Reproduction in a Recently Established Population of Green Crab, *Carcinus maenas*, in Placentia Bay and Juvenile Targeted Mitigation to Prevent Mussel Aquaculture as a Vector for Introduction and Spread

BY

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Abstract

Invasive species are organisms that are introduced to a new environment via natural or anthropogenic means and cause damage to the native biota through interactions with the native species and habitat. Aquatic invasive species (AIS) in marine coastal ecosystems can thrive in coastal areas in their native and non-native ranges as they have fewer natural barriers to contain spread versus terrestrial environments. AIS spread via vessel traffic, movement of industrial and recreational equipment and currents, weather events, and other organisms in their fluid environment. Generally, invasive species have the ability to tolerate extreme and restrictive conditions with means to make adjustments to their survival strategies to survive and establish populations in areas outside of their native ranges. In this study, we look at the reproductive strategies of the European green crab (*Carcinus maenas*) in recently invaded cold-tolerant populations in Newfoundland. We estimate size minimums for physiological maturity in males and females, timing for mating behaviors, duration of each stage of egg development and timing of larval release in females. This information has been used to establish minimum size thresholds for pilot mitigation efforts in the area and will continue to help pinpoint the best times of year to target a particular life stage for this region. Comparisons to other non-native green crab populations in Atlantic Canada are made to elucidate some of the strategic changes they have made in these environments. This information can be used in targeting different life stages in efforts to control already established populations in Newfoundland and prevent spread and establishment to new areas. This information is then used to pinpoint a vulnerable (likely to settle in and around mussel seed lines of the aquaculture industry) life stage of juvenile green crab to target via mitigation. This was investigated by exposing juveniles to a series of heated salt water immersion treatments. Experiments confirmed that exposing juvenile green crab to heated salt

water for no longer than 1 minute at 45°C is sufficient to cull the crab while not causing any significant physiological stress to mussel seed. This information and subsequent control measures are valuable to the mussel aquaculture industry, stakeholders and managers for designing plans for future control of this invasive species.

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1.0 Introduction and Overview

Invasive species are those taxa that have been introduced to new regions and exert substantial negative impacts on native biota, economic values, or human health (Lodge et al. 2006). Of the wide range of aquatic invasive pathogens, plants, vertebrates and invertebrates, the subphylum Crustacea make up the largest group of invasive alien species worldwide (Engelkes and Mills 2011). Marine aquatic invasive species have a much more complex invasion history due to the environmental influences coastal habitats provide in comparison to freshwater and terrestrial invaders which have more natural barriers. Simons (2003) suggested that the invasion process itself may be selective across species, whereby those species that would exhibit 'increased vigor' in a new territory are more likely to colonize than those that would exhibit 'reduced vigor'. A successful invader makes use of a combination of plastic traits, such as reproductive strategies and tolerances to variable environmental conditions which in turn influence behaviors and skills that include, but are not limited to foraging and food competition, predator avoidance and migration patterns.

European Green crab (*Carcinus maenas*) has invaded many regions worldwide, and has proven to be well adapted as an invasive species (Cohen et al. 1995). They possess many of the qualities of successful invaders, such as rapid reproduction, omnivory, and wide tolerances of salinity and temperature ranges (Walton et al. 2002). As a widespread invasive species green crab has been studied with respect to many of these tolerances and traits that suggest a potential for extensive ecosystem alterations through predator-prey interactions, competition, disturbance and indirect effects (Cohen et al. 1995). In all areas where they have invaded, their potential to have

significant impacts on fisheries, aquaculture, and the ecosystem has caused concern (Klassen and Locke 2007, DFO 2010/033).

Reproductive strategy is one of the most influential factors for the ability of an invasive species to successfully invade and establish a population in a new environment. Traits of invasive crustaceans include long reproductive period, early sexual maturity, smaller size at maturity, seasonally early reproduction, short generation and embryonic development time and optimal timing. Other influencing factors include high growth rates and fecundity with high number of small sized eggs per brood and the ability to store sperm to use when conditions are optimal for high survival of offspring. Green crab reproduction has been investigated in many studies and there are many established traits common to invasive crustaceans possessed by green crab. Populations of *Carcinus maenas* have two cohorts of spawning females a year in South Africa (Broekhuysen 1937), a primary and secondary female cycle in South West Ireland (Lyons et al. 2012) and have ovigerous females present all year long in Portugal (Baeta et al. 2005). In northeastern North America they experience early and smaller sexual maturity at 34-45 mm carapace width (CW), sizes much smaller than the local rock crab at 60-69 mm (Scarratt and Lowe 1972) as well as seasonally early reproduction in August (Berrill 1982) versus local rock crab which reproduces in late summer and Fall (Scarratt and Lowe 1972). They have high growth rates with juveniles reaching 9.5mm carapace width (CW) before the end of their first Winter in Sweden, Maine and Nova Scotia (Eriksson and Edlund 1977), high number of small sized eggs per brood at 185,000 (Cohen and Carlton 1995) and the ability to store sperm in spermathecae for up to a year (Broekhuysen 1937).

An example of another invasive crustacean's advantageous reproductive strategy is the amphipod *Dikerogammarus villosus.* When compared to native amphipods it has a longer

reproductive period and matures one to two months earlier. With short generation time three generations are present at the same time versus one of the native and has almost double the number of eggs per brood (Hanfling et al. 2011). Storage of sperm internally as spermathecae is also an advantage and is common amongst brachyuran crabs like the blue swimmer crab *Portunus pelagicus* that can store sperm for a year or more (DASIE 2009).

Tolerance to a wide range of environmental factors also allows for invasive species to take advantage of habitats in which native species would normally not thrive. During various stages of the colonization process, colonists must be able to cope with a range of contrasting environmental conditions including water temperature, oxygen levels, anthropogenic disturbances and salinity (Hanfling and Edwards 2011). Green crab can tolerate a wide range of these conditions, an advantage to successful invasions in many different environments.. They can tolerate temperatures between 0 and 35°C (Hidalgo et al. 2005) feed no lower than 3-4°C in the lab (Williams and Naylor 1967) and 0°C in some Newfoundland waters (pers. observ.). They molt above 10°C in the Pacific Northwest (Behrens Yamada 2005) and lower in Northeast Atlantic waters. Green crab are relatively tolerant to hypoxia as well which is maximized at higher salinities (greater than 10‰) (Legeay and Massabuau 2000). Adults can tolerate salinities from 4 to 52‰ (Cohen and Carlton 1995) with behavioral responses at 9-10‰ (McGaw et al. 1999) with a salinity preference between 10-30‰ (Cohen and Carlton 1995). Adults have even been observed in 0‰ in some Newfoundland rivers (MacNeill pers. comm.) including Deer Brook and Bonne Bay (Hooper pers. comm). Green crab larvae are less tolerant to salinity differences with development stunted at 15‰ (Anger et al. 1998) and a preference between 20-35% (Nagaraj 1993). Adult green crab are also uniquely able to survive out of water for at least five days (Darbyson 2006) and up to two weeks (pers. obs.) which also aids in higher

temperature tolerance as crab can take advantage of evaporative cooling when out of water (Ahsanullah and Newell 1977).

High tolerance to salinity extremes was an advantage for *Eriocheir sinensi* (Chinese mitten crab) in their broad distribution and invasion in tributaries of San Francisco Bay. They are found in intertidal sections of streams with abundant vegetation, made possible by their salinity tolerances (Rudnick et al. 2000). *Procambarus clarkii* (red swamp crayfish) were found to make use of boulders for shelter instead of their usual burrows in a temporary river (Aquilnoi et al 2005) not limiting themselves by habitat availability in a new environment.

A direct advantage to having a wide range of environmental tolerances for invasive species is that more habitats are made available with fewer limitations at different life stages. More habitats give the opportunity to migrate and along with migrations come the need to tolerate environmental changes in those new areas. Invasive crustaceans like many other organisms partake in migratory behavior and can extend their range to new areas when they have more extensive movements and migrations (Weis 2015). Green crab exhibits such migratory behavior during different life stages in the intertidal zone. It has long been recognized that they are migrant on a tidal and seasonal basis (Crothers 1967). Crothers (1967) stated that some adult green crab remain permanently hidden on the shore at low tide exposed to desiccation and more extreme air temperatures, while others forage intertidally on the flood tide and retreat with the ebb, and others remain permanently below the low water mark. Hunter and Naylor (1993) later confirmed that there was more migratory behavior in green crab during falling tides than rising and more foraging during rising tides than falling, no difference during light cycles and a size gradient with smaller crab hidden at the low tide and increasing in size with depth and migratory behaviors. Ovigerous female green crab tend to migrate offshore in the winter to optimize

conditions for egg development (Broekenhysen 1937) then move inshore in the summer to take advantage of increased temperatures for final egg development (Wheatly1981). Larval green crab also use vertical migration to avoid strong currents in estuaries from tides and stream currents which could displace them from the estuaries where they develop before moving offshore and then returning to settle (Queiroga et al. 1997). Conversely, *E. sinensis* juveniles were found to undergo extensive movements and take advantage of in the intertidal area during high tide for an influx of new crabs during each tidal cycle (Gilbey et al. 2008). They also migrate into freshwater to develop as juveniles and then return to saltwater to reproduce, which extends the reach of their invasion strength (Dittel and Epifanio 2009) in comparison to a native crab which cannot withstand these salinity fluctuations.

Many invasive crustaceans have generalized diets and use a wide range of foraging techniques (Weis 2015). They are also often opportunistic and can effectively exploit the most abundant food source available in the invaded habitat (Hanfling et al. 2011). The green crab diet is very broad as with other invasive crustaceans and includes a wide range of species including bivalves, polychaetes, nematodes, gastropods, crustaceans and juvenile fish (Cohen and Carlton 1995). Stomach content analysis reveals that in both native and expanded regions, these predators rely most heavily on bivalve species (Crothers 1967, Elner 1981, Baeta et al 2005).When given a choice between clams, mussels and oysters, green crab in PEI preferred soft shelled clams, then mussels and lastly oysters (Pickering and Quijon 2011). In a Newfoundland study they preferred clams, mussels and lastly scallops (Matheson and McKenzie 2014). Similarly the invasive *E. sinensis* is capable of feeding on a wide range of plants, invertebrates, fish eggs and terrestrially derived detritus with gastropods and bivalves the dominant component of its diet (Dittel and

Epifanip 2009). They use a wide range of foraging techniques which move between surface dwelling to sediment dwelling invertebrates (Rudnick and Resh 2005).

Overall invasive species can be more aggressive and dominate over native species (Weis 2015) which may be one of the main drivers of range expansion of an invader (Rossong et al. 2012). The more behaviorally aggressive an invader the more successful they will be in a confrontation with a smaller, weaker less aggressive native for food or habitat. Green crab make use of both of these techniques and can also remain inactive and rely on camouflage to avoid other crab predators which are tactical hunters at smaller size classes (Lohrer and Whitlach 2002). Other morphometric traits are common in invasive crustaceans like shell robustness seen in green crab with thicker heavier shells in comparison to native crabs to avoid shell damage during confrontation (MacDonald et al. 2007). Aggression is most common during competition for food between green crab and other native species; and in different areas exhibit aggressiveness in competition for both food and habitat. Rossong et al. (2006) found that between juvenile American lobster (*Homarus americanus*) and green crab, the crab were always the first to the food and spent more time with the food than the lobster and also captured and consumed juvenile lobsters in some cases. In Newfoundland waters the native rock crab (*Cancer irroratus*) competes with the green crab for food and habitat. It's been suggested that green crab have an greater impact on smaller rock crab (Matheson and Gagnon 2012b) and during competition green crab reduce foraging in smaller rock crab in higher water temperatures (Matheson and Gagnon 2012a). Rossong et al. (2012) found that adult green crab from Newfoundland when compared to other Atlantic populations was dominant in foraging experiments and was more aggressive.

One of the direct threats a green crab invasion brings to Newfoundland is their preference for bivalves, specifically to mussel aquaculture, particularly since blue mussels are one of their

preferred food. Once green crab inhabit a farm site, the product is at risk of predation during growout as well as the natural beds nearby that provide mussel seed (spat) for farm sites. Newfoundland mussel production is the second largest regional production area in North America, and recently the first ever to receive organic certification. Most of the production currently occurs on the North East Coast (Green Bay and Notre Dame Bay) which is free of green crab. Some smaller farms in Placentia Bay are within the newly established green crab population and are identified as potential seed supply sites for the North East Coast. The transport of seed from Placentia Bay to fill shortages at the higher producing Northern farms has been identified as an additional potential vector for introduction of green crab, especially in the larval and juvenile life stages. Elsewhere in Canada there are many targeted mitigation techniques to prevent spread and proliferation of invasive tunicates like the club (*Styela clava*) and vase tunicate (*Ciona intestinalis*) threatening shellfish aquaculture (not present in Newfoundland waters) including washing with pressurized sea water (Arens et al. 2011) and air drying (Coutts and Forrest 2007) but there are no targeted mitigation methods known for green crab early life stages, anywhere.

Berrill (1982) predicted that because of the periodic occurrence of colder than average temperatures green crab would be restricted to the Gulf of Maine. Green crab were first confirmed in Atlantic Canada in the Bay of Fundy in 1951 (Leim 1951), the Atlantic shore of Nova Scotia in 1954 (MacPhail and Lord 1954), and in communities further north on the coast of Nova Scotia between 1954 and 1966 (Audet et al. 2003), in PEI in 1996 and 2001, then the Magdalen Islands in the Gulf of St. Lawrence in 2004 (Klassen and Locke 2007). Roman (2006) concluded that the population in Northeastern Canada was not an expanded population spread further than Berrill's (1982) temperature limitations but a separate cold tolerant population from

a source population from their Northern Europe native range. Blakeslee et al. (2010) confirmed this by identifying that the Scotian shelf origin was a mixture of genotypes from separate introductions from the Northeast Atlantic introduced in the early 1800's and late 1900's (Norway and Iceland). This is further supported by Ingolfsson (1992) who stated that the rocky shore fauna of Northern Norway, Iceland and the Canadian Maritimes are closely similar with a gradient of similar species decreasing from Northern Norway through Iceland and then sharply to the Maritimes.

It is evident that non indigenous species are a significant stressor and force of change in marine ecosystems (Ruiz et al. 1999). Based on the behaviors and habitat preferences of green crab the damage they cause to an invaded ecosystem is extensive for both ecosystem health and industries based on them. Eelgrass beds provide many of the green crab's preferred prey items, resulting in destruction of this highly productive habitat with predation and burrowing behaviors disrupting the complex lower levels of the intertidal food web. Gotceitas et al. (1996) found that juvenile cod settle and make use of eelgrass beds as habitat along with many other species. Previously mentioned Newfoundland green crab are especially aggressive as a new population and win competitions with lobster and rock crab for food as well as preying on juvenile lobster and smaller rock crab in the lab (Rossong et al. 2012, Matheson and Gagnon 2012b), with observations of an inverse relationship between green and rock crab catches during Newfoundland surveys (McKenzie, pers. comm.). Suggesting that green crab are affecting both native rock crab and lobster populations, and a multitude of other species that use eelgrass beds as habitat for early life stage development and food sources.

Green crab are predators of a broad range of organisms; including most notoriously bivalves (Miron et al. 2005) as previously mentioned and seen in lab experiments (Pickering and Quijon

2011; Matheson and McKenzie 2014) and stomach content analysis (Crothers 1967, Elner 1981, Baeta et al. 2005). Due to this preference green crab also have a significant impact on commercial bivalve fisheries along the northeastern U.S. (Ruiz et al. 1997) and more recently Canada (Floyd and Williams 2004). Bringing urgency for prevention and control of green crab in the Newfoundland bivalve aquaculture industry.

My thesis aims to first understand the life history strategies the new cold tolerant population of green crab has made to establish itself in Placentia Bay, Newfoundland. Secondly to develop strategies and methods for juvenile targeted mitigation to prevent mussel aquaculture as a vector for introduction and spread of this invasive species.

Co Authorship statement

All manuscripts were co-authored with Cynthia McKenzie and Cyr Couturier. In all instances I was the primary contributor to project design, proposal, field and lab execution, analysis of data and preparation of manuscripts.

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Chapter 2: Reproductive strategies of a new population of green crab, *Carcinus maenas,* in Newfoundland

Abstract

Green crab, *Carcinus maenas,* was discovered in North Harbour, Placentia Bay, Newfoundland in 2007. Initially it was predicted that due to the periodic occurrence of colder than average sea surface temperatures (10 °C) green crab would be restricted to the Gulf of Maine. This has not been the case with established populations northeast of the Gulf of Maine including the Maritime Provinces and now Newfoundland where average temperatures are less than 10°C. Reproductive biology and strategies of this Newfoundland cold tolerant population were investigated along with other longer established cold tolerant populations in Atlantic Canada. Placentia Bay reproductive females are smaller, spend a short time in the ovigerous state and release larvae earlier and in colder temperatures, and only do so once annually. Histological and gonadosomatic (GSI) analyses indicated that male green crab are mature at carapace width (CW) 32 mm and females at 37 mm, smaller than other cold water populations in Atlantic Canada. This information is important for government and industry in designing mitigation and prevention plans to either target vulnerable life stages or to avoid transferring life stages when they are most active and better control this invasion or to prevent others.

2.0 Introduction

European green crab, *Carcinus maenas*, is a marine decapod crustacean of the Portunidae family native to the Atlantic coast of Europe, ranging from Norway and the British Isles south to Mauritania (Behrens Yamada 2001).

It has been identified as an invasive species and has successfully invaded and populated areas off the coasts of Australia and Tasmania, Asia, South Africa and the West and East coasts of North and South America (Cohen and Carlton 1995). They pose a very high risk to the ecosystem balance in the areas they invade. Green crab arrived in North America on the East coast of the US by 1817 (Cohen and Carlton 1995) and were first reported in Canada in 1951 in the Bay of Fundy (Leim 1951). Prior to 2007 the most northerly reproducing population was in Nova Scotia in the Bras d'Or Lakes (Cameron and Metaxas 2005).

Previous research in the 1980's hypothesized that low winter temperatures, (below 10°C) would hypothetically limit green crab reproduction and as a result invasions north of the Gulf of Maine. Blakeslee et al. (2010) and Ingolfsson (1992) indicated that a different cold tolerant population of green crab in the Maritimes had origins in Norway and Iceland. These are genetic lineages at the Northern end of the green crab's native range in Europe, which are now persisting in areas that were once thought to be too cold for the original southern invasions front (Roman, 2006). A population discovered in Placentia Bay Newfoundland in 2007 is part of that spread of a secondary introduction of a more cold tolerant population in the Maritimes in the late 1980's (Blakeslee et al 2010).

High propagule pressure through genetic diversity fueled by constant introductions is an asset to invasive species increasing population establishment as better tolerances are reached once one is

deemed unsuitable for survival in a new environment via natural selection. Atlantic Canadian populations of green crab have higher genetic diversity than southern populations, indicating that multiple introductions have occurred in the Maritimes since the 1980's (Roman, 2006). Genetic analysis suggests that the invasion could have been derived from as few as thirty founding individuals (Blakeslee et al. 2010), but with little to no limitations on vessel traffic back and forth to the Scotian Shelf and other areas there are assumed repeat introductions therefore adding to the successful colonization of Newfoundland.

As described by (Broekhuysen, 1937) green crab copulation takes place when the female has just molted and is still soft and the male has a hard shell. Prior to molting females emit a molting pheromone which attracts a male (Broekhuysen, 1937). Males choose a female and engage in amplexus or pre copulatory embrace in which the male carries the female under his abdomen until she molts and they can copulate. The male deposits spermatophores into copulatory pouches and are viable for up to 12 months. When conditions are right the female fertilizes her eggs once her ovaries have developed into a bright orange colour filling the majority of her body cavity and then extrudes them. The eggs are held outside the body on the female's abdominal flap attached to specialized pleopods and this is where they develop over a period of a few months depending on conditions. Once the eggs are developed enough zoea stage 1 larvae are released into the water column for their planktonic larval stages and the female prepares to molt and mate again.

Grosholz and Ruiz (2003) stated that species introduced into a new region frequently undergo changes in size and shape relative to their native range, which can strongly influence the magnitude of the invader. With respect to green crab there was a significant increase in maximum carapace width (CW) in introduced regions as well as a significant difference between

maximum CW in introduced populations with decreasing latitude. Mature green crab have been known to have size as an indicator of reproductive success. Larger older females producing larger clutches of eggs and extruding at optimal conditions for maximum larval survival (Audet et al. 2008). Smaller, younger females with smaller clutches may not extrude at the most optimal of conditions later in the spawning season and in a smaller wave of less successful larvae. These strategies maximize offspring survival and population stability. Such patterns are vulnerable to environmental variables such as changes in water temperature, day length and food availability (Crothers 1967). In some areas of the world, females can produce two egg broods in 12 months and are not restricted by drastic changes in water temperature (Broekhuysen 1937). Some regions have ovigerous females present year round (Baeta et al. 2005). Continuous and restricted breeding are both commonly observed in brachyuran species, in temperate regions tending to use restricted breeding seasons when suitable environmental conditions prevail (Pinheiro & Fransozo, 1998). Another suggestion for retention is proposed by Byers and Pringle (2006) who suggest that spawning over several seasons, larvae with shorter pelagic periods and prodigious larval production improve retention for coastal species.

Variations in reproductive strategies of green crab are seen in long established populations but may also be observed among newly invaded coastlines (Audet et al. 2008). Invasions in European waters have been established long before Eastern Canada, and since 1985 there has been a steady increase in the catch of *C. maenas* in European fisheries (Svane, 1997). Lyons et al. (2012) looked at the frequency and parameters of green crab reproductive biology and size at maturity for a long established population in Irish waters using morphometric and histological techniques and identified some of these reproductive strategies. This study aims to do the same for a much younger Eastern Canadian invasion in the Newfoundland population looking at the

changes in reproductive strategies this population has undergone to be successful in Newfoundland waters. It is hypothesized that there have been changes to population structure, reproductive strategies and size at maturity in contrast to other cold tolerant Eastern Canadian populations. Clarifying these reproductive trends can be used by the fishing and aquaculture industries and recreational coastal users to decrease the movement of vessels and equipment that can relocate life stages required to establish a new invasion in another location. This will also help pinpoint the most effective times to implement strategic and targeted applied management and mitigation.

2.1 Materials and Methods

2.1.1 Study Site and Sampling Methods

Green crab (10 mm-79 mm carapace width) were collected at a coastal site in Goose Cove, North Harbour, Newfoundland Canada (Fig. 2.1). At each sampling a Fukui trap baited with cod filleting discards, frozen herring, or canned tuna was soaked overnight and for no more than 24 hours. Newly settled juveniles can range between 1 and 6 mm CW in Bras d'Or Lakes, Nova Scotia (Cameron and Metaxas 2005) and in Sweden young of the year reach 9.5mm CW by the end of their first winter and 25 mm by the end of their second winter (Eriksson and Edlund 1977). Based on these other population comparisons, CW of the smallest individuals from Fukui pot catches in this study with CW 17 mm and up were considered adults and less than 17 mm considered juveniles. Juveniles were caught by hand in the intertidal zone at low tide. All Fukui pot bycatch was released and green crab placed in a cooler with *Fucus* for live transport to the

laboratory at the Marine Institute. Crabs were collected from September 2008-2011 monthly when possible and bimonthly and trimonthly April to September 2012, from September 2008 through to September 2012 totaling 22 samplings, Tables 2.1, 2.2. Ovigerous female catch data was obtained from the FFAW/DFO experimental mitigation fisheries projects from 2008, 2009 and project work by the Center for Aquaculture and Seafood Development Marine Institute Memorial University (FFAW unpublished data, CASD unpublished data). Ovigerous females were caught as a small percentage (<5% of catches) of heavy fishing pressure during these experimental fisheries, with the majority of them captured after night soaks. Temperature data was obtained from DFO temperature data loggers Vemco miniloggers I and II moored on bottom in the study site as well as Sea Surface Temperature from the ocean observation system SmartBay which collects meteorological and oceanographic data via buoys in Placentia Bay under the Smart Atlantic Alliance.

Fig. 2.1: Map of North Harbour, Placentia Bay, Goose Cove was the primary adult green crab collection site in Area 2 with juvenile collection occurring in Areas 1 and 2.

Table 2.1: Adult green crab (>17mm) sampling dates caught with Fukui pots, total n for GSI, histological analysis, sex ratio and season designation. Histological n is based on number of samples that could be fully analyzed.

Date	**GSI n = 671/453**	**Histology n = 136/129**	**Season**
	male/female	**male/female**	
Sept 28 2008	59/63		Summer
Oct 11 2008	41/24		Fall
Oct 19 2008	16/2		Fall
Nov 2 2008	37/33		Fall
Nov11 2008	83/47		Fall
Nov 22 2008	81/41		Fall
May 23 2009	15/11		Spring
July 14 2009	25/3		Summer
Sept 14 2010	38/22	9/5	Summer
Aug 2 2011	49/13	7/6	Summer
Oct 10 2011	6/7	0/0	Fall
Oct 25 2011	69/35	0/0	Fall
April 8 2012	17/13	16/10	Spring
June 8 2012	15/15	15/15	Summer
July 14 2012	12/5	12/5	Summer
July 22 2012	18/25	18/24	Summer
July 27 2012	15/15	15/14	Summer
Aug5 2012	15/15	15/15	Summer
Aug16 2012	15/17	15/17	Summer
Aug 23 2012	15/15	15/15	Summer
Sept 16 2012	15/15	15/14	Summer
Sept 28 2012	15/17	15/14	Summer
TOTAL	1124	265	

Table 2.2: Juvenile green crab (size <17mm) sampling dates, locations NH = North Harbour SH = Southern Harbour with sediment, air temperature and water salinity.

Date	Site	Air Temp °C	Sediment Temp °C	Water Salinity ppt	N
Aug 10 2012	NH	19	19	18	120
Aug 14 2012	NH	25	22	18	120
Aug 16 2012	NH	19	23	30	80
Aug 19 2012	SH	18	21	32	100
Aug 27 2012	SH	18	22	20	100
Sept 3 2012	SH	19	20	30	100
Oct 15 2012	SH	10	10	31	100
				TOTAL	720

2.1.2 Biological Sampling

In the laboratory a minimum of 15 male and 15 female crabs were analyzed from each collection date, Table 1.1. Carapace width (CW, mm), total body mass (BM, g) and gonad weight (GW, g) were measured to the nearest 0.1 unit, and abnormalities (missing appendages or carapace damage) and carapace colour (red or green) were recorded. The animals were anaesthetized in a freezer (-20˚C) for 5 minutes, carapace removed, gonads excised, weighed (g) for Gonadosomatic Index (GSI) calculation (Audet et al. 2008) then fixed in Davidsons's solution for 24-48 hours for later histological analysis. Female gonads were classified according to a stage maturity scale with the naked eye and notes made on presence or absence of spermathecae, Table 2.3.

Histological techniques were based on Lyons et al. (2012) and samples taken from 2010 -2012 sampling periods. The tissue was removed from fixative and placed in histology cassettes and dehydrated using a Leica TP1020-Automatic Tissue Processor. Once dehydrated, tissues were embedded in paraffin wax and stored until sectioning. Blocks were sectioned and mounted on slides treated with poly-L-Lysine adhesive using procedures from Howard et al. (2004). Blocks were sectioned into 6 µm thick ribbons and mounted on the treated slides. The slides were then stained with haematoxylin and eosin using a Leica Auto Stainer XL and cover slipped. Slides were examined using compound microscopy under 400 x magnification and assigned a histological stage, Tables 2.3, 2.4.

Table 2.3: Stage maturity scale for female gonads adapted from Lyons et al. (2012).

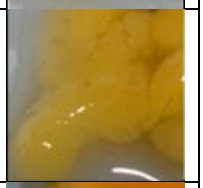
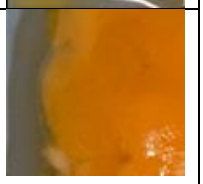
Ovarian Stage	**Maturity**	**Morphometrical**	**Histological**	**Gonad Colour**
0 Unable to Locate	Immature	No gonad tissue could be visually identified	N/A	N/A
1Early Development	Immature	Thin translucent threadlike ovary, hard to distinguish from hepatopancreas	Loosely packed oogonia and primary oocytes, follicle cells are round	
2 Late Development	Immature	Ovary bigger and has more colouration	Oogonia are reduced in number, oocytes increase in number and size and follicles begin to flatten	
3 Mature/Ripe	Mature	Ovary much larger and bright orange	Oogonia absent, larger in size and number and develop a yolky appearance within the cytoplasm, follicles more compressed	
4 Spawning/Spent	Mature	Ovary filling body cavity and darker orange/red colour	Yolky cytoplasm of oocytes become globular and follicle cells begin to round	
5 Spent/Reabsorbing	Mature	Thin translucent threadlike ovary, hard to distinguish from hepatopancreas	Disintegrating mature oocytes with smaller diameter, oogonia and primary oocytes reappear, follicles round	

Table 2.4: Stage maturity scale for male gonads adapted from Lyons et al. (2012). Note there is no morphological description for male gonads.

Testicular Stage	Maturity	Histological Description
0 Unable to locate	Immature	No tissue can be visually identified
1 Developing	Immature	Spermatogonia, spermatocytes and spermatozoa present
2 Mature	Mature	Spermatozoa present in large numbers
3 Spawning/Spent	Mature	Few remaining spermatozoa

2.1.3 Data Analysis

Data was normalized by removing outliers after assessment using Shapiro-Wilk test ($p>0.05$). Population dynamics (n=1124) were evaluated using the variables carapace width (CW) and body mass (BM) for both male and female crabs, and timing of the minimum and maximum of catches which were tested for significance with a Kruskal-Wallis test to determine the season in which the largest and heaviest male and female crab were caught. Analysis of variance ($p<0.01$) was used to compare CW and BM between sexes. A two way analysis of variance ($p<0.01$) was used to evaluate population dynamics the relationships between sex and season with respect to carapace width (CW), body mass (BM), gonad weight (GW) and gonadosomatic index (GSI). The year was separated into four seasons, summer (June to September), fall (October to December), winter (January to March) and spring (April-May). (No Winter data was collected for this work). Correlations were also evaluated for carapace colour and the above variables for a secondary assessment of the relationship. GSI was first calculated using data from all sampling dates between 2008 and 2012 (n=1124) and then further analyzed using only 2012 sampling

dates (n=304) as sampling was more complete in that year. Gonadosomatic Index (GSI) was calculated using Equation 1.

Equation 1: $GSI = \left(\frac{Wet\ Gonad\ weight\ (g)}{CW(cm)}\right) x\ 100$

Analysis of variance ($p<0.01$) was used to investigate if there was a significant difference between GSI of 2012 males (n=152) and females (n=152) and significant difference between seasonal and monthly GSI levels for males and females. Carapace color or new and old shell for males and females was evaluated using data recorded from field samples (n=1124) and combined with multiyear data obtained by DFO St. John's (n=12,582) for animals collected in Goose Cove, North Harbour (McKenzie, unpublished data). This data was used to calculate percent catch red for male and female. All other carapace colour analysis for male and female percentage of catch with red carapace were compared and significant differences for CW, BM, GW and GSI between male and female, red and green grab was analyzed using analysis of variance ($p<0.001$) (n=1124). Correlation was also investigated between percent of red crab catch for male and female with respect to CW, BM, GW and GSI (n=1124) also to further assess the relationship.

CW of ovigerous females was compared to the non ovigerous female CW average using a Friedman test and also to compare the trend in mature gonad stages and CW in female green crab.

A logistic regression model was attempted using histology results for size at maturity to calculate the CW at which 50% of the population was mature. The knife edge relationship between immature and mature crabs was so prominent the standard error and predictions of the model were zero therefore could not explain size at maturity any further than histological results. Data were analyzed using IBM SPSS Statistics22.

2.2 Results

2.2.1 Population Dynamics

Of the green crabs captured in this study (n=1124) 60% were male and 40% female with a male bias (Table 2.5). The mean carapace width CW for males was 53.0 mm, SD=14.0 with a range of 10 – 79 mm. The mean CW for females was 46.7 mm, SD=10.2 with a range of 11-72 mm. The mean BM for males was 41.65 g, SD=27.39 and ranged from 0.34-125.94 g. The mean BM for females was 25.15 g with a range of 0.45-95.87 g. The mean GW for males was 0.30 g, SD=0.28 with a range of 0.01-1.29g. The mean GW for females was 0.80, SD=1.06 with a range of 0.01-6.18g.

Table 2.5: Green crab catch and GSI data descriptive statistics 2008-2012.

	Male	**Female**
N	671	453
% Catch	60	40
CW Range (mm)	10-79	11-72
CW Mean (mm), SD	52.99, 14.05	46.85, 10.24
BM Range (g)	0.34-125.94	0.45-95.87
BM Mean (g), SD	41.65, 27.39	25.15, 12.98
GW Range (g)	0.01-1.29	0.01-6.18
GW Mean (g), SD	0.30, 0.28	0.80, 1.06
GSI (%) Range	2.17×10^{-4} - 1.89	4.76×10^{-3} - 10.84
GSI (%) Mean, SD	0.27, 0.20	1.55, 1.96

Males were significantly larger CW (mean=52.99, SD=14.05) than females CW (mean=46.85, SD=10.24) $F_{(1, 1123)}$=63.59, p<0.001 and significantly heavier BM (mean=41.46, SD=27.39) than females BM (mean=25.76, SD=13.63) $F_{(1,1123)}$=142.81, p<0.001.

Catch ratios by season (summer, fall and spring) were all more than half male with highest ratio of 1.76 males per female in the fall n=522 followed by 1.33 males per female in the spring n=56 and the lowest ratio of 1.29 males per female in the summer n=543.

The largest male crab were captured in September 2012 and largest female captured in July 2009. There was a significant seasonal difference in male CW and BM with the largest and heaviest captured in the summer (Kruskal-Wallis $\chi^{2\,=}$ 10.46 p=0.006, Kruskal-Wallis $\chi^{2\,=}$ 9.147 p=0.010). There was no seasonal difference in female CW or BM (Kruskal-Wallis $\chi^{2\,=}$ 2.808 p=0.246, Kruskal-Wallis $\chi^{2\,=}$ 3.045 p=0.218).

Season had a significant main effect on CW ($F_{(2, 1118)}$=35.86, p<0.001) with a moderate partial eta squared of 0.060. Post-hoc comparisons using the Tukey HSD test indicated that crab caught in the summer (mean=52.32, SD=10.12) were significantly larger than crab caught in the fall (mean=47.24, SD=15.02), were significantly smaller than crab caught in the spring (mean=53.63, SD=10.34), with no significant size difference in crab caught in the summer and spring. Sex also had a significant effect on CW ($F_{(1,1118)}$=36.89, p<0.001) with a small partial eta square of 0.032 with males significantly larger than females. The interaction variable did not have a significant effect on CW ($F_{(2,1118)}$=1.60, p=0.202)

Male crab caught in the summer (mean=56.70, SD=10.99) and Spring (mean=57.56, SD=10.93) were significantly larger than those caught in the fall (mean=49.13, SD=15.70, $F_{(2, 670)}$=26.84, p<0.001), Fig 2.2A). Female crab caught in the summer (mean=49.02, SD=6.81) were

significantly larger than those caught in the fall (mean=43.91, SD=13.14, $F_{(2,452)}$=14.02 , p<0.001), and those caught in the spring (mean=48.38, SD=6.66) were not significantly different from the summer or fall, Fig 2.2(D).

Season had a significant main effect on BM ($F_{(2,1118)}$=16.47, p<0.001) with a small partial eta squared of 0.029. Post-hoc comparisons using the Tukey HSD test indicated that crab caught in the summer (mean=31.28, SD=23.25) were heavier than crab caught in the fall (mean=31.06, SD=24.64) with no significant body mass difference in crab caught in the spring. Sex also had a significant main effect on BM ($F_{(1,1118)}$=69.89, p<0.001) with a moderate partial eta square of 0.059 with males significantly heavier than females. The interaction variable did not have a significant effect on BM ($F_{(2,1118)}$=4.35, p=0.013).

Male crab caught in the fall (mean=35.68, SD=27.41) were significantly lighter by body mass than those caught in the spring (mean=49.00, SD=23.73) and summer (mean=47.37, SD=26.36, $F_{(2,670)}$ =16.45, p<0.001). Fig 2.2(B). Female crab caught in the summer (mean=26.70, SD=10.27) were significantly heavier by body mass than those caught in the fall (mean=22.92, SD=15.82,$F_{(2,452)}$=4.81 , p=0.009), and those caught in the spring (mean=27.04, SD=9.99) were not significantly different from the summer or fall, Fig 2.2(E).

Season had a significant main effect on GW ($F_{(2,1117)}$=6.44, p=0.002) with a small partial eta squared of 0.011. Post-hoc comparisons using Tukey HSD test indicated that crab caught in the fall (mean= 0.42, SD=0.64) have lighter gonads than crab caught in the spring (mean=0.76, SD=0.96) with no significant difference in gonad weight for crab caught in the summer. Sex also had a significant main effect on GW ($F_{(1,1117)}$=93.34, p<0.001) with a moderate partial eta squared of 0.077 with females having significantly heavier gonads than males. The interaction

variable also had a significant effect on GW ($F_{(2,1117)}$=6.87, p=0.001) with a small partial eta squared of 0.012.

Male crab caught in the summer (mean=0.38, SD=0.30) had significantly heavier gonads than those caught in the fall (mean=0.24, SD=0.25, $F_{(2,670)}$ =22.01, p<0.001), and those caught in the spring (mean=0.29, SD=0.18) were not significantly different from the summer or fall. Suggesting that males are prepared to spawn during most of the year (of those seasons sampled), Fig 2.2(C). Female crab had no significant difference in gonad weight between spring, summer and fall, Fig 2.2(F).

Season had a significant main effect on GSI $F_{(2,1118)}$=8.13, p<0.001 with a small partial eta squared of 0.014. Post-hoc comparisons using Tukey HSD test indicated that crab caught in the fall (mean=0.78, SD=1.18) have smaller GSI than crab caught in the spring (mean=1.48, SD=1.93), and GSI for crab caught in the summer are not significantly different from the other seasons. Suggesting that there is some seasonality to GSI but a short lived on as the majority of the year there is no significant difference in GSI. Sex also had a significant main effect on GSI ($F_{(1,1118)}$=130.57, p<0.001) with a large partial eta squared of 0.105 with females having significantly higher GSI than males. The interaction variable also had a significant effect on GSI ($F_{(2,1118)}$=8.62, p<0.001) with a small partial eta squared of 0.015.

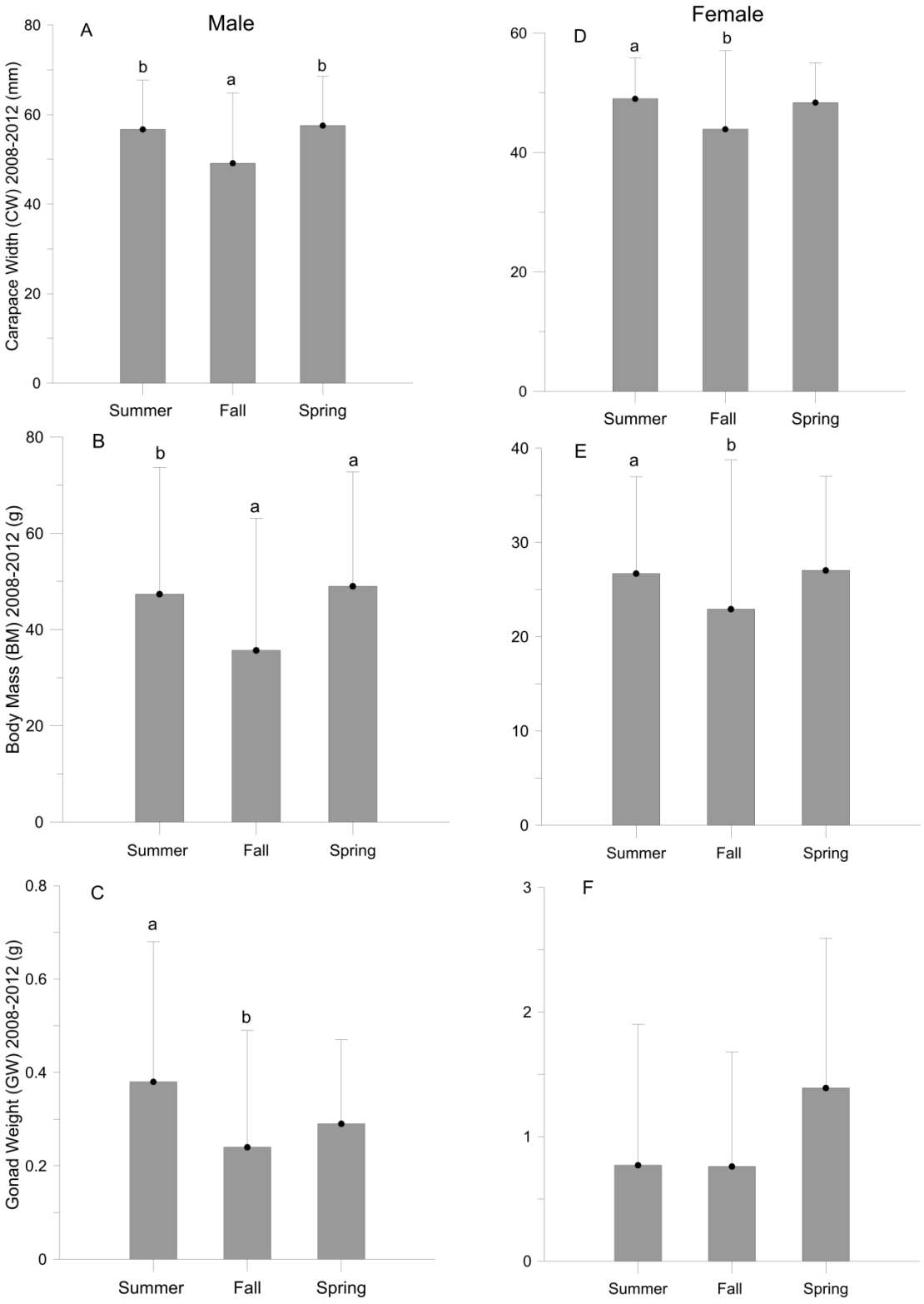


Fig. 2.2: Average (A) male seasonal CW, (B) male seasonal BM, (C) male seasonal GW, (D) females seasonal CW, (E) female seasonal BM, (F) females seasonal GW for crab 2008-2012.

Carapace colour or new and old shell for males and females was recorded from field samples (n=1124) and combined with data obtained by DFO St. John's (n=12,582) from 2008-2012. When all years of catch data were combined there was a female bias with respect to red carapace colour with 26 percent of female crab caught having a red carapace versus 12.4 percent of males. Of the months sampled (April-November) there were differences in ratios for male and female with red carapaces, the highest percent of red males was caught in May and lowest with zero catches in April and June. The highest percent of red females was caught in April and August with the lowest in May and November, Figs 2.3, 2.4. In the 2012 sampling year the highest percentage of red males was caught in mid-September and highest percentage of red females was caught in early August.

Data from 2008-2012, n=1124 individual green crab there was a significantly higher percent catch of red females (mean=26.6, SD=20.2) than males (mean= 12.7, SD=16, $t_{(42)}$=-2.52, p=0.016).

With respect to size red males n=93 (mean=51.24, SD=13.94) were larger than green males n=578 (mean=, SD=, $F_{(1, 670)}$=71.22, p<0.001). Red females n=120 (mean=46.24, SD= 12.09) were not significantly larger or smaller than green females n=333 (mean=47.07, SD=9.49, $F_{(1, 452)}$=0.575, p=0.449).

With respect to total bodyweight red males (mean=65.87, SD=27.44) were significantly heavier than green males (mean=37.76, SD=25.34), $F_{(1, 670)}$=95.70, p<0.001). Red females (mean=25.14, SD=13.12) did not differ from green females (mean=25.15, SD=12.95, $F_{(1, 452)}$=0.000, p=0.994).

With respect to gonad weight red males (mean=0.51, SD=0.27) had heavier gonads than green males (mean=0.27, SD=0.27,$F_{(1, 670)}$=62.72, p=.000). Red females (mean=1.08, SD=1.14) had significantly heavier gonads than green females (mean=0.70, SD=1.01, $F_{(1, 451)}$=11.71, p=0.001).

With respect to reproductive development GSI levels, red males (mean=0.76, SD=0.36) had a higher GSI than green males (mean=0.43, SD=0.39, $F_{(1, 670)}$=56.62, p<0.001). Red females (mean=1.58, SD=1.69) were not significantly different from green females (mean=1.54, SD=2.06, $F_{(1, 452)}$=0.49, p=0.826, Table 2.6.

Table 2.6: Green crab carapace colour catch data, CW and GSI data summary 2008-2012.

	CW range	**Avg CW**	**GSI range**	**Avg GSI**
Red Male n=93	41.5-77.0	43.1	0.1-1.0	0.5
Green Male n=578	32.1-67.7	53.9	0.1-1.1	0.5
Total Male n=671		53.0		0.5
Red Female n=120	.0-67.0	45.4	0.2-6.9	1.7
Green Female n=333	33.6-56.7	47.8	0.3-6.0	1.6
Total Female n=453		46.7		1.9

Carapace colour (green or red), not including sex was evaluated to see if there was any correlation with CW, BM, GSI, and GW. All correlations were positive for red carapace with the CW, BM, GSI and GW all being greater for red carapace crabs with the largest correlation for gonad weight, Table 2.7.

Table 2.7: Correlations between carapace colour (green and red) and variables of interest for all 1124 green crab, at $p < 0.001$.

Variable	Correlation Coefficient r_s
CW	.250
BM	.231
GSI	.292
GW	.302

Ovigerous female catch data were also collected and compiled with previously mentioned sources. Females carrying clutches of varying developmental stages were caught between July 23 and August 16 2008, 2009 and 2010 during an experimental mitigation fishery in North Harbour. With most ovigerous females caught after night soaks. Of these catches the average CW of ovigerous females was 43.0 mm, SD=4.3 with a CW range of 37-55.3 mm. Catches of ovigerous females were 0.11, 0.23 and 0.36 percent for 2008, 2009 and 2010. Ovigerous females average CW was significantly smaller than overall female CW average (Friedman Test CW $\chi^2 = 24.00$, $p < 0.001$).

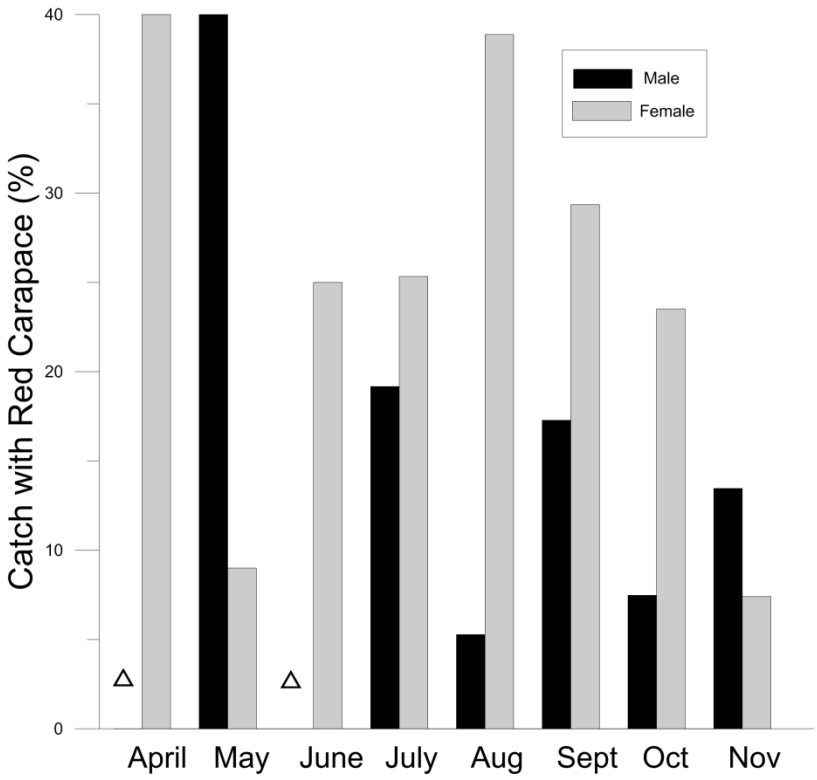


Fig. 2.3: Percentage of catch for red carapace male and female green crab monthly from 2008-2012 (n=1124). Male represented by black bars and female represented by grey bars. Open triangles representing 0 catch of male red carapace crabs.

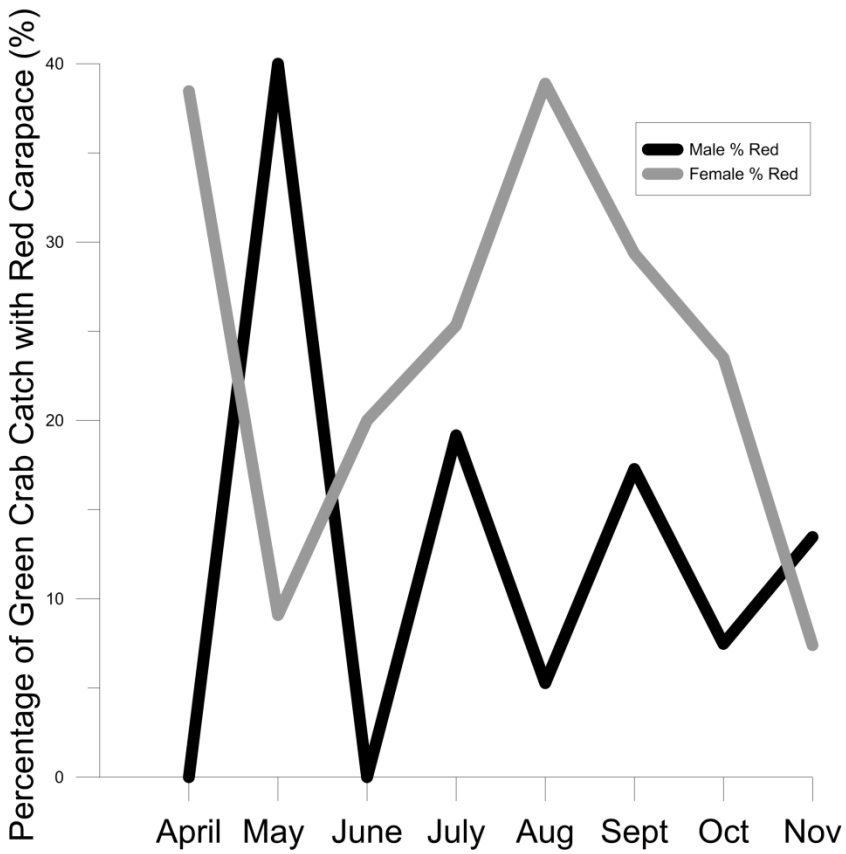


Fig. 2.4: Percentage of catch for red carapace male and red carapace female green crab by month from 2008-2012 (n=1124).

2.2.2 Gonadosomatic Index 2008-2012

Gonadosomatic Index was calculated for 1124 green crab collected between 2008 and 2012. GSI was significantly different between sexes, ANOVA: $F_{(1,1123)}=182.78$, $p<0.001$, with female GSI (mean=1.55, SD=1.96) significantly larger than male GSI (mean=0.49, SD=0.40).

Differences in GSI over seasons (summer, fall, Winter and Spring) were investigated for both sexes. Winter was not included in this analysis as there was no data collected for this season. An analysis of variance ($p<0.001$) concluded that males had significantly different GSI between the seasons $F_{(2,670)}=26.27$, $p<0.001$. Post-hoc comparisons using the Tukey HSD test indicated that male crab caught in the summer (mean=0.61, SD=0.42) had significantly higher GSI than crab caught in the fall (mean=0.39, SD=0.36), and there was no significant difference in GSI for male crab caught in the spring compared to fall and summer, Table 2.8, Fig. 2.5. An analysis of variance ($p<0.001$) concluded that females had significantly different GSI between the seasons $F_{(2,452)}=5.0$, $P=0.004$. Post-hoc comparisons using the Tukey HSD test indicated that female crab caught in the Spring (mean=2.83, SD=2.34) had significantly higher GSI than female crab caught in the summer (mean=1.49, SD=2.08) and Fall (mean=1.47, SD=1.70) and no significant difference in female crab GSI between summer and Fall., Table 2.8, Figure 2.5

Table 2.8: Descriptive statistics for adult male and female green crab seasonal GSI averages 2008-2012. (data for seasons sampled).

	Male			Female		
GSI SeasonalAvg	Mean	SD	N	Mean	SD	N
Summer	0.61	0.42	306	1.49	2.80	240
Fall	0.39	0.36	333	1.47	1.70	189
Spring	0.49	0.40	32	2.83	2.34	24
TOTAL			671			453

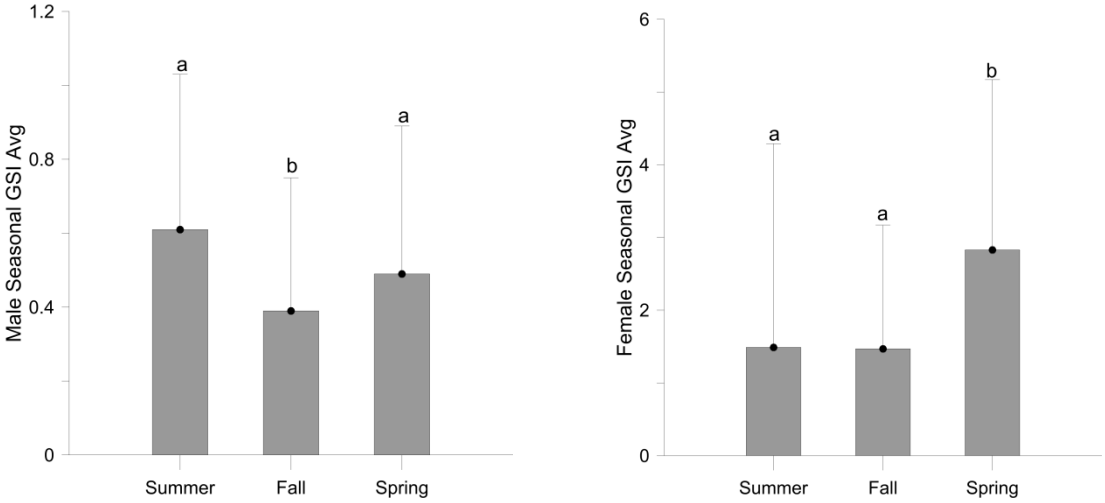


Fig. 2.5: Seasonal average GSI levels for 671 male and 453 female adult green crab sampled from 2008-2012. Bars represent mean +/- SD. Superscripts indicate significant levels at $p<0.01$.

2.2.3 Gonadosomatic Index 2012

The most regularly sampled year was 2012 with 10 sampling dates between April and September 2012 n=304. An analysis of variance of GSI levels between the sex of crab caught in 2012 found that males had a significantly lower GSI (mean=0.79, SD=0.38) than females GSI (mean=1.43, SD=1.94) $F_{(1,303)}=15.78$, $p<0.001$.

Green crab caught in the 2012 sampling year were separated by sex and investigated with respect to season and sampling month. The only seasons sampled in 2012 were spring and summer and from the months April to September 2012. Analysis of variance indicated that males caught in summer 2012 (mean=.83, SD=.38) had a significantly higher GSI than Spring 2012 (mean=0.46, SD=0.21, $F_{(1,151)}=15.60$, $p<0.001$). Analysis of variance indicated that there was no significant difference between female GSI caught between spring and summer of 2012. Table 2.9, figure 2.6.

Table 2.9: Descriptive statistics for adult male and female green crab seasonal GSI averages for 2012 sampling year. (data for seasons sampled).

	Male			Female		
GSI Seasonal Avg	Mean	SD	N	Mean	SD	N
Summer	0.82	0.38	135	0.72	1.17	139
Spring	0.46	0.21	17	0.66	0.48	13
TOTAL			152			152

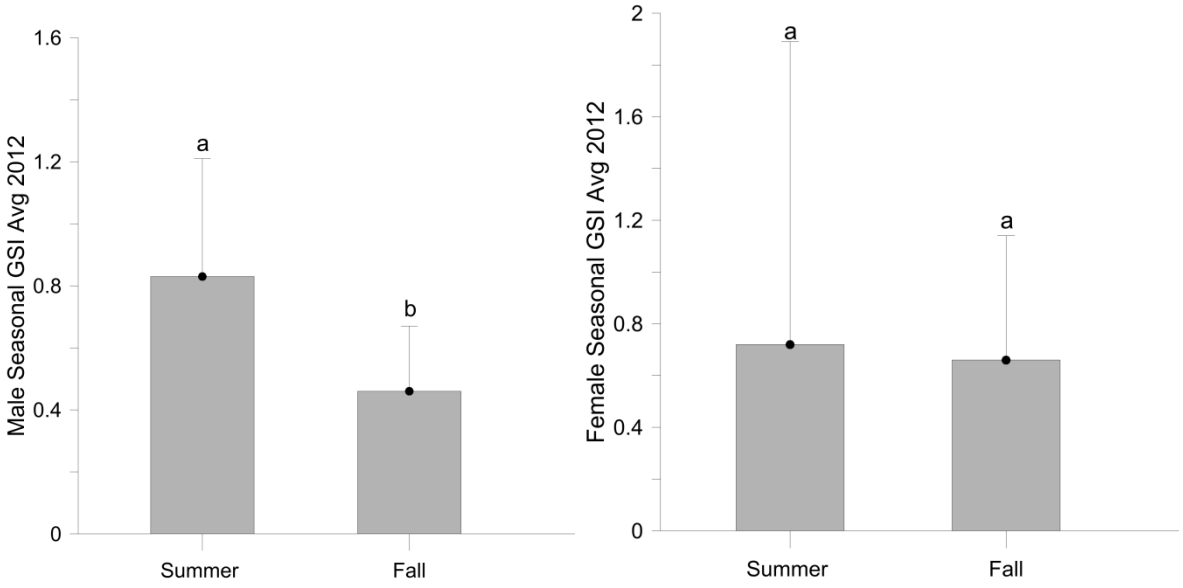


Fig. 2.6: Seasonal average GSI levels for 152 male and 152 female adult green crab sampled from 2012. Bars represent mean +/- SD. Superscripts indicate significant differences at $p<0.01$.

With respect to sampling month an analysis of variance indicated that there was a significant difference for males caught in 2012 $F_{(4,151)}=5.42$, $p<0.001$. Post-hoc comparisons using the Tukey HSD test indicated that males caught in April 2012 (mean=0.46, SD=0.21) had a significantly lower GSI than males caught in June (mean=0.89, SD=0.41), August (mean=0.84, SD=0.37) and September (mean=0.94, SD=0.35) 2012. With respect to sampling month an

analysis of variance indicated that there was a significant difference for females caught in 2012 $F_{(4, 151)}=11.34$, $p<0.001$. Post-hoc comparisons using the Tukey HSD test indicated that females caught in September 2012 (mean=1.75, SD=1.59) had a significantly higher GSI than females caught in June (mean=0.37, SD=0.35), July (mean=0.57, SD=0.79) and August (mean=0.28, SD=0.90) 2012. Table 2.10, Fig. 2.7.

Table 2.10: Descriptive statistics for adult male and female green crab GSI monthly averages 2012 sampling year. (data for months sampled).

	Male			Female		
GSI Monthly Avg 2012	Mean	SD	N	Mean	SD	N
April	0.46	0.21	17	0.66	0.48	13
June	0.89	0.41	15	0.37	0.35	15
July	0.74	0.39	45	0.57	0.79	45
August	0.84	0.37	45	0.28	0.90	47
September	0.94	0.35	30	1.75	1.59	32
TOTAL			152			152

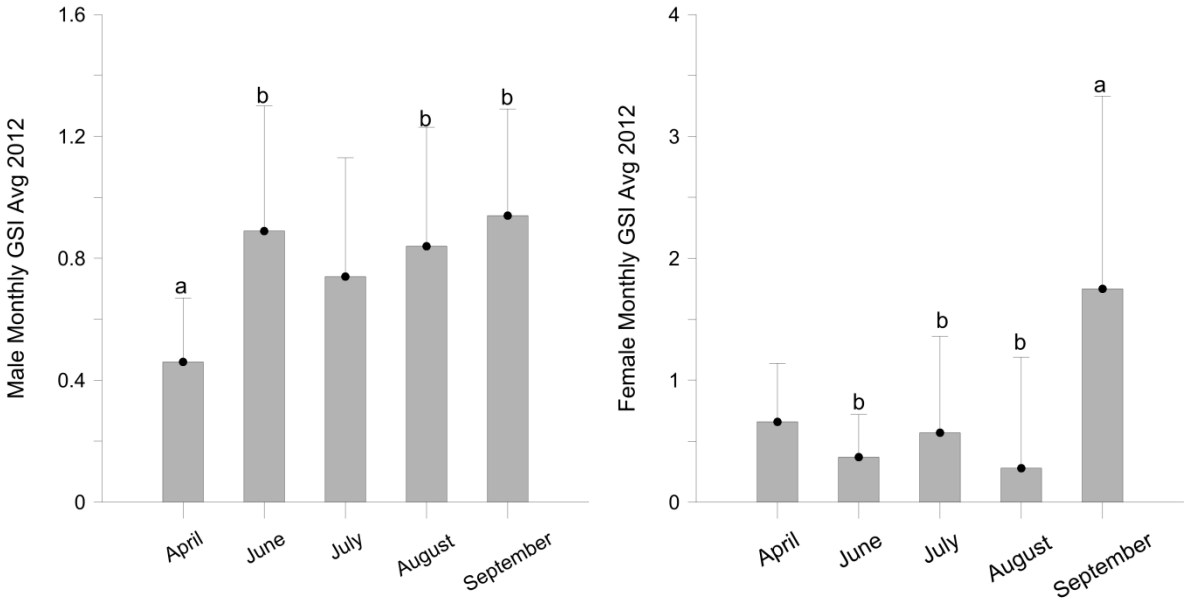


Fig. 2.7: Monthly average GSI levels for 152 male and 152 female adult green crab sampled from 2012. Bars represent mean +/- SD.

2.2.4 Histology

Females

153 female ovaries were analyzed and overall had a range of maturity stages from 1-5. Overall the series were distributed over stages with stage 1=2%, Stage 2=23.5%, Stage 3=24.2%, Stage 4=26.1% and Stage 5=24.2% with seasonality trends. Table 2.3. Females were considered mature at stages 3, 4 and 5 and immature at stage 0, 1 and 2 of ovary development. The smallest mature CW was 37 mm. The largest stage 1 or 2 was 32 mm.

Development stages 1 and 2 had the lowest occurrence but were found in highest percentage in July. The highest percentage of first maturity stage 3 was found in late September. Stage 4 and

ready to extrude eggs had the highest percentage in early June and stage 5 spent had the highest percentage in mid-August, Fig 2.8.

There were sperm plugs or spermatheca found in 12 females within the regular mix of males and females caught on several sampling dates with a CW mean of 54.17 mm range of 42-70 mm and BM mean of 34.33g and range of 16.36-70.55g, GW mean of 0.35g and range of 0.07-0.96g, GSI mean 0.65 and ranging from 0.13-1.57 and all with green carapace colour. Spermatheca were not included in GSI but when compared to total body weight of female crab they ranged from 0.06-5.24 percent of total body weight where gonads in those same females were not drastically different ranging from 0.17-4.03 percent.

Data collected in April indicated that 23 percent of the females were in early development stages having confirmed spermatheca, with less (10 percent of female catch in July and August and 15 percent of female catch in September) demonstrating this in later months. Upon visual interpretation of gonads these individuals were all in immature developmental stages, 58 percent of them were stage 1 and 42 percent of them were stage 2. Histologically there was a wider range of stages, 8.3 percent of female gonads were stage 1, 50 percent stage 2, 33.3 percent stage 3 and 8.3 percent stage 4. Histological stages 1 and 5 are not identifiable by eye. When 4 of these spermatheca were extracted and analyzed they were composed of male spermatophores and of a mature stage. Samples for females with spermatheca were very low and only discovered upon dissection therefore a small n could not offer statistical analysis, in comparison to total catches and were only used as observational data.

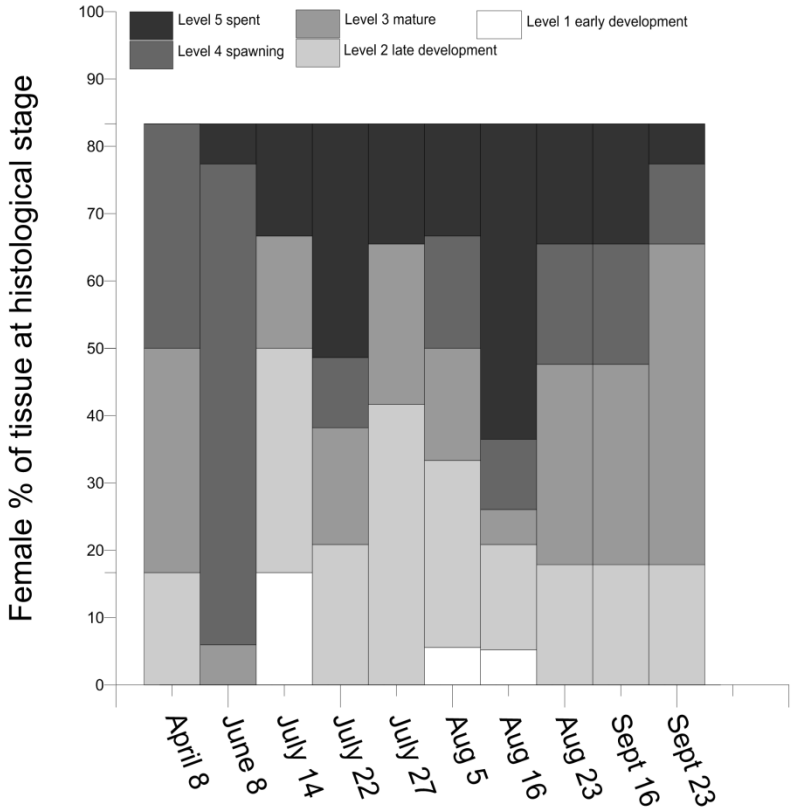


Fig. 2.8: Seasonal patterns in ovary development in female green crab in 2012 determined from histological analysis.

Males

166 testes were analyzed and considered mature at stages 2 and 3 and immature at stages 0 and 1. All histologically evaluated males had a maturity stage of 2 and therefore considered mature. The smallest of these mature males was 32 mm and the largest stage 0 or immature was 29 mm (no stage 1 found), Table 2.4. All males being of the same maturity stage over the entire sampling period indicating that males caught in this area were prepared to spawn at all times during the sampling seasons, sampling bias at Goose Cove may play a part in this finding.

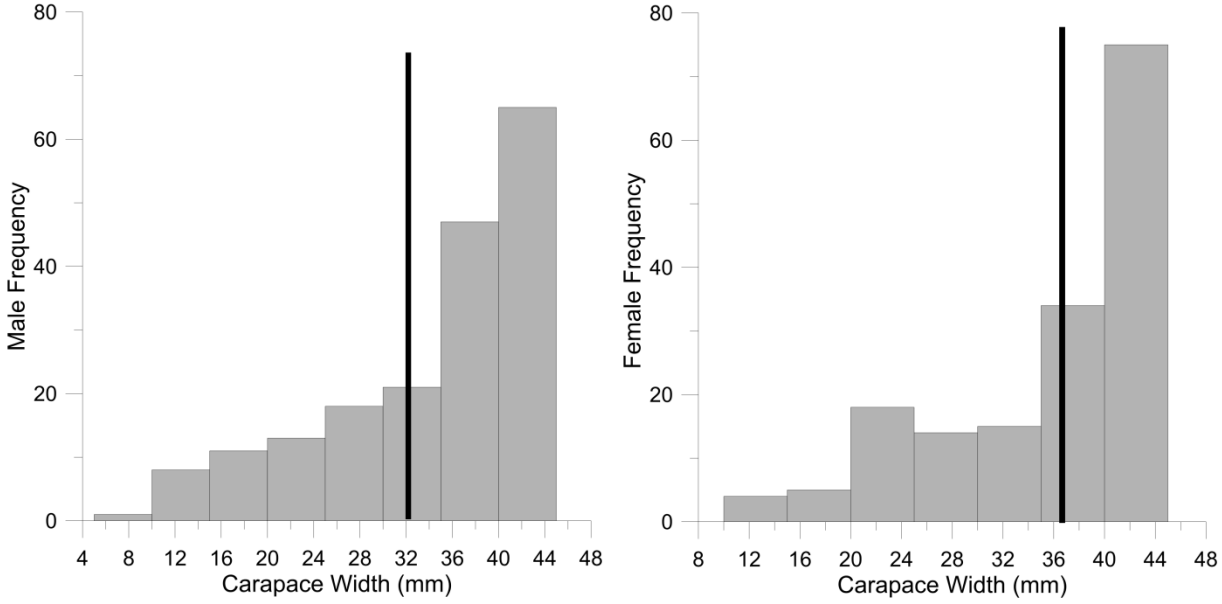


Fig. 2.9: Frequencies of male (n=671) and female (n=453) green crab CW in 5cm bins. Black line indicates maturity based on histology results male mature at 32 mm and female mature at 37mm.

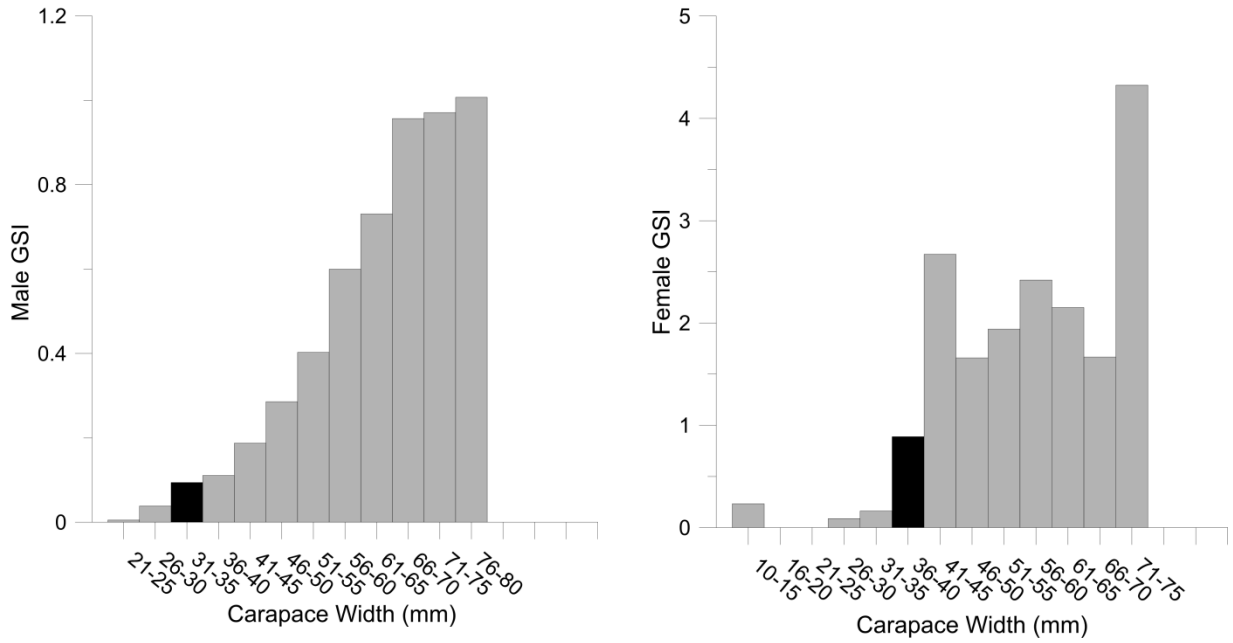


Fig. 2.10: GSI for male (n=671) and female (n=453) green crab by 5 mm CW intervals. The black bar indicates maturity (by bin) based on histology results, male mature at 32 mm and female mature at 37 mm.

In Fig. 2.10 the bar in which the maturity indicator is located sits just before the first large increase in size for both male and female green crab. With respect to GSI and CW we predict that there is a large increase in GSI levels for each sex just after maturation is reached and gonad increases in size relative to the size of the crab. Looking at the 5 mm size bin at which males mature there is a large increase in GSI level but no real large increase in the next size bin. All male GSI levels increase at a steady rate as size increases with a slight leveling off at the highest 3 size bins, Fig. 2.10. Looking at the 5 mm size bin at which females mature there was a large increase in GSI level in the next size bin. There was less of a steady increase for female GSI level compared to males but there is a large jump in level after the mature size bin and then another at the largest size bin, Fig. 2.10. These observations may also be an effect of sample bias

and low n for the outside limits of size bins for both males and females to most accurately represent the population. The catches using Fukui pot size frequencies may not represent the range in sizes of a population as the mesh size is larger than young crab and catchability of smaller crab is low, the population fished, season and conditions fished may not have facilitated catches of the maximum size frequencies for both sexes. All contributing to sample bias and not an accurate estimation of maturation based on CW and GSI for this population.

2.2.5 Life history

Life history for the Newfoundland population of green crab was estimated by pooling all data n=1124, using water temperature, GSI, carapace colour and molting, presence of spermathecae, histological staging, presence of ovigerous females from 2008, 2009 and 2010 and presence of settled juveniles in 2011 and 2012.

Fig. 2.11 displays the monthly relationship between mature female histology stages, GSI levels and average water temperature.

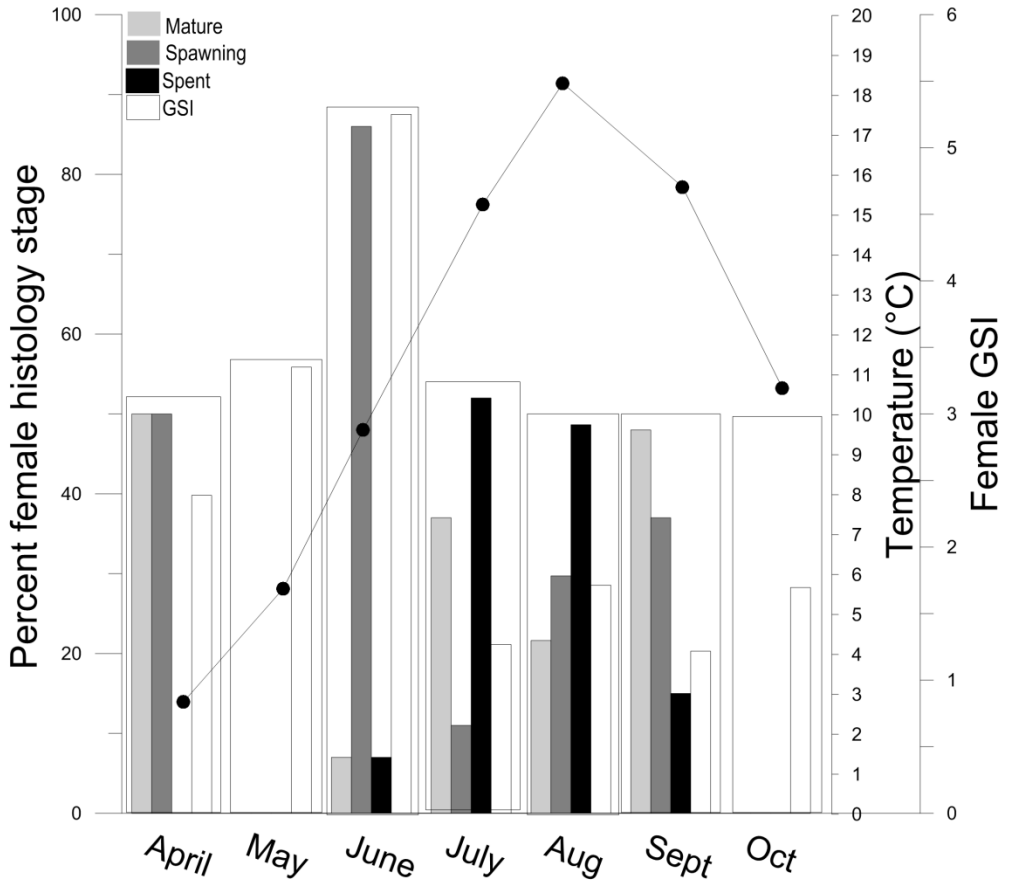


Fig. 2.11: Monthly trends in histologically mature female green crab with corresponding GSI level and average water temperature.

Water temperature increased from April and peaked in August at 18.3°C and then decreased. The GSI level increased from April peaking in June at 5.25 before dropping drastically in July and staying below 2 until October. The percentage of stage 4 or spawning/spent ovaries follows this GSI trend also having the highest percentage in June at 86 percent and dropping in July. These spawning/spent female percentages increase steadily from July to September. Stage 3 or mature

ovaries is highest in April and shows no trend and the stage 5 or spent/reabsorbing ovaries are only present from June till September with the highest levels in July and August.

Fig. 2.12 displays monthly trends in percent of female catch with red carapaces, female GSI levels and the average water temperatures. It also shows symbols representing the presence of ovigerous females and juvenile green crabs.

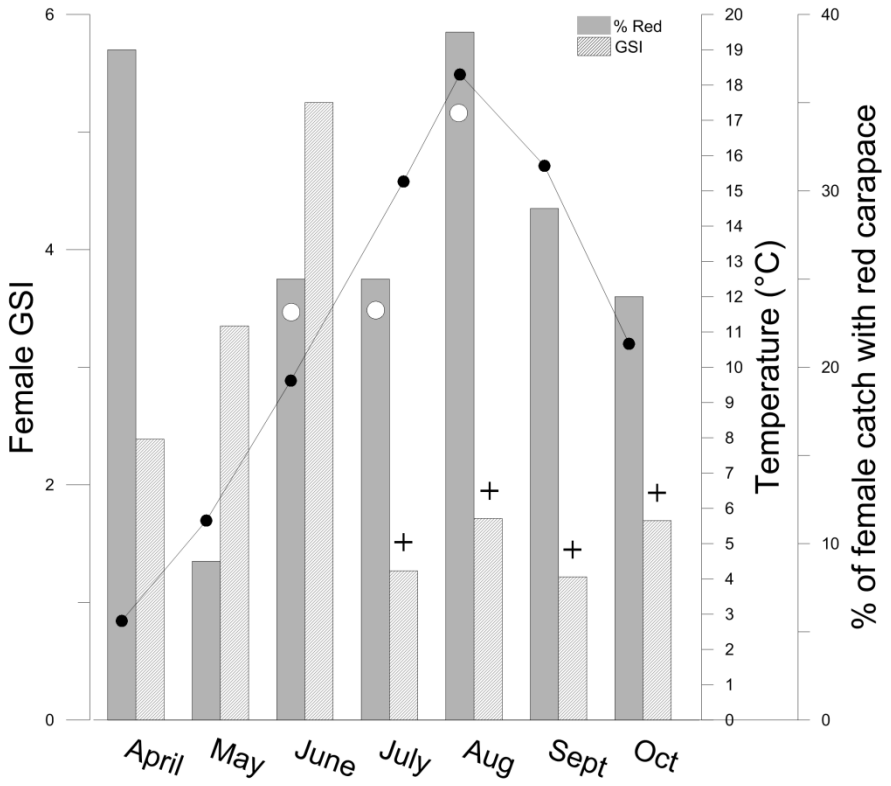


Fig. 2.12: Monthly trends in percent of female catch with red carapaces with corresponding GSI level and average water temperature. Open circles representing presence of ovigerous females, crosses indicating presence of newly settled juveniles in the intertidal.

The percent red females is high in April at 38 percent drops below 10 percent in May then steadily increases to the highest in August at 39 percent and then decreasing in September and October. Ovigerous females were found in pots in June, July and August and juveniles of 5-10 mm CW were found in the intertidal zone from July to October.

Fig. 2.13 displays the monthly relationship between percent of male catch with red carapaces, male GSI levels and the average water temperature.

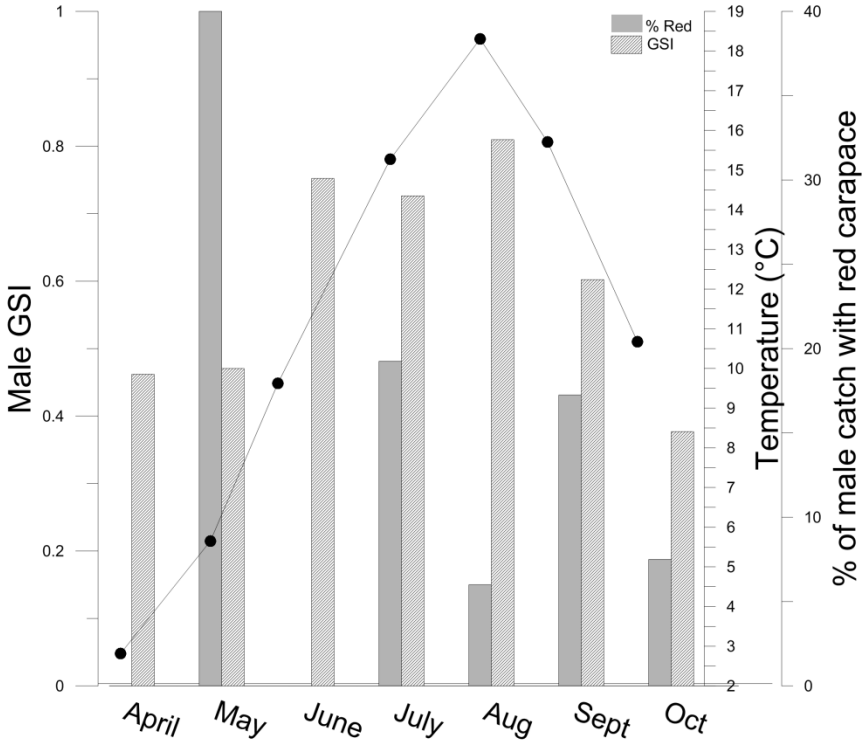


Fig. 2.13: Monthly trends in percent of male catch with red carapaces with corresponding GSI level and average water temperature.

There was no real trend in male GSI as seen previously with no clear development, a peak and then drop off at a main copulation time. There is a peak in percent red males in May at 40 percent and then dropping to 0 and staying below 20 percent from July to October.

2.3 Discussion

Morphometric and histological techniques were used to investigate the reproductive strategies of a Newfoundland population of green crab. As well as looking for changes to population structure, reproductive strategies and maturities in contrast to other northern populations. Crab were collected and analyzed to look at a number of parameters from which trends were established to better understand the life history of the population and when it's is best to target those life stages in attempts to control and slow the invasion of the green crab to new areas of coastal Newfoundland.

2.3.1 Life History

Lyons et al. 2011 states that in order to maximize offspring survival and population stability, marine invertebrates have been reported to possess adaptive reproductive patterns. More specifically green crab populations have been shown to synchronize their life cycle to various seasonal patterns (Audet et al. 2008). Knowledge of when reproductive events like timing and the conditions needed for the different life stages for green crab to reproduce and for larvae to grow through their planktonic stages and settle we can roughly estimate when to target or avoid those high risk events with respect to introduction and spread from in North Harbour, Placentia Bay, Newfoundland.

GSI is the first variable to aid in identifying when spawning occurs, supported by cellular analysis via histology. Males caught in Goose Cove had a seasonality difference in GSI with the lowest in the fall and a steady increase through spring and summer with similar trends in CW, BM, GW, and GSI. GW and BM have similar trends as males are foraging to develop their gonad which is a part of their total body mass in preparation for spring and summer when copulation occurs. The spring copulation coordinates with the prime season for summer larval release and then the larval release molt and subsequent molt. Summer copulation could also occur when the females have their post spawning molt but would not have immediate fertilization. Male catches follow higher CW trends based on the predicted high copulation times in the Spring and summer, with larger male's active and foraging which are more successful in competitions with other males for females and have a higher reproductive index (Styrishave et al. 2004). Regardless of these trends the histological evidence for these males and GSI values confirm they are all mature and ready to spawn regardless of season. This corresponds to the shorter male reproductive cycle in other populations with less time invested in gonad development, each male investing in a week or more in each copulation giving ample time to copulate more than once in a season (Van Der Meeren, 1992).

Females also had a difference in seasonality in GSI also with the lowest in the fall, a large increase to the highest in the spring and then falling off again in the summer with corresponding trends in GW. Females have a much more complex cycle with the percentage of ovary stages showing a trend that as the ovary develops into larger oocytes, gonad increases in size, and GSI increases proportionately. In the fall females are foraging and storing energy for gonad development then as they get closer to spring which corresponds to the critical period to extrude at optimal egg extrusion temperatures. Females have their highest GW, GSI and percentage of

stage 4 ovaries at this time, in late May, early June. Females are ready to fertilize at this time and it can be assumed that females do fertilize and are ovigerous in the following summer months. This is supported by the small catches of ovigerous females in June, July and August in multiple years. These catches are only a small representation of the ovigerous population at these times as when females are carrying eggs they remain buried in the substrate for extended periods of time and feed sporadically, conserving energy and therefore their abundance are likely severely underestimated in the traps (Lyons et al. 2012, Cameron and Metaxas 2005). This trend is also evidence for male dominated catches at seasons when females are inactive and then more sex ratio balanced once most females are finished spawning for the season. The GW, BM and GSI drop back down in the Fall and there is a higher percentage of stage 5 ovaries as those females have released their larvae and are now spent for that summer and are in need of reabsorbing old eggs if any and redeveloping their gonad for the next season over the coming Fall and Winter. Female catches follow CW trends based on the predicted high copulation times in the Spring and summer when larger more successful reproductive females with high reproductive index are more actively foraging in preparation for copulation pre and post egg extrusion (Styrishave et al. 2004).

With respect to histological samples, summer caught females had low GSI as well as the highest percentage of stage 1 and 2 ovaries which are taking up less percentage of total BM, indicating one of 4 possibilities; 1. They were physiologically immature and hadn't had their first spawning yet, or 2. They were making use of spermathecae acquired months prior, and had just skipped that season's fertilization window, 3. They had acquired spermathecae recently in post spawning summer copulation immediately after releasing larvae and molting and would participate in the next fertilization window, or 4. Have finished spawning for the summer and have moved out of

stage 5 spent and began the next development cycle. With the highest percentage of females with spermathecae (n=14) found in April and with the majority with stage 2 ovaries at least a month before egg extrusion is predicted to occur we can infer that these females were involved in summer copulation from the previous summer and would be fertilizing eggs a month later in May with the rest of the population. This is quite possible with green crab females ability to store viable sperm for up to 12 months (Broekhuysen, 1937). Therefore we can assume that the summer caught low GSI stage 1 and 2 ovary females without spermathecae are immature and have not previously spawned, or have finished spawning and moved on to the next development cycle.

There was a male bias in the catches during all seasons, with the highest ratio of males to females in the Fall when both sexes should be foraging; this may be male dominated due to male aggressive tendencies and natural sexual dimorphism in green crab (Berrill & Arsenault, 1982). In the spring and summer this ratio decreases as mating behavior should be intensifying with both sexes engaging in reproductive behaviors.

Molting is a secondary variable to generally estimate when copulation is near for males and females and when larvae have been released for females. Males molt cycle requires that they have a new hard shell to copulate with a female and will molt after copulation to prepare for the next. Female molting occurs just after larval release and corresponds with mating for the next season. In NL samples, when green crabs molt the new exoskeleton is bright green and in some crabs gradually changes into a darker red colour as it becomes older, the mechanism for this change is unknown but it has been suggested that the exoskeleton pigment astaxanthin turns red during photodenaturation as the shell ages (Styrishave et al. 2004). Carapace colour is not a direct indictor of molt stage but of the length an individual has spent in inter-molt (Reid et al.

1997) so can be used in molt estimations. Red carapaces were seen in both male and female green crab in this study, 26 percent of female crab and 12.4 percent of male crab. Red crabs tend to be dominant in the subtidal zone with more consistent water conditions as they less tolerant to environmental stress and green crabs tend to predominate the intertidal zone with more variable conditions as they are more tolerant of environmental stress (Reid et al. 1997). This study's collection site is considered intertidal which could explain the higher green male and female catches there but does not explain why there was a small proportion of red males and females were caught there. Ovigerous females tend to be associated with lower salinities 20 ppt which are better for embryo development (Queiroga, et al. 1997). These females may have been subtidal before egg extrusion but once they extruded moved up to estuarine conditions in this area closer to the intertidal in the lower salinity water for optimal egg development which is more important than combating environmental stress, and were caught in Goose Cove (pers. obs.). There is no known benefit for red males to stay in the estuary aside from mating season but their presence along with females in Goose Cove intertidal area may be in part due to the fact that red crab are less mobile than green morphotypes which are much more likely to express circatidal variations in their physiology and behavior than are red morphotypes and migrate daily to take advantage of foraging in highly productive habitats such as estuaries while at the same time reducing the risk of becoming exposed to desiccation, low salinities and protection from predators (Styrishave et al. 2004).

Red males from Goose Cove were larger, heavier, had heavier gonads and higher GSI than green males which follows findings by Styrishave et al. (2004) and McGaw et al. (1992) making them more likely to win inter-male competitions for mating females, and have healthier reproductive systems which corresponds to a higher reproductive index and higher mating

success. In Goose Cove there was no difference in colour morphs with respect to size, weight, and GSI levels for the red females but they did have heavier gonads than green females. This corresponds to females molting cycle and reproduction, in that before copulation she has the most developed (stage 4), heaviest ovaries, and also has an old shell ready to molt pre copulation (Berill & Arsenault, 1982). A green female can also have heavy stage 4 gonads and are just as able to molt and copulate, just haven't delayed molting as long as a red carapace female and may have a slightly lower success rate when they do reproduce (pers. obs.)

This study saw that there was a significant percentage of red males in May, Fig. 2.3, 2.4. This suggests that there is a primary copulation season in May, corresponding to larger more aggressive high reproductive index males present, followed by males molting after copulation in June which has zero catch of red males. Females had the highest percentage of red carapace in April also corresponding to the pre copulation molting. The season when the larger more successful male and female crab are active are also the same cohort that is most likely to decrease molting frequency and enter a state of prolonged intermolt, channeling energy towards reproductive success thus developing red carapaces. This is also not the rule as green males and females of the same size have no difference in gonadal development (McGaw et al. 1992), these males may just lose some conflicts with larger stronger red males and not get their first pick of large red more productive females. This theory is speculative, based on the present data and one collection site and population and would need further expanded sampling and controlled lab experiments to confirm.

Females that had been fertilized and extruded their eggs were found in June, July and August as temperatures are approaching summer highs, corresponding to increased percentages of stage 4 ovaries and highest GSI levels in June. Keeping in mind that the following conditions are

circumstantial and from a small representative sample of the total population we can make life history estimations. Juveniles detected in the field were assumed to have had at least one molt since settlement and were first found in July (Theil and Dernedde 1994). Back calculating from then with assumed conditions of 7 days for 1 molt and 32 days in the planktonic stages at optimal water temperatures (Broekhuysen, 1937) the estimation can be made that these larvae were released at the latest in early June or earliest in late May and continuing until August. We can estimate that the time spent ovigerous is just less than one month up to three months. Other populations in the East and West Atlantic have ovigerous females present for five to six months in the Netherlands (Brokhuysen 1937), and in Maine (Berrill 1982) and in PEI for less than one month up to three months (Sharp et al. 2003). The Newfoundland population has a similar ovigerous stage length for females to the closer region of PEI which has a comparable environment and a cold tolerant genome. The warmer region populations with ovigerous females for five to six months have longer to develop and more opportunity to release larvae when temperature and food conditions are ideal for survival.

Average water temperature in Basin Head PEI was approximately 20-22°C in July when first ovigerous females were present (Audet et al. 2008) significantly higher than average water temperatures in North Harbour Placentia Bay in June at 10°C when first ovigerous females were found there. With a change in water temperature triggering egg extrusion and providing conditions for healthy egg development while still attached to the female's abdomen (Broekhuysen, 1937) these populations have healthy development at different temperatures. The two populations still having the same duration of ovigerous females in their respective environments may show that the Newfoundland populations' reproductive strategy has

synchronized to a shorter seasonal pattern to start earlier and at a summer water temperature which is significantly colder than the PEI waters.

Maximum summer water temperatures in North Harbour are approximately 18°C and in Basin Head PEI approximately 23°C (Audet et al. 2003). These temperatures contribute to well-timed egg and larval development for green crab for each population to maximize growth and survival. Females are ovigerous in PEI in July with temperatures just below summer maximum at approximately 20°C (Audet et al. 2008) and in Newfoundland females are ovigerous in May/June with temperatures much below summer maximum at approximately 6-10°C. These differences in egg development temperatures with obvious successful development and larval survival in both cold water tolerant populations is evidence that the recently invaded area has resulted in variations in reproduction as they are related to the other Maritime populations. These strategy variations were also found when the Basin Head PEI green crab population was compared to the green crab population in Maine US (Audet et al. 2008). This said, growth and reproduction of crustaceans is highly influenced by environmental factors which further adds to the complexity of these processes (Styrishave et al. 2004), therefore additional analysis of other variables other than temperature would strengthen this conclusion.

Based on female gonad development and presence of juvenile young of the year green crab it can be inferred that the Placentia Bay Newfoundland green crab population has one ovarian development cycle per year and the reproduction cycle is annual, copulating in the Spring and late summer and releasing larvae in June, July and August, which settle between July and October. This observation for this population of green crab needs to be investigated further and in depth to test this hypothesis.

2.3.2 Size at maturity

Different crab species mature physiologically first then morphologically at different rates. It has been found that some species of snow crab, a majid crab, are maturing physiologically before morphometrically successfully mating at smaller than expected sizes in the Gulf of St. Lawrence (Ennis et. al. 1988) as a function of fishing pressure on larger males. Other species like the speckled swimming crab *Areneaus cribrarius* males achieve their secondary sexual characteristics at 55 mm CW and are not physiologically mature until 63 mm CW and females achieve both maturities at 59 mm CW (Pinheiro and Fransozo 1998).

In the investigation of size at maturity for Newfoundland green crab the evaluation of GSI range of both male and females did not give a defined CW in which male and females are mature. Significant trends in seasonal GSI values for females was observed with no such trends for males but did not produce values specific enough to determine a break point for either sex. Deeming GSI inconclusive for determining size at maturity therefore histological techniques were used to clarify. Histology confirmed maturity for males at 32 mm CW and females at 37 mm CW, further confirmed with observing the smallest ovigerous females captured of the same size. The largest immature stage females were 32 mm, but it cannot be confirmed that this female has or has not spawned at least once and is redeveloping ovaries. Abdomen width measurements could confirm the cut off for the size minimum for females that have had morphometrically matured and have wider abdomens for holding egg clutches (Audet et al. 2008), but this was not evaluated in the present study.

Average CW of ovigerous females in Newfoundland was found to be smaller than the total average of non ovigerous females. This is the inverse of the PEI population with an average CW

of ovigerous females larger than non ovigerous. The smallest ovigerous female found in Basin Head had CW of 43.67 mm (Sharpe et al. 2003) and 38.2 mm (Audet et al. 2008), slightly larger than in Newfoundland with 37 mm CW. This suggests that this population may have changed reproductive strategies to mature at a smaller size, perhaps in reaction to environmental conditions, shorter growing season and slower growth rate. This could be a case of an introduced species undergoing changes in sizes and shape relative to their native range, which can strongly influence the magnitude of the impacts of the invader (Grosholz & Ruiz, 2003).

All the individual male gonads examined histologically were of the same development stage, stage 2, mature. This may be explained by probable sample bias based on the study site. Goose Cove is an estuarine area of North Harbour on the western side of the bay and has average salinity of approximately 25 ppt (McKenzie, unpublished data). Conditions are more favorable for reproductive females on this side of the bay because lower salinity is favorable for egg development and the estuarine tidal currents aids with planktonic larval dispersion (Queiroga et al. 1997). Therefore females could spend most of their time there during foraging and egg bearing. As a result of smaller secondary estuaries on that side of North Harbour and Goose Cove and the coriolis effect we assume that the eastern side of Goose Cove is more saline than the western side. Many males who have red carapaces in anecdysis, and are more vulnerable to environmental stress of both natural and anthropogenic origin (Reid et al. 2004). Therefore are sensitive to the lower salinity at Goose Cove. It is shown here that those males, the larger, heavier gonad, highest percentage of red carapace males that are the most reproductively successful had mature gonads when they were caught in Goose Cove. They were tolerating the different environmental conditions for the sake of maximum reproductive success. We also assume that the males that were elsewhere and not available for sampling are in one of two

conditions either have stage 3 testes, are recovering and preparing to molt. Or have stage 1 testes after recovering and are soft shelled and not active enough to be fished. The high number of green males captured in Goose Cove were more than likely there, as part of their tolerance to changing conditions and ability to adapt to flood and ebb in the intertidal with circatidal migration (Styrishave et al. 2004).

More Information on the sex ratios and gonad development for both males and females, migration of red and green carapace males and females as well as more detailed salinity and water chemistry profiles covering more geographical area in the bay can confirm these theories and add to the reproductive strategies for the population.

In other coastal Atlantic populations, physiologically mature females were found in Portugal at 29 mm (Baeta et al. 2005), in Cork Ireland at 38.6 mm (Lyons et al. 2012), ovigerous in the Bras D'Or Lakes, Nova Scotia at 40-60 mm CW (Tremblay et al. 2006), in Basin Head PEI at 42.7 mm (Sharp et al. 2003) and 38.2 mm (Audet et al. 2008) and in Maine USA at 34-45 mm (Berrill 1982). The Placentia Bay Newfoundland physiologically mature females are smaller than all Northwestern Atlantic populations but the Maine population at 37 mm CW. This corresponds with the theory that size at maturity will decrease as latitude increases (Berrill 1982), and that species introduced into a new region frequently undergo changes in size and shape relative to their native range (Grosholz and Ruiz 2003). These confirmed size differences for mature males and females in the Newfoundland population is evidence for newly established invaded coastlines to show variations in reproductive strategies (Audet et al. 2008).

This study has identified adjusted reproductive patterns adopted by green crab in Newfoundland waters. It can be concluded that there is an annual reproductive cycle in which reproductive

males and females are smaller, make use of spermatheca and extrude their eggs and release larvae at cooler temperatures than a similar longer established population under similar environmental factors. Therefore it can be roughly estimated when and under what conditions reproductive events occur in North Harbour, Placentia Bay, Newfoundland and how to best target them in efforts to control spread and new introductions. We also suggest that the life history examined here can be applied to the complex reproductive migration and behavioral system for males and females in the area, that extensive investigation of both sides of North Harbour can offer a better understanding of this system along with other influential environmental factors and comprehensive year round sampling.

Chapter 3: Investigating mitigation of juvenile European green crab *Carcinus maenas* from seed mussels to prevent transfer during Newfoundland mussel aquaculture operations

Abstract

The mussel aquaculture industry has raised concerns following the discovery of green crab, *Carcinus maenas,* in Placentia Bay, Newfoundland, in September 2007. Post-larval green crab have been found in feral mussel beds in high densities in Europe. If this is true for other green crab populations, mussel seed transfers from Placentia Bay could provide a vector for post-larval juvenile crab transfer to other areas. Transport to Notre Dame Bay is a particular concern as provincial mussel aquaculture is concentrated there and to date no green crab have been detected in this area of Newfoundland. Their preferred prey is bivalves, primarily clams and mussels. In this study newly settled green crab juveniles were collected and used in a series of lab scale mitigation trials. Crab and seed mussels were exposed to thermal shocks of a scale that would be applicable and feasible for mussel seed management in Placentia Bay. Crab mortality was measured in the treatments and seed mussels were monitored for stress response using the lysosomal destabilization assay. Exposure to sea water (32-35ppt) heated to 45°C for one minute duration was effective in culling juvenile green crab while causing minimal stress to mussel seed. The method can be effectively employed in mussel seed management and transfer operations where there are concerns related to potential introductions of hitch-hiking green crab.

3.0 Introduction

Newfoundland is the second largest producer of blue mussels in North America; producing 4,400 metric tonnes valued at $14 million CAD in 2012 (DFA 2013). It is a rapidly expanding industry, requiring a good seed supply for its continued growth. The majority of mussel farming takes place in Notre Dame Bay on the Northeast coast of the province with one farm in Placentia Bay (NAIA pers. comm.). Most of these areas collect mussel seed for grow out within the same area as the farm, but with industry expansion in recent years, seed shortages are occurring on farm sites (NAIA pers. comm.). To resolve this problem seed is being collected, harvested, and shipped to different farms. Some of the seed collection is occurring in Placentia Bay with hopes to transfer the mussel seed to farms in Notre Dame Bay (NAIA pers. comm.).

European green crab, *Carcinus maenas,* was found to have an established population in Newfoundland waters in 2007 in Placentia Bay (Klassen and Locke 2007). It is indigenous to European and Northern Africa coasts and estuaries and has high tolerances for temperature and salinity extremes, air exposure, and desiccation. They have a relatively high fecundity and long planktonic larval duration, are voracious omnivores and aggressive competitors. This makes them a successful global invader (Cohen and Carlton 1995, Roman and Palumbi 2004). In all areas where they have invaded, its potential for significant impacts on fisheries, aquaculture, and the ecosystem has caused concern (Klassen and Locke 2007).

C. maenas have had a strong influence on the abundance of natural bivalve populations by virtue of their very aggressive predation patterns (Grosholz and Ruiz 1995). Bivalves are their preferred prey based on stomach content analysis (Elner and Hughes1978). They not only cause a decrease in prey abundance but have also been found to change molluscan defense characteristics like

shell thickness. In areas of high predation, *C. maenas* has induced changes in *Mytilus edulis* including thicker shells; relatively more shell mass, and the mussels were more tightly attached to the substrate (Leonard et al. 1999). In the situation with mussels, the increased shell thickness results in decreased meat yields, and higher losses incurred during processing with an overall lower quality product. Miron et al. (2005) found that adult green crab prefer blue mussels and soft shelled clams over oysters and quahogs in a population in New Brunswick, Canada. Pickering and Quijon (2011) found that *C. maenas* prefer soft shelled clams, and blue mussels then oysters in order of preference as the abundance of each decreased in natural beds in Prince Edward Island, Canada. Matheson and McKenzie (2014) also found that *C. maenas* prefer soft shelled clams and blue mussels over scallops in Newfoundland, Canada.

Not only are blue mussels one of the preferred prey species for green crab, they are also a preferred protective habitat for early life stage *C. maenas*. When pre-settlement megalopae were given habitat choices in the lab they chose mussel substrate over eelgrass and macroalgae for their initial settlement (Hedvall et al. 1998). After megalopae settle onto their preferred habitat on the shore, they remained in that protected area throughout the juvenile stages.

It is evident that green crabs effectively use mussels as a settlement substrate, protective habitat and prey species in native and invaded areas of their distribution. This raises concern for native Newfoundland bivalve populations and the mussel aquaculture industry. With the recent discovery of green crab in Placentia Bay (Klassen and Locke 2007), there is a risk of transferring early life stage *C. maenas* with mussel seed to high value mussel growing areas in the province like Notre Dame Bay, or introducing the crab to a new locale for invasion. Therefore, the development of methods to mitigate mussel seed transport as an aquatic invasive species (AIS) vector, while maintaining a healthy supply of high quality mussel seed, will be critical to the

growth and prosperity of the mussel industry in Atlantic Canada (Vickerson 2009, Vickerson et al. 2011).

Mussel seed transfer is the main vector investigated in this study and is the greatest threat to the Newfoundland mussel industry for dispersal of juvenile green crab. Other human mediated vectors like ballast water, sea chests, shipment of commercial shellfish and aquaculture products, marine construction equipment, fouled hulls, movement of sediment/sand and accidental release from research facilities can threaten unestablished areas (Cohen et al. Carlton 1995; Grosholz and Ruiz 2002). These vectors have been responsible for the transfer of invasive species other than green crab and can be prevented with education to those sectors. Natural dispersal is also a threat as green crab larvae have been documented to transfer in currents up to 50 km per day on the West coast of North America in 1998 (Behrens Yamada and Becklund 2004). Canada has a risk of larval transfer via currents but not from this site of interest in Placentia Bay to Notre Dame Bay in Newfoundland, Fig 3.2 therefore human mediated transfer is the main vector.

There are no specific mitigation techniques targeting green crab on aquaculture sites currently in use to our knowledge. Treatment of oysters for the removal of barnacles, other bivalve spat and seaweed has been established internationally. Solitary and colonial tunicates have been the AIS focus in the Maritime Provinces for years (Howes et al. 2007, LeBlanc et al. 2007, Locke 2009). The techniques used to mitigate these species include treating mussels with fresh water, vinegar, lime (Denny 2008, Vickerson et al. 2011), air drying and high pressure water blasting (Forrest and Blakemore 2006).

Adding a small step to the harvesting and grading of seed can add significant time and cost to the operation before seed transfer, so a simple and cost effective mitigation method is required. The

most simple and available technique considered for this study was immersion in heated salt water (32-35ppt). Other methods of pest removal such as heated freshwater, lime and acetic acid treatments used for some soft bodied invasive species are not appropriate for targeting green crab specifically. The seawater thermal shock technique has been used on oyster species to remove other pests by the Center for the Experimentation and Development of Marine Aquaculture. They removed undesired mussel spat from Pacific oysters *Crassostrea gigas* by immersion in water at 80 and 85°C for 2 and 3 second durations and then slowly cooled to ambient temperature (CREAA 2004). Park et al. (1998) removed a variety of fouling organisms from *C. gigas* by immersion in 60°C water for 10-15 seconds in Korea; Arakawa (1980) did the same in Japan. Forrest and Blakemore (2006) experimented with removal of the kelp *Undaria* from green lipped mussels *Perna canaliculus* using immersion in water heated to 35, 45 and 55 °C for a range of exposure times. They found 55°C for approximately 5 seconds to be the most appropriate and least harmful to the green lipped mussel seed. Smith and MacNair (2000) suggested that blue mussels cultivated in suspended culture have thinner shells than those of oysters and they may be more sensitive to high temperature baths. Therefore the techniques used for removing fouling from oysters may be more harmful to blue mussels. Increased body temperatures can cause short term problems, like stronger byssal thread attachment and shell gaping, and long term effects of high stress levels from temperature shock that can decrease growth rate and overall mussel health during grow out.

The *C. maenas* temperature tolerance information found in the literature relates to adult crab behavioral changes at different temperatures and time. At temperatures above 25°C we can infer that the health of an adult green crab will start to diminish (McGaw and Whiteley 2012), and even more so for a more vulnerable and thinner shelled juveniles. Taylor and Wheatly (1979)

found that *C. maenas* adults will migrate to an area above water once water temperatures in the intertidal reach 28°C so they can actively decrease their body temperature. Kelley et al. (2011) found that crab could live in water temperatures up to 36.2°C starting at 23°C and increasing the temperature 1 degree an hour. We hypothesize that juvenile green crab from Placentia Bay will not tolerate short exposure times using moderate to large thermal shocks.

The goal of this study was to investigate the effectiveness of an environmentally friendly heated salt water immersion technique for preventing *C. maenas* juveniles from being transported with Newfoundland blue mussel seed. We hypothesized that juvenile green crab from Placentia Bay will not tolerate short exposure times using moderate to large thermal shocks and there will be little to no stress response from blue mussels treated with these techniques.

3.1 Materials and Methods

3.1.1 Green Crab Collection

Juvenile green crabs (carapace width (CW) 2.5-16 mm, mean=9.7, SD=3.7, n=190) were collected by hand from the intertidal zone at low tide between August and October 2012 from two sites (North Harbour and Southern Harbour) in the Northern area of Placentia Bay, Newfoundland, Fig. 3.1, 3.2. Each individual was held live in open 20 mL glass vials with water from the collection site. Animals were transported to the laboratory and kept at room temperature (~20° C) for less than 12 hours before experiments were conducted.

Fig. 3.1: Map of North Harbour (Red square), Southern Harbour (Blue Square) Placentia Bay, Goose Cove primary adult green crab collection site in Area 2 with juvenile collection occurring in Areas 1 and 2.

Fig. 3.2: Map of Newfoundland displaying North Harbour Placentia Bay and Notre Dame Bay.

3.1.2 Green Crab Mitigation Trials

Groups of 60 juveniles (triplicates of 10, plus corresponding control animals, 720 total) were subjected to one of 12 treatments, Table 3.1. Juveniles were individually poured into salt water soaked tea bags and then subjected to either room temperature (control) or heated (experimental) salt water baths for either 5 seconds or 1 minute durations using a Fisher Scientific model 2332 water bath. Each animal was then returned to ambient holding vials with clean salt water of the

same salinity they were collected from in the field. Salt water used in trials was obtained from the aquaculture facility, Marine Institute at 32 ppt, filtered and sanitized using UV. After 10 minutes of recovery the activity level and number of limbs lost for each animal were observed and assigned a number, Table 3.2. Overall average for each treatment was calculated and direct comparisons were made for duration times within each temperature and then among all treatments and the controls.

Table 3.1: Mitigation treatments for juvenile green crab.

No.	Water Temperature (°C)	Duration (sec)	Treatment name
1	20	5	20D
2	25	5	25D
3	30	5	30D
4	35	5	35D
5	40	5	40D
6*	40	60	40 1min
7	45	5	45 D
8*	45	60	45 1min
9*	50	5	50D
10*	50	60	50 1min
11*	55	5	55D
12*	55	60	55 1min

*Treatments used on both juvenile crab mitigation trials and NRA

Table 3.2: Juvenile crab responsiveness scale modified from (Forrest and Blackmore 2006). Number coded to activity level response

1	Dead, no antennule activity
2	Critically weak, partial recovery, lethargic movement, unable to right themselves, sporadic antennule activity
3	Unaffected, makes a full recovery, alert posture, consistent antennule activity

3.1.3 Neutral Red Assay

The lysosomal destabilization assay using neutral red has been shown to be a good predictor of thermal stress in a variety of marine invertebrates including blue mussels (Lowe et al. 1995a; Harding et al. 2004; Vickerson 2009). Stress level is a direct measure of bivalve health and can predict future growth success. In unstressed cells lysosomes will accumulate and retain the neutral red dye for an extended period of time. Once destabilized (or stressed) the dye will leak into the cytosol of the cell through the damaged membranes (Moore 1980). Because neutral red is a weak cytotoxic compound, it too will act as a secondary stressor (Lowe et al. 1995b) and all cells will become stressed eventually. The longer the retention time the lower the stress level of the haemolymph cells and the individual organism.

Neutral red assays were conducted on mussels exposed to mitigation techniques identical to those employed on juvenile green crab, to determine if these treatments would cause stress and potential reduced growth in the mussels. Blue mussels, *Mytilus edulis,* with shell lengths 45-55 mm (mussel seed socked in Newfoundland is between 25 and 45 mm and anything under 55 mm is below market size) from South Arm, Bay of Exploits, Newfoundland. The mussels were cleaned and washed and donated to this experiment by Norlantic Processors and held in a Fukui trap suspended in a flow through tank at the Northwest Atlantic Fisheries Center (DFO St. John's, Newfoundland). After a minimum one week acclimation in tanks, (unfed at ambient temperatures and salinities) individual mussels were subjected to either room temperature (control) or heated (experimental) salt water treatments, Table 3.1 using the same methods as for juvenile crab.

The five most lethal treatments of the 12 used on juvenile green crab were applied to seed mussels to test stress levels. Blood samples were taken from the adductor muscle of each mussel immediately after each thermal shock treatment and neutral red assays were performed based on methods from Wyatt et al. (2013) with some modification to the duration of reaction. For each treatment group six mussels were randomly selected and removed using scissors to detach byssal threads from the Fukui trap and treatments were administered using a Fisher Scientific model 2332 water bath. Six mussels per treatment were chosen for the stress index in this experiment. Mussels collected during the late summer and early fall typically have low variability in stress response (Harding 2003).

Approximately 0.1 mL of haemolymph was extracted from the posterior adductor muscle using a 1 mL syringe and 21 gauge needle filled with 0.3 mL of physiological saline corrected to pH 7.2. The needle was removed after extraction to reduce cell damage while removing the solution from the syringe and transferred into an Eppendorf ® tube, inverted and held on ice. Forty µL of the haemolymph solution was pipetted onto slides pretreated with poly-L-lysine (20 µL PLL in 100 µL distilled water) and cells were left to incubate and adhere for 15 minutes on slides in individual humidity chambers. After adhesion, 40 µL of working neutral red dye solution was pipetted onto sides and further incubated for 15 minutes. After the dye has had time to enter haemocytes a cover slip was placed and the first reading was made. By using a compound microscope under 40x magnification, 50 haemocytes were assessed and assigned a score (Table 3.3). Readings were made 15 minutes after the initial reading and then every 30 minutes for up to 120 minutes. Readings continued until 50% of the cells were stressed (level 2 and higher) or there was no change in stress level for two consecutive readings.

Table 3.3: Haemocyte rating system for stress quantification in blue mussels (based on Wyatt et al. 2013).

1	Low stress, lysosomes clearly defined in the cytosol
2	Moderate stress, increase in lysosome size
3	Moderate/high stress, leakage of dye from lysosomes to cytosol
4	High stress, increased membrane degradation and cell lysis

3.1.4 Data analysis

Data were analyzed using IBM SPSS Statistics 19. One-way analysis of variance ($p<0.01$) was used to determine significant treatments for green crab mitigation and paired t test was used to compare neutral red retention times of control and treatment mussels.

3.2 Results

3.2.1 Green Crab Mitigation Trials

The results of the 12 mitigation treatments are shown in Table 3.4 and Fig. 3.3.

Table 3.4: Descriptive statistics of mitigation treatments

Treatment	Mean	SD
Control	3.00	0.00
20D*	3.00	0.00
25D*	3.00	0.00
30D*	3.00	0.00
35D*	3.00	0.00
40D*	3.00	0.00
40 1min	1.83	0.46
45D*	3.00	0.00
45 1min	1.00	0.00
50D	2.03	0.41
50 1min	1.00	0.00
55D	1.00	0.00
55 1min	1.00	0.00

* Not significantly different from the control

Some of the treatments were found to be significantly different (ANOVA; $F_{(11, 348)} = 830.186$, $p < .05$).Tukey post hoc analysis revealed that several treatments differed from one another 40 °C 1 min (mean=1.83, SD= 0.46), 45 °C 1 min (mean =1.00, SD=0.00), 50 °C Dip (mean =2.03, SD=0.41), 50 °C 1 min (mean =1.00, SD=0.00), 55 °C Dip (mean =1.00, SD=0.00) and 55 °C 1 min (mean =1.00, SD=0.00) were significantly different from the control and treatments, Table 3.4.

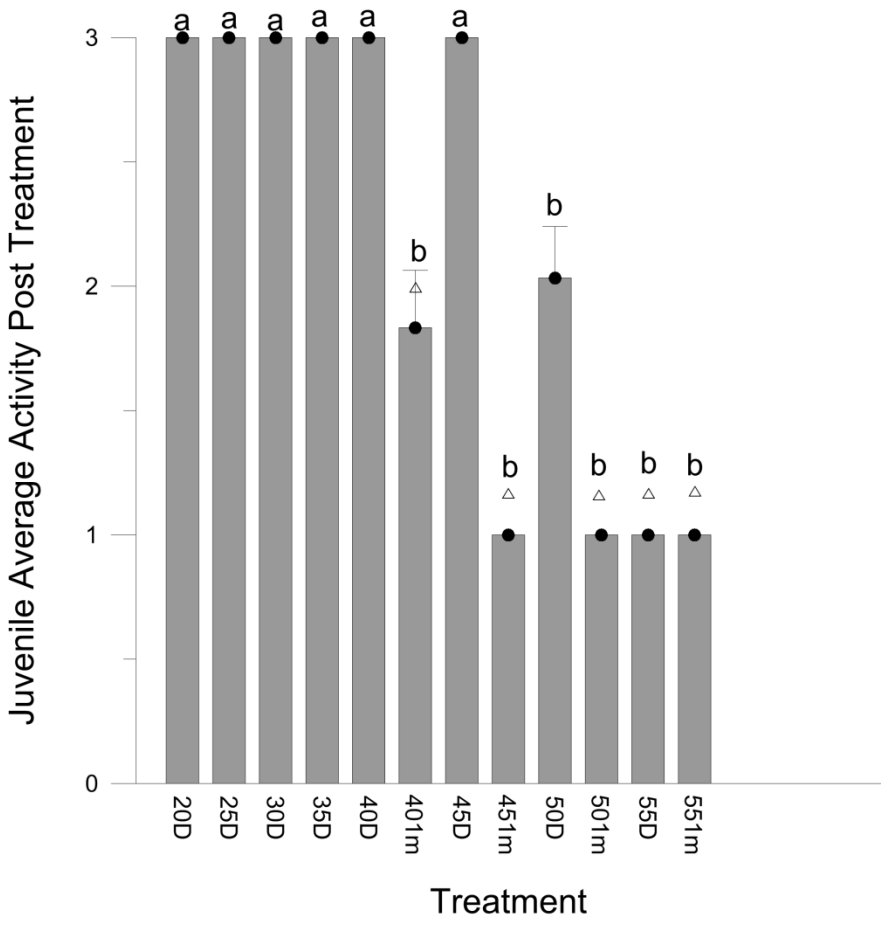


Fig. 3.3: Juvenile crab average activity results post treatment for 12 treatments. D=dip, 1 m=1 minute. Bars representing average response (+/- SD) for n= 30 crabs/treatment. Significant treatments from the control indicated with triangles.

3.2.2 Neutral Red Assay

The results of the five NRA treatments are shown in Fig. 3.4. There was no significant difference in neutral red retention time among the treatment and the control mussels. None of the thermal

stressed mussels reached level 4 stress levels or higher, even after the allotted amount of time (120 minutes).

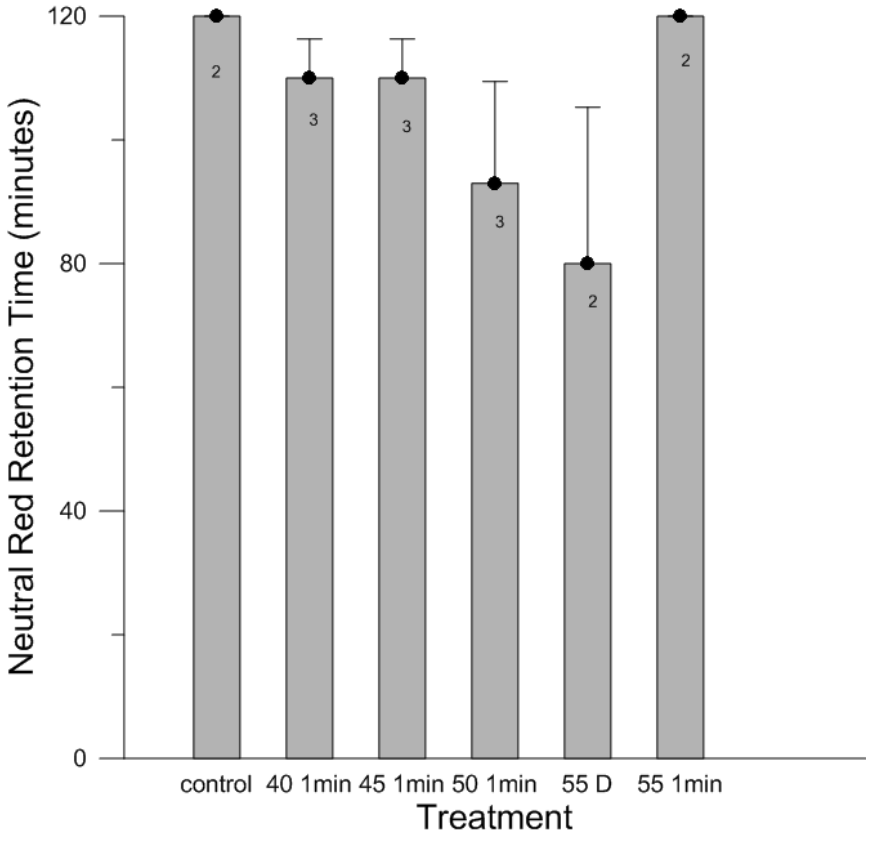


Fig. 3.4: Neutral red retention (NRR) time for control (20D) and five treatments and the highest stress level reached during mussel thermal exposure trials. D=dip, 1 m=1 minute. Bars represent the mean (+/- SD) of N=6 mussels for each treatment. Numbers in each bar represent haemocyte rating.

3.2.3 Combined Results

The final result from this study is the choice of the most suitable treatment while combining the two factors investigated. This is the combination of the juvenile green crab mitigation trials and

then their applicability with regards to stress response on mussel seed that would also be exposed to these treatments in practice when treating seed on a mussel aquaculture site. From Fig. 3.5 we can see that all five effective mitigation techniques for juvenile crab culling did not cause significant stress on mussels. The treatment that had the most effective culling while having the highest neutral red retention time and lowest stress response was 45°C 1 minute, Fig. 3.5.

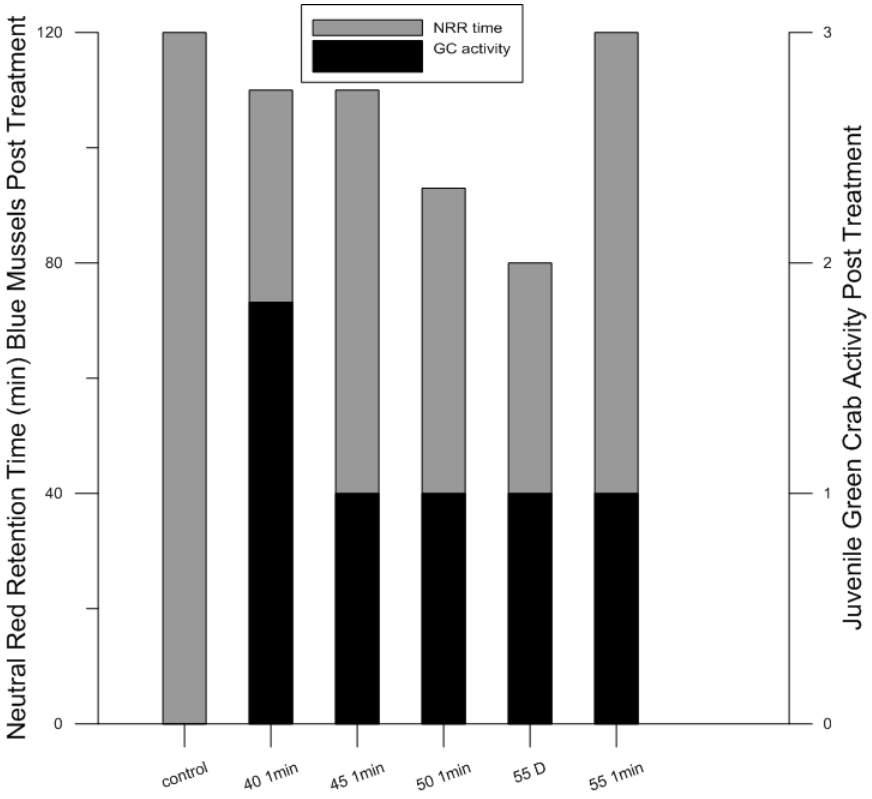


Fig. 3.5: Juvenile green crab average activity post effective treatments and corresponding mussel NRR times. D=dip. N=180.

3.3 Discussion

Green crab temperature tolerances are between -1 and 31°C and the limits for feeding and efficient cardiorespiratory functioning are approximately 5-25°C (Camus et al. 2004). The

animals for this study were collected at ambient temperatures in Placentia Bay ranging between 18 and 25°C, making the animals used here at the upper limit of regular metabolic functions. CTmax or critical thermal maximum increases after heat shock; the stress response was short term in the present study and so would contribute to the ability to withstand short term fluctuations in temperature (Cuculescu et al. 1998). Starting with a higher tolerance range at collection, crab treated with temperatures of 20, 25, 30, 35 and 40°C were not adversely affected in the present study as they can realistically handle this relatively small fluctuation.

Green crab inhabit intertidal areas where other less motile animals have reached summer high temperatures greater than 25°C e.g., limpets reaching 29°C and barnacles reaching 38°C (Taylor and Wheatly 1979). A crab that can tolerate a temperature maximum similar to these other intertidal organisms for a very short time period would not be exposed to a temperate change drastic enough to cause physiological damage and death. When there is a more drastic difference between temperature of acclimation and shock there is a lower CTmax temperature. In the case of crab acclimated to 6°C, CTmax was reached at 34.7°C (at a rate of 1°C /hour) a difference of 27.7 degrees warmer (Kelley et al. 2011). This temperature difference is comparable to the degree difference between the present study's acclimation temperature for juveniles of approximately 20°C and the effective culling temperatures of 45, 50 and 55°C. This is the first study to examine short term temperature shocks on *C. maenas* of this small size. The present study's thermal shock was much more rapid than in Kelley et al. (2011) which would explain why the CTmax here was higher than 34.7°C, at 45°C. This would also explain why trials using 20, 25, 30 and 35°C herein were not drastic enough to cull juvenile *C. maenas* in our experiments. Forrest and Blakemore (2006) found that green lipped mussels could withstand 55°C for five seconds when evaluating mussel health post treatment using gape tests and byssus

reattachment time. This is similar to the findings presented here for another mussel species, the blue mussel.

All organisms in this study trial elicited a similar behavioral response to increasing temperature, which was characterized by initial hyperactivity, then muscular spasms, followed by lack of movement and finally relaxed open abdomens also seen in Kelley et al. (2011). As treatment temperature increased it was observed that animals would immediately start to convulse as soon as they were immersed, no matter if the exposure time was 5 or 60 seconds. This observation is indicative of reaching CTmax (Kelley et al. 2011).

The mussels which were also subjected to these treatments were found to be unaffected with respect to stress levels measured by neutral red retention time. It was hypothesized that there would not be a significant stress response from mussels and, if any, would increase with temperature (decrease in neutral red retention time). This trend did occur for treatments 40°C 1 min, 45°C 1 min, 50°C 1 min and 55°C dip. However 55°C 1 min, the highest temperature and exposure time, had the same stress response as the control which was not exposed to any stressor treatment. This unexpected result may be due to large mean error in responses for individual mussels under this treatment, n = 6 were therefore insufficient to represent this treatment's response. But because all mussels were in the same condition to start we are confident in our findings. Future studies should use a higher treatment number to eliminate this error. The size of mussel seed used in this experiment was on the larger end of seed sizes used for grow out in Newfoundland (25-45mm) and there may be a difference in stress tolerance for smaller sizes than used here. Vickerson (2009) tested seed 30-40 mm and also found low stress levels for mussels temperature shocked for four hours with temperatures 10 degrees above ambient. Considering the effectiveness of each treatment in mitigating or culling 100% of juvenile *C.*

maenas and causing little to no stress on the mussels also exposed to the same treatments, water temperatures of 40, 45 and 50 and 55°C for the duration of one minute and 55°C dip were chosen as the most appropriate due to very high neutral red retention times, and low stress response. None of the neutral red assay treatments were significantly different from the control but the treatment that caused the least amount of stress on the mussels while culling 100% of green crab juveniles was 45°C for one minute, so is the most appropriate treatment from a practical perspective.

Further investigation is needed to see if this mitigation technique is effective when juvenile green crab and blue mussels are treated together in the lab as well as in the field, and to make adjustments to temperature and duration to maintain these parameters. It is also advisable that the feasibility of this technique be tested with mussel growers in Newfoundland with mussels of smaller size. Upon approval of the methods by industry for farm scale operations further design and engineering would be needed to create a system that could easily produce and maintain the mitigation conditions on the farm site for an entire seed harvest.

4.0 Summary

4.1 Overall objective of the study

Invasive species have many strategies that allow them to tolerate conditions in non-native areas. Advantages like better and more aggressive foraging and habitat usage, broad and easily changed diets, better predator awareness, defense and avoidance, more robust size, advantageous migration patterns and adaptive reproductive methods compared to native species. Reproduction is one of the most important factors which permit a population to grow and take hold in a new environment. It has been found in other circumstances that green crab and other invasive crustaceans make adjustments to regular life strategies to take advantage of seasonal or physical conditions in invaded areas. The new population in Newfoundland, as part of the cold tolerant strain of Northern European origin, does share many traits with the other Atlantic Provinces populations. As the presently studied population is the most recent and farthest east in Canada there are many questions as to whether there are some differences for these green crab in this new environment in comparison to its neighboring populations, Therefore, studying the Newfoundland population of green crab reproductive strategies will help to determine if they have made further changes to survive in Newfoundland waters. This information may provide knowledge on how best to combat the damage this population is having on the native biota. Knowing the timing and duration of the different reproductive and early life stages of green crab in Newfoundland can help to avoid the riskiest times of the year for aquaculture transfer, for industry and recreational boating in order to decrease the likelihood of further spread around Newfoundland. This knowledge can then be transferred to spread prevention and management near and around mussel aquaculture sites.

4.2 Green crab reproduction strategies

Chapter 2 investigated the reproductive biology of the North Harbour, Placentia Bay green crab population to pinpoint life history stages and strategies. Estimations of physiological maturity size for both males and females, timing for mating behaviors, time lines to estimate time spent at each stage of egg development and larval release in females were determined. This information has been used to establish minimum size thresholds for pilot mitigation efforts in the area and will continue to help pinpoint the best times to target a particular life stage for this region. When these findings are compared with one of the geographically close cold tolerant populations, Prince Edward Island, males and females are mature at a smaller size, females spend equivalent if not shorter time frames ovigerous and successfully release larvae earlier and in colder water temperatures. This supports the hypothesis that the Newfoundland population has made adjustments for reproduction in comparison to other cold water populations. These findings are partially based on observations of the population and need further experimentation to confirm that inferred timelines are realistic for all stages of reproduction.

4.3 Green crab mitigation

Chapter 3 used conclusions made on the life stages of Placentia Bay green crab to investigate a mitigation technique suitable for removing juvenile green crab from mussel seed. I hypothesized that exposing juveniles to a series of heated salt water immersion treatments would cull and prevent transport with mussel seed. It was confirmed that exposing juvenile green crab to heated salt water for no longer than one minute at 45°C is sufficient to cull the crab. These methods, which in practice will treat the mussels that green crabs are taking shelter in, did not cause any

significant physiological stress to the mussel seed. This is important for mitigation techniques so that mussel seed is not compromised; growth rate and meat yield is maintained while effectively removing the green crab.

4.4 Importance of study

Coastal marine invasive species are very difficult to eliminate and control once they have entered and established a population in a non-native area. With minimal natural barriers and high usage of coastal recreational and industrial vessels, natural spread with currents and weather events, some level of spread is unavoidable. Collecting the most knowledge possible on a new species in its new environment and understanding its biology is the most important factor in designing mitigation and control measures as well as making changes to policy to limit human assisted movement between infected and non-infected areas. By studying the reproduction of the Newfoundland population of green crab it is now known when and how often they reproduce, how long their larvae are in the water, the difficulty in trapping ovigerous females, distribution and size of maturity for males and females.. It can now be shown that if we target mature female crab between June and September catches will be very low as they are ovigerous and inactive. It can also be suggested that the best time to target both males and females are in the early spring months prior to May and in the fall before and after mating behavior occur. It was observed that they will be foraging and developing their gonads and are more likely to be attracted to baited traps. To target newly settled juvenile crab, July to October in intertidal areas at low tide just after settlement was optimal. Knowledge regarding life stage can be used in preventative measures to decrease the spread of early life stages of green crab by the mussel aquaculture

industry. In infected mussel sites we can now advise farmers when to avoid transfer of seed to an uninvaded site or market sized product to the processing facilities in areas that are not currently invaded. Management decisions using advice using treatment methods on mussel product will decrease or prevent the spread of green crab while making regular operations continue as smoothly as possible at the farm site.

4.5 Future directions

This study provides a framework for future green crab reproductive strategy studies. This study focused on water temperatures at collection sites. Future studies that include additional oceanographic parameters, seasonal trends and a wider geographic area would be valuable information for comparison. This study's lab mitigation and mussel stress trials also laid a framework for combined mussel and juvenile crab trials in the lab as well as field trials to further test the feasibility of these methods in practice on the farm site in the short and long term in Newfoundland waters. Additionally this juvenile green crab mitigation method could be used in other areas where aquaculture transfers would be a concern. This study along with others in PEI are the only in depth looks at green crab reproduction the Atlantic provinces of Canada. The methods used in this study could translate into a spatial timing comparison for the greater Atlantic population of green crab. This could add to a best practices framework and prevention plan for the greater region to prevent further introductions of green crab and other aquatic invasive species between the Atlantic Provinces.

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