0.010	$F_{8,225}$ = 21.0	0.844	<0.001	SR = -5.21 (Chance Harbour)
Crusher claw dactyl length	$F_{8,208}$ = 0.55	0.909	0.817	$F_{8,216}$ = 2.21	0.907	0.028	SR = 4.69 (Georgetown)
Pincer claw length	$F_{8,207}$ = 0.89	0.902	0.524	$F_{8,215}$ = 1.19	0.899	0.303	
Pincer claw width	$F_{8,204}$ = 2.20	0.932	0.029	$F_{8,212}$ = 11.0	0.926	<0.001	SR = 5.60 (North Harbour) SR = 4.90 (St. Andrews) SR = -4.21 (Dipper Harbour)
Pincer claw dactyl length	$F_{8,204}$ = 0.83	0.887	0.575	$F_{8,212}$ = 1.15	0.883	0.331	

SR = Studentized residual; R^2 = coefficient of determination

Table 6-2. Test statistics and p-values for group contrasts of adjusted claw size across provinces.

Response variable	**Contrast**	***F*-Statistic**	***P*-value**
Crusher claw length	NB vs NL	$F_{1,226} = 1.77$	0.185
	NB vs PE	$F_{1,226} = 7.67$	0.006
	NL vs PE	$F_{1,226} = 2.23$	0.137
Crusher claw width	NB vs NL	$F_{1,225} = 24.9$	<0.001
	NB vs PE	$F_{1,225} = 126$	<0.001
	NL vs PE	$F_{1,225} = 42.7$	<0.001
Crusher claw dactyl length	NB vs NL	$F_{1,216} = 1.57$	0.212
	NB vs PE	$F_{1,216} = 0.308$	0.580
	NL vs PE	$F_{1,216} = 0.513$	0.475
Pincer claw width	NB vs NL	$F_{1,212} = 51.6$	<0.001
	NB vs PE	$F_{1,212} = 75.1$	<0.001
	NL vs PE	$F_{1,212} = 2.76$	0.098

Table 6-3. Test statistics and p-values for group contrasts of adjusted claw size for each province versus Seal Cove (SC).

Response variable	**Contrast**	***F*-Statistic**	***P*-value**
Crusher claw length	NB vs SC	$F_{1,255} = 0.073$	0.787
	NL vs SC	$F_{1,255} = 1.83$	0.177
	PE vs SC	$F_{1,255} = 5.34$	0.022
Crusher claw width	NB vs SC	$F_{1,254} = 20.7$	<0.001
	NL vs SC	$F_{1,254} = 0.536$	0.465
	PE vs SC	$F_{1,254} = 15.0$	<0.001
Crusher claw dactyl length	NB vs SC	$F_{1,245} = 0.464$	0.496
	NL vs SC	$F_{1,245} = 0.089$	0.766
	PE vs SC	$F_{1,245} = 0.146$	0.703
Pincer claw width	NB vs SC	$F_{1,240} = 43.6$	<0.001
	NL vs SC	$F_{1,240} = 1.11$	0.292
	PE vs SC	$F_{1,240} = 0.023$	0.880

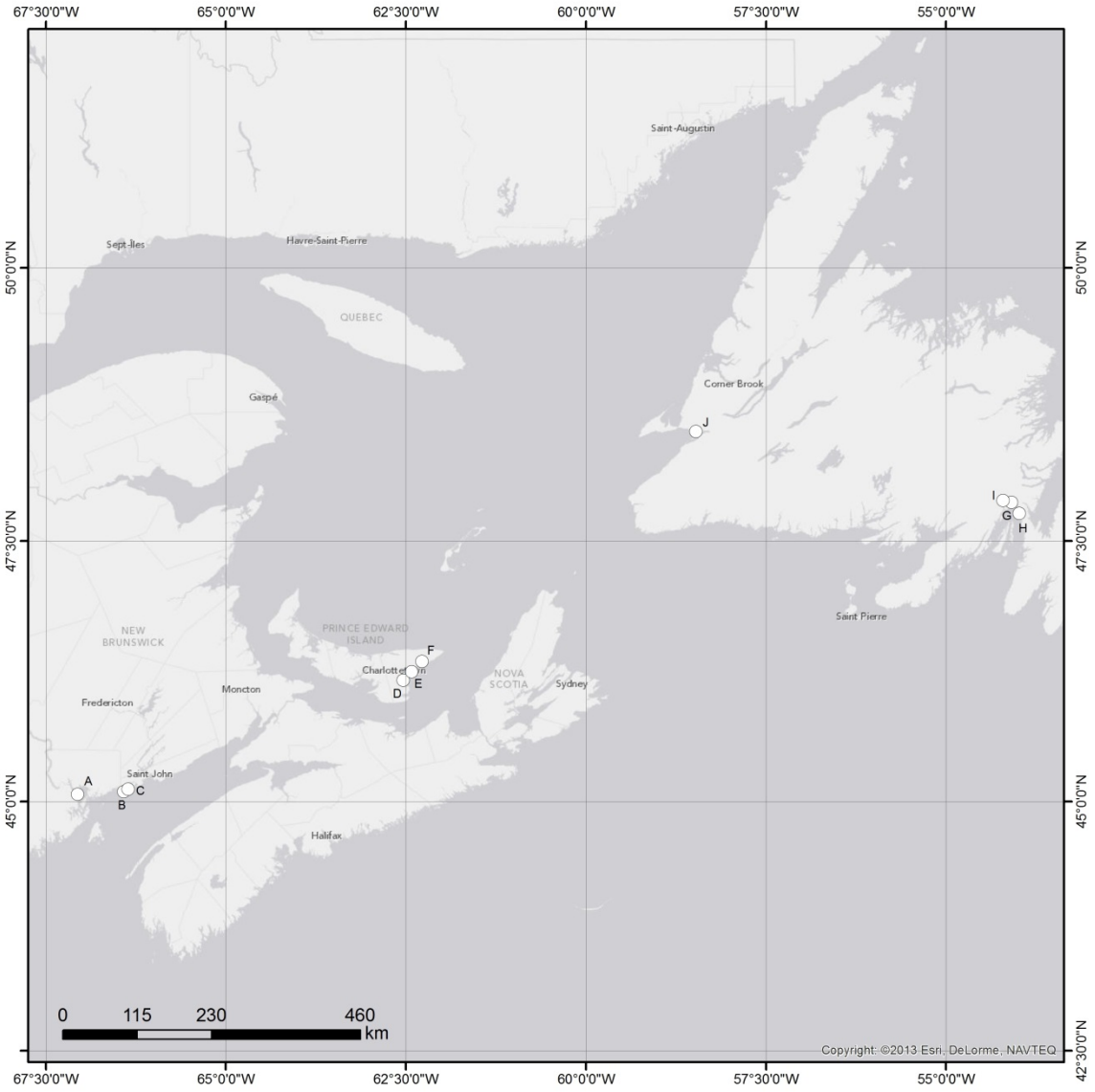


Figure 6-1. Collection sites for green crab from populations in New Brunswick (A = St. Andrews; B = Dipper Harbour; C = Chance Harbour), Prince Edward Island (D = Georgetown; E = Annandale; F = Souris) and Newfoundland (G = North Harbour; H = Arnold's Cove; I = Swift Current; J = Seal Cove).

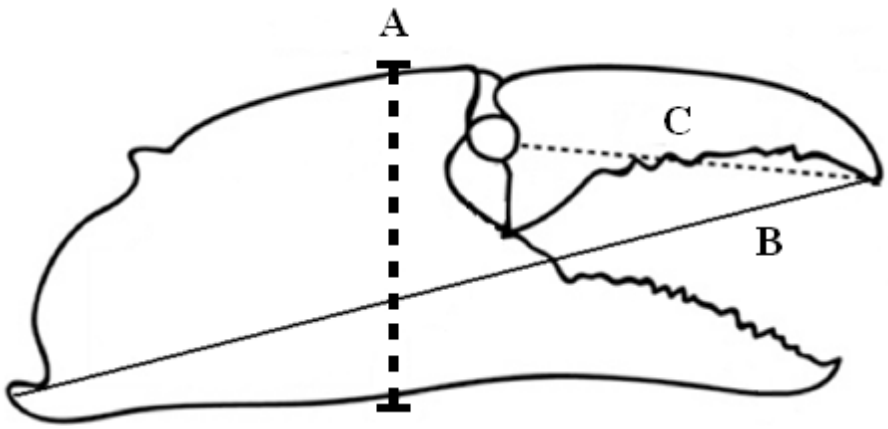


Figure has been modified from Behrens Yamada et al. (2010).

Figure 6-2. Claw measurements taken from the green crab, where A defines claw width (propal height), B defines claw length and C defines dactyl length.

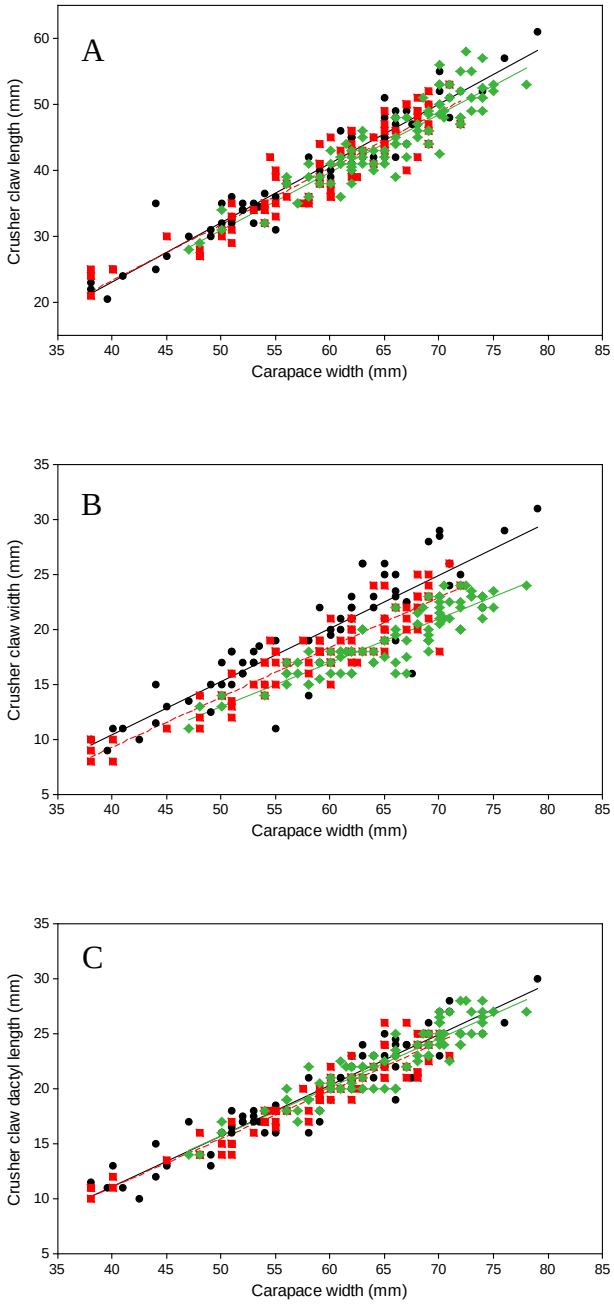


Figure 6-3. Scatterplot and linear regressions of A) crusher claw length versus carapace width, B) crusher claw width versus carapace width, and C) crusher claw dactyl length versus carapace width for male green crab collected in New Brunswick (●), Newfoundland (■), and Prince Edward Island (◆).

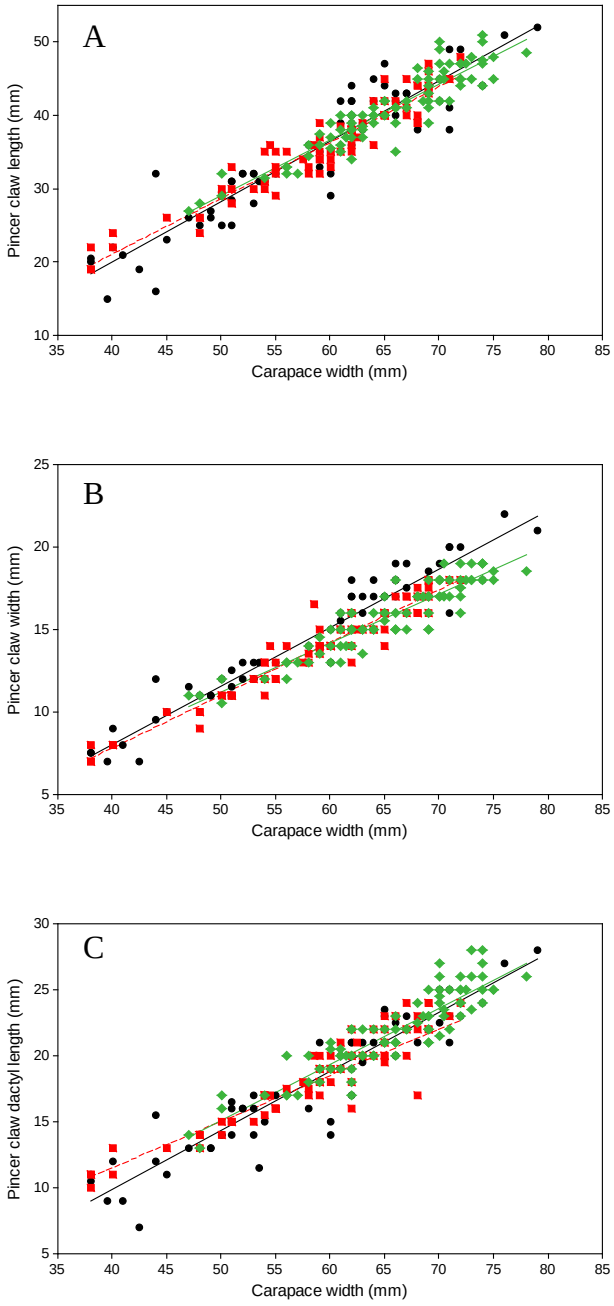
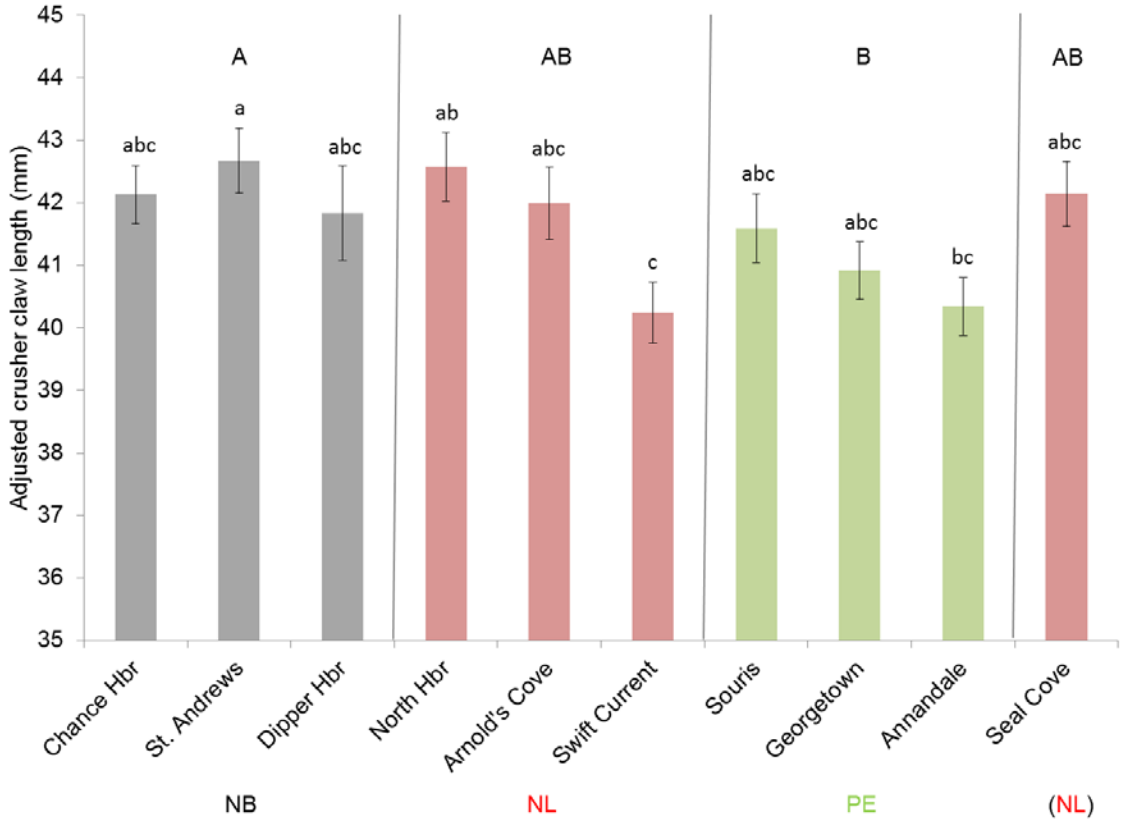


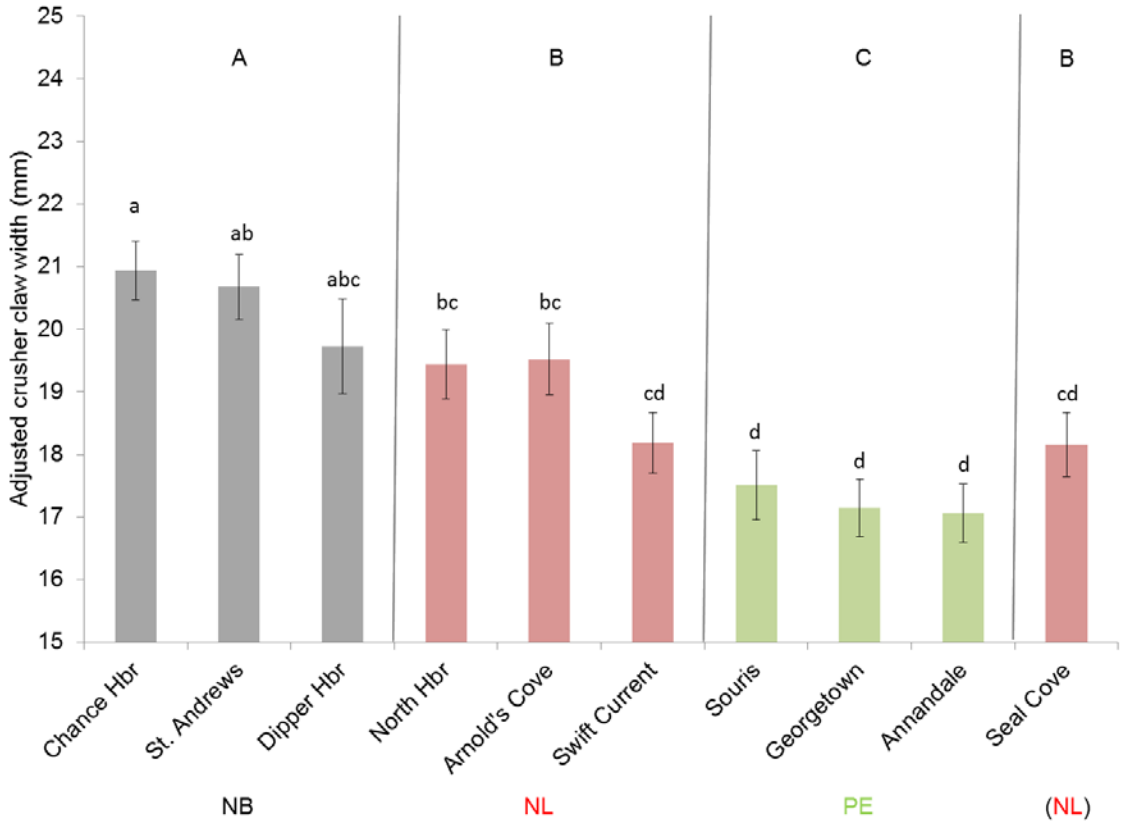
Figure 6-4. Scatterplot and linear regressions of A) pincer claw length versus carapace width, B) pincer claw width versus carapace width, and C) pincer claw dactyl length

versus carapace width for male green crab collected in New Brunswick (●), Newfoundland (■), and Prince Edward Island (◆).



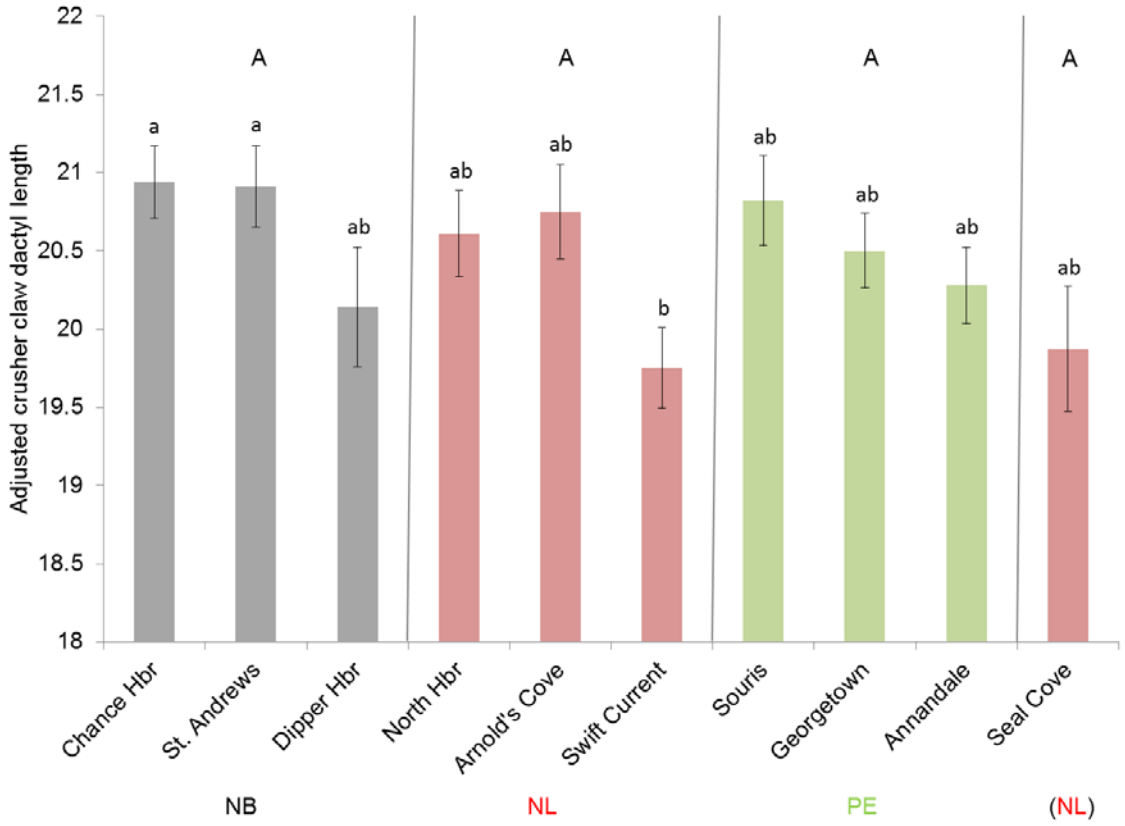
Sampling locations that do not share a letter (lower-case) are significantly different at the α = 0.05 level of significance. Groups sharing a letter (upper-case) are not significantly different at the α = 0.05 level of significance based on contrasts comparing group (province) mean.

Figure 6-5. Least squares means for crusher claw length adjusted to a mean carapace width of 61.3 mm.



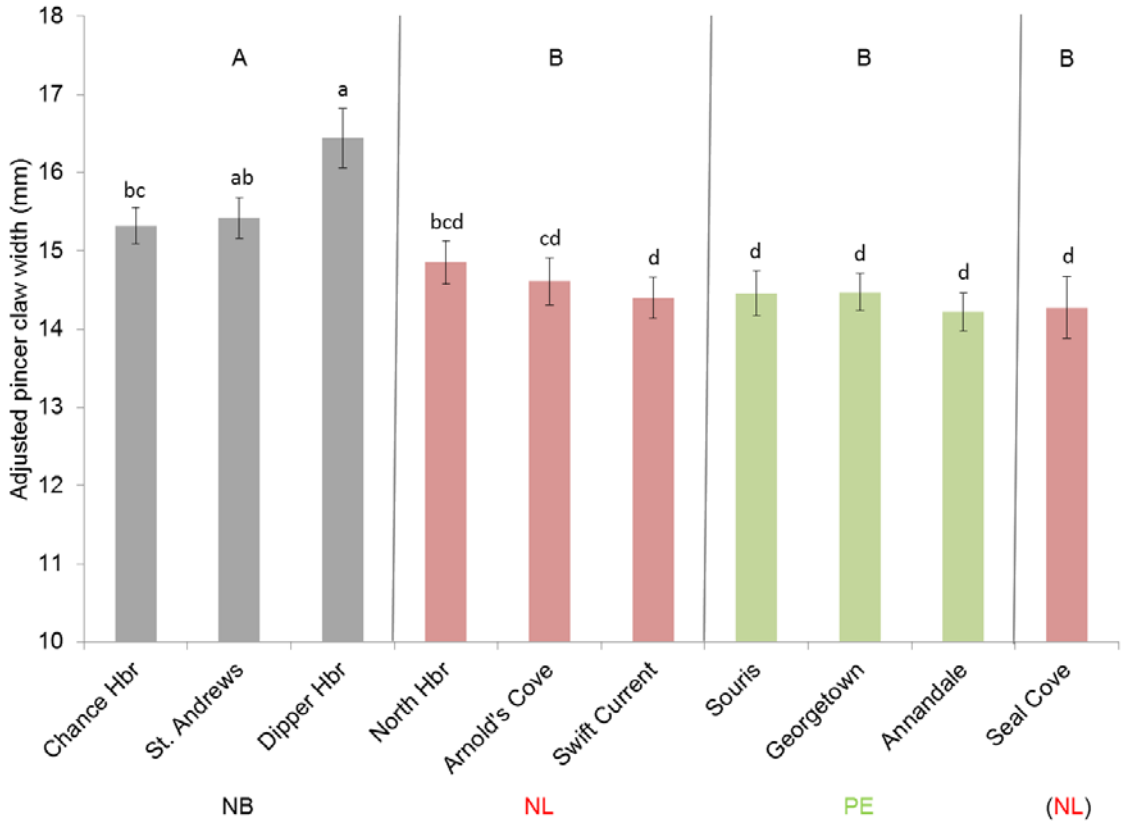
Sampling locations that do not share a letter (lower-case) are significantly different at the $\alpha = 0.05$ level of significance. Groups sharing a letter (upper-case) are not significantly different at the $\alpha = 0.05$ level of significance based on contrasts comparing group (province) means.

Figure 6-6. Least squares means for crusher claw width (mm) adjusted to a mean carapace width of 61.2 mm.



Sampling locations that do not share a letter (lower-case) are significantly different at the $\alpha = 0.05$ level of significance. Groups sharing a letter (upper-case) are not significantly different at the $\alpha = 0.05$ level of significance based on contrasts comparing group (province) means.

Figure 6-7. Least squares means for crusher claw dactyl length (mm) adjusted to a mean carapace width of 61.32 mm.



Sampling locations that do not share a letter (lower-case) are significantly different at the α = 0.05 level of significance. Groups sharing a letter (upper-case) are not significantly different at the α = 0.05 level of significance based on contrasts comparing group (province) means.

Figure 6-8. Least squares means for pincer claw width (mm) adjusted to a mean carapace width of 61.21 mm.

7. Summary and Conclusions

7.1. Thesis Summary

In contrast to most cases of invasive species, the early discovery of the establishment of green crab populations in Newfoundland offered a unique opportunity to follow the initial stages of the invasion and corresponding alteration of the native ecosystem. My research project began shortly after (2009) the initial confirmation of an isolated green crab population in North Harbour in 2007 (Klassen and Locke 2007) and the population has since rapidly expanded throughout Placentia Bay affecting a progressively wider range of organisms and habitats.

The main objectives of my thesis were to assess the impacts of green crab in Placentia Bay on native species through direct field studies and laboratory experiments. I was mainly concerned with evaluating impacts on native species through predation and competition by green crab, and using this information to design experiments to examine those impacts directly and assess differences (if any) of the Placentia Bay population compared to other populations in Atlantic Canada.

The logical first step of my project was to determine which native species were most likely to overlap with green crab diet and habitat use (Chapter 2). Diver transects were conducted to examine both the degree of overlap as well as changes in abundances of green crab, native rock crab, and adult lobster. At one site, the initial survey detected no green crab, allowing comparison of native abundances before and after green crab arrival. After the establishment of green crab at this site, rock crab and lobster distributions both apparently shifted to deeper water. Distributional shifts in native

species often follow the arrival of invasive species and likely reflected avoidance of increased competition or predation levels from this invader (Mooney and Cleland 2001; Bruno et al. 2005; Lockwood et al 2007). However, even if such habitat shifts mitigate agonistic interactions, they often result in tradeoffs such as lower quality food and shelter resources, ultimately reducing overall fitness of native species (Alcock 2009). In my study, these shifts may not necessarily translate into permanent behavioural changes. Indeed, as numbers of green crab potentially decline through intraspecific competition, native species may re-occupy their pre-invasion habitat. Unfortunately, the time and number of generations required to stabilize invasive populations varies among species, and information of this type is unavailable for green crab. In the case of Newfoundland, green crab show no indication of substantial decline in the last six years. The presence of green crab in the native habitat of rock crab and lobster suggests increased competition for resources could have occurred through direct interaction and indirectly through habitat displacement as described above. The major limitation of this study was that species were assessed in water depths of only 1 to 9 m, and sampling methods biased collection towards larger individuals. Small sample size in stomach content analysis could only indicate general dietary overlap and not assess dominant prey items. A better understanding of the impacts of dietary overlap could be achieved by collecting more stomachs at multiple locations, over multiple sampling periods, and at different times of the day. This study could not fully assess the long-term impacts of green crab and further research collected from baseline population data and large-scale field manipulations would aid in understanding overall impacts.

Chapter 3 delved deeper into the impact of green crab populations on American lobster. Specifically, this chapter examined indirect effects of green crab on juvenile lobster. I was able to assess behavioural shifts with respect to both shelter and foraging activity in the presence of green crab using lobster naïve to green crab. In manipulated treatments (i.e. with a caged green crab) contrasted with controls (no green crab), I demonstrated that in the presence of this invader, smaller juvenile lobster spent more time in shelters and less time foraging. Rather than examining the direct effects of one species on another (in terms of predation and competition), I explored a relatively novel approach, namely that adult green crab alone may reduce lobster fitness. Expanding on this idea, further testing might examine chemical rather than physical presence of green crab to evaluate whether general movement of the crab was affecting behaviour. Additionally, future studies could examine behaviour using juvenile lobster that have been exposed to green crab for several generations to determine whether ongoing exposure reduces impacts on foraging. In studies in the Bay of Fundy (Lynch and Rochette 2009), spatial comparisons of lobster and green crab suggest random distribution of individuals of both species, indicating that after a substantially longer invasion period, lobster appear less impacted by the presence of green crab. Whether this pattern will occur in Newfoundland remains uncertain and would be an interesting study direction.

The third study of my thesis (Chapter 4) switched the focus from decapods and potential competitive interactions towards the effects of green crab as predators. Green crab are often described as efficient predators in newly established areas. In Chapter 4, I

examined their impacts on benthic communities in different habitat types (eelgrass, mud, and sand). Benthic communities differed among habitat types as well as between nearby sites with and without green crab. Beyond obvious habitat differences, mud sites exhibited the strongest differences in invaded and control sites, although these differences were also evident in the other two habitats. Unfortunately, the distribution of control and invaded sites limited conclusions regarding green crab impacts because some samples were taken in St. Mary's Bay rather than Placentia Bay, adding potential between-bay differences as an explanatory variable. Recognizing this limitation in the survey data, I designed a caging experiment to try to specifically address the contribution of green crab to the observed spatial habitat differences. The caging study ran for one week and although faunal differences were evident between cages with and without green crab, especially in bivalves and gastropods, my experiment also detected a cage artefact. Increasing the duration of the study would help evaluate the full impacts of green crab on resident fauna but would also likely exacerbate potential artifacts (cf. Quijón & Snelgrove 2005). Furthermore, increased replication would have increased statistical power and thus produced more conclusive results.

Recent studies in Atlantic Canada (Roman 2006), as well as throughout the native and non-native distributional range of green crab, documented different genetic haplotypes in these populations. Based on that information, Chapter 5 examined whether green crab behaviour reflected these genetic differences. In an intraspecific foraging study, green crab behaviour differed depending on the geographic identity of the populations, with green crab from Prince Edward Island (PE) and Newfoundland (NL)

out-competing individuals from New Brunswick (NB) and Nova Scotia (NS). For instance, when foraging with a juvenile lobster, green crab from NL foraged much longer than green crab from NS even though these individuals represent the most genetically similar populations in my study. These results were interpreted to reflect not only a genetic component associated with levels of foraging success, but also differences in invasion time. With higher densities of conspecifics, which typically occur early during invasions, most organisms likely interact for limited resources. Better foragers will be more successful at high population densities, potentially explaining why recent invaders dominated well established and typically less dense populations. Future studies using green crab of known genetic haplotypes would provide better understanding of the role of genetics and behavior, and how these factors translate to invasion success.

Chapter 6 focused on green crab claw sizes (length and width). The main goal of this study was to determine if "weapon" size helps explain differences in dominance reported in Chapter 5. Using crab from similar geographic locations, claw variation apparently depended more on location than on genetic similarity. NB crab, the longest established population in this study, exhibited the largest average crusher claws with smallest claw sizes (PE) in some of the most competitively dominant individuals in Chapter 5. This result likely reflected gradual changes based on food preferences because the more dominant claw reflected the strongest differences. Further direct examination of habitat, food preference and morphology of green crab would allow stronger conclusions regarding the role that claw size plays in invasion success.

7.2. Future Study Directions and Conclusions

Despite the obvious potential damage from the green crab invasion, several mitigating factors must be considered. Initially, during the invader establishment phase, these species often flourish and their population growth appear unconstrained. During this period green crab may impact native prey, competitors, and habitats most severely. However, in some cases green crab populations may self-regulate their numbers after the initial growth phase of the population. Greater competition eventually leads to limiting resources, and once food resources approach depletion green crab tend to move to new areas, or populations plateau or crash.

Green crab invaded northern Nova Scotia (Northumberland Strait) in the late 1990s achieving densities similar to those currently reported for Placentia Bay (>100 crabs/hr in a similar size trap to the standard Fukui currently used in population surveys; Campbell 2001). Over an extended period (approximately 8-10 years) of time, these initial numbers declined and although population numbers have fluctuated they have never reached similar densities (J. Williams pers. comm.). The Nova Scotia invasion was not monitored as carefully as Placentia Bay and the extent of initial impacts were not well documented. However, the decline in Nova Scotia suggests that in some cases populations may eventually be controlled by biological (e.g. predation; cannibalism; Moksnes et al. 1998) or environmental factors (e.g. winter mortality; Welch 1969). Regardless of whether the population stabilizes, impacts on the ecosystem during this initial phase may lead to substantial environmental changes. No research to date has addressed habitat recovery after the decline in green crab population, potentially because

insufficient time has passed for communities to rebound or in some systems the absence of historical data precludes direct comparison.

Sampling and comparison of ecosystem parameters against baseline data offers the most accurate way to determine impacts of an invasion. In most cases such data do not exist. In Newfoundland, natural and anthropogenic pathways increase the likelihood of spread from Placentia Bay to neighboring embayments. Boat traffic moves regularly among bays (e.g. coastal ferries), increasing the chance of spread. Tidal movement, currents, and storm activity transport larvae among the large interconnected bays in Newfoundland, adding further dispersal potential. Increased sampling in these areas where green crab have not yet invaded could provide novel insights into potential impacts and specific mechanisms by which green crab might impact native species in NL.

The data presented in my thesis offer a starting point for the collection of baseline data. Noting that natural variability characterizes benthic communities, well-replicated sampling of a variety of different environments during different time periods and monitoring environmental parameters (e.g. slope, temperature, salinity etc.) offers the most informative approach to understanding change associated with the spread of green crab. My data could complement a more widespread baseline study, however, given the rapid spread in Placentia Bay, studies must sample other embayments at risk for green crab invasion to form a baseline for future comparisons. In addition, more field studies, including large-scale field manipulations to examine various levels of competition, could provide generality regarding conclusions on the specific impacts of green crab. Underwater observations through dive studies and camera deployment, along with

temporal monitoring offer additional approaches in understanding the long-term impacts of this species in its newly expanded range in Newfoundland.

In Placentia Bay, the high densities of green crab raise concern about potential competitive interactions with native species. This thesis demonstrates that green crab forage in similar habitats as both rock crab and lobster, and can consume large numbers of epifauna and infauna across various substrates (e.g. mud, eelgrass, and sand). Besides direct impacts, green crab may reduce fitness of juvenile lobsters and green crab appear more dominant than populations in the other Atlantic Provinces. This thesis points to the need for further research to expand on the knowledge obtained and generate a better understanding of the impacts of green crab on native species in Placentia Bay and Atlantic Canada.

7.3. References

Alcock J (2009) Animal Behavior: An Evolutionary Approach, Ninth Edition. Sinauer Associates.

Bruno JF, Fridley JD, Bromberg KD, Bertness MD (2005) Insights into biotic interactions from studies of species invasions. In: Species Invasions Insights into Ecology, Evolution and Biogeography, ed. DF Sax, JJ Stachowicz, SD Gaines pp. 13-40, Sinauer Associates, Sunderland Mass.

Campbell E (2001) The ecology of the green crab, *Carcinus maenas*, and its invasion in the southern Gulf of St. Lawrence. B.Sc. Hons. Thesis, St. Francis Xavier University, Antigonish, Nova Scotia, Canada.

Grozholz ED, Ruiz GM, Dean CA, Shirley KA, Maron JL, Connors PG (2000) The impacts of a nonindigenous marine predator in a California Bay. Ecology 81: 1206-1224.

Klassen G, Locke A (2007) A biological synopsis of the European green crab, *Carcinus maenas*. Can. Manuscr. Rep. Fish. Aquat. Sci. no. 2818.

Lockwood JL, Hoopes MF, Marchetti MP (2007) Invasion Ecology. Blackwell Publishing, UK.

Lynch BR, Rochette R (2009) Spatial overlap and biotic interactions between sub-adult American lobsters, *Homarus americanus*, and the invasive European green crab *Carcinus maenas*. J. Exp. Mar. Biol. 369: 127-135.

Moksnes PO, Pihl L, van Montfrans J (1998) Predation on postlarvae and juveniles of the shore crab *Carcinus maenas*: importance of shelter, size and cannibalism. Mar. Ecol. Prog. Ser. 166: 211-225.

Mooney HA, Cleland EE (2001) The evolutionary impact of invasive species. Proc. Natl. Acad. Sci. USA 98:5446-5451.

Quijón PA, Snelgrove PV (2005) Polychaete assemblages of a sub-arctic Newfoundland fjord: habitat, distribution, and identification. Polar Biol. 28: 495-505.

Roman J (2006) Diluting the founder effect: cryptic invasions expand a marine invader's home range. Proc. R. Soc. Lond. B. Biol. Sci. 273: 1-7.

Welch WR (1968) Changes in abundance of the green crab, *Carcinus maenas* (L.) in relation to recent temperature changes. Fish. Bull. 67: 337-345.