Surviving winter on the Rock: How European green crab (*Carcinus maenas*) utilizes thermal tolerance and habitat use to tolerate the cold-water temperatures in Newfoundland.

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Abstract

European green crab, *Carcinus maenas*, is a successful invasive species and has established populations on every continent, except Antarctica. To date, the coldest wintertime water temperatures experienced by green crab in the northeastern Atlantic are observed in Newfoundland, where temperatures regularly drop below 0 °C during the winter. Previously, thermogeographic models predicted that Newfoundland winter water temperatures are too cold for green crabs to survive and therefore to colonise Newfoundland. Previous research on thermal tolerance of green crabs has primarily focused on their upper thermal tolerance; and any investigation into their lower thermal tolerance has only considered acute responses. In this thesis, I use laboratory experiments to study multiple physiological and behavioural responses of green crab to long-term exposure (ranging from 6 days to 5 months depending on experiment) to water temperatures at the lower end of their thermal tolerance. I also use field experiments to investigate over wintering movement behaviour, habitat use, and feeding behaviour of green crab in Newfoundland. With this combination of lab experiments and *in situ* field studies, I show that green crab can survive several months exposure to cold water temperatures as low as -1 °C. Temperatures between 4 to 6 °C trigger a torpor-like response, with reduced cardiac and metabolic rates, locomotive activity and feeding behaviour. However, movement and feeding do not completely cease, which indicates that green crab enter a 'torpor-like' state rather than true hibernation. Additionally, green crabs are absent from the intertidal zone during the winter but they do not leave the shallow bay for deeper waters, contradicting hypotheses that green crabs either leave the shallow subtidal zone for deeper, more stable water temperatures, or entered a state of hibernation and bury in the mud or hide under a shelter for the duration of winter. These results

show green crab can tolerate the extreme cold temperatures during Newfoundland winters without altering their behavioural strategies from that used in their native range, suggesting they may be able to survive long periods at even colder temperatures, indicative of potential further northward geographical range expansion.

General Summary

Green crabs have a very wide tolerance of water temperatures from 0 °C to 32 °C. However, water temperatures in Newfoundland, a geographic area green crabs colonized approximately 15 years ago, regularly drops below 0 °C for multiple months during the winter. How green crabs survive the winter here is not known, therefore I used lab experiments and field studies to investigate the physiological and behavioural strategies used by green crabs in Newfoundland to survive the cold winters. I found that crabs avoid intertidal habitats, where they could risk air exposure, and reduce, but not cease, their movement and feeding behaviour, entering a 'torpor-like' state but not a true hibernation.

Co-authorship Statement

All primary intellectual and practical contributions reported in this thesis were completed by Molly L. Rivers and Iain J. McGaw. Two to three publications are planned from the data produced in this thesis. The first data chapter will form one to two publications, with the behavioural and physiological data potentially being split into separate publications. Authors on these papers would be myself and Iain McGaw. The second chapter will form a separate paper, the authors will include: Molly Rivers, Iain McGaw, Cynthia McKenzie, Kyle Matheson, Michael Piersiak, Philip Sargent, Andrew Perry, George Bishop, Qiwu Jiang and Russel Wyeth.

Chapter 2

I, Molly Rivers, identified the research questions, established experimental design, carried out data collection and analysis, created figures and tables, and wrote the chapter. Iain McGaw helped refine the objectives, advised on experimental design and revised all aspects of the chapter.

Chapter 3

I, Molly Rivers, identified research questions, determined experimental designs, carried out data collection and analysis, produced all figures and tables and wrote the chapter. Iain McGaw advised on design and analysis of lab and field experiments and revised the chapter. Kyle Matheson, Michael Piersiak, Philip Sargent, Andrew Perry and George Bishop assisted with designing the acoustic array, and deploying and retrieving the receivers. Michael Piersiak assisted with analysis of acoustic array location and activity data, conducting initial, preliminary analysis and data organization and shared and edited code for further analysis and figure generation. Non-parametric analysis of total

time spent in each habitat, collected from the mesocosm experiment was carried out Qiwu Jiang. Non-parametric analyses of monthly protein serum concentration during the long term survival experiment was conducted by Russel Wyeth.

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Chapter 1

Introduction to green crab biology, invasion history, and thermal tolerance

Introduction

Green crab, *Carcinus maenas* (Linnaeus, 1758), is a small decapod crustacean in the family *Portunidae.* This species reaches a maximum carapace width of ~ 10 cm (McGaw *et al*., 2011) and average lifespan that varies from 3-7 years, depending on geographical location (Klassen and Locke, 2007). Green crabs are native to northwest Europe and northern Africa, but over the last 200 years have expanded their range to include every continent, except Antarctica (Carlton *et al.*, 2003; Klassen and Locke, 2007).

Another morphologically similar species co-exists within the Mediterranean Sea and southern-most part of green crabs' native range, *Carcinus aestuarii* (Nardo, 1847). Amidst much debate, morphological characteristics (Cohen *et al.*, 1995) and molecular genetic techniques (Roman and Palumbi, 2004) have shown these two species to be distinct. Using molecular techniques, invasive populations of *C. aestuarii* have been found in Japan and South Africa and have confirmed that the populations in North America and Tasmania contain only *C. maenas* (Geller *et al.*, 1997). No invasion of *C. austuarii* independent of *C. maenas* has been identified (Darling and Tepolt, 2008). Several studies suggest that the local topography and ocean circulation between the Mediterranean Sea and Atlantic Ocean (Gibraltar Straight) limits the speas of dispersal of larvae, explaining the separation of these two species within the native range, and the formation of two *C. maenas* lineages, originating from the north Atlantic and the Mediterranean populations (Patarnello *et al.*, 2007; Marino *et al.*, 2011). Furthermore, this geographic separation of the two species is observed for the larval stages (Darling and Tepolt, 2008).

Green crabs inhabit sheltered bays with soft sediment and rocky shore intertidal and estuarine habitats (Klassen and Locke, 2007; Amaral *et al.*, 2009). In their native range, they are roughly distributed into three groups: (i) young crabs that spend all their time above the low tide mark and hide under cover when exposed to air at low tide, (ii) crabs that move into the intertidal zone with high tide to feed and retreat below the tide line with the receding tide, and (iii) larger crabs that spend all their time in the shallow subtidal zone (Crothers, 1968). Juvenile green crabs' commonly occur in structurally complex habitats, such as mussel beds, rocky shores, eel grass beds and filamentous algae (Moksnes, 2002).

Reproduction and life cycle

Decapod crustaceans have a hard exoskeleton that they must shed in order to grow. Female crabs must also moult immediately prior to mating (Berrill and Arsenault, 1982). As they get larger, growth rate and frequency of moulting decreases. After moulting the underside of adult green crabs is green in colour, but as the carapace ages it becomes dark red (Wolf, 1998). Therefore, two main colour morphs exist: (i) green, in which rapid growth and frequent moulting is common, and (ii) red, which have greater reproductive activity and longer periods of intermoult. The green morphotypes tend to be better adapted to environmental change, than the red morphotypes (Styrishave *et al.*, 2004). Timing, seasonality, and synchronicity of moulting appear to vary between populations and geographical location (Berrill, 1982; Poirer *et al.*, 2016).

Green crabs are a highly successful invasive species, which is in part, due to their high fecundity and planktonic larvae. The fecundity in an invasive green crab population was estimated at 140,000 – 200,000 embryos per crab (Audet *et al.*, 2008). Green crabs have

two distinct annual mating events, the primary event, in which larger crabs reproduce, occurs during the winter and the secondary event, when smaller crabs mate, occurs in the summer (Lyons *et al.*, 2012). Green crab eggs and larvae have a reduced temperature tolerance compared to adults, thus spawning times may be altered depending on local conditions (Berrill, 1982; Hidalgo *et al*., 2005). For example, in Placentia Bay, Newfoundland, female crabs produce larvae once annually and spend shorter periods in an ovigerous phase than the native populations (Best *et al.*, 2017). Additionally, green crabs in Prince Edward Island reproduce later in the year compared to native and more southernly populations (Audet *et al.*, 2008).

Green crab larvae are planktonic with four zooel stages and one megalopal stage. They remain planktonic for about 90 days, during which time they conduct daily vertical migrations in the water column (Zeng and Naylor, 1996; dos Santos *et al.*, 2008). During the final larval stage, they settle on the benthos before metamorphosis into a juvenile crab occurs (Leignel *et al.*, 2014). In their invasive range the life cycle can vary from that in their native range. For example, green crabs on the coast of Maine, USA, mature slower, have longer larval phases, and have longer life spans, which has been accredited to the colder water temperatures in this region (Berrill, 1982). This extended larval period may allow them to colonize new areas with greater success.

Tolerance to temperature variability

Green crabs are tolerant to a broad range of abiotic environmental conditions. They can tolerate large variations in salinity, hypoxia and temperature, and withstand extended periods of air exposure and starvation (see Appendix 1). They are also resistant to pathogens and diseases (Leignel *et al.*, 2014). This plasticity aids their ability to colonize

new areas and facilitates survival in habitats that experience a broad range of conditions, such as the intertidal zone.

Temperature is undoubtedly the most important environmental factor affecting the distribution, behaviour, and physiology of aquatic ectotherms, including green crabs (Kalssen and Locke, 2007; McGaw and Reiber, 2015). Adult green crabs can survive temperatures from < 0 ˚C to ~38 ˚C (Tepolt and Somero, 2014) but their larvae require water temperatures above 10 ˚C for several months for successful development (Berrill, 1982; Hidalgo *et al*., 2005). Although green crabs continue to invade new locations and expand their range, they have not colonised polar or tropical regions, most likely due to the temperature limitations of their larvae. Minimum seasonal sea surface temperature has been suggested as a highly influential factor in determining the northern range expansion and limit of green crabs (Audet *et al.*, 2003; Hidalgo *et al.*, 2005; Jefferey *et al.*, 2018).

Temperature affects crustaceans in myriad ways, including behavioural functions and metabolic activity (see Appendix 1 for overview of decapod behaviour and physiology), protein synthesis and maintenance, immune function, and membrane structure (Truscott and White, 1990; Somero, 2002). Heart rate and metabolic rate generally increase with temperature (Aagaard, 1996; Styrishave *et al.*, 2003; Willmer *et al.,* 2005; McGaw and Reiber, 2015) until they reach a critical thermal maximum temperature (CT_{max}), the temperature at which locomotive activity is disrupted and animals cannot recover leading to death (Taylor *et al*., 1977; Frederich and Pörtner, 2000; Levington *et al*., 2020). Populations of green crabs that experience varying thermal regimes differ in heat shock protein synthesis and CT_{max} (Kelley *et al*., 2011). Correspondingly, CT_{max} is higher in

crabs that are acclimated to higher temperatures (Cuculescu *et al.*, 1998). *In situ* experiments have shown temperature to be one of the most influential factors for determining cardiac activity; heart rate increases even within a small temperature range (Aagaard, 1996). Metabolic rate similarly increases with temperature in crustaceans (Whiteley *et al.*, 1997). Temperature mediated cardiac and metabolic responses in crustaceans vary depending on rate and magnitude of the change and whether the change is acute or chronic, decreasing or increasing (McGaw and Reiber, 2015).

Temperature also affects behaviour. At high temperatures (28 °C) green crabs exhibit an emersion response, whereby they exit warm water into air (Taylor and Wheatly, 1979). It has been hypothesised that evaporative water loss in air assists in cooling (Taylor and Wheatly, 1979), thus potentially driving this behaviour. Green crabs forage more consistently and efficiently in warmer waters and reach optimum foraging rates at ~ 12 °C (Elner, 1980; Bélair and Miron, 2009a). Foraging rates increase with increasing temperature in the presence of conspecifics and heterospecifics as compared to solitary individuals (Bélair and Miron, 2009a). Movement and agonistic activities decline with decreasing temperature (Bélair and Miron, 2009b). Additionally, after exposure to cold stress green crab acclimated to colder temperatures return to normal activity levels more rapidly than warm acclimated animals (Tepolt and Somero, 2014). However, a few studies have investigated behavioural responses to cold temperatures consider temperatures < 5 °C. Miron *et al.* (2002) found reduced predation on shellfish species at 0 ˚C, and Tepolt and Somero (2013) reported reduced heat tolerance in green crabs acclimated to 0 ˚C compared to warm acclimated crabs. Young *et al.* (2006) reported decreased walking speed with declining temperatures (tested to -2.5 ˚C) and indicate that

green crab enter behavioural torpor at ~ 0 °C and have an increased mortality rate after short exposures to sub-zero temperatures, yet this remains relatively unknown.

Seasonal Behaviour

During the summer months green crabs are more active and forage more successfully (Elner, 1980; Young *et al.*, 2006; Bélair and Miron, 2009a; Matheson and Gagnon, 2012). Torpor has been suggested as a possible over wintering strategy for green crabs, with reduced locomotor activity at ~ 5 °C and cessation at 0 °C (Welch, 1968; Berrill, 1982; Young *et al.*, 2006). Heart rate and metabolic rate also decline at these temperatures, suggesting reduced energy requirements and further supporting a state of torpor at these temperatures (Breteler, 1975; Camus *et al.*, 2004; Tepolt and Somero, 2013; 2014). Additionally, a mark recapture study reported crabs were recaptured closer to their release point in January than in April, suggesting lower rates of movement in the winter (Ameyaw-Akumfi and Naylor, 1987).

During the winter crabs appear to remain in the subtidal zone, with a few small individuals in the intertidal zone (Edwards, 1958; Naylor, 1962). Estuarine populations overwinter (once temperatures fell below 10 °C) in downstream areas, remaining fairly inactive during the winter months (Welch, 1968; Sharp *et al*., 2003; Zarrella-Smith *et al*., 2022). This migration may be caused by a combination of reduced temperature and salinity (Gomes, 1991), noting that in some open coastal locations (with constant near or full salinity) offshore winter migrations do not occur (Naylor, 1962). However, the specific movements and behaviour of green crabs within the subtidal zone during the winter still remains largely unknown.

Burying has also been suggested as an overwintering strategy (Dow and Wallace, 1952; Welch, 1968, see Appendix 1 for more information). Reduced heart rate in buried green crabs is consistent with and suggestive of periods of inactivity (Cumberlidge and Uglow, 1978). Implying that burying may be a strategy for enduring winter conditions. However, the duration and extent to which green crabs remain buried is still unclear.

Global Invasion History

Green crabs are a highly successful invasive species and have now established populations on every continent, except Antarctica (Klassen and Locke, 2007). When green crabs invade a new geographical location, their subsequent spread can be extensive. For example, green crabs were first reported in San Francisco Bay, California, in 1989 (Cohen *et al.*, 1994; Grosholz and Ruiz, 1996) and in 1997 they were collected almost 300 km to the north, in Oregon (Jamieson *et al*., 1998; Behrens Yamada *et al*., 1999). By 1999 they were reported in Barkley Sound, British Columbia, a further 225 km north (Jamieson *et al.*, 2002), extending into the Salish Sea by 2018 (Brasseale *et al*., 2019). On the East coast of North America green crabs were first recorded in Massachusetts in 1817 (Glude, 1955; Grosholz and Ruiz, 1996), by 1937 they had reached Maine and the first record in Canadian waters was in Passamaquoddy Bay, New Brunswick, in 1951 (Scattergood, 1952.). Green crabs now occur from Virginia to Newfoundland, a distance of more than 2000 km (Carlton and Cohen, 2003; Klassen and Locke, 2007).

In contrast, in other locations their spread has been relatively restricted. One such location is the Cape peninsula of South Africa where green crabs were first reported in 1983 but have not been reported outside this locale since (Joska and Branch, 1986; Le Roux

et al.,1990). This lack of dispersal may reflect the high levels of wave exposure in this area (Griffiths *et al.*, 2009). There is some debate over when green crabs first colonised Victoria, Australia, however they were abundant here by 1902 (Fulton and Grant, 1902). Since then, their spread has been limited the southeast coast of Australia and to Tasmania (Thresher *et al.*, 2003; Burden *et al.*, 2014). Green crabs were first observed on the Atlantic coast of Patagonia, in southern Argentina in 2003 (Hidalgo *et al.*, 2005) and have since expanded their range 250 km north along the east coast of Argentina (Torres and González-Pisani, 2016). However, the degree to which this population will spread across the coast of South America is as yet unclear.

Recently, genetic analyses have determined that separate invasion events, originating from different parts of the native range, have occurred. For example, *Carcinus aestuarii* (*C. maenas'* sister species) was reported in Japan in 1984. By the 1990's it had spread across the south coast of Japan (Darling, 2011). However, in 1997 genetic analysis of the Japanese *Carcinus* populations revealed the presence of *C. maenas* haplotypes. This observation suggested a mixed species origin of the Japanese population of *Carcinus* (Geller *et al*., 1997; Darling, 2011). Similarly, genetic analysis of invasive populations revealed at least two *C. maenas* lineages, one originating from northern Europe (referred to as the northern lineage) and the other from Mediterranean Europe and northern Africa (referred to as the southern lineage) (Roman and Palumbi, 2004; Roman, 2006; Darling *et al.,* 2008). At least one hybridized population has also now been reported in southern Nova Scotia (Blakeslee *et al*., 2010).

Invasion Mechanisms

Carlton and Cohen (2003) suggested three major invasion episodes that resulted in the major spread of *C. maenas*: (i) around 1800, (ii) between the 1850's and 1870's, and (iii) between the 1980's and 1990's. The main mechanism for the first two invasion episodes (in the 19th century) likely originated from maritime activity via the ballast water of vessels, hull fouling, or other fisheries products (Cohen *et al.*, 1995; Carlton, 1999; Grosholz and Ruiz, 2002; Klassen and Locke, 2007). In the 1800's trans-continental journeys took weeks, and the lengthy larval period (~90 days) of green crabs means that larvae could survive to reach new coasts. The third invasion episode (1980's to 1990's) had many more potential mechanisms: ship ballast water, aquaculture and fishing industries, aquarium trade, research activities, and intentional or accidental release (Scattergood, 1952; Carlton, 1999; Grosholz and Ruiz, 2002; Klassen and Locke, 2007).

By the first invasion episode (~1800) the transatlantic trade route between Europe and North America was established and became a lucrative business connection by the middle of the eighteenth century (Natkiel and Preston, 1986), which would explain this first invasion of *C. maenas* in the northwest Atlantic. This first introduction resulted in the continued northward range expansion of *C. maenas* northward to Maine, New Brunswick, Nova Scotia and Prince Edward Island (Grosholz and Ruiz, 1996; Carlton and Cohen, 2003). The second major invasion episode worldwide (1850's to 1870's) resulted from the establishment of global trade routes leading to reports of *C. maenas* on the shores of Brazil, Hawaii, Myanmar, Panama, and Sri Lanka, however none of these reports resulted in successful colonisation (Boschma, 1972; Carlton and Cohen, 2003; Klassen and Locke, 2007; Leignel *et al.*, 2014). The established green crab population in Australia is also thought to have originated within this invasion episode (arriving in

Australia no later than 1890) in that they were reported to be highly abundant in 1900 (Fulton and Grant, 1902; Carlton and Cohen, 2003). Few new reports of *C. maenas* were recorded between the second and third invasion episodes. The colonisation of South Africa (1983) and California (1989), as well as the colonisation of Japan by the *C. aestaurii* and *C. maenas* (1984), resulted from the third invasion episode, beginning in the early 1980's (Joska and Branch, 1986; Le Roux *et al.*,1990; Cohen *et al.*, 1994; Grosholz and Ruiz, 1996; Geller *et al*., 1997; Carlton and Cohen, 2003; Darling, 2011).

Invasion History on the East Coast of North America

Green crabs have expanded their range on the East coast of North America over 2000 km in 200 years (Glude, 1955; Grosholz and Ruiz, 1996; Carlton and Cohen, 2003; Klassen and Locke, 2007). Reports and research efforts were scarce for the first hundred years following the first report of *C. maenas* in the northwest Atlantic (Massachusetts) in 1817 (Scattergood, 1952). However, by 1879 they were reported as far south as New Jersey (Smith, 1879; Scattergood, 1952). In 1905 green crabs were reported in southern Maine and roughly 200 km north by 1937 (Rathbun, 1905; Scattergood, 1952). The first instance of green crabs in Canada was reported in southern New Brunswick, in 1951 (Leim, 1951; Scattergood, 1952).

Since their discovery in Canadian waters in 1951, green crabs have established populations in every province in Atlantic Canada (McPhail, 1951; Klassen and Locke, 2007; DFO, 2011) and in the Magdalen Islands in Quebec (Paille *et al.*, 2006). Green crabs were first seen in Nova Scotia in 1953 where they spread rapidly across the south coast (MacPhail, 1953; MacPhail and Lord, 1954; Audet *et al.*, 2003; Klassen and Locke, 2007). The next record of their range expansion was in 1982 in north-eastern Nova

Scotia (Audet *et al.*, 2003). The first record of green crabs in Prince Edward Island was in 1997 on the Eastern coast (Gillis *et al.*, 2000; Audet *et al.*, 2003). Green crabs were later reported on the coasts of the Magdalen Islands, Quebec, in 2004 (Paille *et al.*, 2006), and then on the island of Newfoundland, NL, in 2007 (Klassen and Locke, 2007; DFO, 2011). They were initially sighted in Placentia Bay, on the south coast of Newfoundland and have since spread throughout Placentia Bay and Fortune Bay with a separate second population reported on the west Coast in 2009 (DFO, 2011; Lehnert *et al.*, 2018).

At least two distinct green crab invasion events occurred on the East coast of North America (Darling *et al*., 2008). Both invasions have distinct origins, from either the southern European lineage or northern European lineage (Darling *et al.*, 2008; Darling, 2011; Lenhert *et al.*, 2017; Jeffery *et al*., 2017; 2018). The northern European lineage is broadly defined as deriving from native populations between northern mainland Europe and the northernmost limit of their native range (in Iceland, the Faroe Islands, and Norway). In contrast, the southern European lineage derives from native populations from northern Africa and the Mediterranean (Roman, 2006; Darling *et al*., 2008). The initial invasion of Eastern North America in Massachusetts (in the early 1800's) originated from the southern European lineage. A secondary invasion event occurred some 180 years later in Nova Scotia, consisting of the northern European lineage. A contact zone now exists between southern New Brunswick and southern Nova Scotia where the two populations have hybridized (Darling, 2008; Jeffery *et al.*, 2017).

The two lineages differ in thermal tolerances, with greater cold tolerance in the northern lineage and greater resilience to warm temperatures in the southern (Tepolt and Somero, 2014). Moreover, genetic markers have been used to identify changes in cold tolerance

in both native and invasive green crab populations, suggesting genomic changes may occur over relatively short time periods (Tepolt and Palumbi, 2020). This plasticity in thermal tolerance between the two lineages could help to explain the broad latitudinal range of green crabs both in their native and invasive ranges.

Green crabs' invasion history of Newfoundland

Green crabs were first reported in Newfoundland in North Harbour, Placentia Bay, on the south coast in 2007 (Best *et al.*, 2009; McKenzie *et al.*, 2007). At the time of discovery, researchers inferred that green crabs had already been established in Newfoundland for several years (Blakeslee *et al.*, 2010). By 2010 their range had increased to include the entirety of Placentia Bay and westward into Fortune Bay (DFO, 2010). A separate, different and smaller population (Lehnert et al 2018) was also discovered in St Georges' Bay, some 600 km away, on the west coast (DFO, 2010). The geographic range in these two populations continues to expand, encompassing more of the south and west coast, however, they are yet to have establish a population on the north coast. This is potentially due to barriers to dispersal or due to the extreme cold over-winter sea-water temperatures (Jeffery *et al*., 2018). Their invasion in Newfoundland waters has had negative impacts on native eel grass habitat, commercially important shellfish populations and native crab populations (Matheson *et al*., 2012; 2014; 2016), as seen in other colonized locations (Grosholz *et al.*, 2011; Neckles, 2015).

Successful invasions of green crabs in new geographic locations relate to the degree of genetic variation in the establishing population (Darling *et al*., 2008; Darling, 2011). A genomic study identified the *C. maenas* population in southern Newfoundland as a sec-

ondary hybridized population, containing haplotypes from both the southern and northern lineages (Lenhert *et al.*, 2017; Jeffery *et al*., 2017; 2018). This hybridization event could, therefore, help to explain the successful establishment of green crabs in Newfoundland waters, a location previously predicted to have over-wintering temperatures too cold for their colonization (Compton *et al.*, 2010).

Study Aims

Carcinus maenas is one of the 100 most invasive species, rapidly spreading around the globe (Lowe *et al.*, 2000). Despite a previous assumption that waters of Newfoundland would be too cold for *C. maenas* to persist (Compton *et al.*, 2010) they are flourishing and spreading around the island. In cold years mass die offs of *C. maenas* in other areas have occurred (Crisp, 1964; Welch, 1968; Beukema, 1991), but in Newfoundland such die offs have not occured. Therefore, the aim here was to determine how *C. maenas* deals both physiologically and behaviourally with prolonged cold, typical of the waters around Newfoundland.

The research presented here is split into two broad sections, the first investigating individual level responses to cold exposure representative of the over-wintering sea-surface temperatures in southern Newfoundland. The second section examines the population-level habitat use and *in situ* behaviour of green crabs during the winter in Newfoundland. These two study themes aim to provide a greater understanding of how green crabs successfully colonised the generally cold coastal waters of Newfoundland.

The first chapter aims to (i) investigate the physiological (heart rate and oxygen consumption) and behavioural (locomotor activity and burying behaviour) responses of *C.*

maenas to declining temperatures, and (ii) understand their physiological and behavioural responses during extended periods of exposure to cold temperatures, representative of over-wintering sea-surface temperatures in southern Newfoundland. I hypothesise I will find: (i) a decrease in heart rate, metabolic rate and locomotor activity with decreasing temperature, (ii) reduced heart rate, metabolic rate and locomotor activity in crabs acclimated to winter temperatures (compared to those acclimated to summer temperatures), and (iii) an increase in burying behaviour with declining temperatures.

The second chapter investigates population level survival techniques utilised by *C. maenas* during the winter in Newfoundland. This study aims to: (i) observe *in situ* habitat use and locomotor activity behaviour of *C. maenas* during the winter, and (ii) understand feeding requirements during the winter and the impact this has on physiological condition. Various methodologies were used, including: a mesocosm experiment, long-term starvation trials, monthly catch per unit-effort surveys and acoustic location trackers (attached to green crabs in the field). The hypotheses for this section are (i) that fewer crabs occur in the sub- and inter-tidal zones during winter months compared to summer months, and (ii) that green crabs survive low temperatures and starvation for multiple months at a time (representative of winter conditions and duration in Newfoundland).

Chapter 2

Lower thermal tolerance of *Carcinus maenas*: Physiological and behavioural response to declining temperatures.

Abstract

Green crabs (*Carcinus maenas*) are a highly invasive species of intertidal crustacean that have increased their invasive range to include the majority of the east Coast of North America over the last two centuries. The northern most limit of their range on the east coast of North America is on the south coast of Newfoundland, Canada, where they experience extremely low seasonal water temperatures, reaching temperatures as low as -1 °C. Genomic studies have found two distinct lineages of green crabs in North America, the 'northern' lineage, originating from northern Europe and Scandinavia, and the 'southern' lineage, originating from southern Europe and northern Africa, resulting from multiple invasion events. Green crabs' tolerance of a large range in temperature is presumably one of the most important characteristics accounting for their invasive success, in that populations from the 'northern' lineage likely have greater cold water tolerance whereas populations from the 'southern' lineage have greater warm water tolerance. However, most research into their temperature tolerance has considered their upper thermal range and studies into their lower thermal tolerance have investigated acute responses. The lineage of the population tested have not been considered during most experiments. The hybridised population in southeastern Newfoundland consists of haplotypes from both the northern and southern lineages. Here, I investigated the behavioural and physiological response of green crabs to a temperature reduction regime and cold water exposure over a long time period (6 days) to better understand the survival strategies utilized by this hybridized population of green crabs to survive the extreme cold winter water temperatures in southeastern coastal Newfoundland. Locomotor activity and metabolic parameters declined with decreasing temperature and reduced markedly after long-term exposure to cold temperatures (2 °C) compared to controls

(12 °C). Marked change in both metabolic and locomotive activity at 4 - 6 °C, suggests a torpor-like state occurs around these temperatures, however locomotive activity did not completely cease, even after long term exposure to the lowest test temperature (2 °C). This pattern indicates that crabs remain responsive to their environment and do not enter a true hibernation state. Additionally, time spent buried in the sand increased with declining temperatures, with a slightly lower threshold temperature (2 °C) in which time spent buried significantly increased. Feeding experiments also demonstrated longer response times to food items and less food consumed after long-term acclimation to 2 °C, compared to controls (12 °C). This pattern further supports the presence of a 'torpor-like' state, rather than true hibernation, below 5 °C, in which crabs will continue to actively move and feed, although more slowly and at lower rates. These results suggest that the hybridized population of green crabs in Newfoundland responds to cold temperatures in a similar way (e.g. by reducing their heart, metabolic, and movement rate to reduce energy requirements) to most other populations across their native and invasive range, and they may use other strategies, such as habitat use, to survive the extreme cold water temperatures in Newfoundland during the winter.

Introduction

The European green crab (*Carcinus maenas*) is a small decapod crustacean belonging to the family *Portunidae.* This species reaches a maximum carapace width of approximately 10 cm (McGaw *et al*., 2011) and has an average lifespan that varies from 3-7 years, depending on geographical location (Klassen and Locke, 2007). Green crabs inhabit sheltered bays with soft sediment, rocky intertidal, and estuarine habitats (Klassen and Locke, 2007; Amaral *et al.*, 2009; McKenzie et al. 2022). *C. maenas* is native to northwest Europe and northern Africa, but over the last 200 years have expanded their

range to include every continent, except Antarctica (Carlton and Cohen, 2003; Klassen and Locke, 2007). The main mechanism for their global dispersal is thought to be maritime activity, specifically in ballast water of vessels as larvae/juveniles (Grosholz and Ruiz, 2002; Carlton and Cohen 2003). They have established populations on the east coast of North America in 1817 (Glude, 1955; Grosholz and Ruiz, 1996), in Australia in the 1800's (Fulton and Grant 1902), in South Africa in 1983 (Joska and Branch, 1986; Le Roux *et al.*, 1990), in Japan in 1984 (Darling, 2011), on the west coast of North America in 1989 (Cohen *et al*., 1995; Grosholz and Ruiz, 1996) and in Argentina in 2003 (Hidalgo *et al.*, 2005).

C. maenas' success as an invader partly reflects their broad thermal tolerance (Kern et al., 2002). Adult green crabs can survive in temperatures up to approximately 38 ˚C (Tepolt and Somero, 2014), but can exit water into air when water temperatures increase above approximately 28 ˚C (Taylor and Wheatly, 1979). Information on their lower thermal tolerance is limited, but this species can tolerate acute exposure to temperatures as low as -1 °C without apparent ill-effect (Miron *et al.*, 2002; Young *et al.*, 2006; Tepolt and Somero, 2014). The narrower temperature tolerance of larvae however, require water temperatures to remain above 10 ˚C for several months for successful development to occur (Berrill, 1982; de Rivera *et al.*, 2007; Hidalgo *et al*., 2005). Although *C. maenas* continue to invade new locations and expand their range within established locations, they have not colonised polar or tropical regions, most likely due to the temperature limitations of their larvae. Minimum seasonal sea surface temperature has been suggested as a highly influential factor in determining northern range expansion and limitation of green crabs (Audet *et al.*, 2003; Hidalgo *et al.*, 2005; Jefferey *et al.*, 2018). Thermogeographic models predicting their future range expansion suggest

invasion potential depends on water temperature and origin of the population (from either southern or northern Europe) (Compton *et al.*, 2010).

Ectothermic, including crabs, cannot regulate their body temperature and thus are highly influenced by temperature (Taylor and Wheatly, 1979; Whitely *et al.*, 2001; Hopkin *et al.*, 2006, Leignel *et al.*, 2014). In contrast to considerable work on the distribution, abundance, and migration patterns of *C. maenas* in response to temperature change (Naylor, 1962; Tepolt and Somero, 2014; Young *et al*., 2017), less work has focused on specific behavioural responses, such as foraging success. Green crabs forage more consistently and efficiently during the warmer, summer months (Elner, 1980) and reach optimum foraging rates at approximately 12 ˚C (Bélair and Miron, 2009a). Foraging rates also increase more with increasing temperature in the presence of both conspecifics and heterospecifics as compared to solitary individuals (Bélair and Miron, 2009a).

There has been extensive work on physiological responses of green crabs to temperature increase (Frederich *et al.*, 2000; Madeira *et al.*, 2012; McGaw and Whiteley, 2012; Kelley *et al.*, 2013), heart rate and oxygen consumption as indicators for thermal tolerance in ectotherms (Hochachka & Somero 2002). Heart rate increases with temperature until reaching a critical thermal maximum temperature (CT_{max}), which is estimated to be between 30 ˚C and 38 ˚C for green crabs (Ahsanullah and Newell, 1971; Taylor *et al*., 1977; Frederich and Pörtner, 2000; Cuculescu *et al.*, 1998; Jørgensen *et al.*, 2017). The exact temperature at which CT_{max} occurs depends on the rate of change, geographical origin, and prior acclimation temperature (Cuculescu *et al.*, 1998; Kelley *et al.*, 2011; Tepolt and Somero, 2014; McGaw and Nancollas, 2017; Levinton *et al*., 2020). Heart rate becomes erratic and declines rapidly after reaching CT_{max} (Ahsanullah and Newell,

1971; Lutterschmidt and Hutchison, 1979). Oxygen consumption follows a similar pattern increasing steadily until reaching CT_{max} , with a sharp decline thereafter (Taylor, 1981; Whitely *et al.*, 1997; McGaw and Whiteley, 2012; McGaw and Reiber, 2015).

In comparison to temperature increases, less work has addressed the behavioural and physiological responses of green crabs to declining temperatures, and only a few studies have investigated responses to temperatures < 5 ˚C (Young et al, 2006; Bélair and Miron, 2009; Tepolt and Somero, 2014). Activity levels and agonistic interactions decline with decreasing temperature (Young et al, 2006; Bélair and Miron, 2009) and a reduction in foraging rates occurs at approximately 10 ˚C, ceasing at approximately 0 ˚C (Wallace, 1973; Elner, 1980; Miron et al, 2002; Matheson and Gagnon, 2012a). Likewise, Berrill (1982) observed reduced feeding, lack of mating, and reduced growth at temperatures below 7 ˚C and suggested torpor occurs at approximately 5 ˚C. A significant reduction in both heart rate and metabolism also occurs at 5 ˚C (Breteler, 1975; Camus *et al.*, 2004; Tepolt and Somero, 2013; Tepolt and Somero, 2014), further supporting a state of torpor at these temperatures. Only a few studies have investigated responses below 5 ˚C, and while these do show *C. maenas* can survive acute exposure to temperatures as low as –1 ˚C, (Kelley *et al.*, 2013; Tepolt and Somero, 2014), no address the effects of prolonged cold exposure on the physiological and behavioural responses of this species.

Green crabs were first reported in Placentia Bay Newfoundland, Canada, in 2007 (Blakeslee *et al.*, 2010; DFO, 2011). They have since expanded their range westward into Fortune Bay, and the eastern south coast (DFO, 2011). Water temperatures in coastal regions of southern Newfoundland average approximately 0 to 2 ˚C during the winter (Methven and Piatt, 1991; Colbourne *et al*., 2017); these are harsher conditions

than experienced by *C. maenas* in the entirety of their current range (Compton *et al.*, 2010). Indeed, the invasion of Newfoundland by *C. maenas* defied thermogeographic models as the minimum sea surface temperatures during the winter was predicted to be too low for larval survival (Compton *et al.*, 2010).

Green crabs in Placentia Bay are a hybridised population containing a mix of haplotypes from the southern lineages (originally settling in New England) and a more recent cold-tolerant northern European lineage (Jefferey *et al.*, 2017; Jefferey *et al.*, 2018). Very little is known as to how this population survives during the low winter temperatures characteristic of Newfoundland waters. Local fishermen and scientists theorise that they may move from the intertidal zone and shallow subtidal zone into deeper warmer waters, as occurs in native and more established populations (Naylor, 1962; Sharp *et al.*, 2003). Furthermore, very little is known about the long-term seasonal responses to cold temperatures of *C. maenas* in their native range, let alone the hybridized Newfoundland population which appear to be at their northern limits for cold tolerance. Thus, the present experiment aims to investigate the physiological and behavioural responses of *C. maenas* to the winter sea temperatures in Newfoundland (here tested at 2 ˚C). Physiological (heart rate and metabolic rate) and behavioural (activity, feeding and burying) characteristics were measured in response to an acute decline in temperature and during long-term exposure to cold temperatures. I hypothesised that: (i) physiological responses and locomotor activity of green crabs would decrease with declining temperature and would be modulated by long-term acclimation to cold temperatures, (ii) burying behaviour would increase with declining temperature and after long-term acclimation to cold temperatures, and (iii) green crabs would exhibit reduced feeding activity at cold temperatures.

Materials and Methods

Intermoult adult green crabs (*Carcinus maenas*) were collected between June and October 2019 and 2020 using baited net traps set at multiple locations in northern Placentia Bay, Newfoundland. Large males (carapace width > 5 cm) were brought back to the Ocean Sciences Centre, Memorial University. Female crabs were not used in experiments because protocols to prevent spread of this invasive species precluded their transport and live storage. The crabs were held in flow-through seawater (31-32 ppt) tanks at ambient temperatures ranging -0.5 - 13 ˚C. Air stones in each tank maintained oxygen concentrations above 90% saturation. Cylindrical PVC pipes (10 cm diameter x 12 cm or 24 cm length) were placed on the bottom of the tanks to provide shelter. The crabs were fed herring once a week, and any dead specimens and uneaten fish were removed from tanks promptly to minimize fouling of the water.

Before experiments, we glued foam tag labels to the dorsal surface of the carapace of individual crabs and recorded their wet weight (g), carapace width (mm) and any leg loss. We then moved the crabs into the laboratory and acclimated themin 45 L flow-through seawater tables maintained at either 12 ˚C or 2 ˚C, for a minimum of 2 weeks prior to experimentation (McGaw and Nancollas, 2018). These temperatures represent the average summer and winter sea surface temperatures in Placentia Bay, Newfoundland (Colbourne *et al.*, 2017). Maintaining constant red light in the laboratory minimized disturbance and eliminated any diurnal rhythms, noting that these wavelengths minimally affect crustaceans (Cronin, 1986). Black plastic screens hung around the tanks prevented visual disturbance to the animals. Starving crabs for 3 – 5 days prior to

trials ensured they were in a post-absorptive state (McGaw and Curtis, 2013) and placement in the experimental apparatus > 12 hours before recording began allowed them to settle after handling (Wilson et al., 2021).

Experimental Procedures

In the first experimental series, we assed behavioural and physiological responses during an incremental temperature reduction regime. The physiological characteristics measured were heart rate (treatment: n= 9, control: n = 15), and oxygen consumption (MO_2) (treatment: n = 16, control: n = 10), distance travelled, time spent active (treatment: n = 11, control: n = 11) and time spent buried (treatment: n = 15, control: n = 15) in response to the temperature reduction regime. Crabs that had previously been acclimated to 12 ˚C were placed into the experimental apparatus at a starting temperature of 12 ˚C and recording began following the initial 12 hour settling period in the apparatus. Data collection continued for 24 hours, then at 12 ˚C, after which we lowered the temperature by 2 ˚C (over approximately 30 minutes) and recorded data for a further 24 hours at 10 ˚C. This process was repeated until the minimum test temperature (2 ˚C) was reached (no data collection occurred during the 30 min periods of temperature change). Each experiment included control trials that ran at 12 ˚C for 6 days (time-period for the temperature reduction) ruling out time as a causal factor. Individual crabs were only used once during temperature treatment or control experiments.

In a second series of experiments, we monitored physiological and behavioural responses at a constant temperature (either 12 ˚C or 2 ˚C) for 6 days following acclimation over ~ 2 months to each respective temperature. This experiment mimicked the pro-

longed temperature conditions that green crabs experience during the summer and winter months in Newfoundland. The following number of animals were used in each experimental treatment: heart rate (12 ˚C: n = 15, 2 ˚C: n = 10); MO_2 (12 ˚C: n = 16, 2 ˚C: n = 18); distance travelled (12 ˚C: n = 11, 2 ˚C: n = 11); time buried (12 ˚C: n = 13, 2 ˚C: n = 15), and feeding behaviour (12 ˚C: n = 30, 2 ˚C: n = 30). Data from each 6-day experimental period was analysed in 24-hour blocks.

To measure heart rate (HR), we attached Newshift infrared heart rate monitors (Leiria, Portugal) to the carapace of each crab, directly above the heart, using dental wax and super glue (Burnett *et al.*, 2013). Crabs were then placed inside individual perforated plastic boxes (18 cm x 18 cm x 7 cm depth) and held in a flow through seawater table. They were allowed to adjust to the new setting for at least 12 hours before recording began. Each heart rate monitor was attached to a Newshift AMP03-U heart rate amplifier (Leiria, Portugal), recording the continuous output using ADInstruments LabChart7 software (Colorado Springs, USA). The holding containers were large enough for even the largest crabs to fit comfortably, allowing the animal to turn while preventing excessive movement that can increases HR (Aagaard *et al.*, 1995; McMahon, 1999; McGaw and Nancollas, 2018). We calculate mean HR for each 24-hour period by measuring the HR during the first minute (or the closest minute in which no pause in heart rate occurred) of each hour throughout the trial. We did not calculate HR during the first hour after the temperature changeover because crabs usually react with a startle response that would artificially inflate rates (Taylor, 1976, 1977; McMahon, 1999).

An L-DAQ intermittent flow respirometry system (Loligo systems, Viborg, Denmark) measured oxygen consumption (mg O_2 kg h^{-1}). Individual crabs were placed in separate cylindrical chambers (10 cm diameter x 8 cm height), each equipped with two seawater

pumps. The first pump continually flushed water through the chamber to maintain oxygen saturation during non-measurement periods. During the measurement periods, we turned off this pump and sealed the chamber while the second pump recirculated water within the chamber (10 L/min) (McGaw and Whiteley, 2012). Fiber optic oxygen probes fitted to the respirometry chambers measured oxygen saturation within the chamber during the measurement periods, calculating metabolic rate (MO_2). This fully automated system recirculated water within the chambers for 40 minutes (measurement period) and flushed for 20 minutes every hour, repeating this cycle for the duration of each trial (6 days). During the long-term acclimation trials at 2 ˚C, very low MO_2 led us to increase the recirculation period to 50 minutes (and to decrease the flush cycle to 10 minutes) to allow measurable decline in MO_2 within the chamber. A Loligo data acquisition system (Copenhagen, Denmark) recorded data. Using Loligo Systems AutoResp4 software (Viborg, Denmark), we calculate MO_2 (mg O_2 kg h^{-1}) at hourly intervals resulting in 24 MO_2 measures per specimen per day. We also calculated the mean, maximum (the highest value recorded), and resting (calculated as the average of the lowest five MO_2 values) MO_2 values for every 24-hour period, and estimated energy expenditure of each animal from the total MO_2 over each 24-hour period (using KaleidaGraph software) and standardized to kJ using the conversion factor of 1 mg O_2 = 0.014 kJ (Secor, 2009).

To record locomotor activity and time spent buried, we placed green crabs in individual containers (30 cm length x 20 cm width x 60 cm depth) with a layer of sand (~ 6 cm deep) in the bottom to allow burial. A Brinno TLC200 Pro HDR Time Lapse Video Camera (Taepei City, Taiwan) mounted above the seawater table recorded at 2 frames

per second. For both the temperature reduction trials and the long-term acclimation trials, we obtained the total time each crab spent moving (active), characterized by a change of location, and the total distance travelled (m) during each 24-hour recording period using ImageJ (Animal Tracker plugin). The presence of burying, characterized as when crabs were fully or partially covered in sand and remaining still, was per 24 hours was scored as present or absent. The total time each crab spent buried was measured manually and expressed as a percentage of each 24-hour period.

In a third series of experiments, we investigated feeding behaviour (presence/absence of feeding, time to first feeding event, amount of food consumed) of crabs acclimated to winter- and summer-time temperatures (2 ˚C and 12 ˚C, respectively) after various periods of food deprivation. Crabs were acclimated to the test temperature (either 2 ˚C or 12 ˚C) for ≥ 2 months and deprived of food for either 5 days (fasted) or 28 days (starved) prior to trials. A food deprivation time of 5 days allowed evacuation of all digesta from the gut and for physiological processes associated with digestion to return to baseline levels, but insufficient to cause the physiological effects associated with starvation (Wallace, 1973; McGaw and Whiteley, 2012). The incorporation of different food deprivation times allowed us to determine whether starvation imparts an interactive effect with temperature and causes a change in food consumption rates (Wallace, 1973).

Prior to feeding, we placed crabs in individual plastic containers (30 cm length x 20 cm width x 30 cm depth) at the test temperature and left overnight. Time lapse cameras mounted above the tanks to record feeding behaviour 1-2 minutes after we added a small portion of herring (~10 g). The cameras recorded for 24 hours, capturing the feeding behaviour of each crab and we recorded the presence or absence of feeding and time

taken for the first feeding event to occur for each individual. A feeding event was defined as when a crab handles the food item and was either observed feeding on it or remained holding the food for at least 30 seconds.

We measured the amount of food consumed at 2 ˚C and 12 ˚C following either 5 or 28 days of food deprivation in separate series of experiments (2 ˚C 5-days: n = 15, 2 ˚C 28-days: n = 15, 12 ˚C 5-days: n = 15, 12 ˚C 28-days: n = 15). Prior to feeding we measured crabs' carapace width and wet weight, and collected a haemolymph sample to measure haemolymph protein concentration. The crabs were then placed in individual sealed plastic containers (26 x 16 x 6 cm) and left to acclimate overnight. We cut holes in the containers (2 cm x 2 cm) and covered them in 1 mm mesh screen; this design allowed water circulation while preventing any loss of food. Soaking small pieces of herring (*Clupea harengus)* ~ 10 g, in seawater for ~ 12 hours prior to feeding reduced any changes in weight associated with osmotic water onload. They were then padded dry with a paper towel and weighed to the nearest 0.1g before being offered to the crab. We allowed crabs to feed for 24 hours, after which we removed the remaining fish was removed using forceps. After padding dry remains with a paper towel we re-weighed them to calculate the amount of fish eaten (g) by each crab, expressing weight eaten as a percentage of the wet weight (g) of each crab to account for differences in crab size.

Haemolymph protein concentration can be used as an indication of the crabs' physical condition and nutritional status (Moore *et al.*, 2000; Oliver and MacDiarmid, 2001; Ozbay and Riley, 2002; Wang and McGaw, 2014). In the present study we used it to determine whether haemolymph protein levels affected the amount of food consumed by green crabs. Prior to feeding trials we withdrew approximately 300 ul of haemolymph, using a 1 ml syringe and 16 gauge needle, inserted into the soft tissue between

the joints of the pereiopods. The haemolymph was placed into the sample well of a Brix/RI-Check Digital Pocket Refractometer (Reichert Analytical Instruments, Depew, NY). We calibrated the refractometer with deionized water prior to each haemolymph measurement, recording the Refraction Index (RI) for each crab and converting it to haemolymph protein density (in dg/100 mL) using the equation $HD=510(RI_{water}-RI_{hemo-lymph})-1.81$ (Sunderman, 1944; Wang and McGaw, 2014).

Statistical analysis

To test for differences in heart rate, metabolic rate, and energy expenditure, for both the temperature reduction and long-term temperature acclimation trials, we calculated two-way ANOVAs (in IBM SPSS), adding time as a repeated measures factor (we measured the same individual for the duration of the experiment). Two-way ANOVAs on the temperature reduction trial data tested for total time spent active, total distance travelled, and time spent buried, as described above. Post-hoc pairwise used Fisher's Least Significant Difference (LSD) test. For the long-term acclimation trial we included time as a within factors variable to account for the repeated measures and to rule out time as a causal factor. To compensate for the high proportion of zero values obtained during the long-term 2 ˚C trials (total time spent moving, total distance travelled, and time spent buried), we developed zero-inflated generalised linear mixed models (ziGlmm) in R using the glmmTMB package, applying a negative binomial distribution log link, and including the mixed, nested factor (individual within time).

We analysed the presence and absence of feeding events as a function of temperature and food deprivation duration using a generalised linear fixed effects model with a bi-

nomial distribution log link (in R). Two-way ANOVAs tested the effects of food deprivation duration and temperature on the time taken to first feeding event, where a Tukey's post hoc test evaluated significant differences. We removed one outlier, which yielded a very low P value noting the crab's death shortly after the trial. A linear regression analysis identified any relationship between the pre-trial P values and the time spent before first feed, investigating whether nutritional condition impacted urgency to feed.

Analysis for differences in the amount of fish eaten in relation to temperature and duration of food deprivation used a two-way ANOVA using R-studio (version 2024.04.01). Where we observed significant differences, we applied Tukey's post hoc tests to determine where significant differences occurred among treatments. Additionally, linear regression analysis identified any relationship between the amount of fish consumed and pre-trial haemolymph concentration (taken immediately before trails commenced) (R version 4.2.2, 2022).

Results

The interaction between time and treatment type significantly affected heart rate (mixed factorial ANOVA: $F_{5,110} = 15.889$, $P < 0.001$). Heart rates for the control group (held at 12 ˚C (Fishers LSD $P > 0.05$) remained steady 48 ± 3.35 BPM and 53 ± 2.93 BPM (mean ± 1SD) during the 6-day experimental period (Fig. 1). In contrast, during the temperature reduction treatment, heart rate decreased significantly from 43± 5.19 BPM at 12 ˚C to 14 ± 2.79 BPM at 2 ˚C (Fisher's LSD). We found no difference in heart rate between temperatures of 12, and 8°C however, a marked reduction occurred at 6 ˚C where heart rate declined, on average, by 14 BPM (Fisher's LSD, $P<0.05$). The heart

rates measured at 6 °C and below were significantly lower than those of the control group and at 8 °C and above. At temperatures of 4 and 2 °C heart rates were similar. Neither maximum nor minimum heart rate at 12 ˚C differed significantly different between the control and treatment groups (max: 79 BPM and 64 BPM, respectively; min: 26 BPM and 27 BPM, respectively) (Fig. 1).

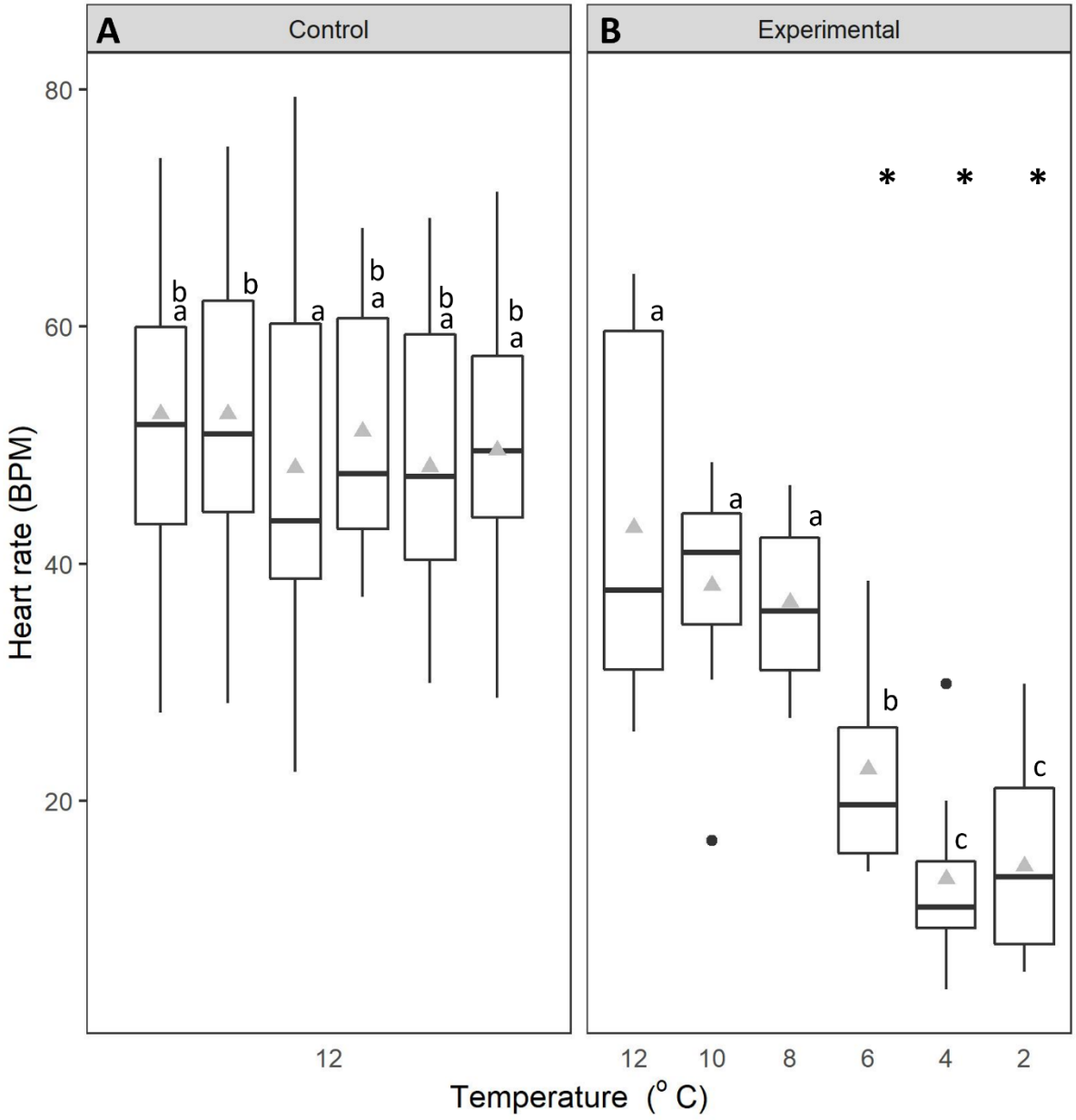


Figure 2.1. Heart rate with declining temperature: Heart rate (beats per minute) of green crabs (control n=15, experimental n=9). A: control crabs, held at 12 ˚C and B: experimental crabs, experiencing a reduction in temperature from 12 ˚C to 2 ˚C. The data is displayed for each 24-hour period over 6 days. Whiskers represent 95 % confidence limits, boxes show upper and lower quartiles, horizontal bar presents median values, black circles denote outlying values and grey triangles denote the mean. * denotes significant changes in the temperature treatment relative the control. Lower case letters denote significant differences within treatment groups.

During the control experiment the MO2, as well as maximal and resting MO2 remained stable over the 6-day trial period (20 ± 2.08 – 24 ± 2.50 mg O_2/kg/h for MO2, 32 ± 3.58 – 43 ± 5.23 mg O_2/kg/h for maximal MO2, and 16 ± 1.62 – 20 ± 1.98 mg O_2/kg/h for resting MO2 (Fig. 2)). In contrast these values all decreased when temperature was lowered from 12 °C to 2 °C (29 ± 2.26 – 7 ± 1.75 mg O_2/kg/h for MO2, 57 ± 6.06 – 15 ± 1.22 O_2/kg/h for maximal MO2, and 18 ± 0.71 – 3 ± 1.07 mg O_2/kg/h for resting MO2).

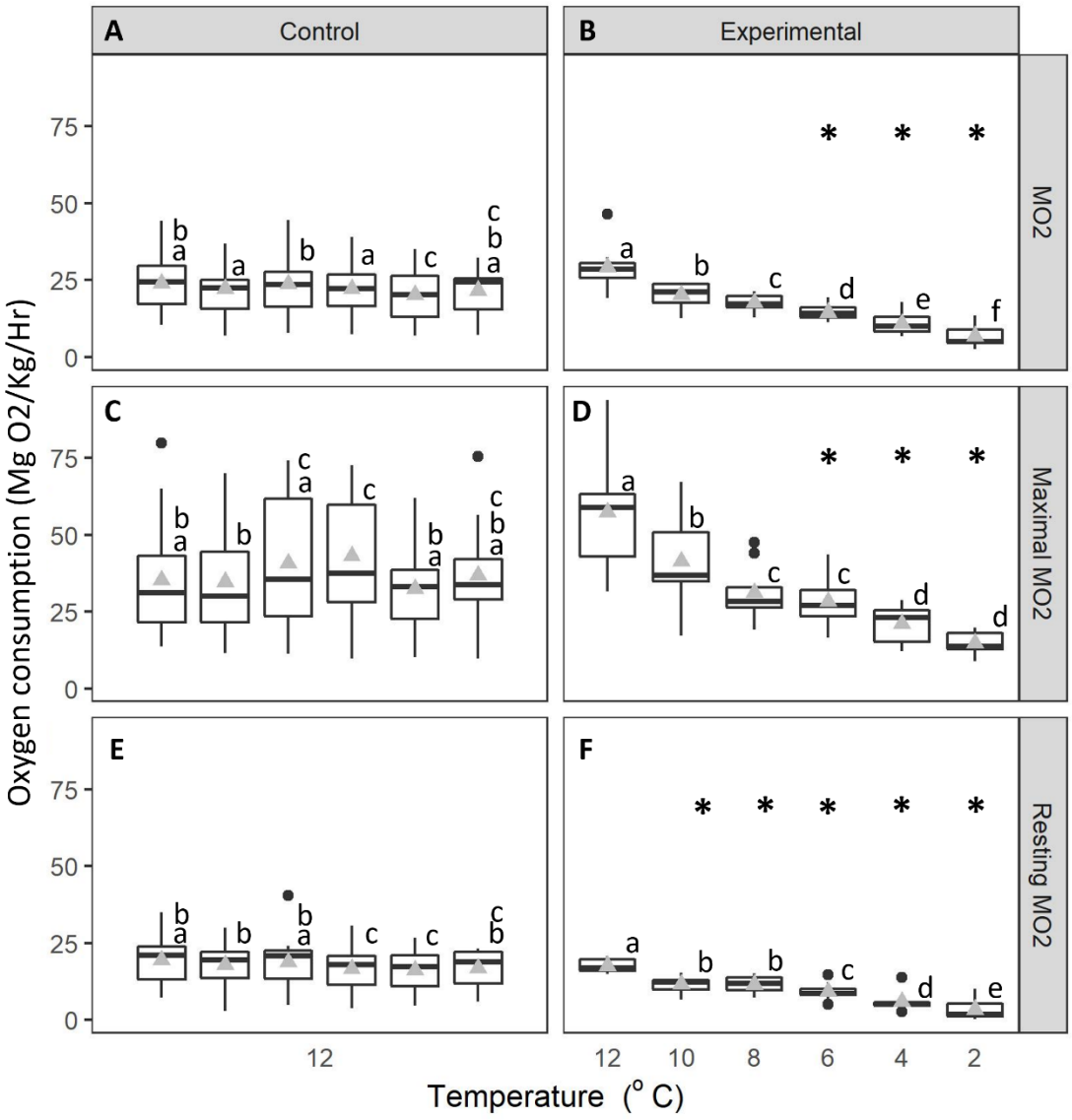


Figure 2.2 Metabolic rate with declining temperature: Oxygen consumption (mg O2/kg/h) of green crab (control n = 16, experimental n = 10). A: MO2 of control crabs (held at 12 ˚C), B: MO2 of experimental crabs (experiencing a reduction in temperature from 12 ˚C to 2 ˚C), C: maximal MO2 of control crabs (highest value recorded per crab), D: maximal MO2 of experimental crabs (highest value recorded per crab), E: resting MO2 of control crabs and F: resting MO2 of experimental crabs. The data is displayed for each 24-hour period over 6 days. Whiskers represent 95 % confidence limits, boxes show upper and lower quartiles, horizontal bar presents median values, black circles denote outlying values and grey triangles denote mean. * denotes where we observed significant changes in the temperature

treatment relative to the control. Lower case letters denote significant differences within treatment groups.

The interaction between temperature and time all significantly affected MO2, maximal, and resting MO2 (mixed effects ANOVAs, MO2: $F_{3,67} = 35.097$, $P < 0.001$; maximal MO2: $F_{3,76} = 15.971$, $P < 0.001$; resting MO2: $F_{3,80} = 16.572$, $P < 0.001$), showing a decline in line with decreasing temperature. MO2 and maximal MO2 differed significantly from the control group at and below 6 °C, while resting MO2 differed significantly at and below 10 °C (Fishers LSD, Fig 2).

The interaction between time and temperature significantly affected the estimated energy expenditure (EEE) of crabs (mixed effects ANOVA: $F_{5,120} = 13.216$, $P < 0.001$; Figure 3). Crabs in the control treatment (12 ˚C for 6 days) maintained a mean EEE of 0.43 and 0.53 ± 0.046 kj/day during the 6-day experimental period (Fisher's LSD). In contrast, the estimated energy expenditure of crabs exposed to a reduction in temperature gradually declined with decreasing temperature (Fisher's LSD). The EEE was significantly reduced in comparison with control individuals (Fig. 3A) held at 12 ˚C at a temperature of 8 ˚C and below (Fisher's LSD).

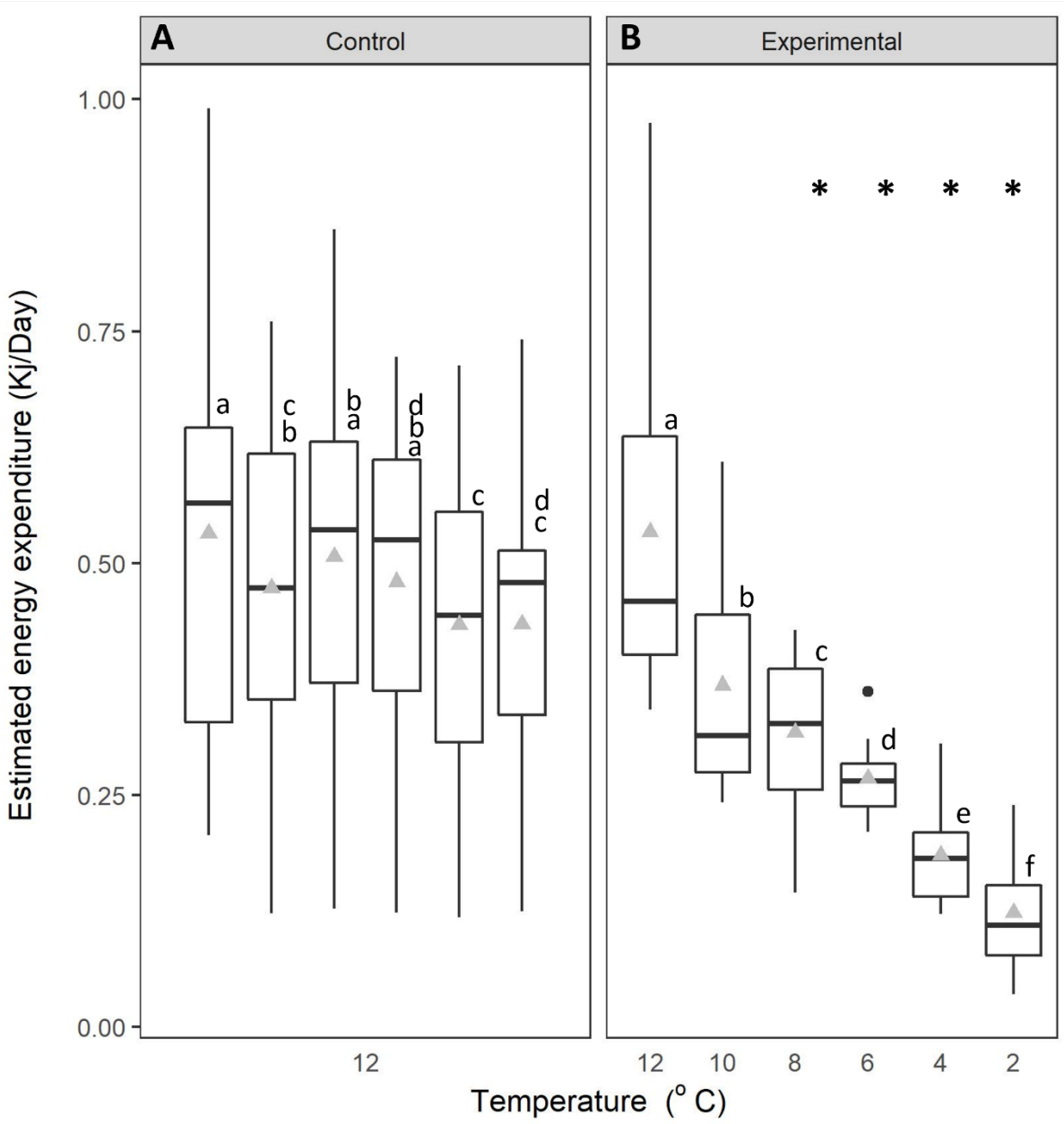


Figure 2.3 Estimated energy expenditure with declining temperature: Estimated energy expenditure (kj/day) of green crabs. A: control crabs, held at 12 C and B: experimental crabs, experiencing a reduction in temperature from 12 ˚C to 2 ˚C. The data is displayed for each 24-hour period over 6 days. Whiskers represent 95 % confidence limits, boxes show upper and lower quartiles, horizontal bar presents median values, black circles denote outlying values and grey triangles denote mean. * denotes significant changes in the temperature treatment relative to mean level in the control. Lower case letters denote significant differences within treatment groups.

Percentage time spent active by green crabs maintained in 12 ˚C ranged between an average of 3.5 and 5.8 % per day over the 6 days, and with no significant difference in these values (Fig. 4A, Fisher's LSD). During the decline in temperature we observed a concomitant decline in mean percentage time active from 4.7 % at 12 ˚C to 0.3 % at 2 ˚C (Fig. 4B). The interaction between time and temperature significantly affected the percentage time spent active (mixed factorial ANOVA: $F_{5,100} = 3.255$, $P = 0.045$). Crabs were significantly less active at and below 4 ˚C (Fisher's LSD). The minimum percentage time spent active by any individual was 0.4 % and the maximum 11.8 %. All individuals, from both treatment groups, were active for some portion of the trial.

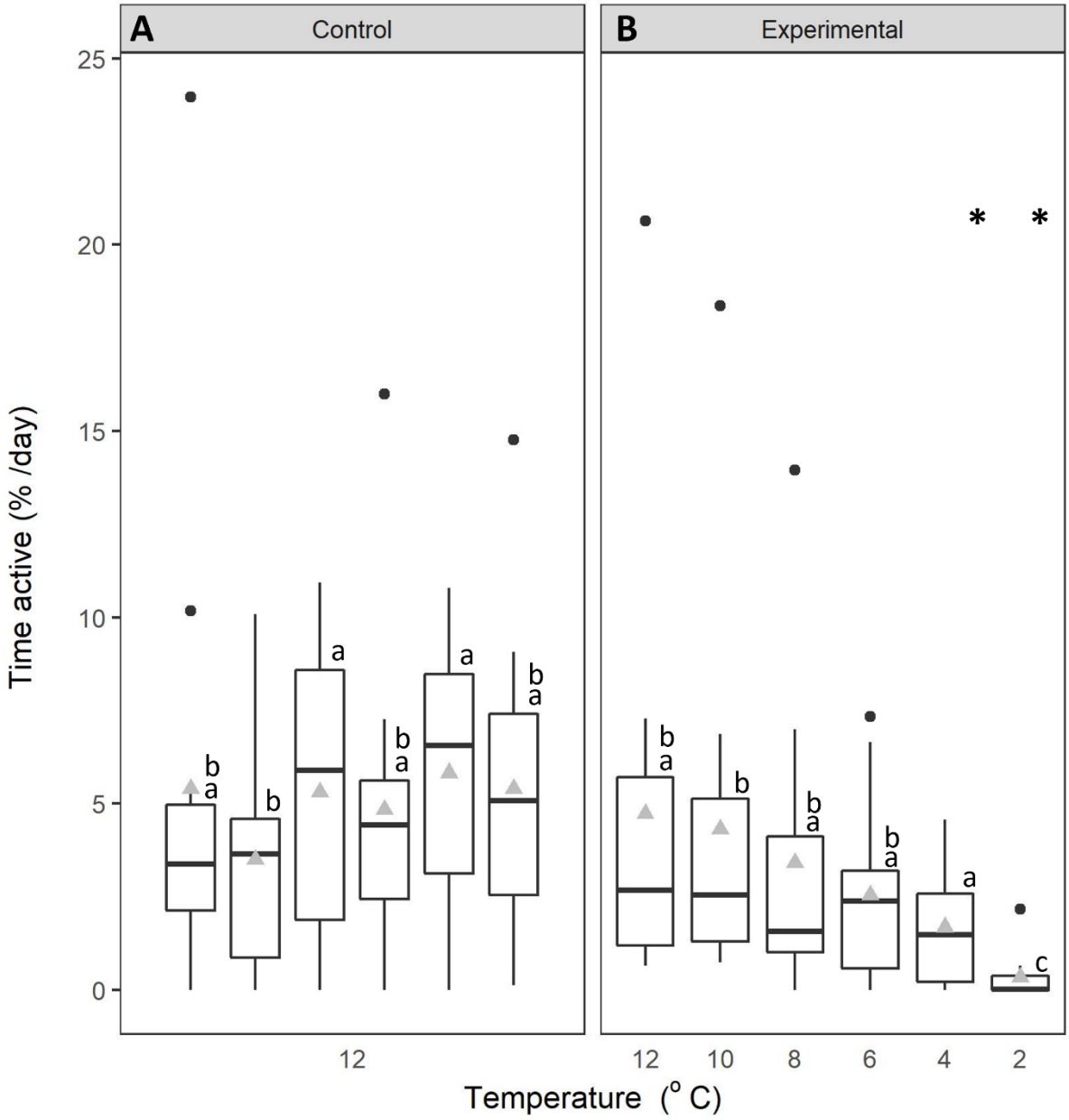


Figure 2.4 Time spent active with declining temperature: Time green crabs spent active (%/day). A: control crabs, held at 12 ˚C, and B: experimental green crabs, experiencing a reduction in temperature from 12 ˚C to 2 ˚C. The data is displayed for each 24-hour period over 6 days. Whiskers represent 95 % confidence limits, boxes show upper and lower quartiles, horizontal bar presents median values, black circles denote outlying values and grey triangles denote mean. * denotes where time active differed significantly from control treatments. Lower case letters denote significant differences within treatment groups.

At 12 ˚C the mean total distance crabs travelled varied between 276 – 425 m per day throughout the 6-day trial duration (Fig. 5A), however, we observed no significant difference between these distances from day to day (Fisher's LSD). However, in the experimental treatment there was an overall decline with temperature in the total distance travelled (mixed factorial ANOVA: $F_{5,100} = 6.764$, $P = 0.003$), from 378.4 $\pm$ 150.0 m per day at 12 ˚C to 24.2 ± 15.20 m at 2 ˚C (Fig. 5B). The total distance travelled was significantly lower at 4 ˚C and 2 ˚C, and the distance travelled at these temperatures also differed significantly from each other (Fisher's LSD). At a temperature of 6 °C and above, the distances travelled were similar among the temperatures (Fisher's LSD), with maximum distance travelled in any experimental individual of 1764.7 m at 12 ˚C and 170 m at 2 ˚C.

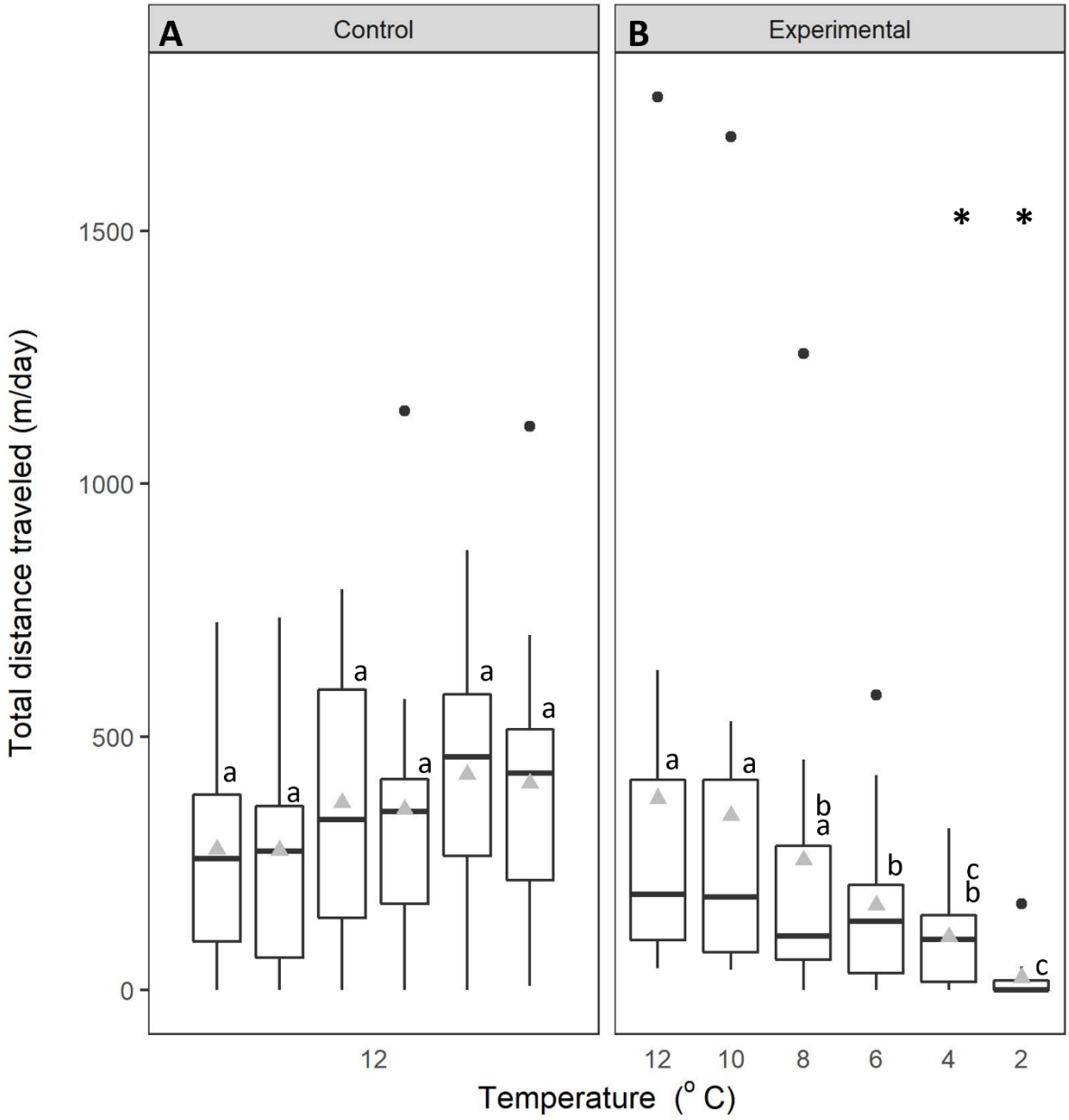


Figure 2.5 Total distance travelled with declining temperature: Total distance travelled (m/day) by green crabs. A: control crabs, held at 12 ˚C and B: experimental crabs experiencing a reduction in temperature from 12 ˚C to 2 ˚C. The data is displayed for each 24-hour period over 6 days. Whiskers represent 95 % confidence limits, boxes show upper and lower quartiles, horizontal bar presents median values, black circles denote outlying values and grey triangles denote mean. * denotes where we observed significant changes in the temperature treatments relative to mean level in the control treatments. Lower case letters denote significant differences within treatment groups.

All crabs, (with the exception of one individual in the 4 °C treatment), spent a portion of their time buried in the sand. Control crabs at 12 °C remained buried for between 66.7 % and 75.2 % of the time per day, with no significant change over the 6 day experimental period (Fisher's LSD). Percentage time spent buried generally increased with decreasing temperature (mixed factorial ANOVA: $F_{5, 145} = 10.309$, $p < 0.001$): mean percentage time buried in experimental crabs almost doubled from 50 % at 12 ˚C to 96 % at 2 ˚C (Fig. 6). Despite this trend, the difference was statistically significant only at 2 ˚C, (Fishers LSD). For the experimental crabs the minimum time spent buried ranged from 5 % at 12 ˚C to 66 % at 2 ˚C, while minimum burying durations in control individuals varied between 6 % and 47 % over the duration of the trial (Fig. 6).

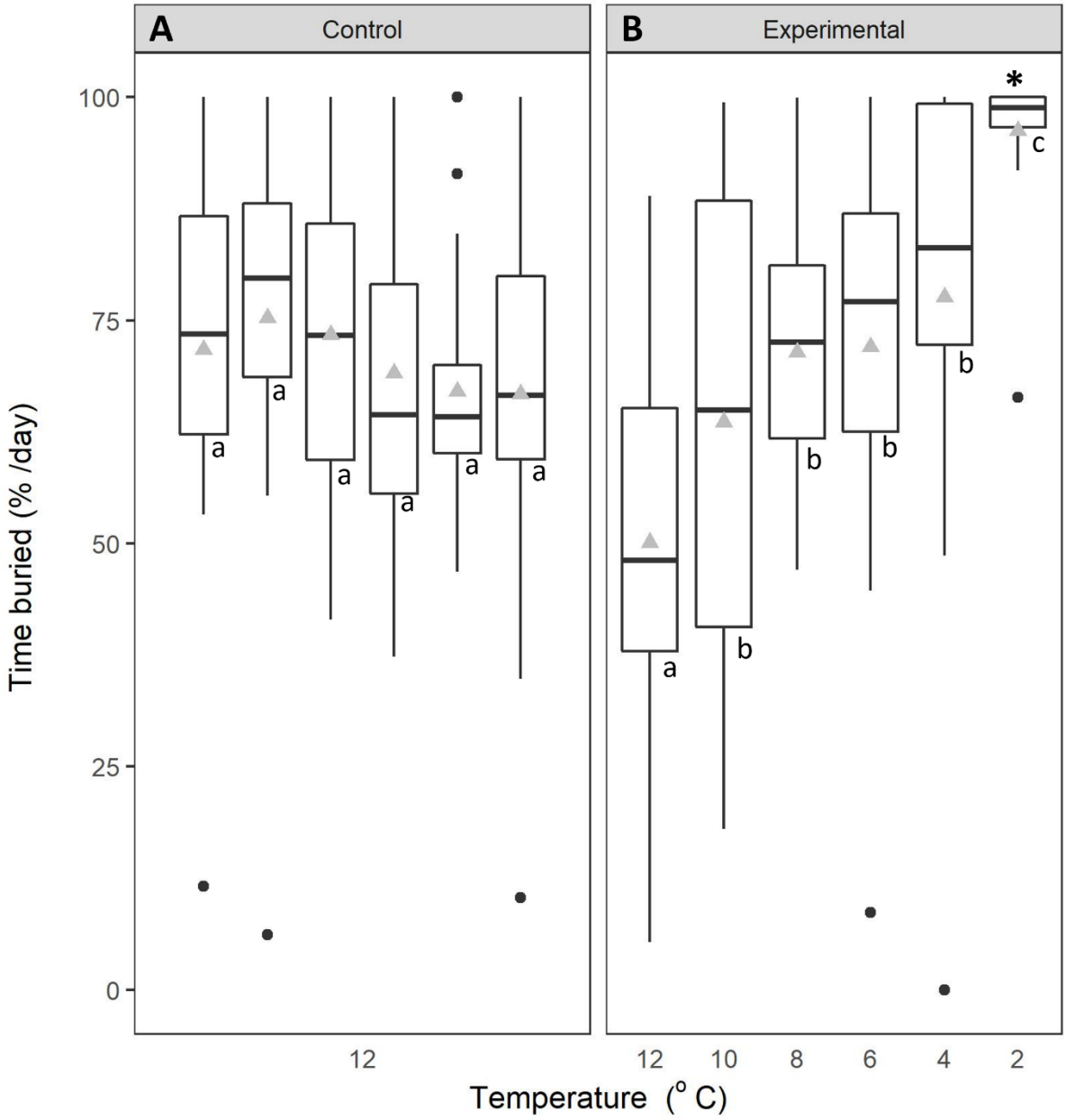


Figure 2.6 Time spent buried with declining temperature: Percentage time green crabs spent buried (%) per 24 hours. A: control crabs, held at 12 ˚C and B: experimental crabs, experiencing a reduction in temperature from 12 ˚C to 2 ˚C. The data is displayed for each 24-hour period over 6 days. Whiskers represent 95 % confidence limits, boxes show upper and lower quartiles, horizontal bar presents median values, black circles denote outlying values and grey triangles denote mean. * denotes significant differences in time spent buried relative to control temperature. Lower case letters denote significant differences within treatment groups.

The mean heart rate of green crabs acclimated (six days) to 12 ˚C ranged between 48 and 53 ± 13 BPM. These values were significantly higher than 24 to 32 ± 12 BPM measured for crabs acclimated (six days) to 2 ˚C (mixed factorial ANOVA: $F_{1,23}$ = 23.807, P < 0.001; Fig. 7). The interaction between temperature and time affected heart rate although temperature remained constant throughout. The 2 ˚C acclimated group in which heart rate reduced significantly on days 5 and 6 (Fisher's LSD) explained this difference.

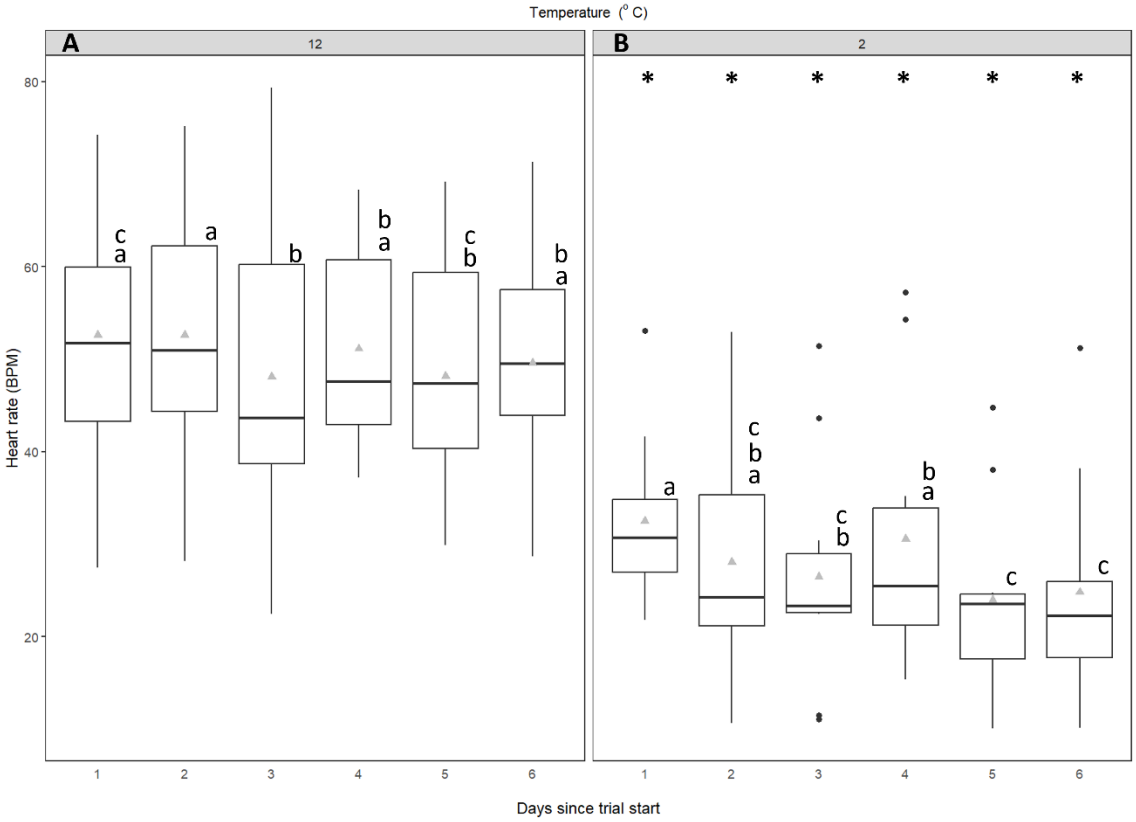


Figure 2.7 Heart rate after long-term cold water acclimation: Heart rate (beats per minute) of green crabs. A: control crabs, held at 12 ˚C, and experimental crabs, held at 2 ˚C. This data is displayed for each 24-hour period over 6 days. Whiskers represent 95 % confidence limits, boxes show upper and lower quartiles, horizontal bar presents median values, black circles denote outlying values and grey triangles denote mean. * denote where time spent buried differed significantly from the control treatment. Lower case letters denote significant differences within treatment groups.

Overall, the MO2, maximal and resting MO_2 were significantly lower at 2 ˚C compared with 12 ˚C (mixed factorial ANOVA: max: $F_{1,33} = 11.113$, $P = 0.002$, mean: $F_{1, 33} = 10.376$, $P = 0.003$, resting: $F_{1,33} = 44.531$, $P < 0.001$). The MO_2 for any 24-hour period at 12 ˚C varied between 20 and 24 mg O_2/kg/h, while at 2 ˚C it varied between 11 and 13 mg O_2/kg/h (Fig. 8). A significant interaction between time and temperature on MO_2 (mixed factorial ANOVA: $F_{5,160} = 2.908$, $P = 0.039$) occurred because the 12 ˚C acclimated group showed a slight decrease in MO_2 (Fisher's LSD), declining from 24 mg O_2/kg/h on day 1 to 21.6 mg O_2/kg/h on day 6, wheras the MO_2 of 2 ˚C acclimated crabs remained stable throughout the 6 day experimental period (Fig. 8). The interaction between time and temperature affected maximal MO2 (mixed factorial ANOVA: $F_{5,160} = 3.729$, $P = 0.017$) reflecting a more variable maximal MO_2 over time at 12 ˚C than at 2 ˚C. Neither time nor its interaction with temperature significantly affected the resting MO2 (mixed factorial ANOVA; interaction: $F_{5,160} = 2.587$, $P = 0.066$ time: $F_{5,160} = 1.968$, $P = 0.132$).

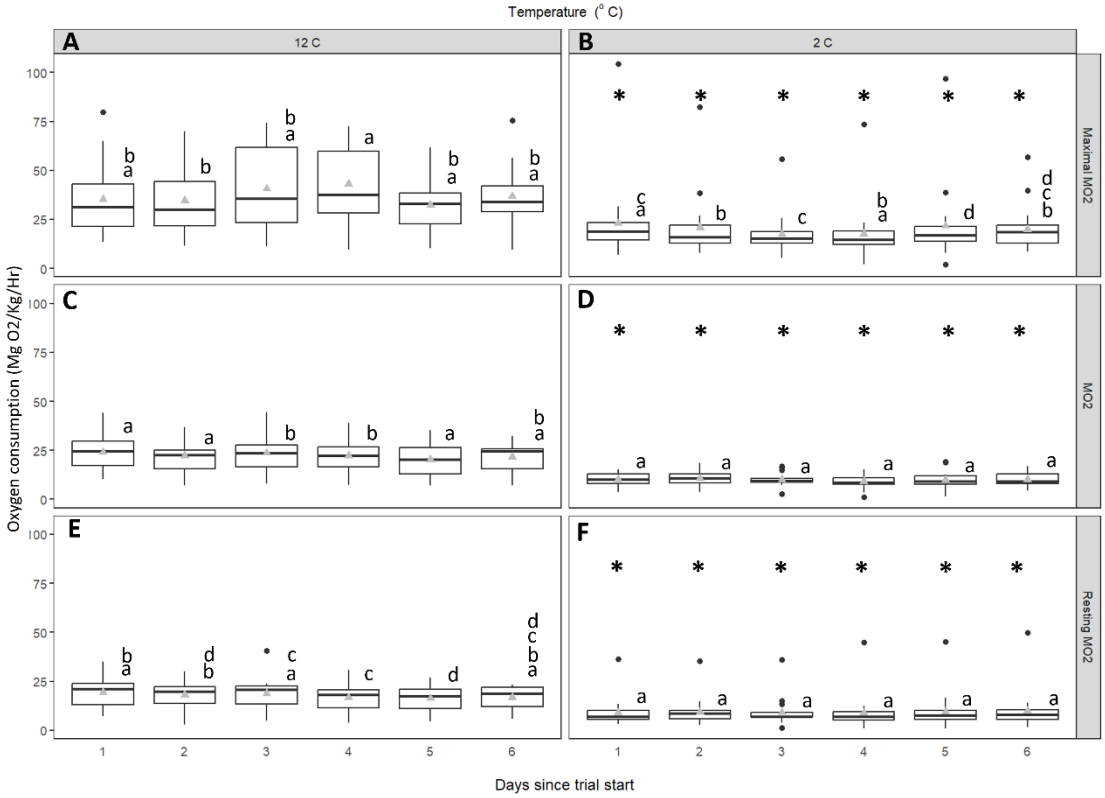


Figure 2.8 Metabolic rate after long-term cold water acclimation: Metabolic rate (mg O_2/kg/h) of green. A: max MO_2 (highest value recorded per crab) of control crabs (held at 12 ˚C), B: max MO_2 (highest value recorded per crab) of experimental crabs (held at 2 ˚C), C: mean MO_2 of control crabs, D: mean MO_2 of experimental crabs, E: resting MO_2 of control crabs and F: resting MO_2 of experimental crabs. The data is displayed for each 24-hour period over 6 days. Whiskers represent 95 % confidence limits, boxes show upper and lower quartiles, horizontal bar presents median values, black circles denote outlying values and grey triangles denote mean. * denotes where time spent buried differed significantly from the control treatment. Lower case letters denote significant differences within treatment groups.

Estimated energy expenditure of green crabs acclimated to 12 ˚C (0.48 kj/h) was more than double that of the crabs acclimated to 2 ˚C (0.20 kj/h) (mixed factorial ANOVA: $F_{1,32} = 39.407$, $P < 0.001$; Fig. 9). Time also affected energy expenditure (mixed factorial ANOVA: $F_{5,160} = 4.216$, $P = 0.008$); crabs in the 12 ˚C acclimated group exhibited lower energy expenditure on days 5 and 6 (Fisher's LSD). EEE of crabs acclimated to 2 ˚C remained stable between 0.18 – 0.22 kj/h. Additionally, mean energy expenditure at 12 ˚C varied more than that measured at 2 ˚C (Fig. 9).

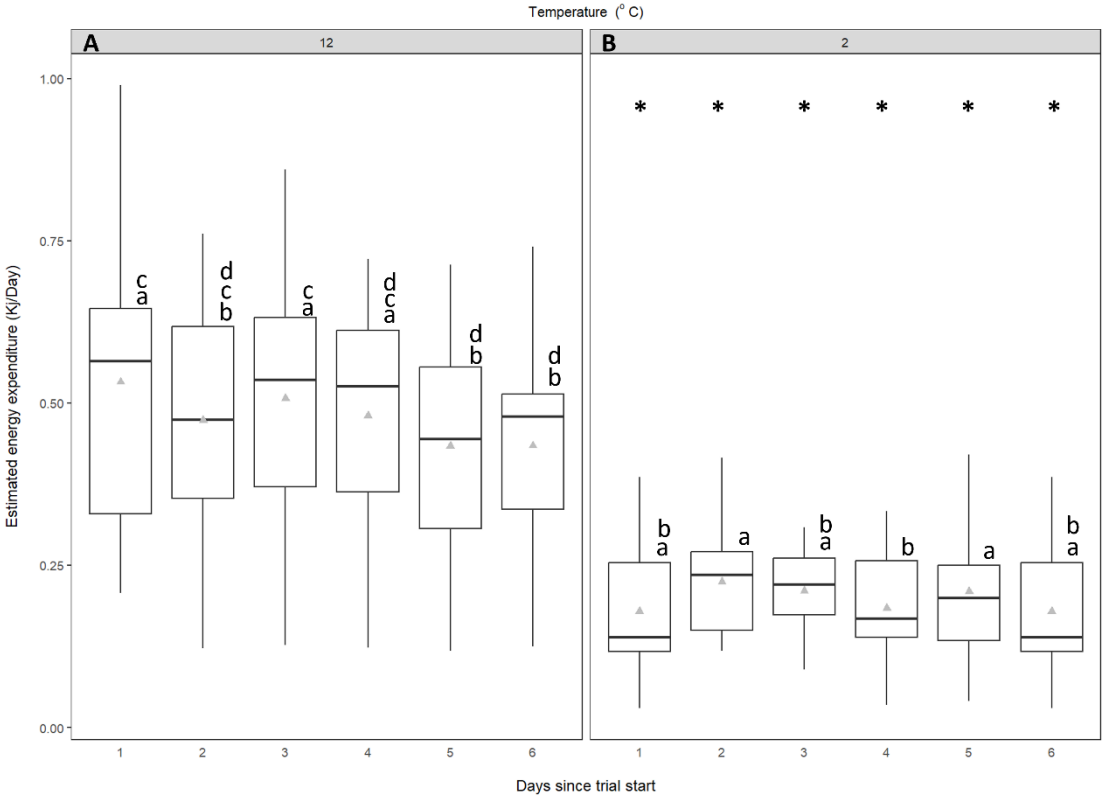


Figure 2.9 Estimated energy expenditure after long-term cold water acclimation: Estimated energy expenditure (kj/day) of green crabs. A: control crabs, held at 12 ˚C and B: experimental crabs, held at 2 ˚C. The data is displayed for each 24-hour period over 6 days. Whiskers represent 95 % confidence limits, boxes show upper and lower quartiles, horizontal bar presents median values, black circles denote outlying values and grey triangles denote mean. * denotes where time spent buried differed significantly from the control treatment. Lower case letters denote significant differences within treatment groups.

Green crabs acclimated to 12 ˚C were more active than those acclimated to 2 ˚C (ziGlmm: $\chi^2_{1, 192} = 11.33$, $p < 0.001$, Fig. 10). Percentage time active per day for crabs acclimated to 12 ˚C ranged between 3.5 % and 5.8 %, whereas those acclimated to 2 ˚C were active for between 0.4 % and 3.2 %, of the time. It was noteworthy that, 9 of the 12 individuals held at 2 ˚C, did not move for at least one full day, and one individual showed no movement during the entire 6-day trial (all crabs were alive at the end of the experiment). In contrast only two individual crabs held at 12 ˚C showed 0 % activity during a given day. Although time did not have a significant effect, we observed a significant interaction between time and treatment (ziGlmm: $\chi^2_{5, 192} = 25.06$, $p < 0.001$). This difference occurred in the 2 ˚C acclimated crabs, which were less active on the last two days of the experiment, while no such decline occurred for 12 ˚C acclimated crabs (Fig. 10).

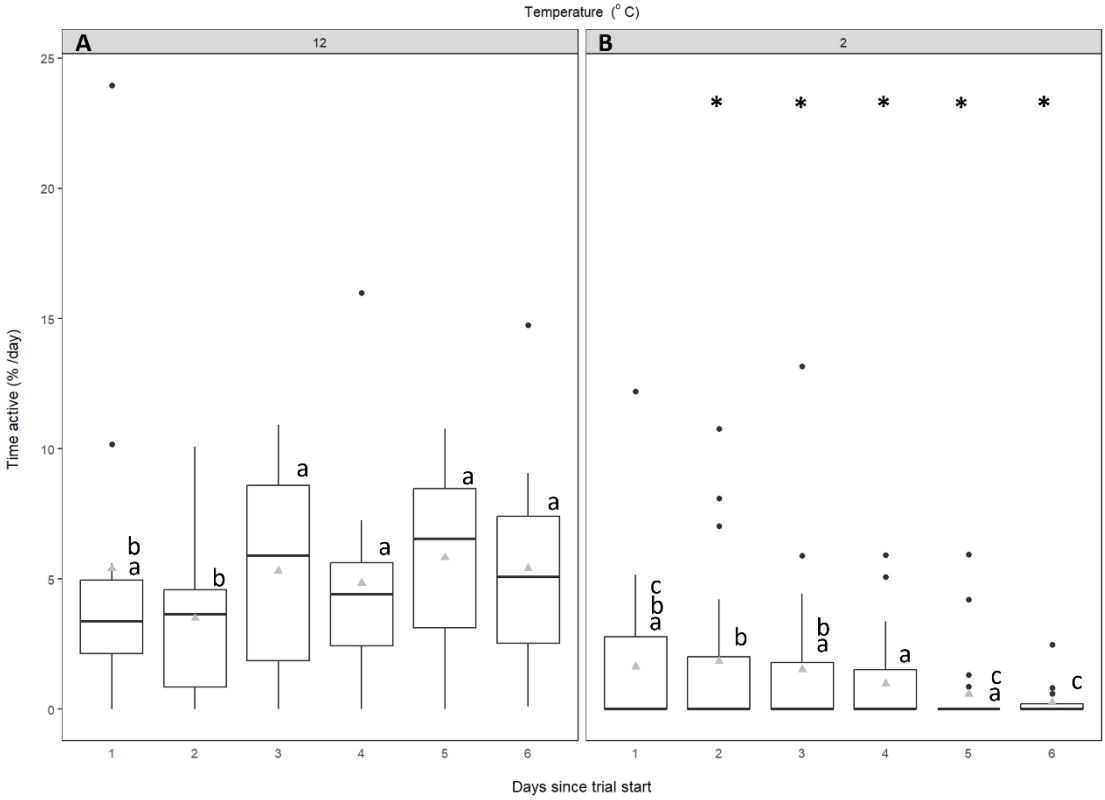


Figure 2.10 Time spent active after long-term cold water acclimation: Percentage time green crabs spent active (% /day). A: control crabs, held at 12 ˚C and B: experimental crabs, held at 2 ˚C. The data is displayed for each 24-hour period over 6 days. Whiskers represent 95 % confidence limits, boxes show upper and lower quartiles, horizontal bar presents median values, black circles denote outlying values and grey triangles denote mean. * denotes where time spent buried differed significantly from control treatments. Lower case letters denote significant differences within treatment groups.

The mean total distance moved per day also differed significantly between acclimation temperatures (ziGlmm: $\chi^2_{1, 192} = 48.09$, $p < 0001$). Green crabs acclimated to 12 ˚C travelled a mean total distance of 352 m per 24 hours, wheras 2 ˚C acclimated crabs only travelled an average of 7 m per 24 hours (Fig. 11). The interaction between time and treatment also significantly affected total distance travelled (ziGlmm: $\chi^2_{5, 192} = 14.41$, $p < 0.013$). This difference was largely related 2 ˚C acclimated crabs decreasing their activity over the course of the experiment (Fig. 11). In contrast, the mean total distance travelled by 2 ˚C acclimated crabs decreased overall from 9.90 ± m to 6.75 ± m during the 6-day experimental period.

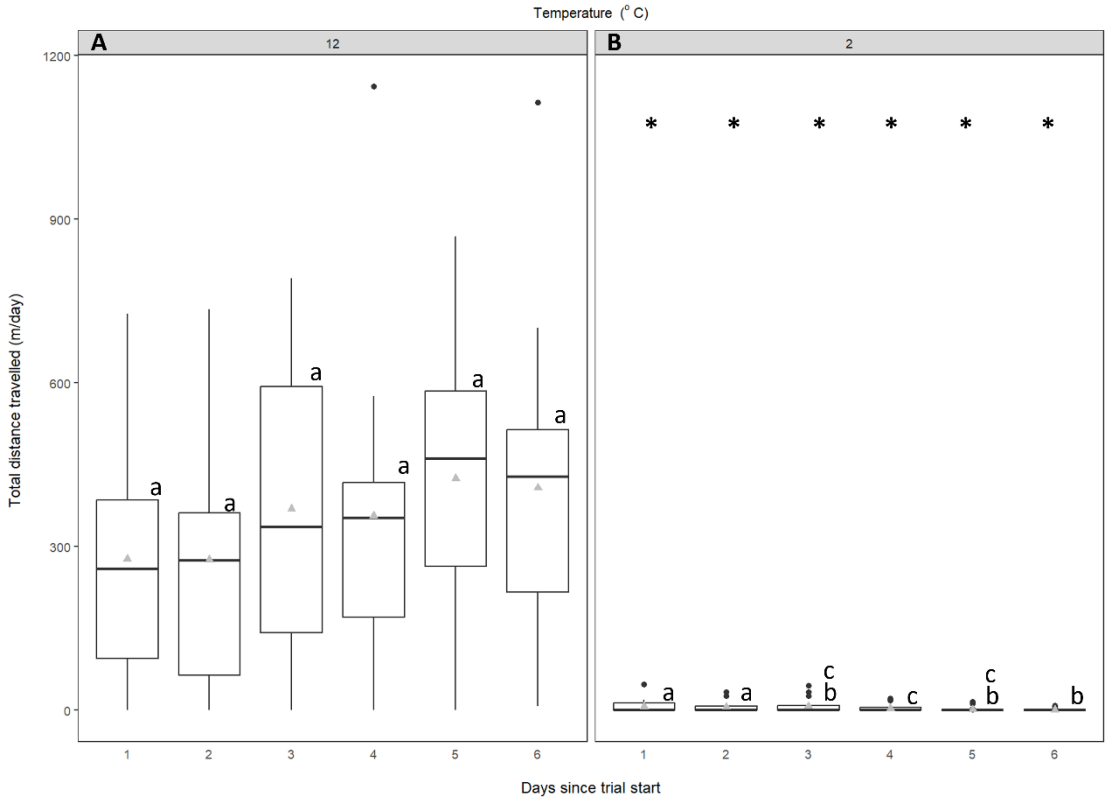


Figure 2.11 Total distance travelled after long-term cold water acclimation: Total distance travelled (m/day) by green crabs. A: control crabs, held at 12 ˚C and B: experimental crabs, held at 2 ˚C. The data is displayed for each 24-hour period over 6 days. Whiskers represent 95 % confidence limits, boxes show upper and lower quartiles, horizontal bar presents median values, black circles denote outlying values

and grey triangles denote mean. * denotes where time spent buried differs significantly from the control treatment. Lower case letters denote significant differences within treatment groups.

All crabs in both treatments spent a portion of each 24-hour period buried. The mean percentage time spent buried was lower in individuals acclimated to 12 ˚C (ziGlmm: $\chi^2_{1, 168} = 12.96$, $p < 0.001$), at 70.5 % at 12 ˚C and 92.6 % at 2 (Fig. 12). The interaction between treatment and time was also significant (ziGlmm: $\chi^2_{5, 168} = 11.90$, $p = 0.036$), the percentage time spent buried in 2 ˚C acclimated crabs increased over the course of the trial from 88.3 % to 94.8 %, while in 12 ˚C acclimated crabs the time spent buried declined from 71.7 % to 66.7%.

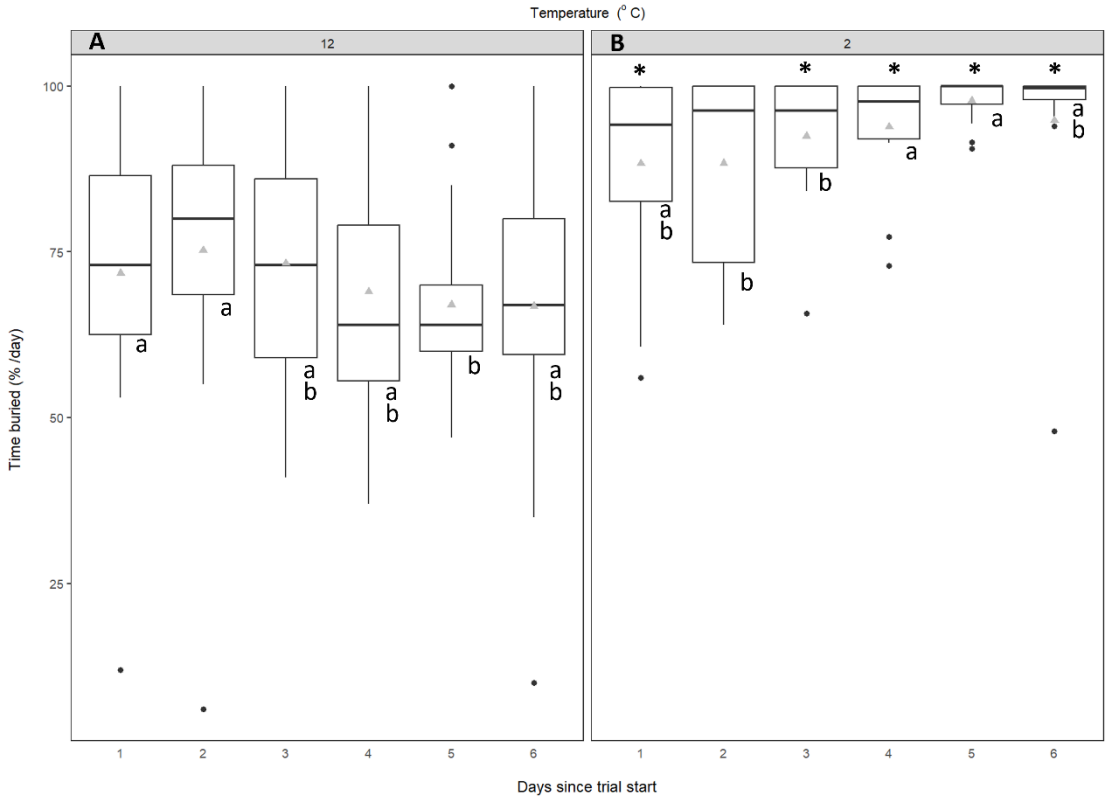


Figure 2.12 Time spent buried after long-term cold water acclimation: Percentage time green crabs spent buried (% /day). A: control crabs, held at 12 ˚C and B: experimental crabs, held at 2 ˚C. The data is displayed for each 24-hour period over 6 days. Whiskers represent 95 % confidence limits, boxes show upper and lower quartiles, horizontal bar presents median values, black circles denote outlying values

and grey triangles denote mean. * denotes where time spent buried differed significantly from the control treatments. Lower case letters denote significant differences within treatment groups.

Acclimation temperature significantly affected feeding (generalised linear fixed effects model: $\chi^2_{1, 60} = 4.34$, $p = 0.037$) with fewer crabs feeding at 2 ˚C than at 12 ˚C (Fig. 13). All individuals acclimated at 12 ˚C fed during the trial while 4 crabs (out of 15 crabs) acclimated to 2 ˚C did not feed during the 24-hour trial period. This number included 3 crabs in the 5 day food deprivation group, and one crab that had been deprived of food for 28 days (Fig. 13). Despite this apparent difference, the starvation duration had no statistically significant effect on feeding (generalised linear fixed effects model: $\chi^2_{1, 60} = 0.377$, $p = 0.539$).

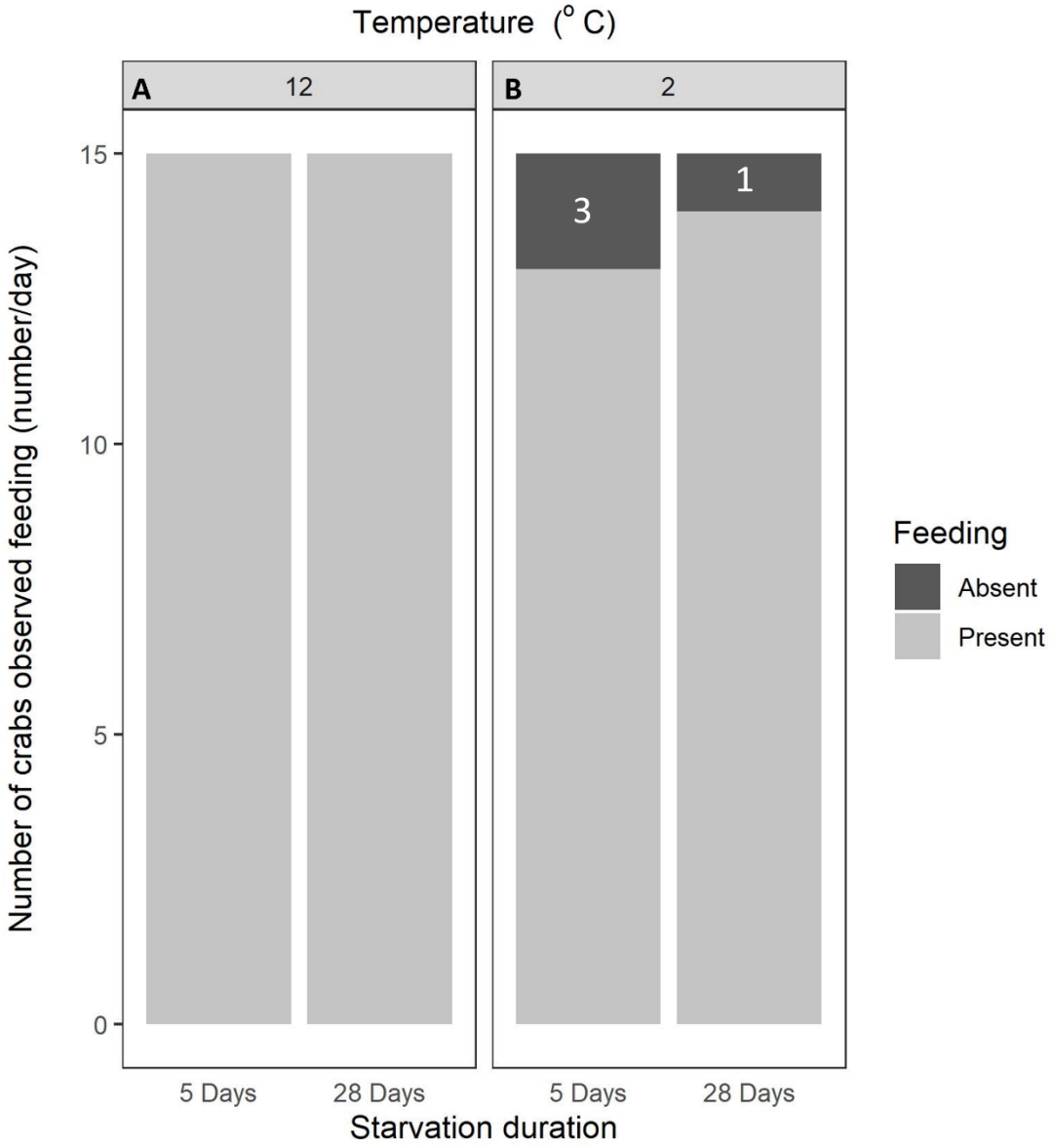


Figure 2.13 Presence and absence of feeding events: Presence and absence of feeding in green crabs after food deprivation of either 28 or 5 days. A: crabs acclimated to 2 ˚C and B: crabs acclimated to 12 ˚C. This data is displayed for each 24-hour period over 6 days. Size of the dark grey bars indicate proportion of crabs that did not feed and size of light grey bars show the number of crabs that did feed. Numbers on bars show number of crabs that did not feed.

The time taken for the first feeding event ranged from less than 30 seconds to over 13 hours (Fig. 14). This time was significantly lower in crabs acclimated to 12 ˚C, averaging 1.0 minute compared to the 211.4 minute mean for crabs acclimated to 2 ˚C (two-

way ANOVA: $F_{1,60} = 6.58$, $p = 0.013$). The duration of food deprivation (5 days or 28 days) did not affect time for first feeding, however, the crabs deprived of food for 28 days generally fed more rapidly (28 d, mean = 53.3 min vs. 5 d, mean = 147.1 minutes). Time to first feed in crabs acclimated to 2 ˚C varied more than those acclimated to 12 ˚C, which was s only seen for 5 days food deprived crabs (Fig. 14).

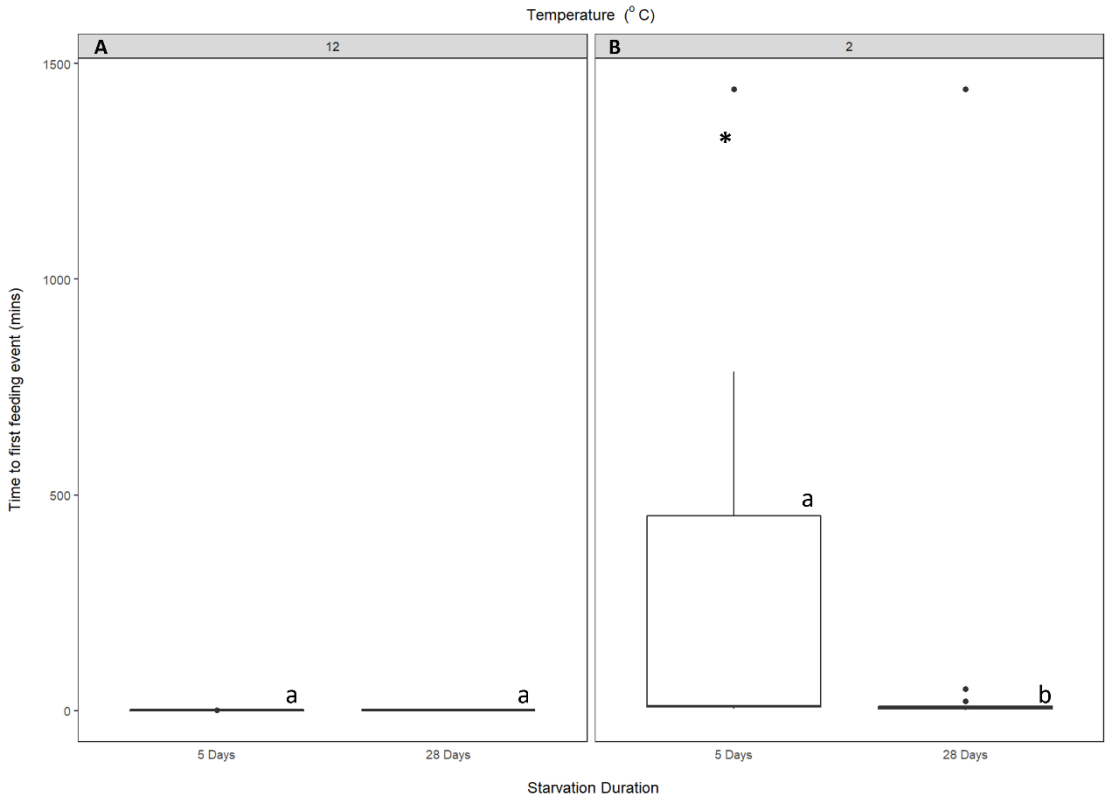


Figure 2.14 Number of feeding events per crab: Time taken by green crabs to first feed (mins) after food deprivation of either 28 or 5 days. A: crabs held at 2 ˚C and B: crabs held at 12 ˚C. The data is displayed for each 24-hour period over 6 days. Whiskers represent 95 % confidence limits, boxes show upper and lower quartiles, horizontal bar presents median values, and black circles denote outlying values. * denotes where time spent buried differed significantly from the control treatments. Lower case letters denote significant differences within treatment groups.

The amount of food consumed by green crabs (standardized for crab mass) within 24 hours was consistently greater at 12 ˚C (4.77 ± 2.41 % /g) than at 2 ˚C (0.99 ± 0.82 %

/g) (two-way ANOVA: $F_{1,70} = 70.65$, $P < 0.001$). The duration of food deprivation had no significant impact on the amount consumed (two-way ANOVA: $F_{1,70} = 0.158$, $P = 0.693$; Fig. 15).

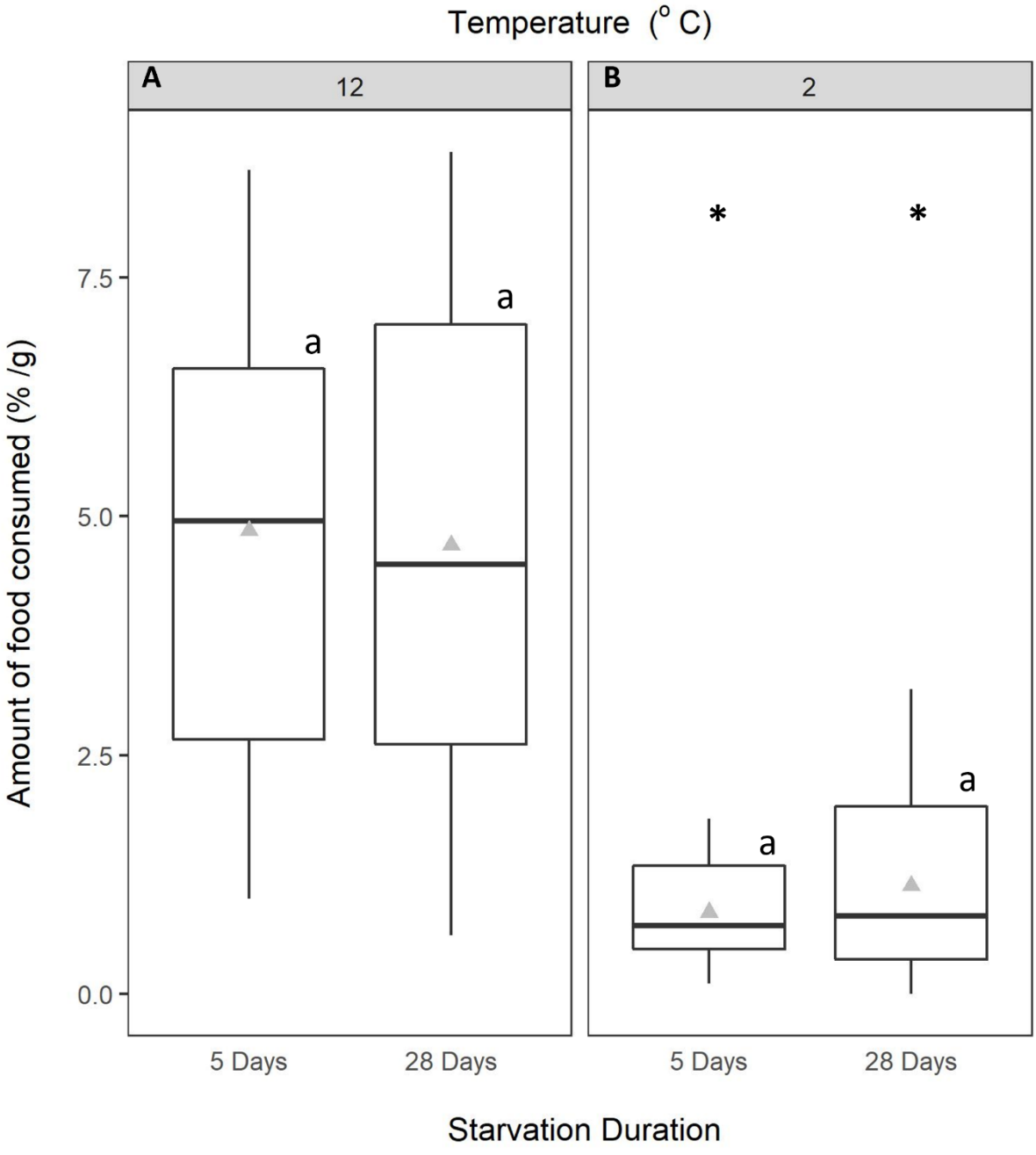


Figure 2.15 Amount of food consumed: Amount of food consumed by green crabs as percentage of crab weight (% /g) after food deprivation of either 5 or 28 days. A: crabs held at 12 ˚C and B: crabs held at 2 ˚C. The data is displayed for each 24-hour period over 6 days. Whiskers represent 95 % confidence

limits, boxes show upper and lower quartiles, horizontal bar presents median values, and grey triangles represent means. * denotes where amount of food consumed differed significantly between temperature treatments. Lower case letters denote significant differences within treatment groups.

The interaction between temperature and food deprivation duration significantly affected protein serum concentration [P] of each crab measured after food deprivation of 5 days and 28 days (multiple regression: $F_{1,67} = 9.462$, $P = 0.003$; Fig. 16). Post hoc tests (Tukey) showed that the greatest difference in [P] occurred between 12 ° C and 2 ° C acclimated crabs starved for 5 days ($p < 0.001$), indicating much higher protein serum concentrations when acclimated to 12 ° C. Crabs acclimated to 12 ° C also show a reduction in protein serum concentration when starved for 28 days compared to 5 days (Tukeys post-hoc: $p = 0.011$). [P] values were not related to time taken to feed (linear regression: $F_{1,58} = 0.693$, $P=0.409$) or to the amount of food consumed (linear regression: $F_{1,67} = 2.664$, $P = 0.1073$).

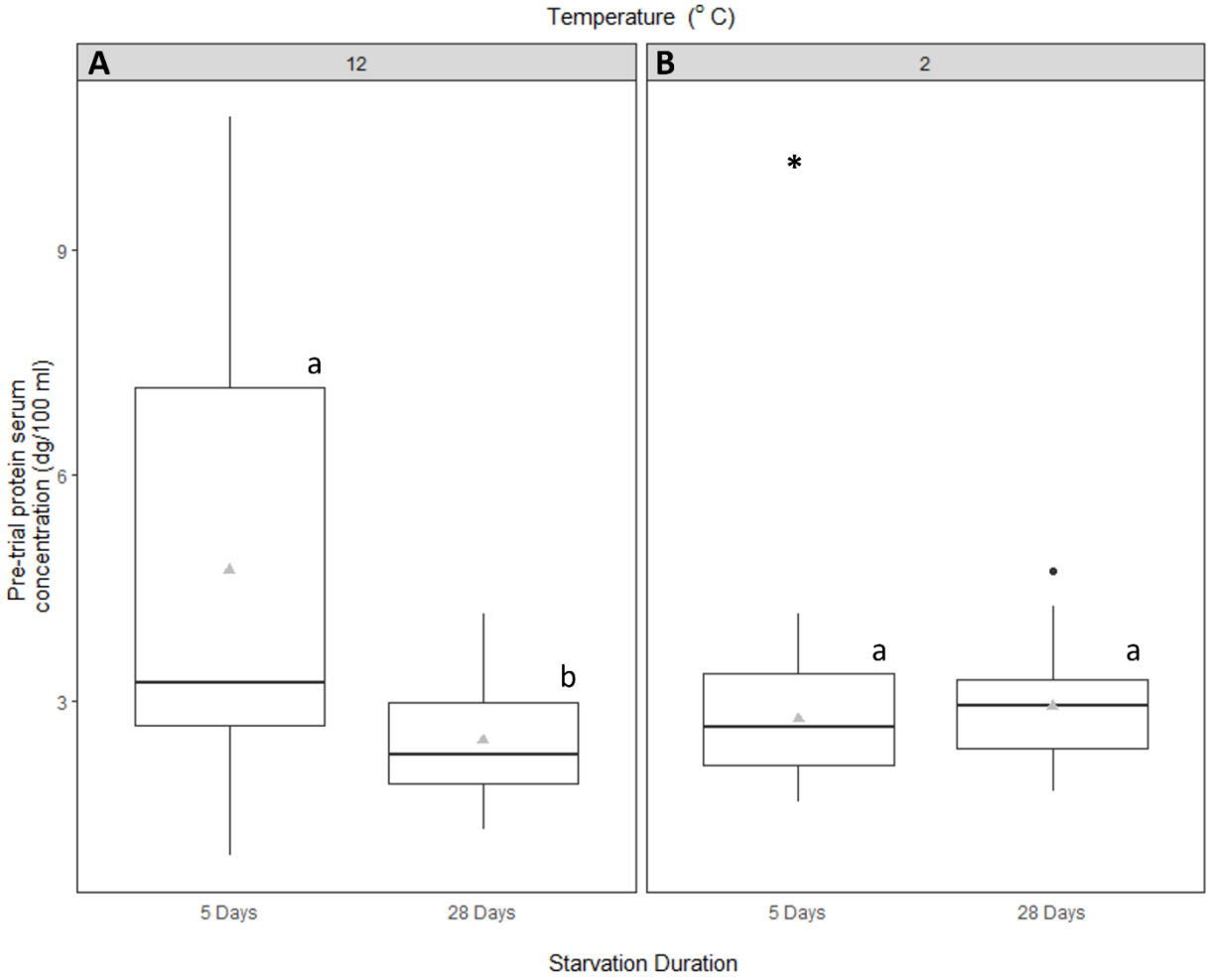


Figure 2.16 Protein serum concentration after food deprivation: Protein serum concentration (dg/100 ml) of green crabs after food deprivation of either 5 or 28 days and acclimation to A: 12 ˚C or B: 2 ˚C. Whiskers represent 95 % confidence limits, boxes show upper and lower quartiles, horizontal bar presents median values, and grey triangles represent means. * denotes where protein serum concentration differed significantly between temperature treatments. Lower case letters denote significant differences within treatment groups.

Discussion

The over-wintering temperatures in Newfoundland (Methven and Piatt, 1991; Colbourne *et al.,* 2017) are some of the coldest experienced by *Carcinus maenas*

throughout their global range (Compton *et al.*, 2010;). Green crabs in southeastern Newfoundland (Placentia Bay and Fortune Bay) contains haplotypes from both the northern and southern lineages (Lenhert *et al.*, 2017; Jeffery *et al*., 2017a,b; 2018). Several studies suggest that the 'northern lineage', originating from north-west Europe and Scandinavia, are more cold tolerant than the 'southern lineage', originating from south western Europe and northern Africa (Roman, 2006; Darling *et al*., 2008; Compton *et al.*, 2010). However, these lineage-specific tolerance hypotheses are based on observed genomic spatial structure and have not been experimentally validated (Jeffery *et al.*, 2018; Lehnert *et al.*, 18; Coyle *et al.*, 2019).

When exposed to a decline in water temperature green crabs reduced their in activity (time spent moving and distance travelled) and metabolic parameters (heart rate, oxygen consumption, estimated energy expenditure). Time spent buried, however, increased with decreasing water temperature, this gradual decline in physiological responses differed significantly (from control values at 12 °C). A similar trend was observed for the behavioural responses, but statistical differences occurred at a lower temperature, of 4 °C. Both the heart rate and oxygen consumption of crustaceans correlated positively with locomotor activity (Hamilton and Houlihan, 1992; De Wachter and McMahon, 1996; Rose *et al.*, 1998). Nevertheless, how heart rate and oxygen consumption are coordinated with the onset and termination of exercise, and how this relates to temperature responses, remains unclear. MO_2 in exercising green crabs remains elevated for around 6 minutes post exercise, returning to resting levels approximately 30 minutes after exercise has ceased (Hamilton and Houlihan, 1992) and increases in heart rate in decapods occur both before (up to 10 seconds) and after (1 -3 minutes) exercise commences (Aagaard, 1995; O'Grady *et al.*, 2001). Additionally, factors such as crab size

and moult stage can influence the relationship between MO_2 and exercise (Pennoyer et al., 2016; Houlihan and Innes, 1984). These observations support a dissociation between heart rate and MO_2 with locomotor activity. Therefore, one might not expect heart rate and MO2 to show the exact same responses at the same temperature. In addition, behavioural responses were monitored continually throughout the trials, whereas we calculated heart rate and oxygen once at hourly intervals. This difference in measurement timing could contribute to the differences in temperature responses in that green crabs could have been active during periods in which oxygen consumption and heart rate were not monitored. Different individuals were also used for each experiment, necessitating caution when interpreting the relationship between heart rate, oxygen consumption, and locomotor activity.

Overall, these results suggest that temperatures between 4 to 6 °C represent an important switch-over range for green crabs. This inference is supported by the findings of Berrill (1982) that green crabs enter a torpor-like state at approximately 5 °C. Torpor in endotherms is an energy conservation response to cold temperatures, characterized by reduced heart rate, metabolic rate, and locomotor activity (Clark, 1998). Ectotherms body temperature changes directly in-line with water temperature, thus they experience a natural decline in physiological and behavioural reactions with water temperature (Wieser, 1973). They also revive rapidly and feed, albeit at a lower rate, and they can move extensively during the winter (Chapter 3). Therefore, characterizing this behaviour as torpor must include the caveat that, only endotherms display true torpor (hibernation), and this behaviour could instead be described as ‘torpor-like’.

Reduced activity and responsiveness during exposure to colder temperatures could leave the crabs vulnerable to predation. Note that the time C. *maenas* spent buried gradually increased with reduced temperature, a response previously suggested but never directly tested within their native range (Dow and Wallace, 1952; Welch *et al*., 1968). Several different species of crustaceans bury themselves as a refuge from predators and/or adverse environmental conditions. Some crabs also bury during moulting, reproduction, and/or feeding (Nye, 1974; Barshaw and Able, 1990; Bellwood, 2002; McGaw, 2004; Bellwood, 2002). Similar behaviour may also be important for *C. maenas* to avoid predation or sudden weather events. When buried, cancrid crab cardiac output declines with interspersed episodes of cardiac arrest and pauses in ventilation of the branchial chambers (McGaw, 2004). Thus, energy conservation offers another explanation for burial in crabs (McGaw, 2005). Burying could allow *C. maenas* to maintain a state of reduced energy usage (torpor-like state) during the winter while also avoiding predation and adverse environmental conditions (Nye, 1974; Barshaw and Able, 1990; Bellwood, 2002; McGaw, 2004).

The 2 °C per day rate of temperature decline in the temperature reduction experiments exceeds what green crabs would experience during the seasonal change from summer to winter in Placentia Bay, Newfoundland, which vary on average by 10 °C. Most studies of responses of crustaceans to temperature use much more rapid temperature changes (> 1 °C/h) and tend to investigate temperature increases (Stillman and Somero, 2000; Jost *et al.*, 2012; Madeira *et al.*, 2012; 2014). Of the few studies investigating response to declining temperature, our regime is the most representative of seasonal temperature change, in that most other researchers have used much more drastic and rapid reductions in temperature (Kelley *et al.*, 2013; Tepolt and Somero, 2013). Thermal

equilibrium rates in aquatic crustaceans (time taken for core body temperature to match that of external temperature upon change) of ~ 5 minutes, means that even in the rapid temperature change experiments (> 1 °C/h) body temperature would have equilibrated with the environment (Payette and McGaw, 2003). However, heart rate and neural signal response times decrease with increased rate of change, until they reach a threshold rate of 2.5 °C per minute (Jury and Watson, 2000). Additionally, range of temperature change also affects behavioural responses, with greater ranges of change causing larger but slower responses (Young *et al.*, 2006). Similarly, movement activity of crayfish (*Astacus astacus*) is most stable at an intermediate temperate range (10-19 °C) with greater impacts by similar degrees of change at either end of its temperature range (6-10 °C and 19-24 °C) (Kivivuori, 1983; Lehti-Koivunen and Kivivuori, 1994). Although body temperature rapidly equilibrates with the ambient environment, biochemical and neural, and thus physiological and behavioural reactions, may be slower to adjust to temperature change, therefore the slower rate of temperature change used in our experiments likely produces more accurate results.

Although the experimental regime used here, encompassed the full temperature change experienced by green crabs *in situ* in coastal waters in Newfoundland, with slower change than in previous studies, our rate of change nonetheless greatly exceeded the typical seasonal changes (Chapter 3). Therefore, we also investigated the responses of *C. maenas* after long-term acclimation to typical winter (2 °C) and summer (12 °C) temperatures in Newfoundland.

Long-term acclimation to cold temperatures (2 °C), led to reduced energy requirements (lower heart rates, oxygen consumption, estimated energy expenditure) and locomotor activity in green crabs and longer periods buried. However, locomotor activity did not

completely cease, indicating that green crabs maintain some responsiveness to their environment. This interpretation aligns with previous studies showing highly reduced green crab movement below 5 °C, with complete inactivity around 0 °C (Young *et al.*, 2006; Young *et al*., 2017). The minimum 2 °C test temperature in our study was the winter water temperature in Newfoundland remains at approximately 2 °C, and only drops to 0 °C for ≤ 1 month during the winter (Chapter 3: Fig 3.7A, 3.8A). In addition, we encountered logistical problems in keeping sea water at 0 °C for extended periods of time in the lab. Although green crabs in Newfoundland appear to undergo a torpor-like state with a significant reduction in activity between 6 to 4 °C, they do not shut-down completely as occurs in some animals in the region, such as cunner, even after months of exposure to cold water. Green crab remain responsive to their environment, which could allow for opportunistic feeding or predator evasion.

Acclimation time, the time animals are held at test temperature for the days to weeks before experiments commence, strongly influences physiological and behavioural responses (Newell and Bayne, 1973; Matveev and McGaw, 2022). An acclimation time of approximately four weeks has been recommended for experiments using green crabs (Bowler, 1963; Ahsanullah and Newell, 1971). After long-term exposure to 2 °C, we found that heart rate, time spent moving and distance traveled decreased with time, whereas time spent buried increased with time. In contrast, the control animals, held at 12 °C for the same 6-day period, did not change over time. This difference in response could indicate that colder temperatures necessitate longer acclimation times, potentially impacting behavioural traits more than physiological traits, noting that we did not see a difference with MO2 and EEE.

Acclimation time, the time animals spend in their test apparatus before experiments commence, also plays an important role. Matveev and McGaw (2022) recommend a minimum of 16 hours acclimation time for *Cancer irroratus* feeding behaviour trials. Experiments on handling stress of *C. maenas*, suggest an acclimation time of ≥ 12 hours to eliminate the impact of handling (Wilson *et al.*, 2021). These experiments were conducted at 10 – 12 °C and therefore considered the ability of decapods to deal with environmental change at thermal conditions typical for this species. Physiological trials tend to use acclimation times of approximately 12 hours, whereas behavioural trials, especially those investigating foraging behaviour, generally use much shorter acclimation times of < 1 hour (Taylor and Wheatly, 1979; Sneddon *et al.*, 1997; Robertson *et al.*, 2002; Rossong *et al.*, 2006; Matheson and McKenzie, 2014). Our results, however, showing heart rate to decline from a mean of 32 BPM to 25 BPM and percentage time spent buried increase from 88 % to 95 % over the course of the experiment at 2 °C. These changes raise the question whether low acclimation temperatures require longer acclimation times, especially when conducting behavioural trials.

Previous studies report green crabs feeding at temperatures down to 4 °C, although at reduced rates (Bélair and Miron, 2009; Matheson and Gagnon, 2012); we observed a similar pattern at 2 °C. Lower metabolism and energy expenditure at reduced temperatures in green crabs (McGaw and Reiber, 2015; Fig. 2,3,8,9), would result in lower food requirements to maintain metabolic processes. In optimal conditions crabs tend to eat until their foregut is full (McGaw and Curtis, 2013; McGaw and Penney, 2014) and this certainly appeared to be the case in 12 °C. However, at 2 °C they ate less than half the amount consumed at 12 °C treatments. This difference indicates that energy requirement determines the amount of food consumed, which is lower in cold water, rather

than by gut stretch receptors indicating stomach fullness (McGaw and Curtis, 2013; McGaw and Penney, 2014).

The lower activity rates meant reduced reaction time to the presence of food at 2 °C, but only after 5 days of food deprivation. After 28 days of food deprivation in 2 °C, reaction times to food decreased, and the crabs were also more likely to feed. At optimal temperature ranges crabs generally undergo "starvation" after 28 days, whereby they start to break down protein stores in the body for energy (Wallace, 1973; Sánchez-Paz *et al.*, 2006). This pattern was echoed by a decrease in protein serum concentration in 12 °C. However, protein serum concentration [P] at 2 °C was unaffected by food deprivation duration, which also supports the low energy usage theory. Therefore, use of body energy reserves (as indicated by a decline in serum protein levels) apparently does not create an impetus to feed at 2 °C. Indeed, *C. maenas* can survive up to 5 months at 2 °C without feeding (Chapter 3). The fact that green crabs actively continued to feed, albeit less frequently and with lower intake, after prolonged exposure to cold suggests that they likely feed opportunistically during the winter, especially if food is in their vicinity (Chapter 3). Lower catches of green crabs in winter might not necessarily be because the crabs are absent, but rather a reduced need for food slows their effort to locate food (Chapter 3). Our results also support the idea that the crabs are dormant, or in a torpor-like state, rather than undergoing complete torpor/hibernation as per some anecdotal evidence from local fish harvesters.

An unexpected, but consistent trend across all the traits (physiological and behavioural) was greater inter-individual variation in warmer temperatures. For example, the range of EEE at 12 °C was more than double that at 2 °C (Fig. 9) and the range in total distance travelled at 12 °C was 20 times larger than at 2 °C (Fig. 11). Inter-individual variation

in metabolic rate might reflect variations in "personality" (defined as consistent individual differences in behavior, with bold and shy individuals) and occur in multiple species (Careau *et al.*, 2003; Réale *et al.*, 2007; Biro and Stamps, 2008; 2010; Metcalfe *et al*., 2016). Such variation in personality within populations can impact the response of labile traits to temperature changes (Biro *et al.*, 2010). Few studies have addressed personalities and the impact of inter-individual variation on the response to environmental change in crustaceans (Matveev, 2021). Our results suggest that the physiological requirements to reduce energy usage and undergo torpor after prolonged exposure to highly reduced temperatures cause reduced inter-individual variation in warmer temperatures. However, our study did not investigate consistent inter- or intra-individual differences with temperature, and the high degree of variation seen 12 °C could be incidental, suggesting a need for caution when interpreting these results.

Conclusion

Our experiments indicate significant reduction in physiological parameters and behavioural responses in green crabs at water temperatures between 4 and 6 °C. These responses to changing temperature in green crabs from the southeastern coast of Newfoundland parallel those in other populations of green crabs in both their native and invasive range (Breteler, 1975; Berrill, 1982; Young et al, 2006; Bélair and Miron, 2009; Camus *et al.*, 2004; Tepolt and Somero, 2013, 2014). This consistently suggests that the hybridized Newfoundland population retains a similar temperature induced torpor-like response to low temperatures as most other populations of green crabs. Previous studies hypothesized increased larval cold tolerance in the northern lineage (Hines *et al.*, 2004; Coyle *et al.*, 2019), but our study did not address cold tolerance in the

larvae of the hybridized population. However, the critical response temperatures, at least for the adult stages, may persist across populations.

Although we observed a significant reduction in behavioural responses crabs retained a level of activity in which they remained responsive to their environment and fed opportunistically. Classifying this behaviour as a true torpid state or hibernation requires caution in that the term primarily applies to endotherms that actively downregulate body temperature, and in a few ectotherms such as cunner (*Tautogolabrus adspersus*) during winter in Newfoundland. Cunner completely cease locomotor activity and feeding during the winter, and reduce their resting metabolic rate and cardiac output, characterised as winter dormancy (Costa *et al.*, 2013; Speers-Roesch *et al*., 2018; Knight, 2022). We found no evidence of such dormancy in green crabs, and parameters appeared to be reduced simply because their physiological responses largely reflected reduced metabolism at colder temperatures. A "torpor-like state" might therefore provide a more apt description of green crab cold response, to differentiate it from true torpor. This lack of a full torpor at these temperatures imply green crab could tolerate lower seasonal temperatures which could therefore indicate further northward range expansion is possible.

Chapter 3

Behaviour and habitat use of European green crabs (*Carcinus maenas*) during winter in Newfoundland, Canada

Abstract

European green crabs (*Carcinus maenas*) are a highly invasive coastal decapod native to Europe and northern Africa. Minimum seasonal water temperature has been hypothesized to limit green crab range expansion. Green crabs were first found in Newfoundland in 2007; this location experiences the coldest water temperatures throughout the hybrid populations' range. The hybridized population in Newfoundland contains haplotypes from both the 'northern' and 'southern' lineage. Here, I combined lab experiments and *in situ* monitoring to document green crab seasonal behaviour and habitat use, and thus better understand survival strategies in Newfoundland during the winter. Crabs were absent from the intertidal zone during the coldest months (Dec – Mar), but remained in the subtidal zone during the winter, likely to avoid exposure to air when temperatures commonly fall below 0 °C. Similarly, catch rates in monthly subtidal surveys using baited traps declined during the winter months, with a catch rate of zero in February and March, when water temperatures were lowest. *In situ* acoustic monitoring showed crabs remained within a sheltered bay during the winter and did not migrate outside the bay into deeper water, challenging theories that green crabs move to deeper waters during winter. During long term exposure (28 days) to 2 °C in a laboratory mesocosm, green crabs spent most of their time under shelter, with a concomitant reduction in locomotor and feeding activity. The *in situ* acoustic observations in the field confirmed lab experiments that crabs reduce locomotor activity during the colder months, but do not cease completely. Intermittent activity during winter likely represents feeding, because although lab experiments showed some crabs could survive 5 months at 2 °C without feeding, a decrease in protein serum and increase in mortality rates occurred after 3 months. Green crabs remain in the shallow subtidal zone during the extreme cold

temperatures in Newfoundland, and continue to move and feed at a lower rate supporting the idea that they enter a 'torpor-like state' rather than a complete hibernation.

Introduction

European green crab (*Carcinus maenas*) is a small decapod crustacean that reaches a maximum carapace width of approximately 10 cm (McGaw *et al*., 2011). It has an average lifespan that varies from 3-7 years, depending on geographical location (Klassen and Locke, 2007) and inhabit sheltered bays with soft sediment, rocky intertidal and estuarine habitats (Klassen and Locke, 2007; Amaral *et al.*, 2009; McKenzie et al. 2022). *C. maenas* are native to northwest Europe and northern Africa, but over the last 200 years have expanded their range to include every continent, except Antarctica (Carlton *et al.*, 2003; Klassen and Locke, 2007). The main mechanism for their widespread dispersal is thought to be maritime activity, specifically transport in ballast water of vessels as larvae/juveniles (Grosholz and Ruiz, 2002; Carlton and Cohen 2003). *C. maenas* success as an invader partly reflects their broad thermal tolerance (Kern et al., 2002). They can survive in temperatures up to approximately 38 ˚C and can tolerate acute exposure to temperatures as low as –1 ˚ C without apparent ill-effect (Miron et al., 2002; Young et al., 2006; Tepolt and Somero, 2014). Their larvae, however, have a narrower temperature tolerance and require water temperatures above 10 ˚C for several months for successful development (Berrill, 1982; de Rivera *et al.*, 2007; Hidalgo *et al*., 2005). Although *C. maenas* continue to invade new locations and expand their range within established locations, they have not colonised polar or tropical regions, most likely because of the temperature limitations of their larvae. Minimum seasonal sea surface temperature has been suggested as a highly influential factor that may determine northern range expansion and limit of green crabs (Audet *et al.*, 2003; Hidalgo *et al.*,

2005; Jefferey *et al.*, 2018). The origin of the population also affects the ability of green crabs to tolerate low temperatures with higher cold tolerance in populations from northern Europe (Norway and Iceland), than southern (southern Europe and north Africa) populations (Compton *et al.*, 2010).

Numerous studies have documented responses of green crabs to temperature increases (Frederich *et al.*, 2000; Madeira *et al.*, 2012; McGaw and Whiteley, 2012; Kelley *et al.*, 2013). In general crabs increase in metabolic rate and heart rate with increasing temperature (Ahsanullah and Newell, 1971; Whitely *et al.*, 1997; McGaw and Whiteley, 2012; Jørgensen *et al.*, 2017). This increase occurs until reaching a critical thermal maximum, followed by a sharp decline in MO_2 and heart rate thereafter, until death ensues (Taylor, 1981; Whitely *et al.*, 1997; Cuculescu *et al.*, 1998). In addition, locomotor activity increases with temperature and green crabs forage more consistently and efficiently during the warmer, summer months (Elner, 1980), reaching optimum foraging rates at approximately 12 ˚C (Bélair and Miron, 2009a). In contrast, fewer studies have considered the responses of green crabs to declining temperatures. Berrill (1982) observed reduced feeding, lack of mating, and reduced growth at temperatures below 7 ˚C and suggested torpor occurs at approximately 5 ˚C. A significant reduction in heart rate, metabolism, feeding, and locomotor activity also occurs at around 5 ˚C (Breteler, 1975; Camus *et al.*, 2004; Young *et al.*, 2006; Tepolt and Somero, 2013; Tepolt and Somero, 2014; Chapter 2), further supporting the potential existence of a state of torpor at these temperatures. However, little information exists on the effects of prolonged cold exposure < 5 °C (as experienced during the winter) on the physiological and behavioural responses of this species.

In addition to acute responses to temperature change, green crabs respond slower to seasonal changes. As the water warms during the spring, they move into shallow sheltered bays, the rocky shore intertidal zone, and estuarine habitats. During the colder months they appear to be largely absent from these habitats (Klassen and Locke, 2007; Amaral *et al.*, 2009). Previous studies report overwintering migration from the intertidal zone into the subtidal zone in green crabs in their native range (Naylor, 1962; Atkinson and Parsons, 1973). Departure of crabs from the intertidal zone occurs in November when water temperature consistently falls below 10 °C and they return to the intertidal zone in May when ocean temperatures reach about 10 °C (Atkinson and Parsons, 1973). Daily movements, from the shallow subtidal to the intertidal zone, also cease during the winter months (Atkinson and Parsons, 1973). This strategy may reduce the risk of exposure to cold air in the intertidal zone and individuals may seek less variable temperature regimes (Edwards, 1958; Naylor, 1962; Sharp *et al*., 2003; Zarrella-Smith *et al*., 2022). However, these earlier studies were carried out used baited traps, which primarily attract feeding crabs and therefore, do not definitively prove that crabs leave the intertidal zone during the winter, only that fewer crabs respond to bait. A seasonal reduction in feeding activity could also result in reduced catches in winter (Breteler, 1975; Berrill, 1982; Chapter 2). Advances in technology acoustic telemetry has effectively enhanced assessments of both short-term (Lynch and Rochette, 2007), and longer-term seasonal movements of green crabs within an estuarine system (Zarrella-Smith *et al*., 2022). The latter study reported that most crabs restrict their movements within an area of 300 – 600 m^2, regardless of season. However, increased directional movement downstream is associated with a drop in temperature < 10 °C and some of the individuals overwinter in the downstream areas.

Previous studies suggest burying as an overwintering strategy for avoiding the coldest part of the winter (Dow and Wallace, 1952; Welch, 1968, see appendix 1 for more information). Crabs bury themselves in the sediment, coupled with a reduced heart and metabolic rate (Atkinson and Taylor, 1988; Bellwood, 2001; McGaw, 2004; Chapter 2). A similar reduction in energy expenditure also occurs with torpor (Cumberlidge and Uglow, 1978), which green crab could use in combination with burying. However, the duration and extent to which green crabs may remain buried and any seasonal differences in this behaviour remain unclear.

Green crabs were first reported in Placentia Bay Newfoundland, Canada, in 2007 (Blakeslee *et al.*, 2010; DFO, 2011). They have since been sighted in Fortune Bay and the west coast of Newfoundland (DFO, 2011). Water temperatures in coastal regions of southern Newfoundland average approximately 0 to 2 ˚C during the winter (Methven and Piatt, 1991; Colbourne *et al.*, 2017), these are harsher conditions than experienced by *C. maenas* in the entirety of their current range (Compton *et al.*, 2010). Indeed, the invasion of Newfoundland by *C. maenas* defied thermogeographic models that predicted the minimum sea surface temperatures during the winter are too low for larval green crab survival (Compton *et al.*, 2010).

Green crabs in Placentia Bay are a hybridised population containing a mix of haplotypes from the southern lineages (originally settling in New England) and a more recent cold-tolerant northern European lineage (Blakeslee et al. 2010; Jefferey *et al.*, 2017; Jefferey *et al.*, 2018). This population appears to be on their northern limits of cold tolerance, despite this very little is known about how this population survives during the low winter temperatures characteristic of Newfoundland waters. Local fishermen and scientists theorise that they may move from the intertidal and shallow subtidal zone into deeper

warmer waters, as occurs in native and more established populations (Naylor, 1962; Sharp *et al.*, 2003), but at present no studies have validated these theories.

My study aimed to gain a greater understanding of the long-term behavioural responses of green crabs to prolonged exposure to cold temperatures characteristic of Newfoundland. I, therefore, combined laboratory and field studies to investigate (i) any seasonal differences in *in situ* habitat use, (ii) whether green crabs need to feed when spending multiple months at low temperatures, (iii) difference in movement, burying and feeding behaviour at 12°C and 2 °C, and (iv) *in situ* over-wintering movement and habitat use, to determine whether green crabs move to deeper waters during the winter.

Methods

Adult male green crabs (*Carcinus maenas*) were collected from July to September 2020 using baited net traps from multiple locations in northern Placentia Bay, Newfoundland. Large males (carapace width > 5 cm) were transported to the Ocean Sciences Center, Memorial University. Female green crabs were not used in experiments because protocols to prevent spread of this invasive species precluded their transport and live storage. The crabs were held in 45 L flow-through seawater tanks (salinity 31-32 ppt) at ambient temperatures ranging between -0.5 - 13 ˚C. Air stones in each tank maintained oxygen concentration > 90% saturation. PVC pipes (10 cm diameter x 12 cm or 24 cm length) were placed in tanks to provide shelter. I fed crabs herring once a week, removing any dead specimens and uneaten fish from tanks promptly to minimize fouling of the water.

To investigate the seasonal *in situ* abundance of green crabs in both the intertidal zone and the low intertidal/shallow subtidal zone of southern Newfoundland, I conducted

monthly catch per unit effort surveys (CPUE). The low intertidal/shallow subtidal collections were carried out along three wharfs (September 2020 – August 2021) in Southern Harbour, Placentia Bay, NL (Fig. 1). Ten Promar TR-303 (Gardena, USA) collapsible fish and crab traps (90 cm x 60 cm x 50 cm) were set off the wharfs in the same locations every month (Fig. 2) and bottom I recorded water temperature at each location using an Aqua Vu (Gen 2) camera and digital thermometer (Crosslake, MN, USA) on each survey date. The depth of each location at high tide varied between 0.5 and 7.6 m. Traps were set at roughly the same time each month (~ 9 - 10 am), although tide state at deployment differed. I baited each trap with 2 herring, cut into pieces and placed them in bait pots prior to soak times of 4 – 5 hours.

Hand collections assessed the presence of green crabs in the intertidal zone. Once each month I surveyed crab abundance for 30 minutes in the rocky intertidal zone in Little Harbour East (LHE), Placentia Bay, (January 2021 – December 2021). Conducting the surveys within 15 minutes either side of low tide allowed me to overturn and move aside rocks and seaweed to search for green crabs. Surveys were conducted in a zig-zag pattern moving from the water's edge to the upper edge of algae cover (Fig. 3), while moving horizontally along the water line.

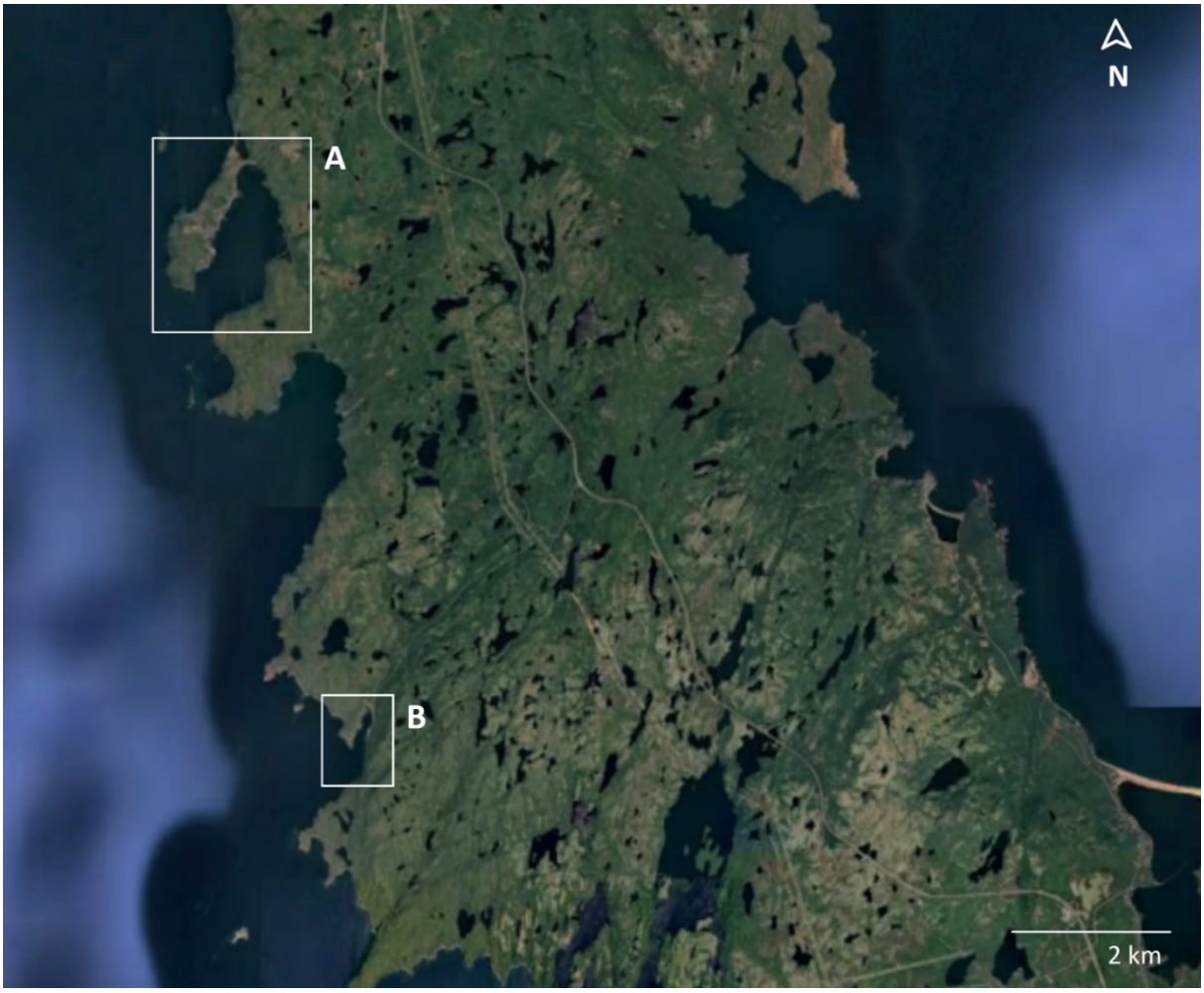


Figure 3.1 Catch per unit effort survey site locations: Map of The Isthmus of Avalon, Newfoundland, joins the Avalon Peninsula to Central Newfoundland. Survey sites marked by white, lettered, squares: A: Southern Harbour, site of low intertidal/shallow subtidal surveys, and B: Little Harbour East, site of intertidal surveys.

We attached six HOBO Pendant MX Water Temperature Data Loggers (Bourne, USA) to concrete blocks (~ 30 x 10 x 5 cm) to record air and water temperature in the intertidal zone, placing them at different locations in the intertidal zone so that they were either directly exposed to the air on the low shoreline or sheltered (under a rock) at mid shore. The loggers recorded ambient temperature every 15 minutes to capture all tide condi-

tions while ensuring battery life for the full 12-month deployment. Unfortunately, I recovered only one logger, however, this logger provided good temperature coverage of both air and water temperatures within the intertidal zone because I placed it in the mid shore under a rock shelter. Because the logger recorded the temperature during the day and night every 15 minutes, and at low and high tide the interaction between these factors made it impossible to accurately determine when it was emersed and immersed. Therefore, I calculated a single mean temperature for both air and water combined. For both monthly surveys I recorded the number, sex, and size of crabs (see Table 1 for description of these categories). I also recorded abdomen colour of each crab as either green, orange or red (McGaw *et al.*, 1992).

Table 3.1 Green crab size categories: Description of size categories recorded for all green crabs caught during Catch Per Unit Effort surveys.

Size categories	
Males	Females
S: < 50 mm	S: < 40 mm
M: 50 – 60 mm	M: 40 – 50 mm
L: 60 – 80 mm	L: 50 – 60 mm
XL: > 80 mm	XL: > 60 mm

For each of these experiments, I calculated catch per unit effort, separately, for each month by dividing the total number of crabs caught by the number of hours of sampling effort (total amount of time traps were deployed/hand collection performed). I also recorded size and sex of green crabs, but some months contained very few individuals per

category. Therefore, I calculated and present only total monthly CPUE (Table S2.1 lists individual categories) for the intertidal and subtidal data. For the low intertidal zone (trap) collections I averaged the bottom water temperature recordings at each trapping location for each month. For the intertidal survey I calculated the mean temperature from both air and water temperature using data from two weeks before and after the survey date (it was necessary to combine air and water temperature because often we were unable to determine when the tags were emersed as temperatures were similar to one another). I then contrasted the CPUE data for the intertidal and subtidal surveys considering air and water temperatures across time/season.

Figure 3.2 Subtidal catch per unit effort sample site locations: Map of Southern Harbour, Newfoundland (N47.425424, W53.574382). Showing locations of wharfs (A, B and C) used for subtidal CPUE surveys and trap positions (1 – 10) for monthly surveys at each wharf. A: the first wharf sampled with traps during each survey, consisting of traps 1 to 3. B: the second wharf sampled with traps during each survey consisting of traps 4 to 6. C: the third wharf sampled with traps during each survey, consisting of traps 7 to 10.

Figure 3.3 Intertidal survey site: Photograph of the rocky shore in Little Harbour East (N47.383053, W53.560688) annotated to show the categorisation of upper and lower shore used for recording shore location of green crabs found during monthly intertidal surveys. The upper limit of the intertidal zone as the edge of algae cover and the lower limit t as the edge of the water line at low tide.

To investigate how long-term exposure to winter temperatures affects the physical condition of *Carcinus maenas* I housed crabs (n = 96) in individual plastic covered wire mesh chambers (chambers: 30cm x 30 cm x 15 cm, mesh: 1 cm^2) in two 45 L flow through tanks (served from the same sea-water source pumped from 40m adjacent Logy Bay). The experiment was conducted at ambient temperature for 5 months from January 12th to June 12th. During this time the ambient water temperature gradually climbed from ~ 0.5 ˚C to 7 ˚C, following the typical seasonal change in water temperature from winter to spring in Newfoundland (Colbourne *et al.*, 2017).

Before the trial commenced the crabs were acclimated to 2 ˚C for 2 weeks and I recorded the following information: mass (g), carapace width (to the nearest mm), any leg loss, and haemolymph protein concentration. Crabs were checked twice weekly; any mortalities were recorded and removed. To determine the importance of feeding during winter months, the crabs were separated into two groups, depriving half of food for the entire period while feeding the others herring once per week (~ 5 g per crab) for the duration of the trial. I used mortality rate, for both the starved and fed group, to determine the impact of starvation, and thus the importance of feeding on survival during the temperatures typical of the winter months in Newfoundland.

Once per month, I removed each crab from the cage to collect a haemolymph sample for measurement of protein concentration, at this time I weighed them. The haemolymph protein concentration was provided as an indication of crab physical condition and nutritional status (Moore *et al.*, 2000; Oliver and MacDiarmid, 2001; Ozbay and Riley, 2002; Wang and McGaw, 2014). Haemolymph was withdrawn using a 1 ml syringe and 16-gauge needle, inserted into the soft tissue between the joints of the pereiopods. The haemolymph (300 ul) was placed into the sample well of a Brix/RI-

Check Digital Pocket Refractometer (Reichert Analytical Instruments, Depew, NY), which I calibrated with deionized water prior to each haemolymph measurement. The Refraction Index (RI) was recorded for each crab and converted to haemolymph protein density (in dg/100 mL) using the equation $HD=510(RI_{water}-RI_{hemolymph})-1.81$ (Sunderman, 1944; Wang and McGaw, 2014).

To monitor the behaviour of *C. maenas* over a prolonged time period, I set up a laboratory mesocosm experiment using a large tank (450 cm x 200 cm x 150 cm), designed to mimic natural conditions as closely as possible. Structures added to the tank allowed natural behaviours: two trays filled with sand (35 x 30 x 5 cm) for burying, 15 shelters (30 x 13 cm) constructed from transparent PVS and rocks (mean size: 12 cm x 8 cm x 3 cm) for sheltering, and a tray of live blue mussels (*Mytilus edulis*) (~ 200 mussels) for feeding (Fig. 3). The flow through (2-4 L/min) system maintained water temperature at 1 - 2 ˚C, salinity at 31 - 32 ppt, and air stones maintained the oxygen concentration above 90% saturation. A natural light regime was maintained using photocell control. During the day fluorescent tubes of approximately 300 lux lighted the tank. During hours of darkness the lights were turned off and red-light bulbs (approximately 100 lux) were used: this strategy allowed me to record video footage without disturbing the crabs, as they cannot detect red light (Cronin, 1986). The experiment ran for 4 weeks during the winter from February 9th to March 10th. I then conducted a separate control experiment in the same apparatus with a separate group of crabs (acclimated to 12 °C) and maintained water temperature at 11-12 ˚C from March 22nd to April 15th, to compare green crab behaviour in winter and spring/summer conditions.

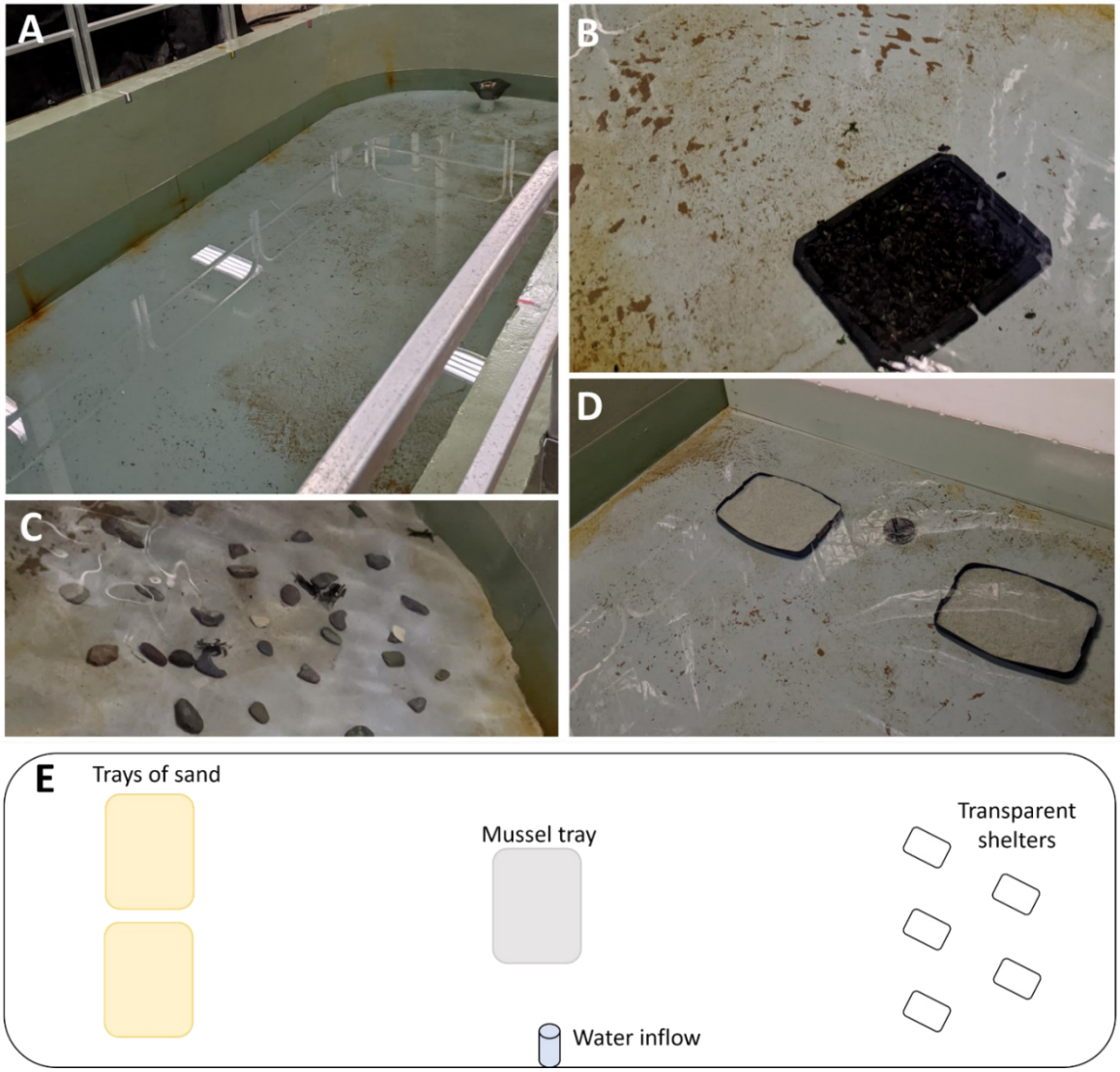


Figure 3.4 Mesocosm experimental setup: Images of the mesocosm experimental setup and schematic of tank layout. A: the full length of the tank used (~ 2.5 m) filled to 35 cm depth, as used in experiment. B: experimental tank with tray of mussels placed in the centre. C: experimental tank with shelters made of clear PVC and rocks placed at one end, showing green crabs in the tank. D: experimental tank with two sand boxes placed at one end. E: representation of the layout of the tank and relative positions of the trays of sand, mussel tray and shelters (not to scale).

I recorded carapace width, weight, any leg loss and haemolymph protein concentration for each crab (experimental group: n=20, control group: n= 21) and glued a large unique foam letter/number (~ 3 cm^2) to the carapace of each crab, to enable easy identification

on the video footage, before placing them in the tank. Crabs were left to acclimate in the tank for 2 days before recording began. A Brinno TLC200 Pro HDR Time Lapse Video Camera (Taipei City, Taiwan) mounted above the tank captured the whole tank (1 frame every 30 seconds), recording continuously throughout the experiment. A dark plastic sheeting draped around the tank prevented visual disturbance.

Using the foam tags to identify individuals, I recorded the behaviour of each crab for the full 4-week duration of the experiment, scoring behaviours as stationary (remaining still, some positional alterations, but the animal did not move location), sheltered (remaining still with all or part of their body under a shelter), active (using pereiopods to reposition their body) or buried (fully or partially covered in sand and remaining still). I then calculated the percentage of time green crabs spent performing each behaviour over the entire experimental period (28 d). This experiment aimed to understand behaviour and habitat use in relation to seasonal changes in temperature, therefore the experimental design did not consider effect of light. Additionally, the video recording method, made calculation of percentage time per day and night cycle impractical, and I recorded these percentages per entire experimental period. Sheltered and stationary behaviours were further categorised as 'solitary' (remaining stationary while not in physical contact with any other individuals) or 'aggregated' (remaining stationary while in physical contact with at least one other individual). This categorization reflected the fact that animals often congregate in groups while remaining immobile for long periods of time.

In addition to these behaviours I also recorded the number of feeding events for each crab. The actual time spent feeding was challenging to measure, because individuals would often spend many hours on the feeding tray, but determining when feeding events

began and finished was almost impossible. Therefore, I recorded the number of individual feeding events. A feeding event was considered as an approach to the mussel tray, or a food item which may have been displaced from the tray.

An acoustic positioning array deployed in the bay of Little Harbour East (LHE) monitored crab movement and habitat use during the winter months in Newfoundland. LHE is a small (~0.7 km length x ~0.22 km width), sheltered bay in southern Newfoundland (Fig. 4), with one small main dock and limited boat traffic. A mix of sand and rocky surfaces with a large amount of kelp cover seafloor (4 – 20 m depth). I chose this location for the acoustic monitoring based on its small size, sheltered location, and lack of winter scallop dredging activity (a common activity in many other local harbours).

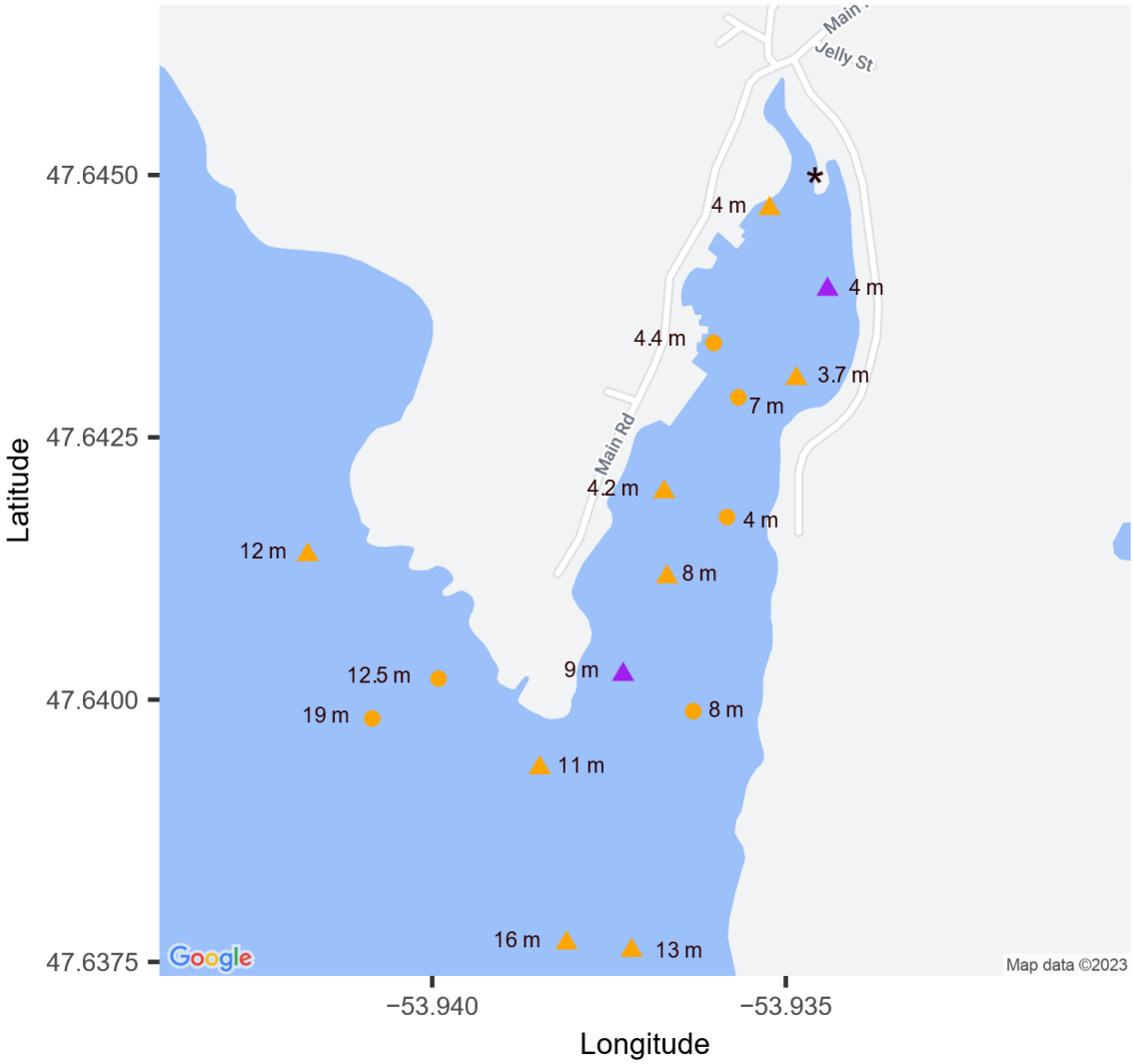


Figure 3.5 Acoustic array receiver positions: Locations of moorings set to form acoustic array within Little Harbour East (N47.383246, W53.560938). Moorings were set in December 2020 and retrieved during June 2021. Each mooring was constructed as in Fig 6, every mooring had a receiver, some moorings had an additional temperature logger, some had an additional coded transmitter and some had both. Purple points indicate moorings with temperature loggers attached and orange points indicate those without. Triangles indicate moorings without coded transmitters attached and circles indicate those without. Figure shows depth at location where I set each receiver. * indicates the crab release location.

Sub-surface moorings (Fig. 5) comprised Vemco VR2W-69 kHz Acoustic Receivers (Nova Scotia, Canada) attached ~ 1 m from the bottom. Small buoys attached to the top of the mooring kept the receiver upright. I used 17 moorings, 10 VEMCO V969 kHz

(Nova Scotia, Canada) coded transmitters attached above the receivers to act as sync tags for data accuracy. I attached two HOBO Pendant MX Water Temperature Data Loggers (Bourne, USA) to separate moorings, one within the bay and the other outside, to record water temperature for the duration of the receiver deployment. The moorings were placed in triangular arrays (as much as possible) within the bay of LHE, and along the east and west coastline just outside the bay (Fig. 4) on 25th November 2020.

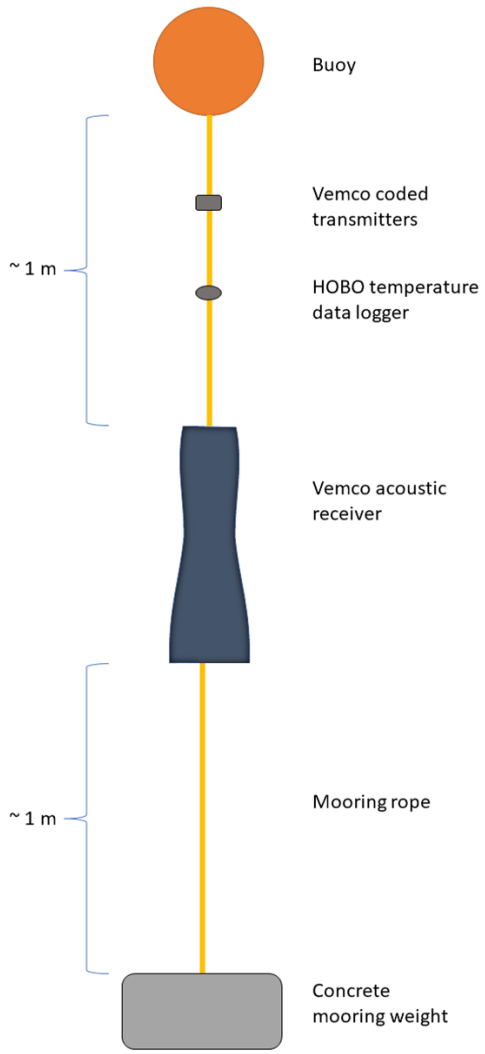


Figure 3.6 Acoustic array receiver mooring: Diagram of acoustic mooring set up. Small buoy attached at the top of the mooring, followed by a VEMCO V969 kHz coded transmitters (in some cases) and a HOBO Pendant MX Water Temperature Data Logger (in some cases), then a Vemco VR2W-69 kHz

Acoustic Receiver and finally a concrete mooring weight. Each component was attached to mooring rope using knots, zip ties, and electrical tape. Mooring total length varied between 3.5 - 4 m.

I captured twenty-five large (> 55 mm carapace width) male crabs at the site and attached VEMCO V7 69 kHz coded acoustic transmitters (12 mm diameter x 40 mm, 1.5 g) to the carapace using super glue (Morse and Rochette, 2016). Green crabs moult during the late summer when water temperatures increase, and because this study was carried out between November and June this reduced any loss of transmitters given that moulting would not occur during this study (Poirier *et al.*, 2016). I recorded carapace width, weight, and abdomen colour and released the crabs within 4 hours of capture and acoustic tagging at the site on 27th November (point marked on Fig. 5).

The moorings and receivers remained in the bay until mid-June 2021 spanning the whole of the Newfoundland winter and ensure that water temperatures had started to increase before retreiving the receivers. Once retrieved, I offloaded the raw data from receivers and sent it to the manufacturer (InnovaSea) for initial positioning analysis. Each time an array of receivers detected a tagged crab, this data was stored and the crab's position calculated. I then superimposed the GPS position of each detection over an outline of LHE bay. Colour coding of the monthly movements enabled visualization of the spatial movements for each crab. The movement patterns of all 25 crabs (November to June) were compiled on one figure and the location and mean depth at each transmitter is shown (Fig. 17). Individual plots for each tag were also created to more clearly show activity change with time and the individuality of movement behaviour during the winter (Fig. 18). Hydrophones won't detect sound pulses emitted by the tags when crabs shelter under rocks or bury in the sand, we assume here that when a tag is detected the crab is out in the open and likely active (Morse and Rochette, 2016). The number of

detections per day per crab were therefore calculated and the sum per month plotted against time to represent the seasonal change in activity (Fig. 19).

To ensure accurate positioning, we included only positions with horizontal position error (HPE) values of less than 20 (Espinoza *et al.*, 20011; Scheel and Bisson, 2012; Furey *et al.*, 2013). Additionally, we removed all positions on land (could not plausibly be underwater due to tides) because they represented errors in positioning (green crabs are not on land during the winter months; Fig. 8). This filtering retained 94 % of the data, so I selected this HPE cutoff because it removed the majority of on-land positions, which are ecologically known to be inaccurate, while retaining a large proportion of the data (Meckley *et al.*, 2014). Our study did not aim to identify fine-scale movement of individual green crabs but rather to understand their general location and movement activity during the winter months. We therefore felt this level of filtering to preserve data retention was sufficient.

Statistical analysis

A Kaplan-Meier Log rank survival test compared survival rates between fed and unfed crabs and overall survival rate. To assess whether lack of food affected mortality rate, we ran separate linear regressions on both fed and unfed groups to assess the effect of time on mortality. A non-parametric analysis for longitudinal data (nparLD) and Tukey post-hoc test compared monthly protein serum concentrations between fed and unfed crabs and between months (Noguchi *et al.*, 2012; Wilson *et al.*, 2022). All analyses were conducted in R version 2022.7.1.554 and nparLD version 2.2 package using Rstudio.

Due to numerous zeros in the data it necessitated, a non-parametric, permutational multivariate analysis of variance (PERMANOVA: Anderson, 2014) to identify differences

in green crab habitat use (total time spent in each habitat) depending on temperature (two factors: temperature, habitat type). If we observed an overall significant impact of temperature we followed with an independent one-way ANOVAs or Kruskal-Wallis rank sum tests (depending on results of Shapiro-Wilk normality tests: ANOVA = normally distributed, Kruskal-Wallis = non-normally distributed) to assess the effect of temperature on the total time spent using each habitat (sheltered, buried, out in open, moving). Holm-Sidak adjusted *post hoc* tests enabled multiple pairwise comparisons. We calculated percent time spent aggregated and solitary from total time spent sheltered and out in the open. Kruskal-Wallis rank sum test analysed the effect of habitat choice (sheltered or out in open) and temperature (2 °C or 12 °C) on the proportion of time spent aggregated or solitary. A Kruskal-Wallis rank sum test was used to assess the difference in the total number of feeding events between the experimental runs at 12 °C and 2 °C.

Results

The highest catch per unit effort (CPUE), 20 crabs per hour, was recorded in August which corresponded with the highest sea water temperature, 22 °C (Fig. 7). The lowest CPUEs occurred from January (0.09 crabs/hour) to March (0.06 crabs/hour), and we captured no crabs in February. In January water temperature had dropped below 5 °C, and the lowest water temperature occured in March (mean temperature 0 °C). Incremental changes occurred in CPUE over time (Fig. 7A), forming clusters: January to April (0 – 1 crab per hour), May to July (9.3 – 16.3 crabs per hour), August to September (16.7 – 19.4 crabs per hour) and October to December (4.7 – 6.7 crabs per hour). Water temperatures however, gradually changed seasonally (Fig. 7B), with the greatest change in water temperature occurring between March and April (increasing by ~ 5.5 °C).

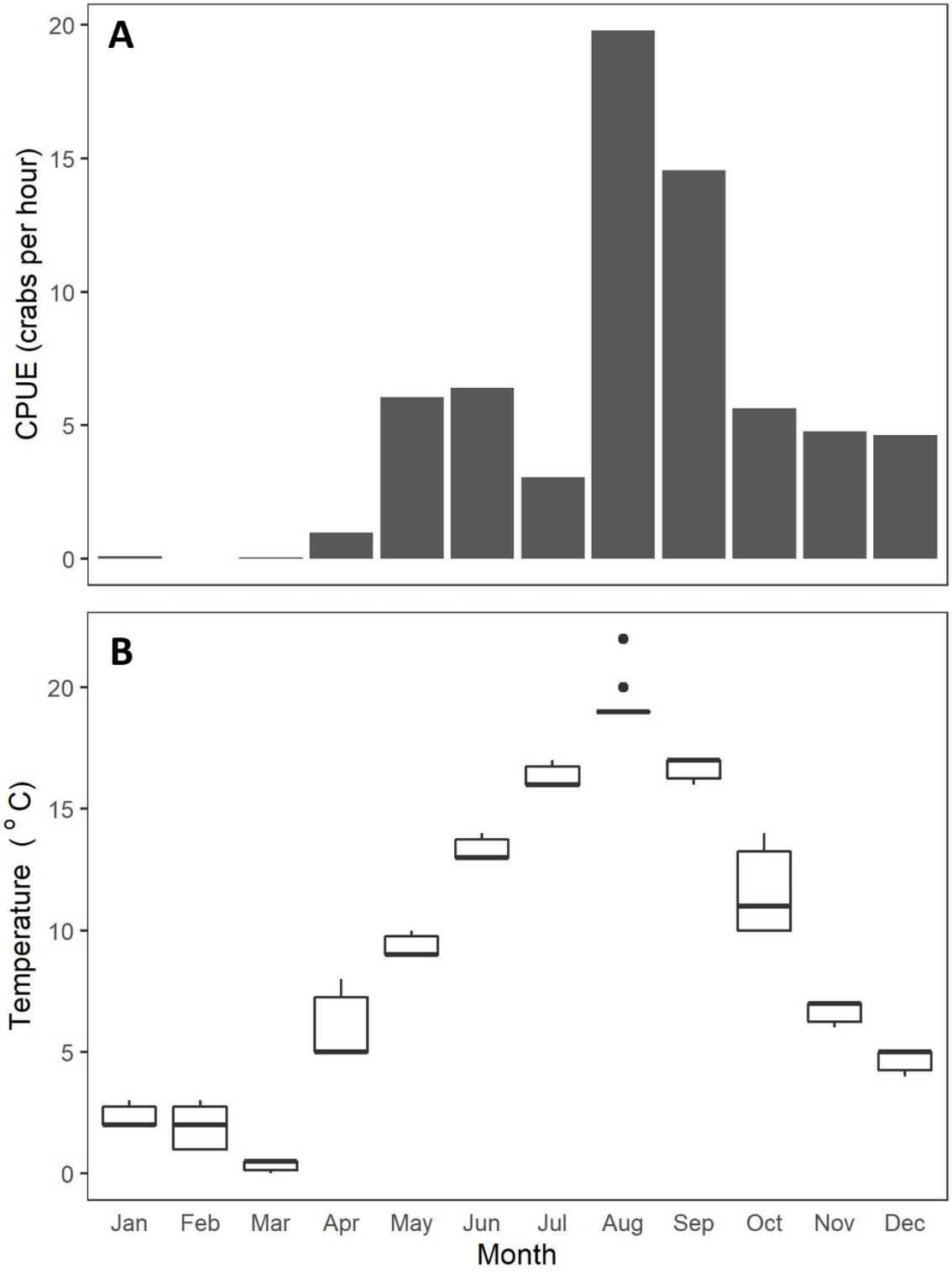


Figure 3.7 Subtidal trap catch per unit effort results and temperature: Catch per unit effort (CPUE) of green crabs and water temperature in subtidal Southern Harbour, Newfoundland, per month. A: CPUE (number of crabs per hour) per month, and B: water temperature (°C) per month, whiskers represent 95 % confidence limits, boxes show upper and lower quartiles, horizontal bar presents median values and black circles denote outlying values. The

surveys were run from September 2020 to August 2021, displayed here from January to December to more clearly show seasonal change.

No green crabs were found in the non-trap surveys in the intertidal zone during the coldest months of the year (December-April) (Fig. 8A). The crabs first appeared in small numbers in the intertidal zone in May. However, CPUE remained low from May to August (< 15 crabs per hour), although temperature gradually increased each month from a mean of 7 °C to 17 °C (the highest mean temperature) during this time. In September CPUE increased noticeably to 86 green crabs per hour, whereas mean temperature had decreased slightly (from an August maximum of 17 °C) to 15 °C (Fig. 8). A significant drop in CPUE followed in October (36 crabs per hour) and November (11 crabs per hour) and no crabs were observed in December. The temperatures measured in the intertidal zone varied more than those in the subtidal zone as they represent the full range of temperatures experienced in the intertidal zone throughout the year, including air and water temperatures (Fig. 8B).

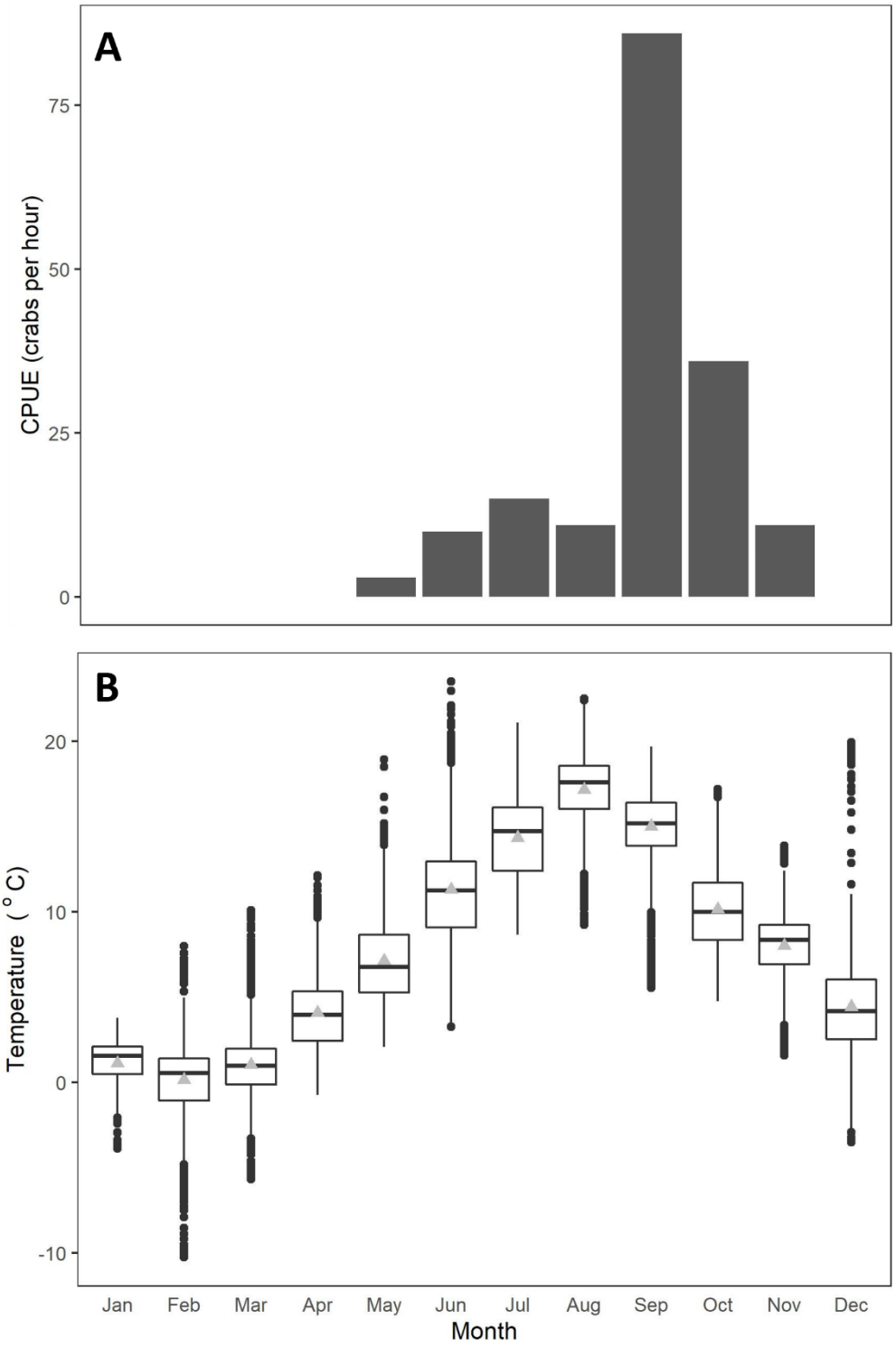


Figure 3.8 Intertidal survey results and temperature: Catch per unit effort (CPUE) of green crabs and water/air temperature in the intertidal zone in Little Harbour East, Newfoundland, per month. A: CPUE (number of crabs per hour) per month, and B: air and water temperature (°C) per month, whiskers represent 95 % confidence limits, boxes

show upper and lower quartiles, horizontal bar presents median values, black circles denote outlying values and grey triangles denote the mean.

The proportion of surviving green crabs declined during the 5-month experimental period for both groups (fed and unfed; Fig. 9), with no difference in the survival rates (time to 50% mortality) of fed and unfed crabs (Kaplan-Meier survival log-rank$_{1,96}$ = 0.037, P = 0.847), and similar survival proportions by the end of the trial for both fed (0.375) and unfed (0.291) crabs. The median time to 50 % mortality in fed crabs was 104 days (67 % of total trial duration) and 118 days (76 % of total trial duration) in unfed crabs.

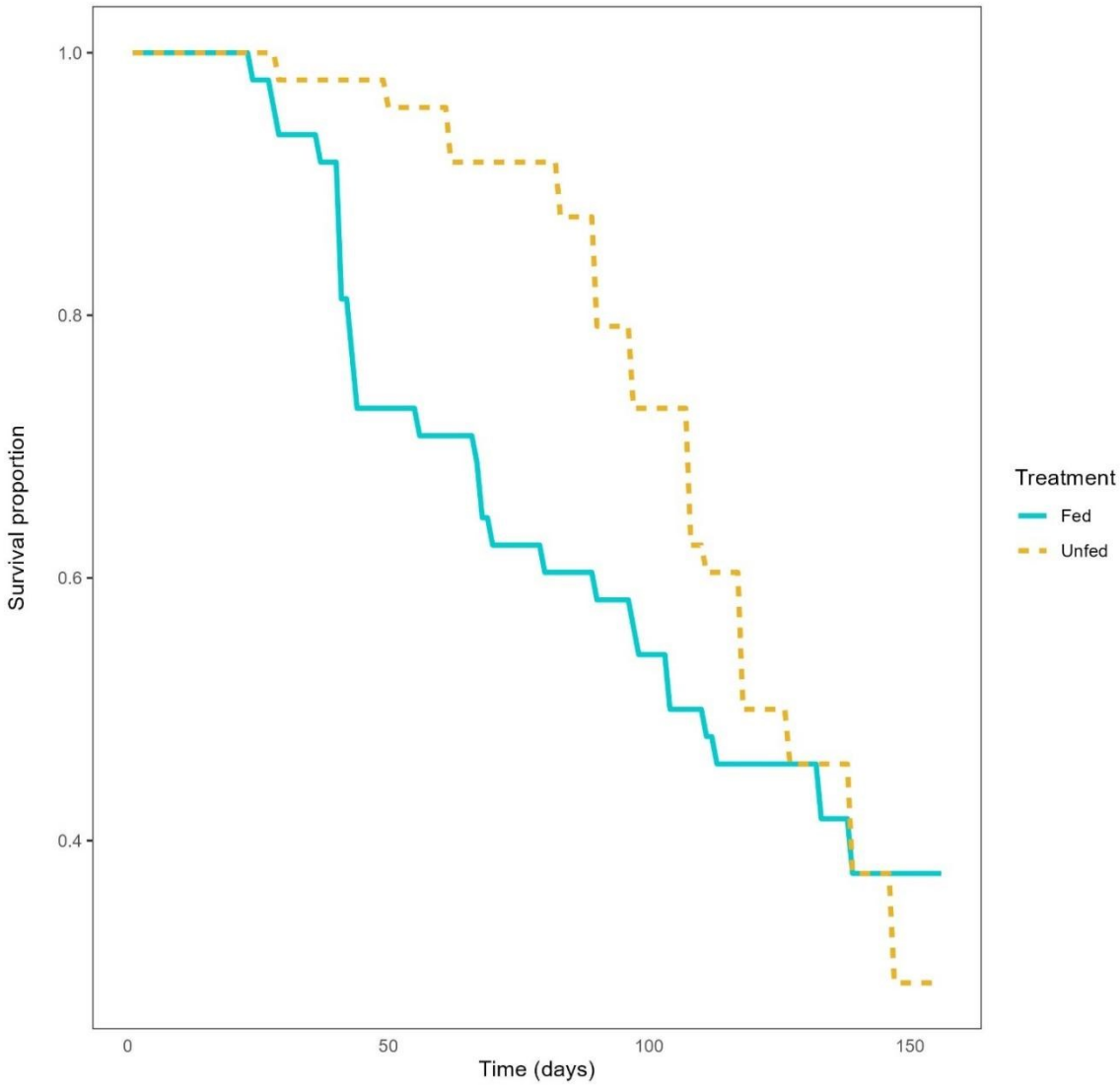


Figure 3.9 Survival with time: Survival proportion for green crabs held at 2 °C over 150 days (5 months), when fed weekly (solid/blue) or deprived of food for the trial duration (dashed/yellow).

The Kaplan-Meier survival test examines the time to 50% mortality but does not account for changes with time. Linear regression analysis showed a more gradual decline in survival in unfed crabs than fed crabs (Fig. 10). A steady mortality rate throughout the trial characterized fed crabs (linear regression: $F_{1,48} = 0.074$, $P = 0.789$), in contrast to an increasing rate of mortality in unfed crabs (linear regression: $F_{1,48} = 5.974$, $P = 0.0346$).

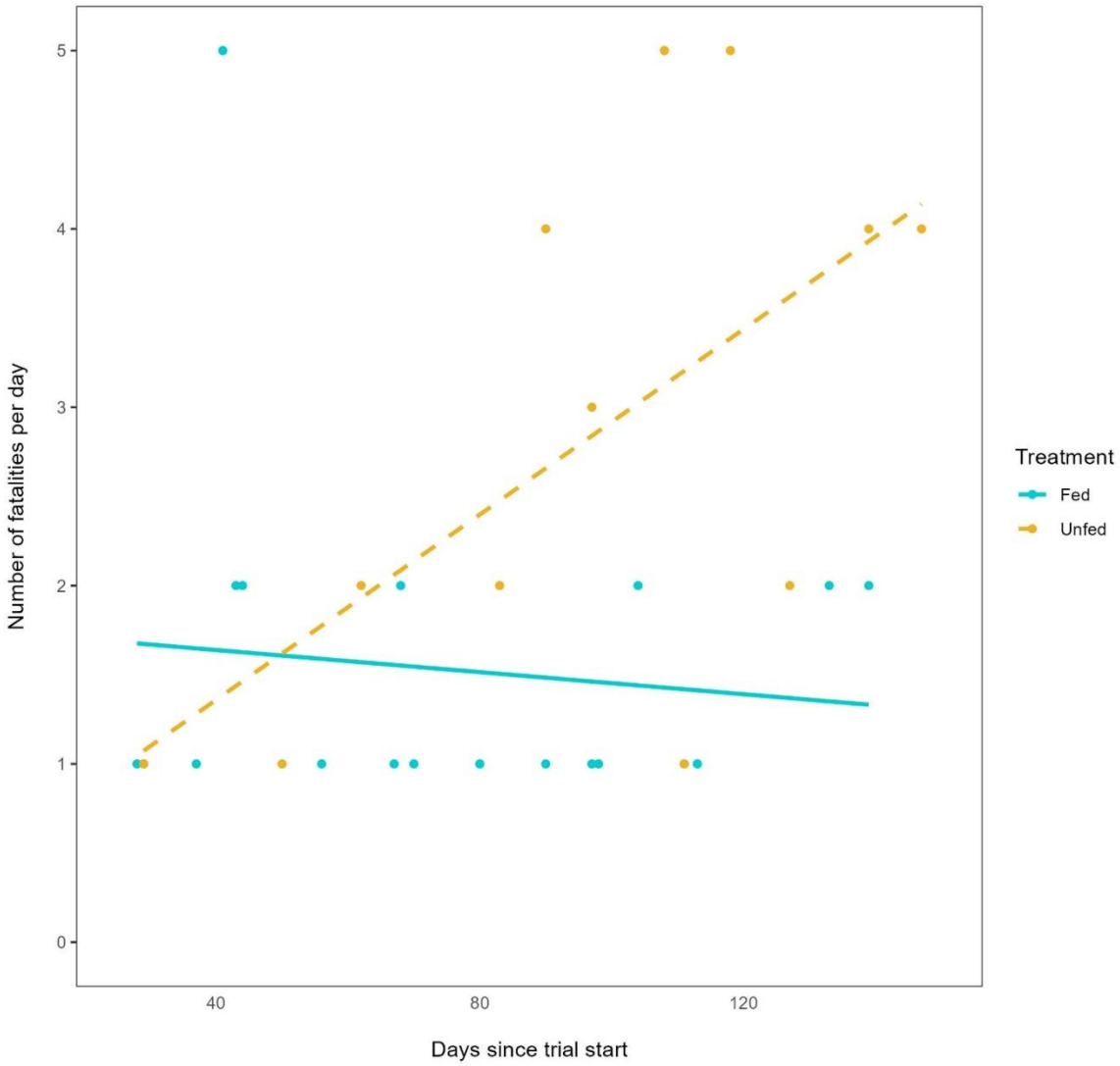


Figure 3.10 Mortality rate with time: Daily mortality rate of green crabs per day when held at seasonal temperatures (0.5 - 7° C) and either fed weekly (solid/blue) or deprived of food for the trial duration (dashed/yellow). Lines show best fit.

Monthly protein serum concentration [P] was significantly affected by the interaction between time and treatment (nparLD: $F_{5,95} = 25.908$, $P < 0.001$). The [P] of fed crabs remained relatively unchanged throughout the trial, while the [P] of unfed crabs declined with time (Fig. 11). [P] of unfed crabs was significantly lower than that of fed crabs by month 3 (March) and [P] also became significantly lower than initial levels

during month 3 (Tukey post-hoc test). A further subsequent decline occurred during the last month in unfed crabs (Fig. 11).

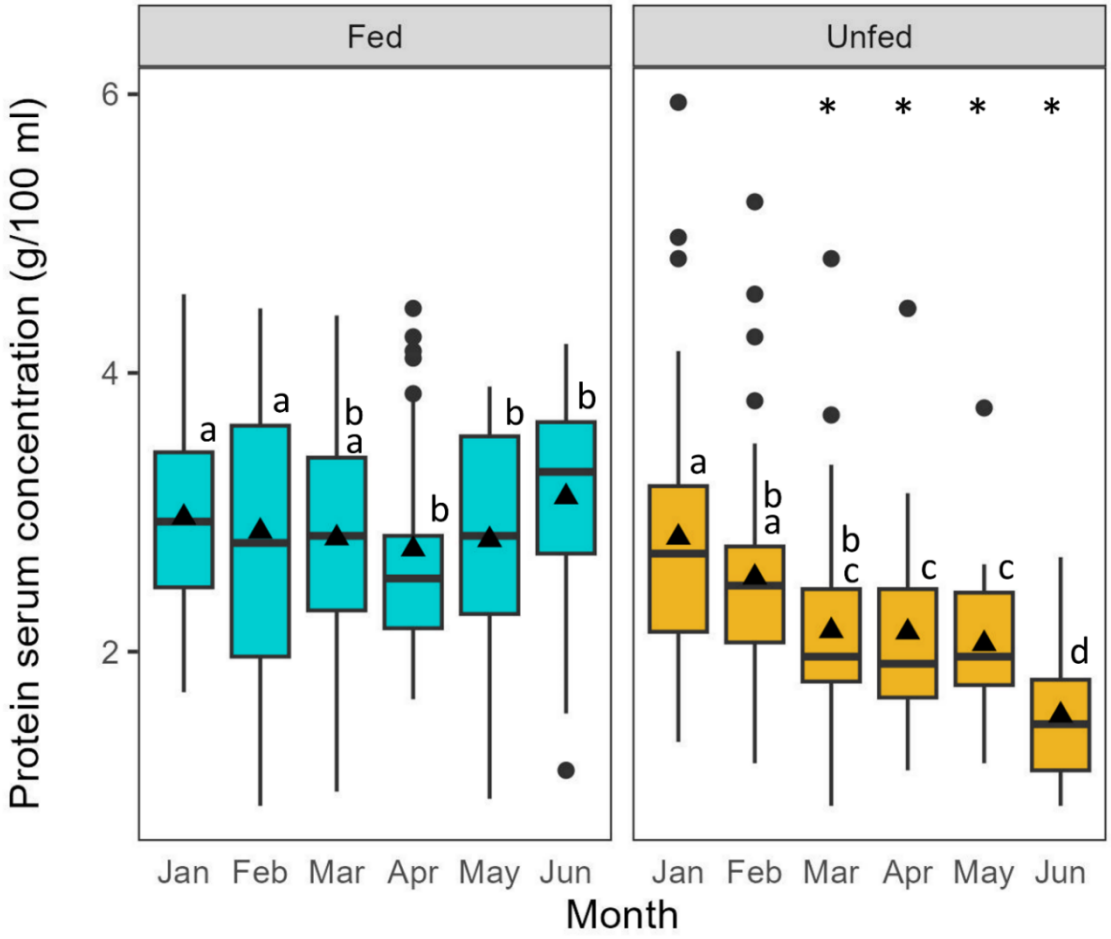


Figure 3.11 Protein serum concentration with time: Protein serum concentration of green crabs held at 2 ° C measured each month when fed weekly (blue) or deprived of food (yellow) for the duration of the trial. Whiskers represent 95 % confidence limits, boxes show upper and lower quartiles, horizontal bar presents median values, circles denote outlying values and black triangles denote mean. * denotes significant changes in treatment (unfed) relative to mean level in control (fed). Lower case letters denote significant differences within treatment groups.

Habitat use was significantly affected by treatment temperature (PERMANOVA: $F_{1,39} = 7.257$, $P < 0.001$; Fig. 12). Total time spent using each habitat, except being out in the (Kruskal-Wallis rank sum test: $\chi 2\ 1,39 = 0.153$, $P = 0.696$), was affected by temperature (one-way ANOVA: moving: $F_{1,39} = 39.632$, $P < 0.001$; Kruskal-Wallis rank sum test:

burying: $\chi 2$ 1,39 = 6.127, P = 0.026; one-way ANOVA: sheltering: F1,39 = 9.068, P = 0.015). Less time was spent moving and burying in the 2 ° C trial than in the 12 ° C trial, crabs spent more time sheltering in the 2 ° C trial (Fig. 12).

In the 12 ° C trial the crabs spent the greatest amount of time buried in the sand (mean = 196 h), followed by remaining out in the open (mean = 151 h), and then sheltering (mean = 112 h; Fig. 13). In contrast, in the 2 ° C trial crabs spent the greatest amount of time sheltering (mean = 251 h), followed by time out in the open (157 h), and then buried in the sand (mean = 102 h). In both 12 ° C and 2 ° C trials crabs spent the least amount of time actively moving (mean: 12 ° C trial = 98 h, 2 ° C trial = 28 h).

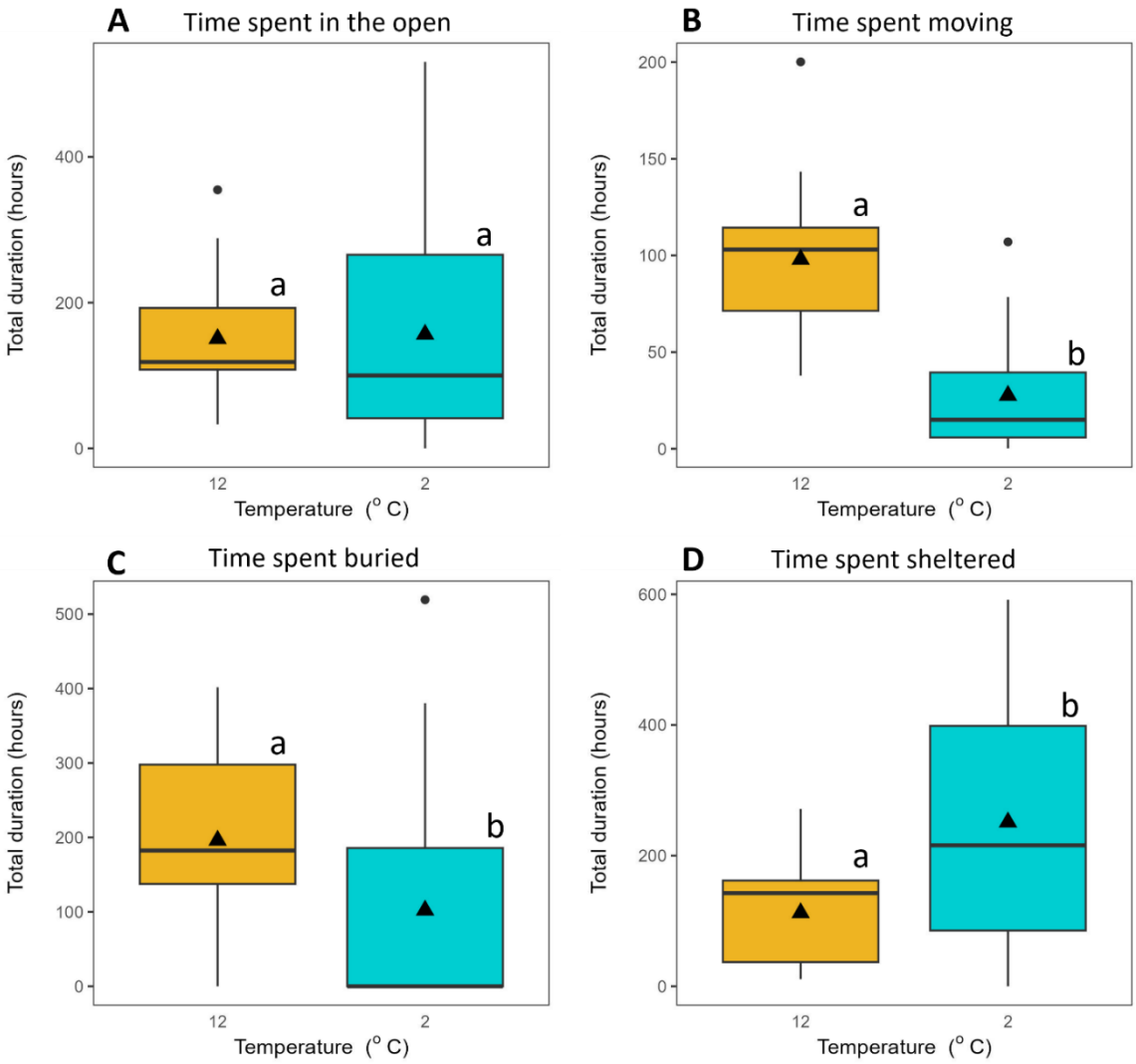


Figure 3.12 Time spent in each habitat type: Total time spent using each habitat: A) out in the open, B) moving, C) buried, and D) sheltered in the 12 ° C trial (yellow) and in the 2 ° C trial (blue). Whiskers represent 95 % confidence limits, boxes show upper and lower quartiles, horizontal bar presents median values, black circles denote outlying values and black triangles denote mean. Lower case letters denote significant differences in the use of each habitat between temperature treatments.

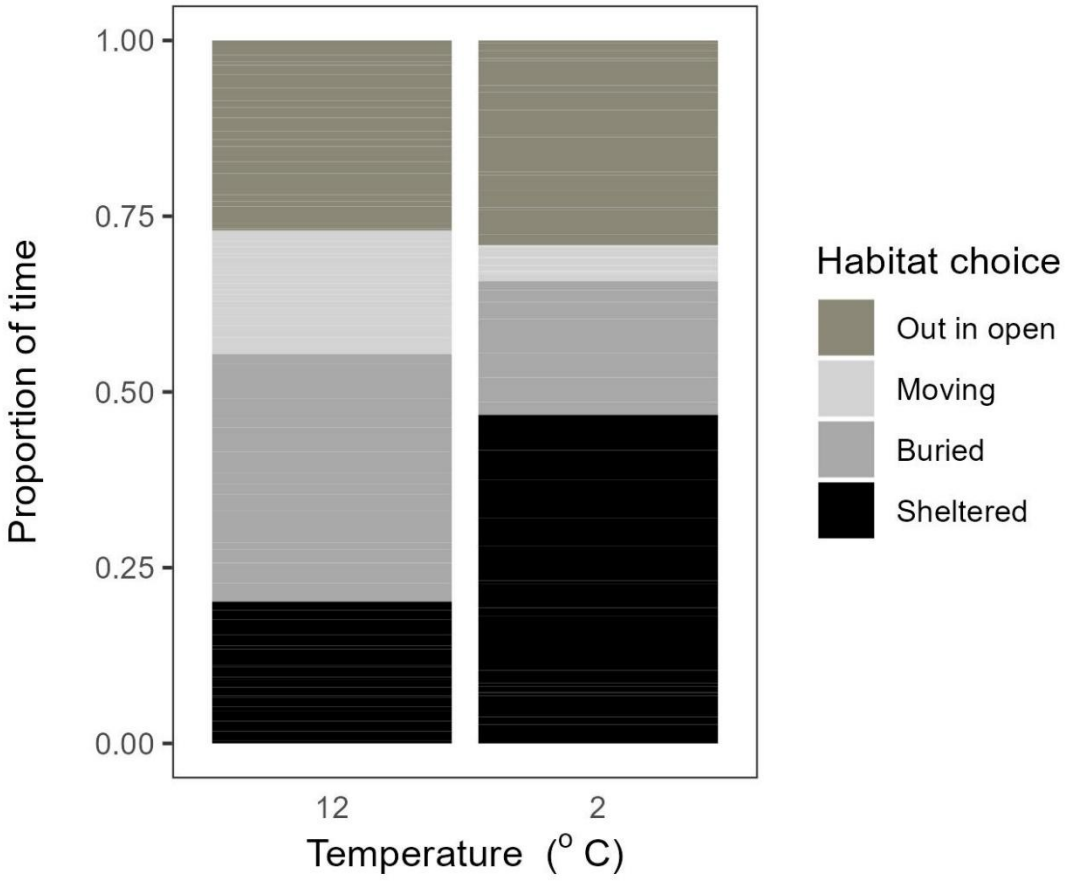


Figure 3.13 Proportion of time spent in each habitat type: Time spent in each habitat as a proportion of total time in the 12 ° C trial and in the 2 ° C trial for all crabs. Figure shows proportion of time out in the open (brown), moving (light grey), buried (dark grey) and sheltered (black).

When crabs were sheltered or out in the open, they showed a propensity to aggregate in groups. Therefore, the amount of time spent in either of these habitats (sheltered or out in open) was further divided by whether the crabs aggregated (physically touching at least one other crab) or remained solitary (Fig. 14). As a proportion of time spent in each habitat, habitat choice significantly affected total time spent aggregated or solitary (Kruskal-Wallis: $\chi 2$ 1,81 = 41.894, $P < 0.001$). Crabs were more likely to aggregate when under a shelter than when out in the open (Fig. 14). However, temperature did not affect time spent aggregated or solitary for either habitat (Kruskal-Wallis: sheltered: $\chi 2$ 1,39 = 0.557, P = 0.456; out in open: $\chi 2$ 1,39 = 2.217, P = 0.137).

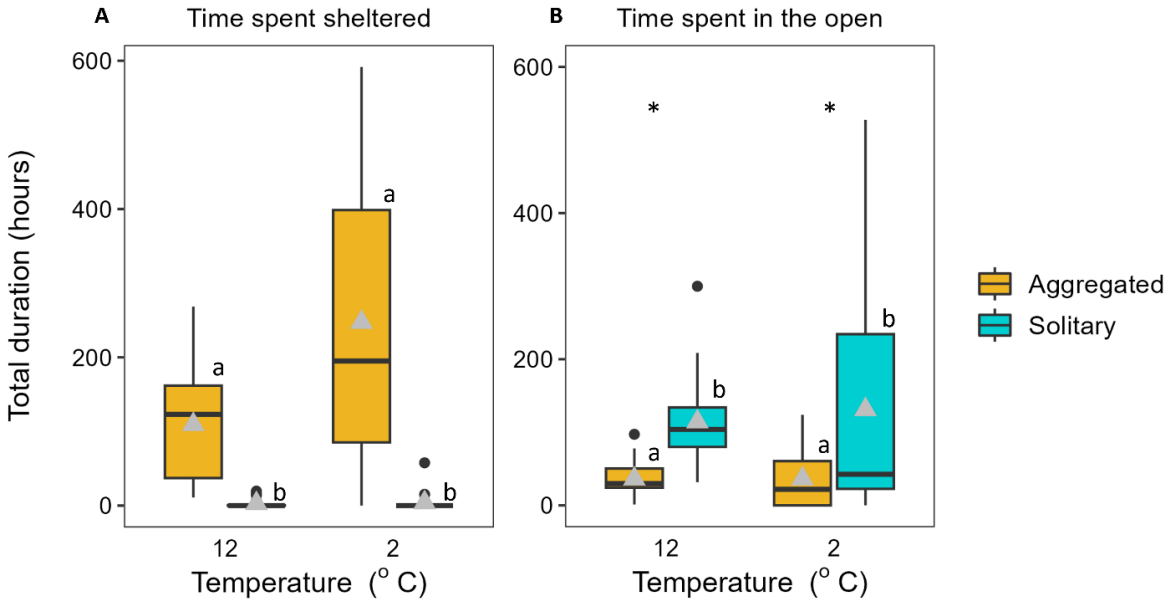


Figure 3.14 Time spent aggregated or solitary: Total time spent aggregated (yellow) or solitary (blue) in the 2 °C trial or in the 12 °C trial when A) under a shelter or B) out in the open. Whiskers represent 95 % confidence limits, boxes show upper and lower quartiles, horizontal bar presents median values, black circles denote outlying values and grey triangles denote mean. * denotes significant differences in time spent aggregated or solitary as a function of habitat choice. Lower case letters denote significant differences as a function of temperature treatment for each habitat.

The total number of feeding events of green crabs throughout the trial differed significantly between temperature treatments (Kruskal-Wallis: $\chi^2_{1,39} = 27.343$, $P < 0.001$). Crabs held in the 12 ° C trial fed much more frequently than those held in the 2 ° C trial (Fig. 15), showing an average number of feeding events ten times that of crabs held in the 2 ° C trial (50.4 and 5.4, respectively).

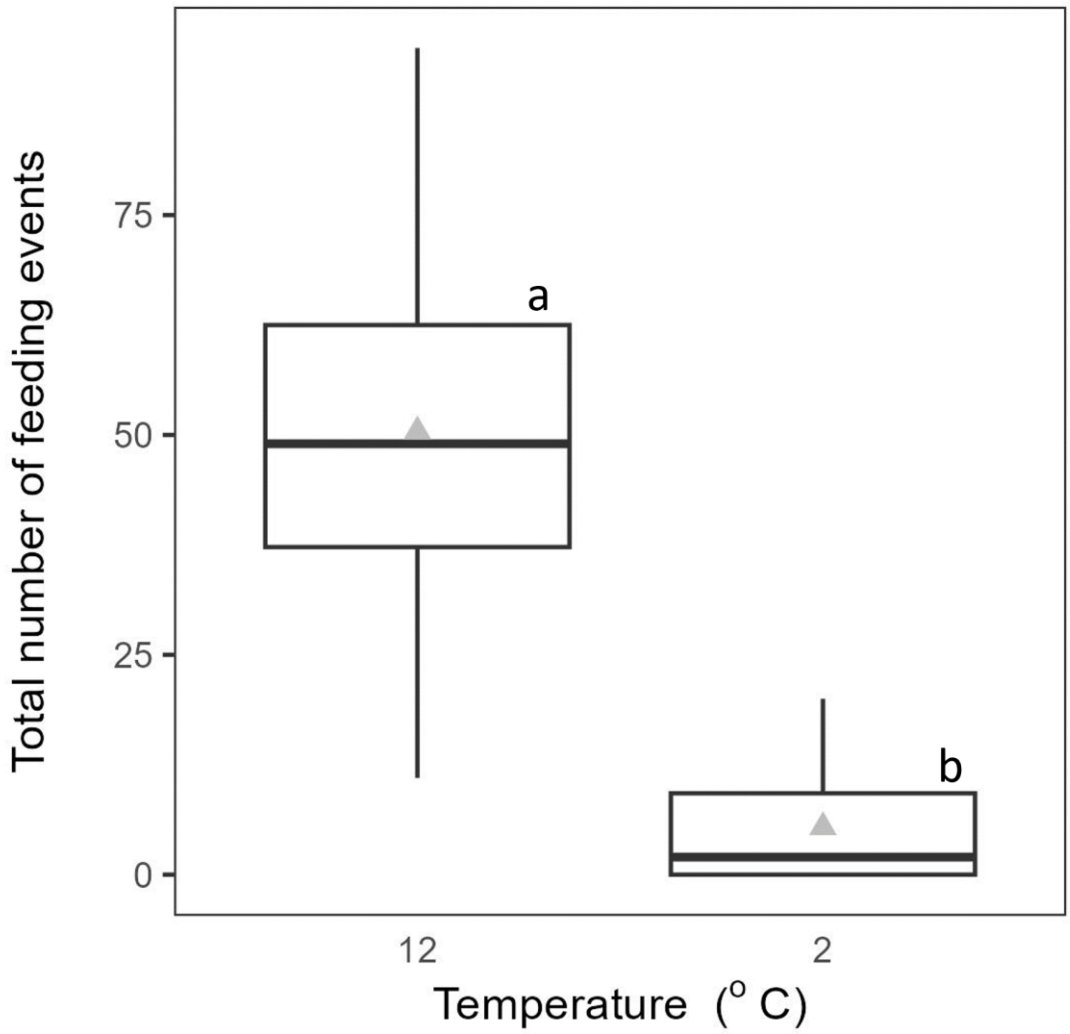


Figure 3.15 Number of feeding events: Total number of feeding events (recorded as approaches to the feeding tray) for crabs held in the 12 ° C trial and in the 2 ° C trial. Whiskers represent 95 % confidence limits, boxes show upper and lower quartiles, horizontal bar presents median values, black circles denote outlying values and grey triangles denote mean. Lower case letters denote significant differences between treatment group.

Little Harbour East (LHE) is a small, sheltered bay in Newfoundland. The length of the bay, measured from the mouth to the tip of the bay, is 0.8 km with a maximum width of 0.25 km. The bay is relatively shallow averaging 4 m depth in most of the bay and reaching 9 – 10 m depth towards the mouth of the bay. Outside the mouth of the bay the depth drops to 12 – 20 m. However, there is a trench that runs through the center of

the bay from the mouth of the bay to the main dock, creating an area of deeper water (7 – 8 m depth) surrounded by shallower zones (Fig. 5).

Water temperature inside and outside of the bay were similar each month, varying by a maximum of 0.8 °C in May (temperature was higher inside the bay; Fig. 16). From January to April, the average water temperature, both inside and outside the bay dropped below 2 °C, with temperatures of 0 to -2 °C in March (Fig. 16). Water temperature was consistently above 4-5 °C in November and December and rose again over 4-5C between May and June.

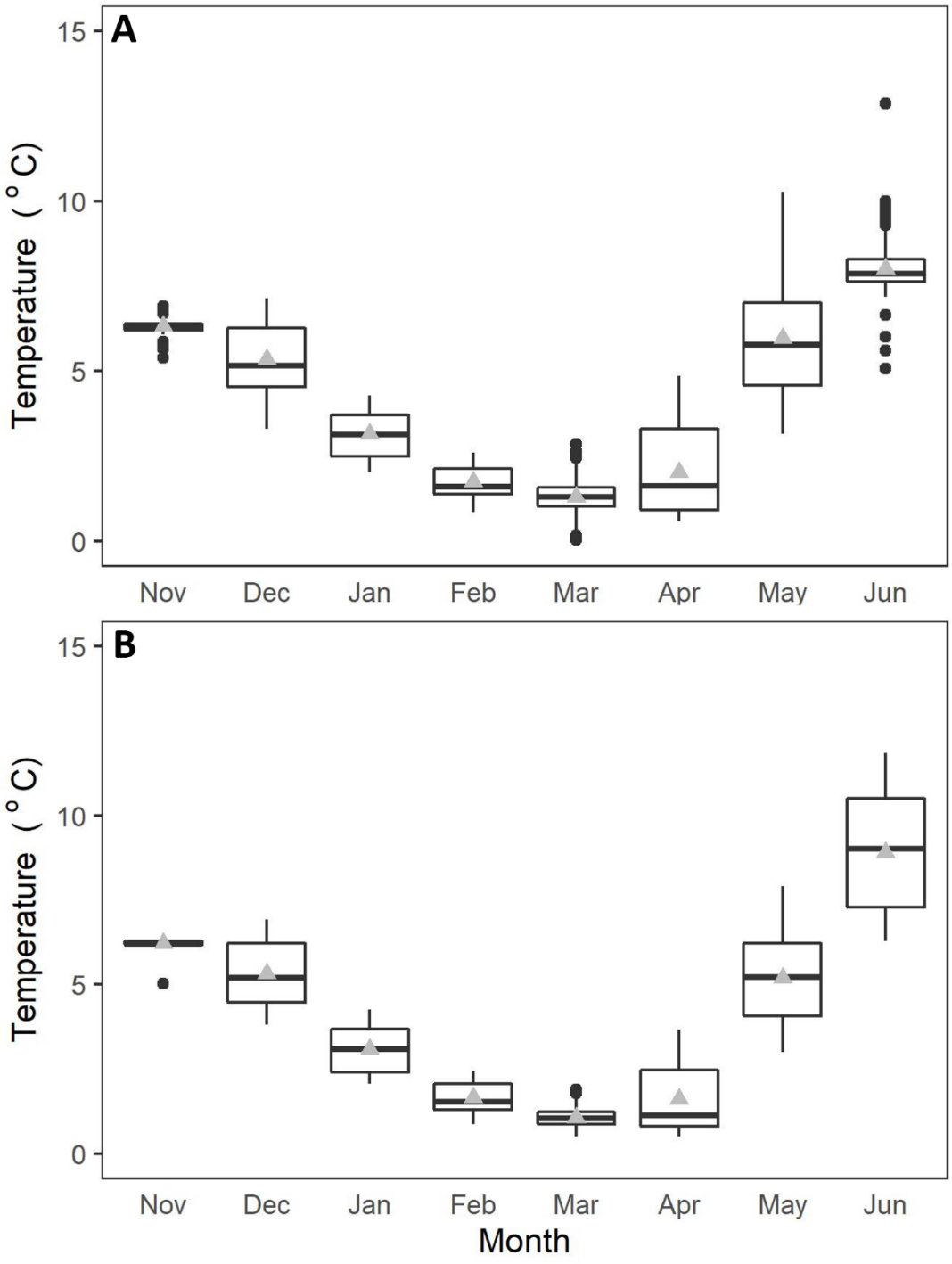


Figure 3.16 Temperature observations in the acoustic array over time: Water temperature (°C) per month, measured by two temperature tags attached to receiver buoys in LHE. A: situated within the bay at 4 m depth, and B: situated at the mouth of the bay at 9 m depth (see figure 5 for temperature tag locations). Whiskers represent 95 % confidence limits, boxes show upper and lower quartiles, horizontal bar presents median values, black circles denote outlying values and grey triangles denote mean.

Detections ceased after 2-3 months in two of the tags, showing no movement out of the array or a reoccurrence of detections at warmer temperatures (42061 and 42072), likely indicating a fault with the tag or that the crabs themselves died or were eaten (Fig. 19J,U). Four of the tags (42057, 42067, 42070 and 42073) were not detected for 3-4 months (December to March or February to April in the case of 42070) during the winter but were detected again in May and June (Fig. 19D,P,S,V). The resumption of detections in April, May, and/or June makes it unlikely that these individuals died, instead suggesting they likely spent several months during the winter either buried in the mud or hiding under a rock, or otherwise in a location where none of the receivers could detect the signal from their transmitter. Two tags (42064 and 42076) were detected so sporadically throughout the duration of the study that suggests a fault with the tag or that these individuals potentially spent the duration of the study in a location blocking transmitter detection by the receivers, e.g. buried in the mud or under a rock (Fig. 19M,Y).

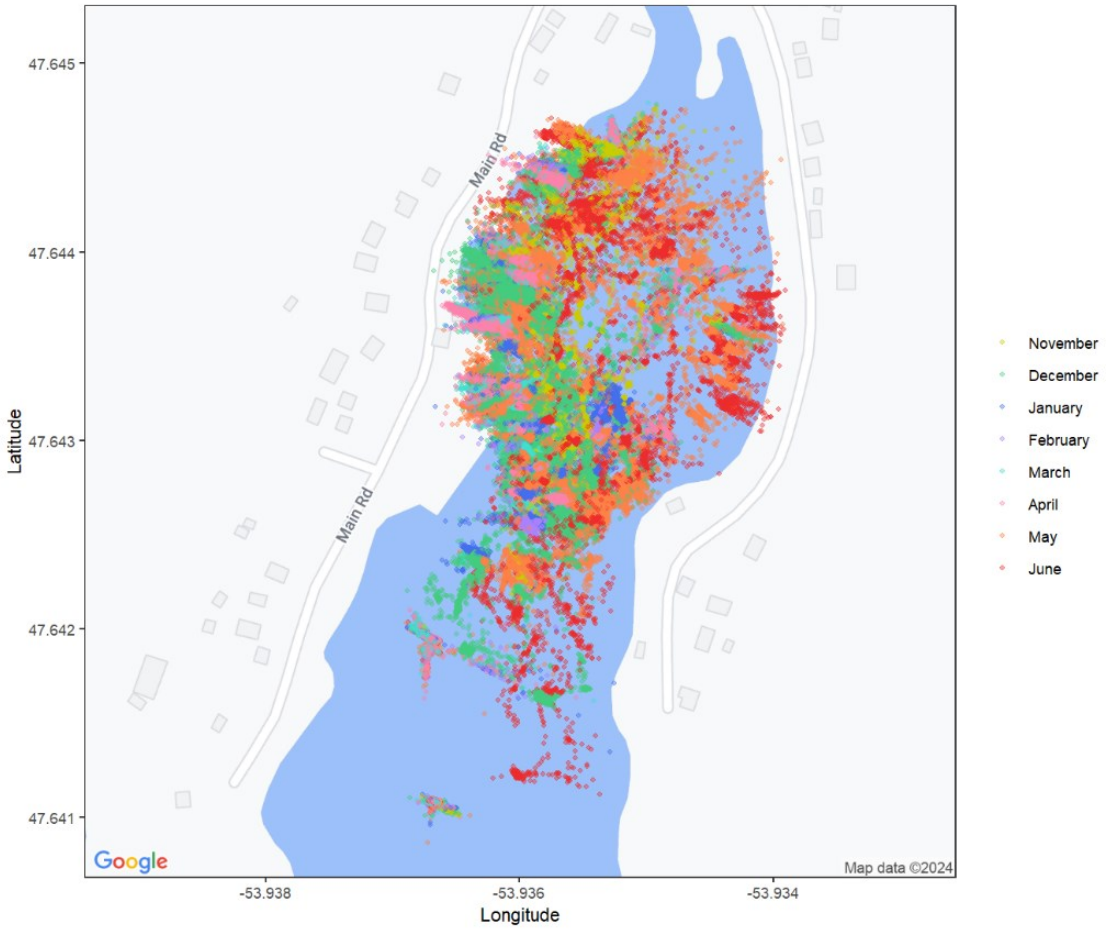
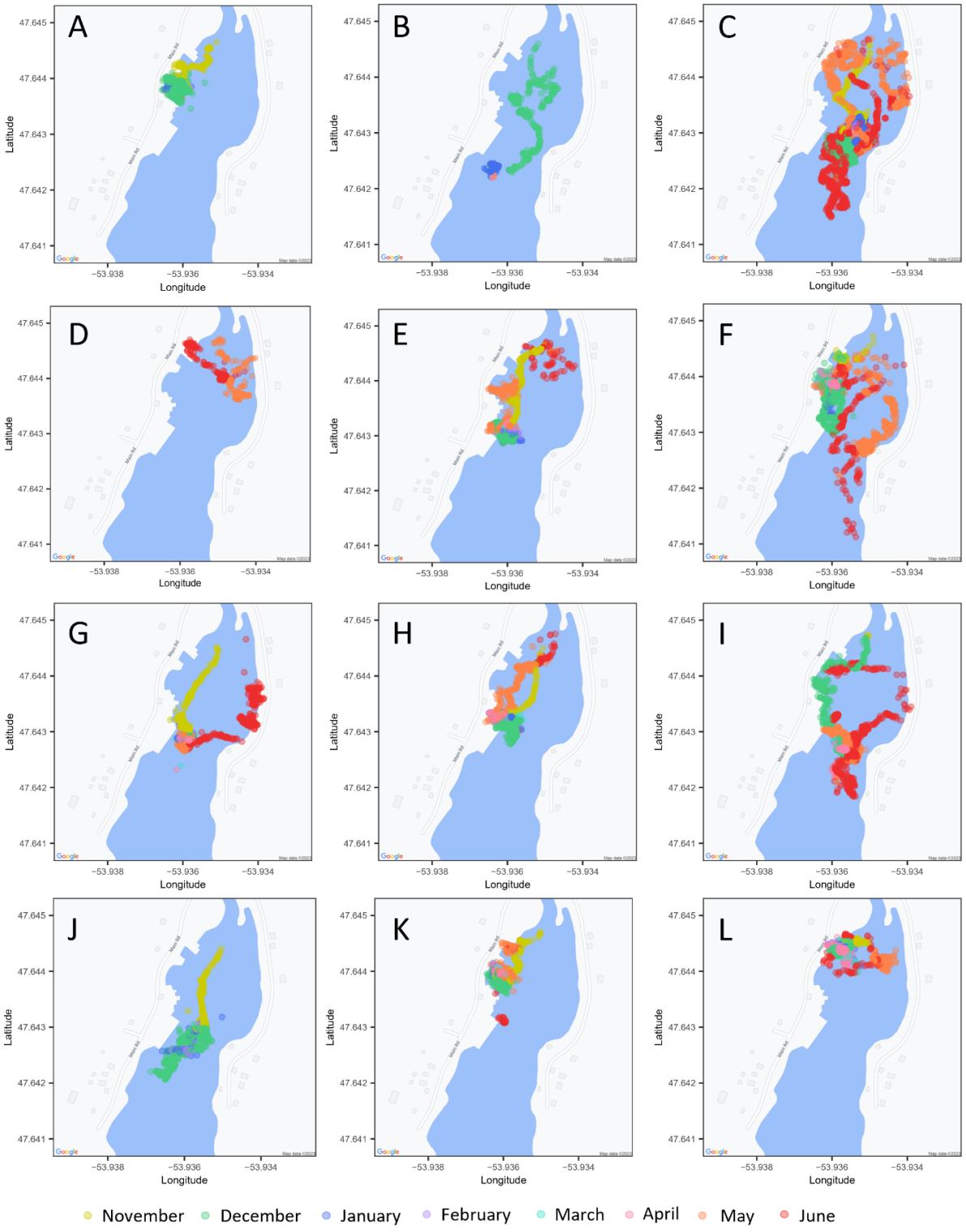


Figure 3.17 All detection locations: Detections of all tags combined from the acoustic receiver array deployed from December 2020 to June 2021 in Little Harbour East, Newfoundland, Canada. Colour depicts month, Yellow = November, Green = December, Blue = January, Purple = February, Turquoise = March, Pink = April, Orange = May and Red = June.

The position data produced by the telemetry study in LHE showed that crabs mostly remained within relatively shallow water, < 5 m depth, in the upper and central areas of the bay throughout the study period (Fig. 17). Five individuals (tags: 40636, 42059, 42060, 42071, 42074) moved towards the mouth of the bay (8 – 9 m depth), with most of this activity occurring in June (expect tag 42071, which moved toward the mouth of the bay in December; Fig. 18C,F,I,T,W). Crabs were more or less randomly distributed within the bay with most residing in shallow zones near the beach, in the middle of the bay and near the main dock (Fig. 17,18).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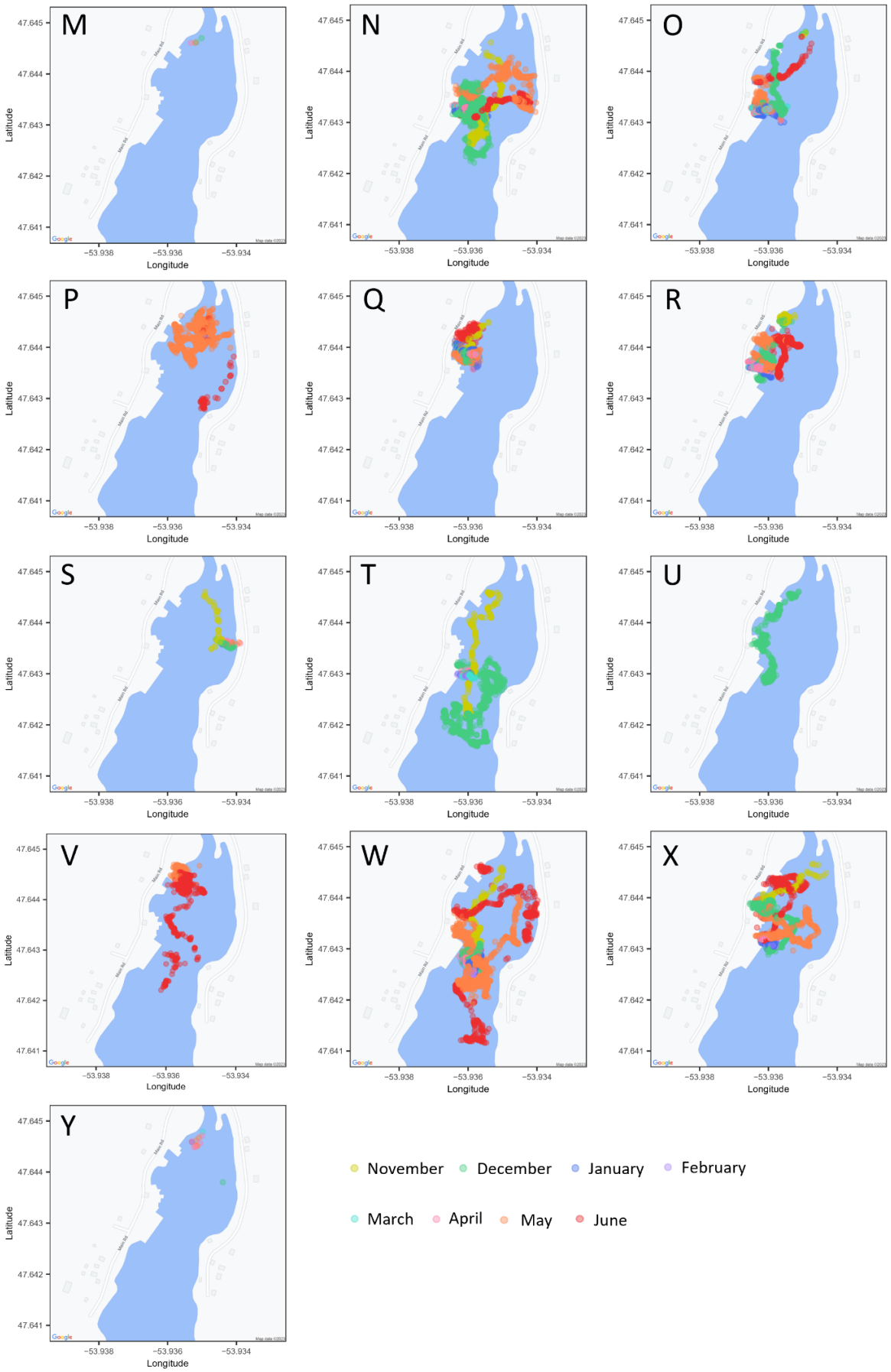
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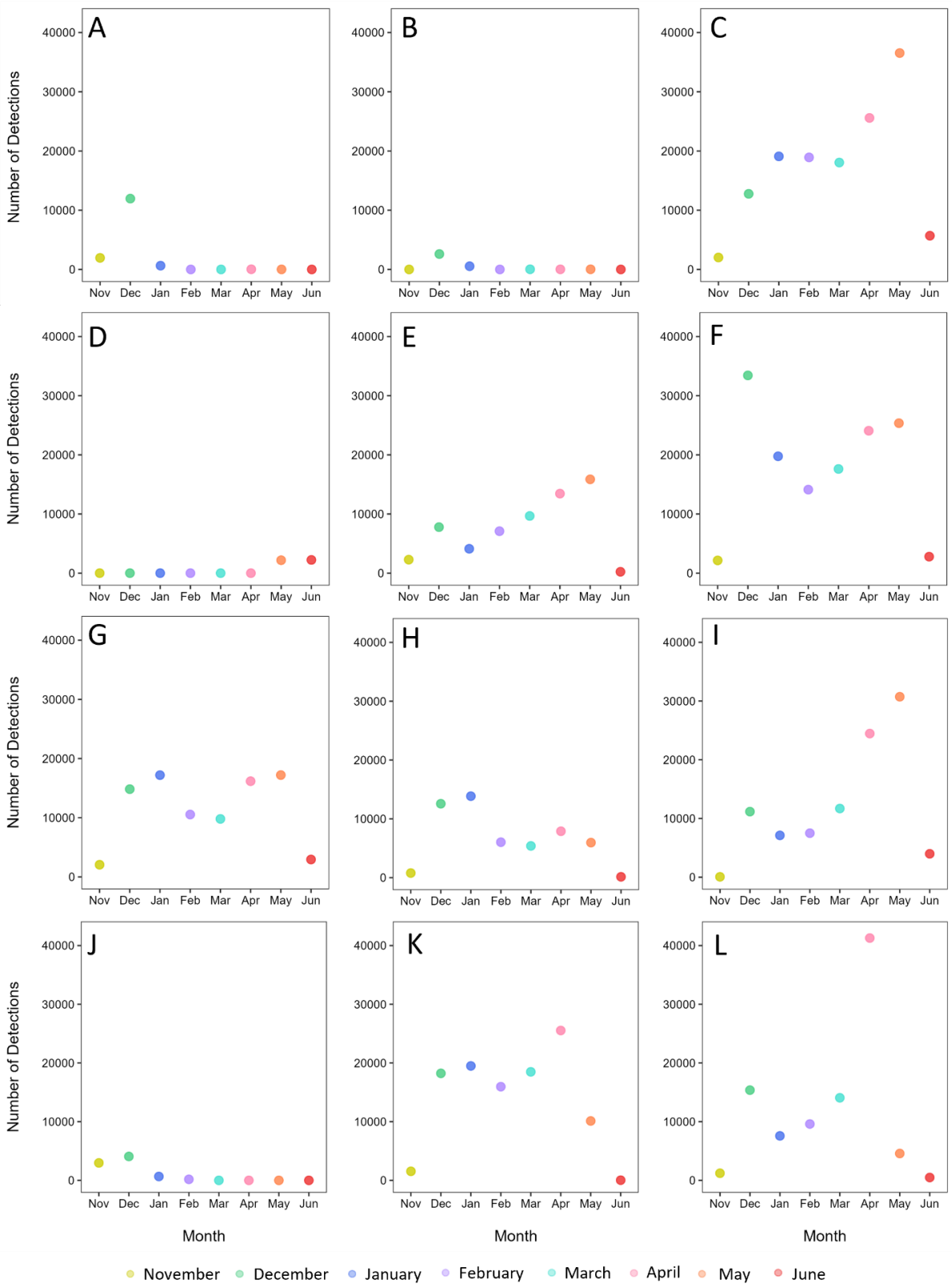
Figure 3.18 Detection locations for each crab: Detections of each tag from the acoustic receiver array deployed from December 2020 to June 2021 in Little Harbour East, Newfoundland, Canada. Each map showing one tag placed on a large, male green crab, with colour depicting month. A: tag 40632, B: tag 40635, C: tag 40636, D: 42057, E: tag 42058, F: tag 42059, G: tag 40633, H: tag 40634, I: 42060, J: tag 42061, K: tag 42062, L: tag 42063, M: tag 42064, N: tag 42065, O: tag 42066, P: 42067, Q: tag 42068, R: tag 42069, S: tag 42070, T: tag 42071, U: tag 42072, V: tag 42073, W: tag 42074, X: tag 42075, Y: tag 42076. Yellow = November, Green = December, Blue = January, Purple = February, Turquoise = March, Pink = April, Orange = May and Red = June.

The majority of the tagged crabs were detected throughout the duration of the study, including the winter months (Fig. 19). The fewest number of detections occurred in November and June, reflecting the start and stop times of the study part way through these months. The second fewest detections were recorded in March and January with an average of 6,197 and 6,762 detections per month, respectively (Fig. 19). The greatest number of detections occurred in May (average 10,722 detections), followed by April (average 10,073 detections). The second and third highest water temperatures of the study period, occurred during these months (highest water temperatures were recorded in June; Fig. 16).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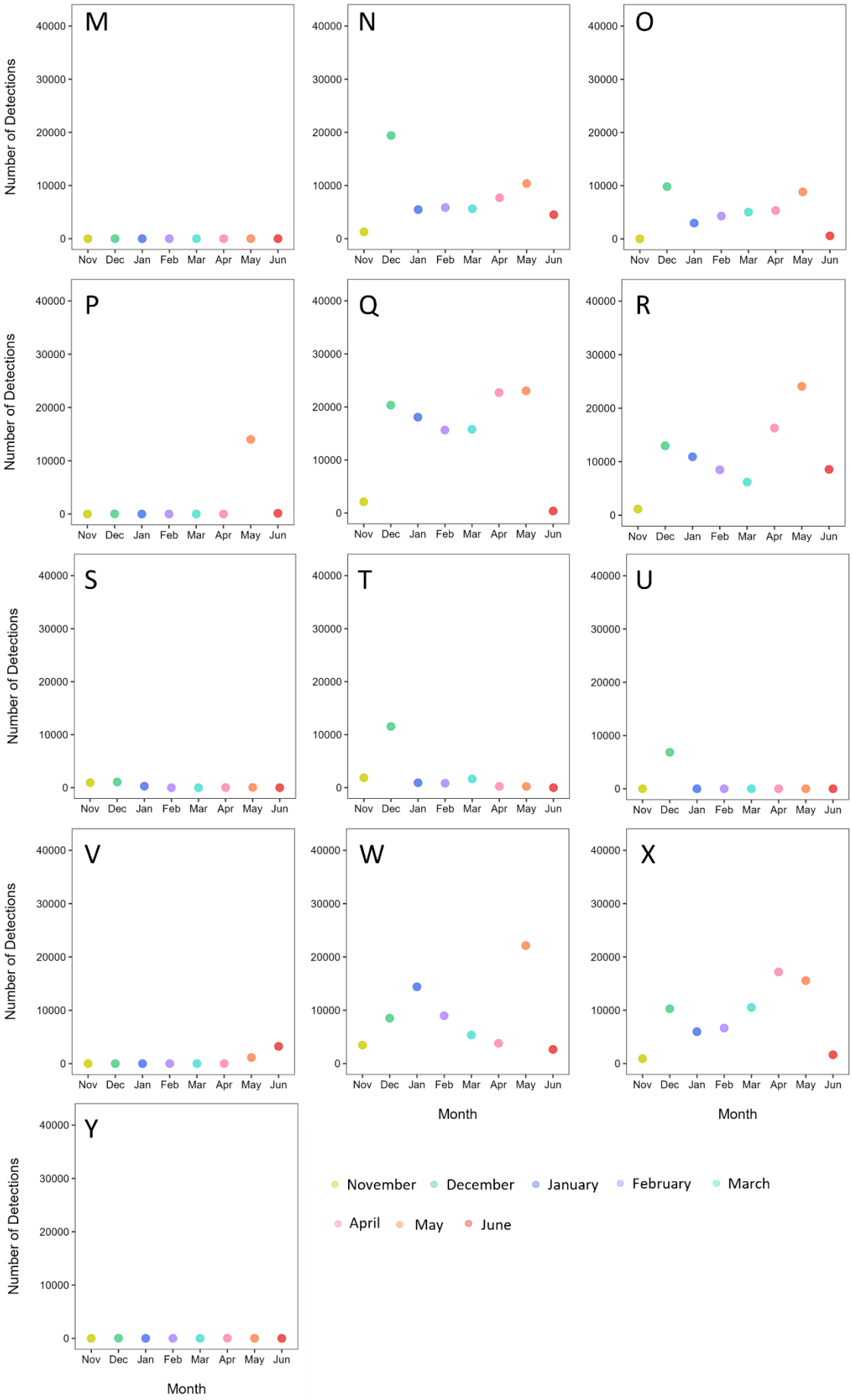
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Figure 3.19 Number of detections for each crab: Total number of detections per month of each tag from the acoustic receiver array deployed from December 2020 to June 2021 in Little Harbour East, Newfoundland, Canada. Each map shows one tag placed on a large, male green crab, with colour depicting month. A: tag 40632, B: tag 40635, C: tag 40636, D: 42057, E: tag 42058, F: tag 42059, G: tag 40633, H: tag 40634, I: 42060, J: tag 42061, K: tag 42062, L: tag 42063, M: tag 42064, N: tag 42065, O: tag 42066, P: 42067, Q: tag 42068, R: tag 42069, S: tag 42070, T: tag 42071, U: tag 42072, V: tag 42073, W: tag 42074, X: tag 42075, Y: tag 42076. Yellow = November, Green = December, Blue = January, Purple = February, Turquoise = March, Pink = April, Orange = May and Red = June.

Discussion

There was a temperature related seasonal shift in behavior and habitat use during the winter period in Newfoundland. Green crabs occurred in the intertidal zone in Little Harbour East in the months of May to November but were absent December to April. In September, when water temperatures ranged between 16 – 17 °C, t the number of green crabs present in the intertidal zone peaked (Fig. 8). This peak consisted almost entirely of very small individuals, most with a carapace width < 5mm, suggesting a short-lived insurgence of newly settled crabs. Green crabs have planktonic larvae with four zooel stages and a final megalopal stage, in which they settle on the substrate before metamorphosis into juveniles occurs (Zeng and Naylor, 1996; Leignel *et al.*, 2014). The duration of the planktonic larval stage is approximately 90 days (Rice and Ingle, 1975; Leignel *et al.*, 2014). A carapace width of approximately 1 - 6 mm in newly settled crabs, in Atlantic Canada, (Cameron and Metaxas, 2005), corresponds to the size range of crabs seen during September in the intertidal zone and indicates mating toook place in early summer (May – June). In their invasive range, green crab reproductive cycles can vary relative to those in their native range. For example, longer larval durations characterise the northern limit of their range on the east coast of North America, which take longer to mature and become reproductively active later in the year (Audet *et al.*, 2008; Best *et al.*, 2017). A reduced thermal tolerance in larvae compared to the adults,

might influence spawning time and a potentially limit their northward range expansion (Berrill, 1982; Hidalgo *et al*., 2005). Additionally, in their native range, crabs have two reproductive events, one in the summer, when larger crabs reproduce, and one in the winter, when smaller crabs reproduce (Lyons *et al.*, 2012). Our study suggests, that in Newfoundland, this secondary mating event in the winter does not occur, in that we observed no smaller crabs in the intertidal zone during the winter. Mating requires moulting, because only soft-shelled females can mate successfully (Berril and Arsenault, 1982; Hayden *et al.*, 2007), therefore, lower thermal limits of newly moulted crabs could underlie the apparent elimination of the secondary mating event. However, seasonal reproduction was not the focus of this study, and only large crabs were used in the acoustic tracking experiment, due to the size and weight of tags, so the seasonal movement of juvenile or newly hatched crabs was not investigated. Thus, our study offers no definitive conclusions on the geographic variations in reproduction.

Most of the crabs observed in the intertidal zone were smaller crabs (CW < 50 mm), with only a few larger males (CW ~ 60 mm) found during the year. In their native range, smaller crabs (CW < 30mm) remain in the intertidal zone year-round, where there is more shelter and fewer predators. Larger crabs occur more commonly within the subtidal zone and migrate daily up the shore with the tide (Naylor, 1962; Crothers, 1968; Hunter and Naylor; 1993; Moksnes, 2002; Baeta *et al.*, 2005). However, unlike in the native range, green crabs in Newfoundland did not remain in the intertidal zone during the winter. Winter ocean temperatures in Newfoundland are the coldest experienced within the entirety of the green crab range (Compton *et al.*, 2010). However, even at -1 °C (recorded in March; Fig. 8) crabs can probably endure this temperature range (Kelley *et al.*, 2013; Tepolt and Somero, 2014); because they are iso-osmotic within seawater

and their body fluids would not freeze until the water itself froze at -1.9 °C. However, the air temperatures did drop lower than -1.9 °C and crabs remaining in the intertidal zone during the winter would risk freezing of tissues, causing dehydration, rupture, and death of cells (Hochachka and Somero, 2002b).

Many studies use baited traps to assess presence and/or abundance of decapods in coastal zones and to assess and mitigate the spread of invasive marine decapods, such as green crabs (Sharp *et al.*, 2003; Poirer *et al.*, 2020; McKenzie et al. 2022). Although crabs were present in the subtidal zone, we caught very low numbers in baited traps during the coldest months (Fig. 1). The lack of crabs in trap catches during the winter, however, does not prove they are absent. Acoustic monitoring results confirmed green crabs remained in the subtidal zone of the bay throughout the winter, suggesting that decreased need to feed and reduced locomotor activity resulted in fewer crabs within traps (Chapter 2). Movement speed decreases in the cold, with rates of 0.02 ms^{-1} at 0 °C and an average of 6.75 m travelled during a day at 2 °C compared to 0.07 ms^{-1} at 10 °C and 352 m per day at 12 °C (Young *et al.*, 2006; Chapter 2). The baited traps were deployed for 5 hours, which may reduce the number of crabs reaching the traps during the winter, even if attracted to the bait. Additionally, crabs have been shown to have much slower response times to prey items at 2 °C, with some individuals not responding for > 2 hours (Chapter 2). Temperature effects on green crab catch rate in baited traps, recommend against using them (Murray and Seed, 2010). Here we provide further evidence that care should be taken when planning CPUE or other population abundance surveys using baited traps, and the importance of considering season and water temperature.

Green crabs appeared able to survive without food for approximately 3 months, after which time the haemolymph protein serum concentration [P] declined gradually in unfed crabs as they used their protein reserves to survive (Matozzo *et al.*, 2011). This decrease was paralleled by increased mortality in unfed crabs after 3 months. We found a [P] reduction of 55 % after 5 months starvation but no overall decline in fed crabs during this same period. This trend resembles that in fed and unfed crabs at warmer temperatures, after 4 weeks starvation crabs [P] declined by an average of 27 % while fed crabs reduced by 9 % during this same period (Uglow, 1969). These results might suggest that the crabs can survive without feeding or cease feeding during the coldest months (December to April). However, we did not measure [P] *in situ*, therefore cannot confirm whether they stop feeding in the wild. The fact that the field data and the mesocosm data showed movement even during the coldest months suggests that they probably do not stop feeding entirely, but feed at a lower rate. In addition, a decline in [P] makes animals more vulnerable to stress and increases mortality (Wang and McGaw, 2016), so, although they could survive the extended Newfoundland winters without feeding, it would not be without cost.

In cold temperatures, green crabs reduce their locomotor activity and spend more time inactive (Tepolt and Somero, 2014; Chapter 2), however, their preferred habitat type and *in situ* habitat use, while remaining inactive, is as yet unclear. The crabs were inactive and slower at 2 °C, meaning that when they occupy inshore/intertidal areas during low temperature periods, it could make them more vulnerable to predators, such as cod and lobsters which remain active at lower temperatures (Ennis, 1973; Astthorsson and Pálsson, 1987). Another clear finding was that crabs spent more time hiding, which would reduce their vulnerability to predators. The mesocosm experiments offered two

choices: bury in sand or hide under rocks. The bay of LHE comprises mostly rocks with patches of sand and gravel. The mesocosm experiment compared behaviour and habitat use at 2 °C and 12 °C despite some difference in the amount of time spent buried or under shelter at 2 °C and 12 °C, but crabs in both cases spent over 50% of their time "hiding". Either way we consider burying and sheltering as an opportunistic hiding-type behaviour favoured at 2 °C compared to 12 °C. The design of this experiment was aimed at observing the 'natural' behaviour of green crabs at winter temperatures compared to summer temperatures, therefore, all 25 crabs were placed in a mesocosm experiment together. This does, however, result in these individuals not being 'true' replicates, so trials are compared here with caution.

Green crabs are known to adhere to social conformity, in which they will conduct similar behaviour to other individuals when in a group (Fürtbauer and Fry, 2018). During the mesocosm experiment, green crabs congregate in groups when under a shelter. Crabs spent most of their time while sheltered in an aggregation, regardless of temperature, while the opposite was evident for crabs out in the open, where they spent most of this time alone. This difference could potentially reflect spatial constraints, in that experimental shelters accounted for approximately 7 % (0.6 m^2) of tank space, in contrast to the 89% occupied by open space (8 m^2). Therefore, there was more space available for crabs to remain solitary when out in the open when compared to that available under a shelter.

In Newfoundland anecdotal evidence from fishermen and scientists suggested green crabs migrate offshore in winter and buried into the mud. However, no study has addressed how far "offshore" and how deep they went. During winter, various crustaceans make offshore migrations. Decapods, such as spiny lobsters (*Panulirus argus*) and

American lobsters (*Homarus americanus*), can migrate into deeper water for the winter, travelling 10 -12 km offshore (Cooper and Uzmann, 1971; Herrnkind *et al.*, 1973; Uzmann *et al.*, 1977). This migration coincides with, and may be triggered by, seasonal changes in light, temperature, and ocean turbulence (Herrnkind and McLean, 1971; Herrnkind, 1980; Kanciruk and Herrnkind, 1978; de Lestang and Caputi, 2015). A shorter seasonal migration (~ 1 km) has also been hypothesised to occur in green crabs in the northern most limits of their native range, with crabs most commonly occupying the subtidal zone during the winter and the intertidal zone in summer (Naylor, 1962; Atkinson and Parsons, 1973). The results of our over-winter acoustic tracking study refuted this within a small, sheltered bay in southern Newfoundland. Green crabs tended to move away from the intertidal areas close to shore but occurred within shallow areas of the bay throughout the winter and did not move out past the mouth of the bay, offshore into deeper waters (December to June). Outside the mouth of the bay, the depth drops drastically, the receiver locations outside the mouth of the bay ranged between depths of 11 m to 20 m, while within the bay depths ranged from 3.5 m to 9 m. Water temperature was monitored throughout the study at two locations: at the mouth of the bay (9 m depth) and in the centre of the bay (4 m depth). The average temperature range across this bay was from 12.4 °C to 0 °C during the study period (Nov to Jun). Despite the slightly more variable temperature, most crabs were detected within the central area of the bay, than at the mouth of the bay and there was no evidence that any left the bay. This pattern indicates that crabs do not seek out deeper offshore locations that have more stable or warmer water temperatures during the winter. In addition, the topography of this bay includes a central trench running through the outer half of the bay, crabs 40637, 42058, 40633, 40634, 42071, 42074 were the closest to this trench (depth 7m)

but did not move seasonally into it in that many of them spent the warmer months in the same location (Fig. 18C,E,G,H,J,T,W). In any case, these results further imply that seasonal migration to deeper waters does not occur in green crabs in southern Newfoundland. This behaviour is not unexpected as green crabs move more slowly below 5 °C and although deeper waters in Newfoundland do not drop to the -1 °C, temperatures that occur in shallow waters remain at a constant 2 to 4 °C. This temperature would still drastically reduce the rate of crab movement and leave them vulnerable to predation during a potentially long migration.

Although crabs moved out of the intertidal zone during the winter and crabs spent less time moving and feeding at 2 °C, these activities did not completely cease. Longer intervals between detections when temperatures were lower, further suggest a reduction, but not complete absence, of movement at colder temperatures. This response differs from the hibernation in cunner (*Tautogolabrus adspersus*), during the winter in Newfoundland. This species completely ceases locomotor activity and feeding during the winter, and reduces the resting metabolic rate and cardiac output, characterised as winter dormancy (Costa et al., 2013; Speers-Roesch et al., 2018; Knight, 2022). Previous studies have shown green crab reduce heart rate, metabolic rate and movement in cold water and suggests they enter a state of torpor at approximately 5 °C (Breteler, 1975; Berrill, 1982; Camus *et al.*, 2004; Tepolt and Somero, 2013; Tepolt and Somero, 2014; Chapter 2). Our study showed that a torpor-like state (a temperature induced reduction in metabolism and heart rate, and a reduced response to stimuli) likely occurs, rather than a full hibernation occur during the winter, and crabs remain responsive to their environment (Wang, 1989; Ruf and Geiser, 2015).

Conclusions

The green crab population inhabiting southern Newfoundland, is a hybridised population, containing haplotypes from both the cold-tolerant, northern lineage and the heat-tolerant, southern lineage (Roman, 2006; Darling *et al*., 2008; Compton *et al.*, 2010; Lenhert *et al.*, 2017; Jeffery *et al*., 2017; 2018). Despite predictions of greater thermal tolerance in this population, it presented a similar torpor-like response to reduced temperatures, triggering at a temperature (4 to 6 °C) comparable to that of native populations (Berrill, 1982; Chapter 2). Our study has found differences in the overwintering location and habitat use of this population in comparison to populations in the northern parts of their native range. Specifically, we found that no green crabs, of any size, remained in the intertidal zone during the winter. In addition, we found no evidence of a seasonal migration to deeper water during the winter (Figs. 8, 18), as reported in some native populations (Naylor, 1962; Atkinson and Parsons, 1973; Crothers, 1968; Hunter and Naylor; 1993; Moksnes, 2002; Baeta *et al.*, 2005). This difference may imply that although green crabs enter a similar temperature induced torpor-like state, regardless of genetic lineage, their habitat interactions may vary at cold temperatures. Short-term and acute cold water tolerance experiments typically miss such differences (Young et al, 2006; Bélair and Miron, 2009; Tepolt and Somero *et al.*, 2014; Chapter 2), suggesting a need for *in situ* monitoring and long-term laboratory experiments to identify the population and lineage specific differences in over wintering behaviour and habitat use.

Chapter 4

Final conclusions on the cold tolerance and over wintering strategy of European green crabs (*Carcinus maenas*) in New-foundland

Summary

European green crabs (*Carcinus maenas*) are a highly successful invasive species of intertidal decapod crustacean native to Europe and northern Africa. To date, they have colonized every continent except Antarctica, and during the last century they colonised the majority of the east Coast of North America (Carlton and Cohen, 2003; Klassen and Locke, 2007). In 2007 they colonized Newfoundland, Canada, which remains the northern most limit of their range on the east coast of North America (Blakeslee *et al.*, 2010; DFO, 2011). Here they experience the coldest seasonal water temperatures across the entirety of their range, living in temperatures as low as -1 °C. Genomic studies identified two distinct lineages of green crabs in North America, the 'northern' lineage originating from northern Europe and Scandinavia and the 'southern' lineage originating from southern Europe and northern Africa, resulting from multiple invasion events (Roman, 2006; Darling *et al*., 2008; Compton *et al.*, 2010). The hybridized southeastern population in Newfoundland, one of the few to exist, contains haplotypes from both the 'northern' and 'southern' lineage of green crabs (Jefferey *et al.*, 2017a,b; 2018).

Many researchers consider the ability of green crabs to tolerate a wide temperature range as one of the key characteristics accounting for their invasive success, and hypothesized that populations from the 'northern' lineage likely have greater cold-water tolerance whereas populations from the 'southern' lineage have greater warm water tolerance. Most research into their temperature tolerance has focussed on the upper limits of their thermal tolerance, of the few studies investigating their lower thermal tolerance, most have concentrated on acute responses (Miron *et al.*, 2002; Young *et al.*, 2006; Tepolt and Somero, 2014). Additionally, most studies have not considered the lineage of the population being tested.

In the first data chapter of this thesis, I investigated the behavioural and physiological responses of green crabs at the lower limit of their thermal tolerance. I used a temperature reduction regime from average coastal water temperature in Newfoundland during summer and winter to study their responses to longer-term exposure (6 days) to these same average temperatures. This study provided the first experimental data on winter survival strategies of this hybridized population of green crabs exposed to the extreme cold winter water temperatures in coastal Newfoundland. During these lab experiments I found that locomotor activity and metabolic parameters declined with decreasing temperature from 12 °C to 2 °C and that these characteristics differed significantly after long-term exposure to cold temperatures (2 °C) compared to control treatments (12 °C). I found a marked change in both metabolic and locomotive activity between 4 to 6 °C, which suggests a torpor-like state occurs at these temperatures. However, even after long term acclimation to 2 °C, locomotive activity did not completely cease, indicating that green crabs remain alert to their environment even at the lower end of their temperature tolerance. In addition, crabs increased in time spent buried with declining temperature but this reduction was most obvious at 2 °C, a lower threshold than for metabolic and locomotive characteristics. Feeding experiments also showed longer response times to food and a lower rate of food consumption after acclimation to 2 °C, compared to control treatments (12 °C). These results confirm the presence of a 'torpor-like' state below 5 °C, in which crabs continue to move and feed actively, although more slowly and at lower rates, which parallels a similar response to other populations of green crabs.

The second data chapter of this thesis further explored the cold-water tolerance and seasonal behaviour and habitat use of green crabs in Newfoundland using a combination of even longer-term lab experiments and *in situ* surveys. After long-term exposure (28

days) to winter-time temperatures (2 °C) crabs spent most of their time under shelter, reducing time spent moving and feeding. However, again I found movement and feeding persisted, albeit more slowly and at a lower rate, when exposed to 2 °C for long periods, compared to the control treatments (12 °C). *In situ* acoustic positioning data, also showed that crabs maintained locomotive activity during the winter, but at a reduced rate. Together, these results challenge theories that green crabs move offshore during the winter or bury in the mud or hide under a shelter, remaining completely inactive throughout the winter. Intertidal presence/absence surveys however, found no crabs in the intertidal zone during the coldest months (Dec – Mar), indicating they remain in the subtidal zone during the winter, likely to avoid air exposure. *In situ* acoustic monitoring showed that crabs did not move out of the bay to deeper waters, and monthly subtidal surveys using baited traps found catch rates declined during the winter months, with catch rates of zero in February and March, when water temperatures were lowest. These *in situ* results support lab experiments showing reduced feeding and movement rate in crabs at cold temperatures; even if crabs responded to the bait during winter, their reduced movement speed would reduce the likelihood of them entering traps during the soak time. Finally, lab experiments testing survival at 2 °C with and without feeding, found crabs could survive without feeding for 5 months, but their protein serum concentration (a measure of stored proteins) steadily declined during this period of starvation. Moreover, when given the opportunity, crabs would feed during long-term exposure to 2 °C, and these crabs maintained steady protein serum levels. Together, these findings suggest that green crabs remain in the subtidal zone during the winter but lower their energy requirements by reducing locomotive activity and metabolic parameters to survive cold-water temperatures. However, they continue to move and feed at a lower

rate, further supporting the presence of a 'torpor-like' state in contrast to a fully torpid state seen in some marine vertebrates in Newfoundland (Costa *et al.*, 2013; Speers-Roesch *et al*., 2018; Knight, 2022).

Collectively, these results support a 'torpor-like' state for green crabs at temperatures below 5 °C, in which they continue to actively move and feed, although more slowly and at lower rates. This response parallels that seen in other populations of green crabs across their native and invasive range, and suggest that they may use other strategies, such as habitat cover to survive the extreme cold-water temperatures in Newfoundland during winter. *In situ* acoustic monitoring apparently disproved a migration offshore and a complete cessation of activity, associated with burying in the mud or hiding under shelters for extended periods. However, the results may support intermittent burying and sheltering with opportunistic feeding occurring throughout the winter, though confirmation requires further research.

Importance of results

This thesis demonstrates a maintained physiological and behavioural over-wintering strategy in green crabs across their invasive and native range, and also across lineages, including the hybridised population in Newfoundland. Green crabs can survive winter water temperatures in Newfoundland for extending periods without having to alter their physiological or behavioural responses. Previous studies had only considered acute responses to such cold temperatures, and here I show that green crabs can maintain their responses to cold temperatures for months, although not without cost. The long-term survival study, investigating the ability of green crabs to survive long term cold exposure without feeding showed that green crabs will use their protein reserves to survive

without feeding, but if food is available, they maintain these protein reserves. This strategy suggests that they may be at the limit of their cold-water tolerance in Newfoundland, in terms of water temperature and winter duration, which represent greater extremes than in most of their current range.

Green crabs are an internationally invasive species that have negative impacts on ecosystems, native species, and fisheries across the globe (Lowe *et al.*, 2000). They can outcompete native decapod species, cause swift declines in commercially important bivalve populations, and cause destruction of ecologically important eel grass habitats (Grosholz *et al.*, 2011; Matheson and Gagon, 2012; Matheson and McKenzie, 2014; Matheson *et al*., 2016; Neckles, 2015). Therefore, these characteristics punctuate the importance of understanding their winter survival and the relationship this may have with their northern range expansion both on the east coast of North America, and the west coast, where they have recently invaded and displayed a rapid range expansion (Cohen *et al.*, 1994; Grosholz and Ruiz, 1996; Jamieson *et al*., 1998; Behrens Yamada *et al*., 1999; Jamieson *et al.*, 2002; Brasseale *et al.,* 2019). Geothermal modelling previously predicted Newfoundland and Alaska are too cold for green crabs to colonise (Compton *et al.*, 2010), however, these predictions have been disproved in Newfoundland, with concerns regarding the spread of green crabs throughout Alaska, noting they have already invaded British Columbia and already occur on the southern coasts of Alaska (Jamieson *et al.*, 2002; Alaska Department of Fish and Game Press Release #22–3413). Understanding their physiological and behavioural winter survival strategies in the coldest regions of their invasive range, will aid future predictions of their northward range expansion in northern America and potentially help inform mitigation efforts in these regions.

The ability of 'northern' and 'southern' lineages to hybridise and theoretically have the potential to withstand more extreme thermal ranges, both at the lower and upper end of their thermal limits (Tepolt and Somero, 2014; Tepolt and Palumbi, 2020), illustrates a greater potential threat from these species to increase their invasive ranges. Therefore, the results presented here, that imply the physiological and behavioural responses of the hybridised population of green crabs to the extended winters in Newfoundland resemble that of most other populations, both in their native and invasive range, indicating that they lack a different strategy for surviving extremely cold winters; therefore hybrid green crabs may be at the limit of their cold-water tolerance in this location.

All lab experiments, including the temperature reduction and long-term acclimation physiology, behaviour and feeding experiments, the long-term survival experiments and the mesocosm habitat use experiments, used a minimum test temperature of 2 °C. This choice reflected logistical issues with lowering the water temperature below this level in a lab environment. Although 2 °C is the average wintertime temperature of coastal waters in Newfoundland, and therefore a good representative test temperature, *in situ* water temperatures drop as low -1 °C for several weeks during the winter. During these weeks, green crabs may differ physiologically and/or behaviourally in ways not captured by the lab experiments conducted during this study. Therefore, further experiments investigating the physiological and behavioural responses to long-term exposure to temperatures below 2 °C would increase our understanding of how green crabs survive the extreme wintertime temperatures in Newfoundland.

Field work in Newfoundland during the winter is challenging due to weather conditions, reduced hours of daylight and the tendency of the surface of sheltered bays to freeze over. These logistical issues resulted in limiting soak times of the baited traps during

the monthly subtidal surveys to four hours. This abbreviated soak time likely reduced the efficacy of the surveys to trap crabs during the winter because green crabs move at a much slower rate, thus a longer soak time would increase accuracy of estimates of presence and activity level of green crabs during the winter. The intertidal surveys were similarly limited during the winter by an even smaller window in which to conduct surveys due to the requirement for low tide. Although I conducted surveys as clos to low tide as possible, greater shore exposure occurred in some months relative to others. Conducting subtidal and intertidal surveys multiple times a month would help to minimize these limitations to winter sampling, by increasing the sample size of the surveys and allowing for greater variation in weather and tide times. Multiple surveys each month, might make it possible to leave the baited traps submerged overnight, vastly increasing the soak time and therefore the likelihood of catching green crabs during the winter.

The *in situ* acoustic monitoring of green crabs in Little Harbour East aimed to observe the over wintering movements of tagged green crabs, to test theories that crabs either move toward the mouth of the bay during the winter where the water is deeper and air exposure is less likely, or that they bury in the mud and remain inactive for the duration of the winter. Therefore, the acoustic array reached from the head of the bay to outside of the mouth of the bay, to ensure it captured any seasonal migration. Individual crabs also had to be caught and tagged with devices that produce acoustic 'pings' that the receivers detect within the array. These tags are a few centimetres long and weigh less than 10 g, but this is still too large for many of the smaller crabs, therefore limiting the tagging programme to the largest crabs (> 50 mm CW), which meant that all tagged crabs were male, noting they are consistently larger than female crabs. Therefore, my

study could not resolve any sex or age determined variation in over wintering habitat use. Deploying camera traps in addition to the acoustic monitoring system to allow for visual confirmation of the acoustic data would offer one possible solution. However, such an effort would increase the cost of an already fairly expensive study.

Future work

Given the findings of the studies presented here, future research could focus on understanding *in situ* habitat use of green crabs during the winter in Newfoundland. Using a combination of techniques, such as acoustic monitoring, camera traps, and trapping surveys, in a variety of different locations across the southern coast of Newfoundland, would help to understand where green crabs go during the winter, why they are caught much less frequently in baited traps during the winter, and what they do during the winter. This knowledge would further aid future predictions of their ability to extend their range northward on to the coast of Labrador or Alaska. Additionally, studies on the variation in temperature tolerance in green crabs' populations from different lineages, 'northern', 'southern' or a hybridisation of both, would aid in understanding of whether these lineages differ in temperature tolerances and the causes of this difference.

References

Aagaard, A., 1996. In situ variation in heart rate of the shore crab Carcinus maenas in relation to environmental factors and physiological condition. *Marine Biology*, *125*(4), pp.765-772.

Aagaard, A., Warman, C.G., Depledge, M.H. and Naylor, E., 1995. Dissociation of heart rate and locomotor activity during the expression of rhythmic behaviour in the shore crab Carcinus maenas. *Marine & Freshwater Behaviour & Phy*, *26*(1), pp.1-10.

Ahsanullah, M. and Newell, R.C., 1971. Factors affecting the heart rate of the shore crab Carcinus maenas (L.). *Comparative Biochemistry and Physiology Part A: Physiology*, *39*(2), pp.277-287.

Amaral, V., Cabral, H.N., Jenkins, S., Hawkins, S. and Paula, J., 2009. Comparing quality of estuarine and nearshore intertidal habitats for Carcinus maenas. *Estuarine, Coastal and Shelf Science*, *83*(2), pp.219-226.

Ameyaw-Akumfi, C. and Naylor, E., 1987. Spontaneous and induced components of salinity preference behaviour in Carcinus maenas. *Marine Ecology Progress Series*, *37*, pp.153-158.

Ansell, A.D., 1973. Changes in oxygen consumption, heart rate and ventilation accompanying starvation in the decapod crustacean Cancer pagurus. *Netherlands Journal of Sea Research*, *7*, pp.455-475.

Arudpragasam, K.D. and Naylor, E., 1964. Gill ventilation and the role of reversed respiratory currents in Carcinus maenas (L.). *Journal of Experimental Biology*, *41*(2), pp.299-307.

Astthorsson, O.S. and Pálsson, O.K., 1987. Predation on euphausiids by cod, Gadus morhua, in winter in Icelandic subarctic waters. *Marine Biology*, *96*, pp.327-334.

Atkinson, R.J.A. and Parsons, A.J., 1973. Seasonal patterns of migration and locomotor rhythmicity in populations of Carcinus. *Netherlands Journal of Sea Research*, *7*, pp.81-93.

Atkinson, R.J.A., Taylor, A.C., 1988. Physiological ecology of burrowing decapods. Symp. Zool. Soc. Lond. 59,201–226

Audet, D., Davis, D.S., Miron, G., Moriyasu, M., Benhalima, K. and Campbell, R., 2003. Geographical expansion of a nonindigenous crab, Carcinus maenas (L.), along the Nova Scotian shore into the southeastern Gulf of St. Lawrence, Canada. *Journal of Shellfish Research*, *22*(1), pp.255-262.

Audet, D., Miron, G. and Moriyasu, M., 2008. Biological characteristics of a newly established green crab (Carcinus maenas) population in the southern Gulf of St. Lawrence, Canada. *Journal of shellfish research*, *27*(2), pp.427-441.

Baeta, A., Cabral, H.N., Neto, J.M., Marques, J.C. and Pardal, M.A., 2005. Biology, population dynamics and secondary production of the green crab Carcinus maenas (L.) in a temperate estuary. *Estuarine, Coastal and Shelf Science*, *65*(1-2), pp.43-52.

Baeta, A., Cabral, H.N., Marques, J.C. and Pardal, M.A., 2006. Feeding ecology of the green crab, Carcinus maenas (L., 1758) in a temperate estuary, Portugal. *Crustaceana*, pp.1181-1193.

Barshaw, D.E. and Able, K.W., 1990. Deep burial as a refuge for lady crabs Ovalipes ocellatus comparisons with blue crabs Callinectes sapidus. *Mar. Ecol. Prog. Ser*, *66*, pp.75-79

Behrens Yamada, S., Hunt, C. and Richmond, N., 1999. The arrival of the European green crab, Carcinus maenas in Oregon estuaries. *In: Pederson J (ed) Marine Bioinvasions: Proceedings of the first national conference* (pp. 94-99).

Bélair, M.C. and Miron, G., 2009a. Predation behaviour of Cancer irroratus and Carcinus maenas during conspecific and heterospecific challenges. *Aquatic Biology*, *6*, pp.41-49.

Bélair, M.C. and Miron, G., 2009b. Time budget of Cancer irroratus (Say) and Carcinus maenas (L.) under various temperature and prey density conditions during conspecific and heterospecific challenges. *Journal of Shellfish Research*, *28*(4), pp.923-930.

Bellwood, O., 2002. The occurrence, mechanics and significance of burying behaviour in crabs (Crustacea: Brachyura). *Journal of Natural History*, *36*(10), pp.1223-1238.

Berrill, M. and Arsenault, M., 1982. Mating behavior of the green shore crab Carcinus maenas. *Bulletin of Marine Science*, *32*(2), pp.632-638.

Berrill, M., 1982. The life cycle of the green crab Carcinus maenas at the northern end of its range. *Journal of Crustacean Biology*, *2*(1), pp.31-39.

Best, K., McKenzie, C.H. and Couturier, C., 2009. Early life stage biology of a new population of green crab, Carcinus maenas. *Placentia Bay: implications for mussel*

culture in Newfoundland. Agriculture Association of Canada Special Publication, *15*, pp.48-50.

Best, K., McKenzie, C.H. and Couturier, C., 2017. Reproductive biology of an invasive population of European green crab, Carcinus maenas, in Placentia Bay, Newfoundland. *Management of Biological Invasions*, *8*(2), p.247.

Beukema, J.J., 1991. The abundance of shore crabs Carcinus maenas (L.) on a tidal flat in the Wadden Sea after cold and mild winters. *Journal of Experimental Marine Biology and Ecology*, *153*(1), pp.97-113.

Biro, P.A. and Stamps, J.A., 2008. Are animal personality traits linked to life-history productivity?. *Trends in ecology & evolution*, *23*(7), pp.361-368.

Biro, P.A. and Stamps, J.A., 2010. Do consistent individual differences in metabolic rate promote consistent individual differences in behavior?. *Trends in ecology & evolution*, *25*(11), pp.653-659.

Biro, P.A., Beckmann, C. and Stamps, J.A., 2010. Small within-day increases in temperature affects boldness and alters personality in coral reef fish. *Proceedings of the Royal Society B: Biological Sciences*, *277*(1678), pp.71-77.

Blakeslee, A.M., McKenzie, C.H., Darling, J.A., Byers, J.E., Pringle, J.M. and Roman, J., 2010. A hitchhiker's guide to the Maritimes: anthropogenic transport facilitates long distance dispersal of an invasive marine crab to Newfoundland. *Diversity and Distributions*, *16*(6), pp.879-891.

Boschma, H., 1972. On the occurrence of Carcinus maenas (Linnaeus) and its parasite Sacculina carcini Thompson in Burma, with notes on the transport of crabs to new localities. *Zoologische Mededelingen*, *47*(11), pp.145-155.

Bowler, K., 1963. A study of the factors involved in acclimatization to temperature and death at high temperatures in Astacus pallipes. I. Experiments on intact animals. *Journal of cellular and comparative physiology*, *62*(2), pp.119-132.

Breteler, W.K., 1975a. Food consumption, growth and energy metabolism of juvenile shore crabs, Carcinus maenas. *Netherlands Journal of Sea Research*, *9*(3-4), pp.255-272.

Brasseale, E., Grason, E.W., McDonald, P.S., Adams, J. and MacCready, P., 2019. Larval transport modeling support for identifying population sources of European green crab in the Salish Sea. *Estuaries and coasts*, *42*, pp.1586-1599.

Breteler, W.K., 1975b. Oxygen consumption and respiratory levels of juvenile shore crabs, Carcinus maenas, in relation to weight and temperature. *Netherlands Journal of Sea Research*, *9*(3-4), pp.243-254.

Broekhuysen, G.J., 1937. On development, growth and distribution of Carcinides maenas (L.). *Archives néerlandaises de Zoologie*, *2*(1), pp.257-400.

Burch, A. and Seed, R., 2000. Foraging behaviour of Carcinus maenas on Mytilus edulis: the importance of prey presentation. *Journal of the Marine Biological Association of the United Kingdom*, *80*(5), pp.799-810.

Burden, C.T., Stow, A.J., Hoggard, S.J., Coleman, M.A. and Bishop, M.J., 2014. Genetic structure of Carcinus maenas in southeast Australia. *Marine Ecology Progress Series*, *500*, pp.139-147.

Cameron, B. and Metaxas, A., 2005. Invasive green crab, Carcinus maenas, on the Atlantic coast and in the Bras d'Or Lakes of Nova Scotia, Canada: larval supply and recruitment. *Journal of the Marine Biological Association of the United Kingdom*, *85*(4), pp.847-855.

Campbell, R.T., Baring, R.J. and Dittmann, S., 2019. Cracking the cuisine: Invasive European shore crabs (Carcinus maenas) select a menu of soft-shelled mussels over cockles. *Journal of experimental marine biology and ecology*, *517*, pp.25-33.

Camus, L., Davies, P.E., Spicer, J.I. and Jones, M.B., 2004. Temperature-dependent physiological response of Carcinus maenas exposed to copper. *Marine Environmental Research*, *58*(2-5), pp.781-785.

Careau, V., Thomas, D., Humphries, M.M. and Réale, D., 2008. Energy metabolism and animal personality. *Oikos*, *117*(5), pp.641-653.

Carlton, J.T., 1999. Molluscan invasions in marine and estuarine communities. *Malacologia*, *41*(2), pp.439-454.

Carlton, J. T., & Cohen, A. N. (2003). Episodic global dispersal in shallow water marine organisms: The case history of the European shore crabs Carcinus maenas and C. aestuarii. *Journal of Biogeography*, *30*(12), 1809–1820. https://doi.org/10.1111/j.1365-2699.2003.00962.x

Carr, W.E., 1998. Of the Behavior of Aquatic Animals. *From Structure To Information In Sensory Systems-Proceedings Of The International School Of Biophysics*, *5*, p.3.

Chakravarti, L.J. and Cotton, P.A., 2014. The effects of a competitor on the foraging behaviour of the shore crab Carcinus maenas. *PLoS One*, *9*(4), p.e93546.

Clarke, A., 1998. Temperature and energetics: an introduction to cold ocean physiology. In: *Portner, H.O., Playle, R. (Eds.), Cold Ocean Physiology. Cambridge University Press, Cambridge*, pp. 3–30.

Cohen, A.N., Carlton, J.T. and Fountain, M.C., 1994. Introduction, dispersal and potential impacts of the green crab Carcinus maenas in San Francisco Bay, California. *Marine Biology*, *122*(2), pp.225-237.

Colbourne, E.B., Holden, J. , Snook, S., Han, G., Lewis, S., Senciall, D., Bailey, W., Higdon, J., and Chen, N., 2017. *Physical oceanographic conditions on the Newfoundland and Labrador Shelf during 2016*. DFO Can. Sci. Advis. Sec. Res. Doc. 2017/079. v + 50 p.

Compton, T.J., Leathwick, J.R. and Inglis, G.J., 2010. Thermogeography predicts the potential global range of the invasive European green crab (Carcinus maenas). *Diversity and Distributions*, *16*(2), pp.243-255.

Cooper, R.A. and Uzmann, J.R., 1971. Migrations and growth of deep-sea lobsters Homarus americanus. *Science*, *171*(3968), pp.288-290.

Costa, I.A., Driedzic, W.R. and Gamperl, A.K., 2013. Metabolic and cardiac responses of cunner Tautogolabrus adspersus to seasonal and acute changes in temperature. *Physiological and Biochemical Zoology*, *86*(2), pp.233-244.

Coyle, A.F., Voss, E.R., Tepolt, C.K. and Carlon, D.B., 2019. Mitochondrial genotype influences the response to cold stress in the European green crab, Carcinus maenas. *Journal of Experimental Biology*, *222*(17), p.jeb203521.

Crisp, D.J., 1964. The effects of the severe winter of 1962-63 on marine life in Britain. *Journal of Animal Ecology*, *33*(1), pp.165-210.

Cronin, T.W., 1986. Photoreception in marine invertebrates. *American Zoologist*, *26*(2), pp.403-415

Crothers, J.H., 1968. The biology of the shore crab, Carcinus maenas (L.). 2. The life of the adult crab. *Field Stud.*, *2*, pp.579-614.

Cuculescu, M., Hyde, D. and Bowler, K., 1998. Thermal tolerance of two species of marine crab, Cancer pagurus and Carcinus maenas. *Journal of thermal biology*, *23*(2), pp.107-110.

Cumberlidge, N. and Uglow, R.F., 1977. Heart and scaphognathite activity in the shore crab Carcinus maenas (L.). *Journal of Experimental Marine Biology and Ecology*, *28*(1), pp.87-107.

da Silva Vianna, B., Miyai, C.A., Augusto, A. and Costa, T.M., 2020. Effects of temperature increase on the physiology and behavior of fiddler crabs. *Physiology & behavior*, *215*, p.112765.

Dall, W.H. and Moriarty, D.J., 1983. Functional aspects of nutrition and digestion. *The biology of Crustacea*, *5*, pp.215-261.

Dare, P.J. and Edwards, D.B., 1981. Underwater television observations on the intertidal movements of shore crabs, Carcinus maenas, across a mudflat. *Journal of the Marine biological Association of the United Kingdom*, *61*(1), pp.107-116.

Darling, J.A. and Tepolt, C.K., 2008. Highly sensitive detection of invasive shore crab (Carcinus maenas and Carcinus aestuarii) larvae in mixed plankton samples using polymerase chain reaction and restriction fragment length polymorphisms (PCR-RFLP). *Aquatic Invasions*, *3*(2), pp.141-152.

Darling, J.A., 2011. Interspecific hybridization and mitochondrial introgression in invasive Carcinus shore crabs. *Plos One*, *6*(3), p.e17828.

Darling, J.A., Bagley, M.J., Roman, J.O.E., Tepolt, C.K. and Geller, J.B., 2008. Genetic patterns across multiple introductions of the globally invasive crab genus Carcinus. *Molecular Ecology*, *17*(23), pp.4992-5007.

Daum, B.S. and Chang, A., 2014. Habitat Preference of the Introduced Green Crab Carcinus maenas [Poster]. CalPoly, STAR Researcher and Teacher Programme. https://digitalcommons.calpoly.edu/star/288.

Dawirs, R.R., 1983. Respiration, energy balance and development during growth and starvation of Carcinus maenas L. larvae (Decapoda: Portunidae). *Journal of Experimental Marine Biology and Ecology*, *69*(2), pp.105-128.

de Lestang, S. and Caputi, N., 2015. Climate variability affecting the contranatant migration of Panulirus cygnus, the western rock lobster. *Marine biology*, *162*(9), pp.1889-1900.

De Wachter, B.D. and McMahon, B.R., 1996. Haemolymph flow distribution, cardiac performance and ventilation during moderate walking activity in Cancer magister (Dana)(Decapoda, Crustacea). *Journal of Experimental Biology*, *199*(3), pp.627-633.

DeFur, P.L. and Mangum, C.P., 1979. The effects of environmental variables on the heart rates of invertebrates. *Comparative Biochemistry and Physiology Part A: Physiology*, *62*(2), pp.283-294.

Depledge, M.H., 1985. The influence of nutritional state on the circulatory and respiratory physiology of the shore crab, Carcinus maenas. *Journal of the Marine Biological Association of the United Kingdom*, *65*(1), pp.69-78.

Depledge, M.H., Lundebye, A.K., Curtis, T., Aagaard, A. and Andersen, B.B., 1996. Automated interpulse-duration assessment (AIDA): a new technique for detecting disturbances in cardiac activity in selected macroinvertebrates. *Marine Biology*, *126*(2), pp.313-319.

Derby, C.D. and Weissburg, M.J., 2014. The chemical senses and chemosensory ecology of crustaceans. *The natural history of the Crustacea*, *3*, pp.263-92.

Derby, C.D. and Zimmer, R.K., 2012. Neuroecology of predator-prey interactions. In *Chemical ecology in aquatic systems* (pp. 158-171). Oxford University Press.

Derivera, C.E., Hitchcock, N.G., Teck, S.J., Steves, B.P., Hines, A.H. and Ruiz, G.M., 2007. Larval development rate predicts range expansion of an introduced crab. *Marine Biology*, *150*(6), pp.1275-1288.

DFO., 2011. Ecological assessment of the invasive European Green Crab (Carcinus maenas) in Newfoundland 2007–2009. *DFO Canadian Science Advisory Secretariat Science Advisory Report 2010/033.*, p.10.

dos Santos, A., Santos, A.M.P., Conway, D.V., Bartilotti, C., Lourenço, P. and Queiroga, H., 2008. Diel vertical migration of decapod larvae in the Portuguese coastal upwelling ecosystem: implications for offshore transport. *Marine Ecology Progress Series*, *359*, pp.171-183.

Dow, R.L. and Wallace, D.E., 1952. Observations on green crabs (C. maenas) in Maine. *Maine Dept. Sea Shore Fish., Fish. Circular*, *8*, pp.11-15.

Eastman, L.B., Thiel, M. and Watling, L., 2015. Foraging behavior of crustacean predators and scavengers. *The Natural History of the Crustacea*, *2*, pp.535-56.

Edwards, R.L., 1958. Movements of individual members in a population of the shore crab, Carcinus maenas L., in the littoral zone. *The Journal of Animal Ecology*, pp.37-45.

Elner, R.W., 1980. The influence of temperature, sex and chela size in the foraging strategy of the shore crab, Carcinus maenas (L.). *Marine & Freshwater Behaviour & Phy*, *7*(1), pp.15-24.

Engelkes, T. and Mills, N. J. (2011) 'A conceptual framework for understanding arthropod predator and parasitoid invasions', *BioControl*, 56(4), pp. 383–393. doi: 10.1007/s10526-011-9377-3.

Ennis, G.P., 1973. Food, feeding, and condition of lobsters, Homarus americanus, throughout the seasonal cycle in Bonavista Bay, Newfoundland. *Journal of the Fisheries Board of Canada*, *30*(12), pp.1905-1909.

Espinoza, M., Farrugia, T.J., Webber, D.M., Smith, F. and Lowe, C.G., 2011. Testing a new acoustic telemetry technique to quantify long-term, fine-scale movements of aquatic animals. *Fisheries Research*, *108*(2-3), pp.364-371.

Fairchild, E.A., Rennels, N. and Howell, W.H., 2008. Predators are attracted to acclimation cages used for winter flounder stock enhancement. *Reviews in Fisheries Science*, *16*(1-3), pp.262-268.

Fraser, P.J., Bevengut, M. and Clarac, F., 1987. Swimming patterns and the activity of identified equilibrium interneurones in the shore crab, Carcinus maenas. *Journal of experimental biology*, *130*(1), pp.305-330.

Frederich, M., DeWachter, B., Sartoris, F.J. and Pörtner, H.O., 2000a. Cold tolerance and the regulation of cardiac performance and hemolymph distribution in Maja squinado (Crustacea: Decapoda). *Physiological and biochemical zoology*, *73*(4), pp.406-415.

Frederich, M., Sartoris, F.J., Arntz, W.E. and Portner, H., 2000b. Haemolymph Mg (2+) regulation in decapod crustaceans: physiological correlates and ecological consequences in polar areas. *Journal of experimental biology*, *203*(8), pp.1383-1393.

Fulton, S.W. and Grant, F.E., 1902. Some Little Known Victorian Decapod Crustacea with Description of a New Species. *Journal of the Royal Dublin Society*, *1*, p.1S58.

Furey, N.B., Dance, M.A. and Rooker, J.R., 2013. Fine-scale movements and habitat use of juvenile southern flounder Paralichthys lethostigma in an estuarine sea-scape. *Journal of Fish Biology*, *82*(5), pp.1469-1483.

Fürtbauer, I. and Fry, A., 2018. Social conformity in solitary crabs, Carcinus maenas, is driven by individual differences in behavioural plasticity. *Animal behaviour*, *135*, pp.131-137.

Geller, J.B., Walton, E.D., Grosholz, E.D. and Ruiz, G.M., 1997. Cryptic invasions of the crab Carcinus detected by molecular phylogeography. *Molecular Ecology*, *6*(10), pp.901-906.

Gillis, D.J., MacPherson, J.N. and Rattray, T.T., 2000. The status of green crab (Carcinus maenas) in Prince Edward Island in 1999. *PEI Department of Fisheries and Tourism Fisheries and Aquaculture Division, report*, *255*.

Glude, J.B., 1955. The effects of temperature and predators on the abundance of the soft shell clam, Mya arenaria, in New England. *Transactions of the American Fisheries Society*, *84*(1), pp.13-26.

Gomes, V., 1991. First results of tagging experiments on crab Carcinus maenas (L.) in the Ria de Aveiro Lagoon, Portugal. *Ciencia Biologica Ecology and Systematics*, *11*(1-2), pp.21-30.

Gotceitas, V., Fraser, S. and Brown, J.A., 1997. Use of eelgrass beds (Zostera marina) by juvenile Atlantic cod (Gadus morhua). *Canadian Journal of Fisheries and Aquatic Sciences*, *54*(6), pp.1306-1319.

Griffen, B.D., Guy, T. and Buck, J.C., 2008. Inhibition between invasives: a newly introduced predator moderates the impacts of a previously established invasive predator. *Journal of animal ecology*, *77*(1), pp.32-40.

Griffiths, C.L., Robinson, T.B. and Mead, A., 2009. The status and distribution of marine alien species in South Africa. In *Biological invasions in marine ecosystems* (pp. 393-408). Springer, Berlin, Heidelberg.

Grosholz, E., Olin, P., Williams, B. and Tinsman, R., 2002. Reducing predation on Manila clams by nonindigenous European green crabs. *Journal of Shellfish Research*, *20*(3), pp.913-919.

Grosholz, E., Lovell, S., Besedin, E. and Katz, M., 2011. Modeling the impacts of the European green crab on commercial shellfisheries. *Ecological Applications*, *21*(3), pp.915-924.

Grosholz, E.D. and Ruiz, G.M., 1996. Predicting the impact of introduced marine species: lessons from the multiple invasions of the European green crab Carcinus maenas. *Biological Conservation*, *78*(1-2), pp.59-66.

Grosholz, E.D. and Ruiz, G.M., 2003. Biological invasions drive size increases in marine and estuarine invertebrates. *Ecology Letters*, *6*(8), pp.700-705.

Hamilton, N.M. and Houlihan, D.F., 1992. Respiratory and circulatory adjustments during aquatic treadmill exercise in the European shore crab Carcinus maenas. *Journal of Experimental Biology*, *162*(1), pp.37-54.

Handy, R.D. and Depledge, M.H., 1999. Physiological responses: their measurement and use as environmental biomarkers in ecotoxicology. *Ecotoxicology*, *8*(5), pp.329-349.

Hänfling, B., Edwards, F. and Gherardi, F. 2011. 'Invasive alien Crustacea: Dispersal, establishment, impact and control', *BioControl*, 56(4), pp. 573–595. doi: 10.1007/s10526-011-9380-8.

Harding, J.M., 2003. Predation by blue crabs, Callinectes sapidus, on rapa whelks, Rapana venosa: possible natural controls for an invasive species? *Journal of Experimental Marine Biology and Ecology*, *297*(2), pp.161-177.

Hayden, D., Jennings, A., Müller, C., Pascoe, D., Bublitz, R., Webb, H., Breithaupt, T., Watkins, L. and Hardege, J., 2007. Sex-specific mediation of foraging in the shore crab, Carcinus maenas. *Hormones and behavior*, *52*(2), pp.162-168.

Hazel, J.R. and Prosser, C.L., 1974. Molecular mechanisms of temperature compensation in poikilotherms. *Physiological reviews*, *54*(3), pp.620-677.

Herreid II, C.F., Lee, L.W. and Shah, G.M., 1979. Respiration and heart rate in exercising land crabs. *Respiration Physiology*, *37*(1), pp.109-120.

Herrnkind, W.F., 1980. Spiny lobsters: patterns of movement. *Biology and management of lobsters. Physiology and behavior*, *1*, pp.349-407.

Herrnkind, W.F. and McLean, R., 1971. Field studies of homing, mass emigration, and orientation in the spiny lobster, Panulirus argus. *Annals of the New York Academy of Sciences*, *188*(1), pp.359-376.

Herrnkind, W. F., Kanciruk, P., Halusky, J. and Mclean, R. (1973). Descriptive characterization of mass autumnal migrations of spiny lobster, *Panulirus argus*. *Proc. Gulf Caribb. Fish. Inst.*25, 78–98.

Hidalgo, F.J., Baron, P.J. and Orensanz, J.M., 2005. A prediction come true: the green crab invades the Patagonian coast. *Biological Invasions*, *7*, pp.547-552.

Hines, A.H., Ruiz, G.M., Hitchcock, N.G. and deRivera, C., 2004. Projecting range expansion of invasive European green crabs (Carcinus maenas) to Alaska: Temperature and salinity tolerance of larvae. *Prince William Sound Regional Citizens' Advisory Council: Anchorage, AK, USA*; p. 22.

Hochachka, P.W. and Somero, G.N., 2002. *Biochemical adaptation: mechanism and process in physiological evolution*. Oxford university press.

Hopkin, R.S., Qari, S., Bowler, K., Hyde, D. and Cuculescu, M., 2006. Seasonal thermal tolerance in marine Crustacea. *Journal of Experimental Marine Biology and Ecology*, *331*(1), pp.74-81.

Hopkin, S.P. and Nott, J.A., 1980. Studies on the digestive cycle of the shore crab Carcinus maenas (L.) with special reference to the B cells in the hepatopancreas. *Journal of the Marine Biological Association of the United Kingdom*, *60*(4), pp.891-907.

Houlihan, D.F. and Innes, A.J., 1984. The cost of walking in crabs: aerial and aquatic oxygen consumption during activity of two species of intertidal crab. *Comparative Biochemistry and Physiology Part A: Physiology*, *77*(2), pp.325-334.

Hughes, R.N. and Elner, R.W., 1979. Tactics of a predator, Carcinus maenas, and morphological responses of the prey, Nucella lapillus. *The Journal of Animal Ecology*, pp.65-78.

Hunter, E. and Naylor, E., 1993. Intertidal migration by the shore crab Carcinus maenas. *Marine Ecology-Progress Series*, *101*, pp.131-131.

Iftikar, F.I., MacDonald, J. and Hickey, A.J., 2010. Thermal limits of portunid crab heart mitochondria: Could more thermo-stable mitochondria advantage invasive species?. *Journal of experimental marine biology and ecology*, *395*(1-2), pp.232-239.

Jamieson, G.S., Grosholz, E.D., Armstrong, D.A. and Elner, R.W., 1998. Potential ecological implications from the introduction of the European green crab, Carcinus maenas (Linneaus), to British Columbia, Canada, and Washington, USA. *Journal of Natural History*, *32*(10-11), pp.1587-1598.

Jamieson, G.S., Foreman, M.G.G., Cherniawsky, J.Y. and Levings, C.D., 2002. European green crab (Carcinus maenas) dispersal: The Pacific experience. *Crabs in cold water regions: biology management, and economics. Alaska Sea Grant College Program AK-SG-02-01-2002*.

Jeffery, N.W., DiBacco, C., Van Wyngaarden, M., Hamilton, L.C., Stanley, R.R., Bernier, R., FitzGerald, J., Matheson, K., McKenzie, C.H., Nadukkalam Ravindran, P.

and Beiko, R., 2017a. RAD sequencing reveals genomewide divergence between independent invasions of the European green crab (Carcinus maenas) in the Northwest Atlantic. *Ecology and Evolution*, *7*(8), pp.2513-2524.

Jeffery, N.W., DiBacco, C., Wringe, B.F., Stanley, R.R., Hamilton, L.C., Ravindran, P.N. and Bradbury, I.R., 2017b. Genomic evidence of hybridization between two independent invasions of European green crab (Carcinus maenas) in the Northwest Atlantic. *Heredity*, *119*(3), pp.154-165

Jeffery, N.W., Bradbury, I.R., Stanley, R.R., Wringe, B.F., Van Wyngaarden, M., Lowen, J.B., McKenzie, C.H., Matheson, K., Sargent, P.S. and DiBacco, C., 2018. Genomewide evidence of environmentally mediated secondary contact of European green crab (Carcinus maenas) lineages in eastern North America. *Evolutionary Applications*, *11*(6), pp.869-882.

Jimenez, A.G. and Kinsey, S.T., 2015. Energetics and metabolic regulation. *The natural history of the Crustacea*, *4*, pp.391-419.

Jørgensen, L.B., Overgaard, J. and MacMillan, H.A., 2017. Paralysis and heart failure precede ion balance disruption in heat-stressed European green crabs. *Journal of thermal biology*, *68*, pp.186-194.

Joska, M.A.P. and Branch, G.M., 1986. The European shore-crab-another alien invader. *African Wildlife*, *40*(2), pp.63-65.

Jost, J.A., Podolski, S.M. and Frederich, M., 2012. Enhancing thermal tolerance by eliminating the pejus range: a comparative study with three decapod crustaceans. *Marine Ecology Progress Series*, *444*, pp.263-274.

Kanciruk, P. and Herrnkind, W., 1978. Mass migration of spiny lobster, Panulirus argus (Crustacea: Palinuridae): behavior and environmental correlates. *Bulletin of Marine Science*, *28*(4), pp.601-623.

Kelley, A.L., de Rivera, C.E. and Buckley, B.A., 2011. Intraspecific variation in thermotolerance and morphology of the invasive European green crab, Carcinus maenas, on the west coast of North America. *Journal of experimental marine biology and ecology*, *409*(1-2), pp.70-78.

Kelley, A.L., de Rivera, C.E. and Buckley, B.A., 2013. Cold tolerance of the invasive Carcinus maenas in the east Pacific: molecular mechanisms and implications for range expansion in a changing climate. *Biological invasions*, *15*(10), pp.2299-2309.

Kern, F., Grosholz, E. and Ruiz, G., 2002. Management plan for the European green crab. *Aquatic Nuisance Species Task Force. http://www. anstaskforce. gov/Green-CrabManagementPlan. pdf*.

Klassen, G. and Locke, A. 2007. 'A Biological Synopsis of the European Green Crab, Carcinus maenas', *Canadian Manuscript Report of Fisheries and Aquatic Sciences*, 2818, pp. 1–82. doi: 10.1007/BF00348935.

Knight, K., 2022. Cold winter fish slow down naturally rather than by depressing metabolism. *J Exp Biol* (2022) 225 (8).

Kuramoto, T. and Ebara, A., 1984. Neurohormonal modulation of the cardiac outflow through the cardioarterial valve in the lobster. *Journal of Experimental Biology*, *111*(1), pp.123-130.

Kuramoto, T. and Kuwasawa, K., 1980. Ganglionic activation of the myocardium of the lobster, Panulirus japonicus. *Journal of comparative physiology*, *139*(1), pp.67-76.

Le Roux, P.J., Branch, G.M. and Joska, M.A.P., 1990. On the distribution, diet and possible impact of the invasive European shore crab Carcinus maenas (L.) along the South African coast. *South African Journal of Marine Science*, *9*(1), pp.85-93.

Lehnert, S.J., DiBacco, C., Jeffery, N.W., Blakeslee, A.M., Isaksson, J., Roman, J., Wringe, B.F., Stanley, R.R., Matheson, K., McKenzie, C.H. and Hamilton, L.C., 2018. Temporal dynamics of genetic clines of invasive European green crab (Carcinus maenas) in eastern North America. *Evolutionary applications*, *11*(9), pp.1656-1670.

Leignel, V.S.J.H., Stillman, J.H., Baringou, S., Thabet, R. and Metais, I., 2014. Overview on the European green crab Carcinus spp.(Portunidae, Decapoda), one of the most famous marine invaders and ecotoxicological models. *Environmental Science and Pollution Research*, *21*(15), pp.9129-9144.

Leim, A.H. 1951. Unusual marine species on the Atlantic coast in 1951. In: Needler, A.W.H. Fisheries Research Board of Canada, Report of the Atlantic Biological Station for 1951, p. 138-140.

Levinton, J.S., Volkenborn, N., Gurr, S., Correal, K., Villacres, S., Seabra, R. and Lima, F.P., 2020. Temperature-related heart rate in water and air and a comparison to other temperature-related measures of performance in the fiddler crab Leptuca pugilator (Bosc 1802). *Journal of thermal biology*, *88*, p.102502.

Lindström, M. and Fortelius, W., 2001. Swimming behaviour in Monoporeia affinis (Crustacea: Amphipoda)—dependence on temperature and population density. *Journal of experimental marine biology and ecology*, *256*(1), pp.73-83.

Lorenzon, S., Martinis, M. and Ferrero, E.A., 2011. Ecological relevance of hemolymph total protein concentration in seven unrelated crustacean species from different habitats measured predictively by a density-salinity refractometer. *Journal of Marine Biology*, *2011*.

Lundebye, A.K. and Depledge, M.H., 1998. Molecular and physiological responses in shore crabs Carcinus maenas following exposure to copper. *Marine environmental research*, *46*(1-5), pp.567-572.

Lutterschmidt, W.I. and Hutchison, V.H., 1997. The critical thermal maximum: history and critique. *Canadian Journal of Zoology*, *75*(10), pp.1561-1574.

Lyons, L.J., O'Riordan, R.M., Cross, T.F. and Culloty, S.C., 2012. Reproductive biology of the shore crab Carcinus maenas (Decapoda, Portunidae): a macroscopic and histological view. *Invertebrate Reproduction & Development*, *56*(2), pp.144-156.

MacPhail, J.S. 1953. Abundance and distribution of the green crab – a clam predator. . In: Needler, A.W.H. Fisheries Research Board of Canada, Report of the Atlantic Biological Station for 1953. p. 33-34.

MacPhail, J.S., and E.I., Lord. 1954. Abundance and distribution of the Green Crab. *Pp 28, In J.L. Hart (Ed.). Report of the Atlantic Biological Station for 1954*. Fisheries Research Board of Canada, Ottawa, ON, Canada. 171 pp.

Madeira, D., Narciso, L., Cabral, H.N. and Vinagre, C., 2012. Thermal tolerance and potential impacts of climate change on coastal and estuarine organisms. *Journal of Sea Research*, *70*, pp.32-41.

Madeira, D., Narciso, L., Cabral, H.N., Diniz, M.S. and Vinagre, C., 2014. Role of thermal niche in the cellular response to thermal stress: Lipid peroxidation and HSP70 expression in coastal crabs. *Ecological indicators*, *36*, pp.601-606.

Madeira, D., Narciso, L., Cabral, H.N., Vinagre, C., 2012. Thermal tolerance and potential impacts of climate change on coastal and estuarine organisms. Journal of Sea Research.

Marino, I.A., Pujolar, J.M. and Zane, L., 2011. Reconciling deep calibration and demographic history: Bayesian inference of post glacial colonization patterns in Carcinus aestuarii (Nardo, 1847) and C. maenas (Linnaeus, 1758). *PLoS One*, *6*(12), p.e28567.

Matheson, K. and Gagnon, P., 2012*a*. Effects of temperature, body size, and chela loss on competition for a limited food resource between indigenous rock crab (Cancer irroratus Say) and recently introduced green crab (Carcinus maenas L.). *Journal of Experimental Marine Biology and Ecology*, *428*, pp.49-56.

Matheson, K. and Gagnon, P., 2012*b*. Temperature mediates non-competitive foraging in indigenous rock (Cancer irroratus Say) and recently introduced green (Carcinus maenas L.) crabs from Newfoundland and Labrador. *Journal of Experimental Marine Biology and Ecology*, *414*, pp.6-18.

Matheson, K. and Mckenzie, C.H., 2014. Predation of sea scallops and other indigenous bivalves by invasive green crab, Carcinus maenas, from Newfoundland, Canada. *Journal of Shellfish Research*, *33*(2), pp.495-501.

Matheson, K., McKenzie, C.H., Gregory, R.S., Robichaud, D.A., Bradbury, I.R., Snelgrove, P.V.R. and Rose, G.A., 2016. Linking eelgrass decline and impacts on associated fish communities to European green crab Carcinus maenas invasion. *Marine Ecology Progress Series*, *548*, pp.31-45.

Matozzo, V., Gallo, C. and Marin, M.G., 2011. Effects of temperature on cellular and biochemical parameters in the crab Carcinus aestuarii (Crustacea, Decapoda). *Marine Environmental Research*, *71*(5), pp.351-356.

Matveev, E. and McGaw, I.J., 2022. Effects of laboratory holding time and diet type on labile traits in the crab Cancer irroratus Say, 1817 (Decapoda: Brachyura: Cancridae). *Journal of Crustacean Biology*, *42*(1), p.ruab076.

Matveev, Eugueni, 2021. *Assessment of intra-individual consistency of physiology and behaviour in brachyuran crabs (Carcinus maenas and Cancer irroratus).* Masters thesis, Memorial University of Newfoundland.

McGaw, I.J. and Curtis, D.L., 2013. A review of gastric processing in decapod crustaceans. *Journal of Comparative Physiology B*, *183*(4), pp.443-465.

McGaw, I.J. and Nancollas, S.J., 2018. Experimental setup influences the cardiovascular responses of decapod crustaceans to environmental change. *Canadian Journal of Zoology*, *96*(9), pp.1043-1052.

McGaw, I.J. and Naylor, E., 1992. Salinity preference of the shore crab Carcinus maenas in relation to coloration during intermoult and to prior acclimation. *Journal of experimental marine Biology and Ecology*, *155*(2), pp.145-159.

McGaw, I.J. and Penney, C.M., 2014. Effect of meal type on specific dynamic action in the green shore crab, Carcinus maenas. *Journal of Comparative Physiology B*, *184*, pp.425-436.

McGaw, I.J. and Reiber, C.L., 2015. Circulatory physiology. *The natural history of crustaceans*, *4*, pp.199-246.

McGaw, I.J. and Whiteley, N.M., 2012. Effects of acclimation and acute temperature change on specific dynamic action and gastric processing in the green shore crab, Carcinus maenas. *Journal of Thermal Biology*, *37*(8), pp.570-578.

McGaw, I.J., 2001. Impacts of habitat complexity on physiology: purple shore crabs tolerate osmotic stress for shelter. *Estuarine, Coastal and Shelf Science*, *53*(6), pp.865-876.

McGaw, I.J., 2004. Ventilatory and cardiovascular modulation associated with burying behaviour in two sympatric crab species, Cancer magister and Cancer productus. *Journal of experimental marine biology and ecology*, *303*(1), pp.47-63.

McGaw, I.J., 2005. Burying behaviour of two sympatric crab species: Cancer magister and Cancer productus. *Scientia Marina*, *69*(3), pp.375-381.

McGaw, I.J., 2007. The interactive effects of exercise and feeding on oxygen uptake, activity levels, and gastric processing in the graceful crab Cancer gracilis. *Physiological and Biochemical Zoology*, *80*(3), pp.335-343.

McGaw, I.J., Kaiser, M.J., Naylor, E. and Hughes, R.N., 1992. Intraspecific morphological variation related to the moult cycle in colour forms of the shore crab *Carcinus maenas*. *Journal of Zoology*, *228*(2), pp.351-359.

McGaw, I.J., Edgell, T.C. and Kaiser, M.J., 2011. Population demographics of native and newly invasive populations of the green crab Carcinus maenas. *Marine Ecology Progress Series*, *430*, pp.235-240.

McKenzie, C.H., Baines, T., Best, K., Boland, R., Dawe, E., Deibel, D., Drover, D., Johnson, E., Kenny, S., Macneill, S. and Mouland, D., 2007. The European green crab, Carcinus maenas. *Placentia Bay, Newfoundland AIS Survey 2007. Proceedings of Aquatic Invasive Species Newfoundland Work-shop. St. John's, NL*.

McKenzie, C.H., Matheson, K., Sargent, P., Piersiak, M., Bernier, R.Y., Simard, N., Therriault, T.W. 2022. Trapping Methods for the Invasive European Green Crab in Canada. DFO Can. Sci. Advis. Sec. Res. Doc 2022/063 iv + 47 p

McLay, C.L. and Osborne, T.A., 1985. Burrowing behaviour of the paddle crab Ovalipes catharus (White, 1843)(Brachyura: Portunidae). *New Zealand Journal of Marine and Freshwater Research*, *19*(2), pp.125-130.

McLeese, D.W. and Wilder, D.G., 1958. The activity and catchability of the lobster (Homarus americanus) in relation to temperature. *Journal of the Fisheries Board of Canada*, *15*(6), pp.1345-1354.

McMahon, B.R. and Burnett, L.E., 1990. The crustacean open circulatory system: a reexamination. *Physiological Zoology*, *63*(1), pp.35-71.

McMahon, B.R. and Wilkens, J.L., 1983. Ventilation, perfusion and oxygen uptake. *Internal anatomy and physiological regulation*, *5*, pp.289-372.

McMahon, B.R., 1999. Intrinsic and extrinsic influences on cardiac rhythms in crustaceans. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *124*(4), pp.539-547.

McMahon, B.R., 2001. Control of cardiovascular function and its evolution in Crustacea. *Journal of Experimental Biology*, *204*(5), pp.923-932.

Metcalfe, N.B., Van Leeuwen, T.E. and Killen, S.S., 2016. Does individual variation in metabolic phenotype predict fish behaviour and performance?. *Journal of fish biology*, *88*(1), pp.298-321.

Methven, D.A. and Piatt, J.F., 1991. Seasonal abundance and vertical distribution of capelin (Mallotus villosus) in relation to water temperature at a coastal site off eastern Newfoundland. *ICES Journal of Marine Science*, *48*(2), pp.187-193.

Miron, G., Landry, T. and MacNair, N., 2002. *Predation potential by various epibenthic organisms on commercial bivalve species in Prince Edward Island: Preliminary Results*. Can Tech. Rep. of Fish. Aqua. Sci. 2392: ix + 33 p.

Moksnes, P.O., 2002. The relative importance of habitat-specific settlement, predation and juvenile dispersal for distribution and abundance of young juvenile shore crabs Carcinus maenas L. *Journal of Experimental Marine Biology and Ecology*, *271*(1), pp.41-73.

Moksnes, P.O., Pihl, L. and van Montfrans, J., 1998. Predation on postlarvae and juveniles of the shore crab Carcinus maenas: importance of shelter, size and cannibalism. *Marine Ecology Progress Series*, *166*, pp.211-225.

Moore, L.E., Smith, D.M. and Loneragan, N.R., 2000. Blood refractive index and whole-body lipid content as indicators of nutritional condition for penaeid prawns (Decapoda: Penaeidae). *Journal of Experimental Marine Biology and Ecology*, *244*(1), pp.131-143.

Moore, P.G. and Howarth, J., 1996. Foraging by marine scavengers: effects of relatedness, bait damage and hunger. *Journal of Sea Research*, *36*(3-4), pp.267-273.

Morse, B. and Rochette, R., 2016. Movements and activity levels of juvenile American lobsters Homarus americanus in nature quantified using ultrasonic telemetry. *Marine Ecology Progress Series*, *551*, pp.155-170.

Morton, B., 2011. Predator–prey-scavenging interactions between Nucella lapillus, Carcinus maenas and Eulalia viridis all exploiting Mytilus galloprovincialis on a rocky shore recovering from tributyl-tin (TBT) pollution. *Journal of Natural History*, *45*(39-40), pp.2397-2417.

Murray, L.G. and Seed, R., 2010. Determining whether catch per unit effort is a suitable proxy for relative crab abundance. *Marine Ecology Progress Series*, *401*, pp.173-182.

Natkiel, R. and Preston, A., 1986. Atlas of maritime history. Facts on File. *Inc., New York*.

Naylor, E., 1962. Seasonal changes in a population of Carcinus maenas (L.) in the littoral zone. *The Journal of Animal Ecology*, pp.601-609.

Naylor, J.K. and Taylor, E.W., 1999. Heart rate and gill ventilation in ovigerous and non-ovigerous edible crabs, Cancer pagurus: The effects of disturbance, substrate and starvation. *Marine & Freshwater Behaviour & Phy*, *32*(2-3), pp.129-145.

Neckles, H.A., 2015. Loss of eelgrass in Casco Bay, Maine, linked to green crab disturbance. *Northeastern Naturalist*, *22*(3), pp.478-500.

Newell, R.C. and Bayne, B.L., 1973. A review on temperature and metabolic acclimation in intertidal marine invertebrates. *Netherlands Journal of Sea Research*, *7*, pp.421-433.

Newell, R.C., Ahsanullah, M. and Pye, V.I., 1972. Aerial and aquatic respiration in the shore crab Carcinus maenas (L.). *Comparative Biochemistry and Physiology Part A: Physiology*, *43*(2), pp.239-252.

Noguchi, K., Gel, Y.R., Brunner, E. and Konietschke, F., 2012. nparLD: an R software package for the nonparametric analysis of longitudinal data in factorial experiments.

Nye, P.A., 1974. Burrowing and burying by the crab Macrophthalmus hirtipes. *New Zealand journal of marine and freshwater research*, *8*(2), pp.243-254.

O'Grady, D.F., Jury, S.H. and Watson III, W.H., 2001. Use of a treadmill to study the relationship between walking, ventilation and heart rate in the lobster Homarus americanus. *Marine and freshwater research*, *52*(8), pp.1387-1394.

Oliver, J.S. and Slattery, P.N., 1985. Effects of crustacean predators on species composition and population structure of soft-bodied infauna from McMurdo Sound, Antarctica. *Ophelia*, *24*(3), pp.155-175.

Oliver, M.D. and MacDiarmid, A.B., 2001. Blood refractive index and ratio of weight to carapace length as indices of nutritional condition in juvenile rock lobsters (Jasus edwardsii). *Marine and Freshwater research*, *52*(8), pp.1395-1400.

Ozbay, G. and Riley, J.G., 2002. An analysis of refractometry as a method of determining blood total protein concentration in the American lobster Homarus americanus (Milne Edwards). *Aquaculture Research*, *33*(8), pp.557-562.

Paille, N., Lambert, J., Simard, N. and Pereira, S., 2006. Le crabe vert (Carcinus maenas): revue de littérature et situation aux Îles-de-la-Madeleine. *Rapp. can. ind. sci. halieut. aquat*, *276*.

Patarnello, T., Volckaert, F.A. and Castilho, R., 2007. Pillars of Hercules: is the Atlantic–Mediterranean transition a phylogeographical break?. *Molecular ecology*, *16*(21), pp.4426-4444.

Payette, A.L. and McGaw, I.J., 2003. Thermoregulatory behavior of the crayfish Procambarus clarki in a burrow environment. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *136*(3), pp.539-556.

Pennoyer, K.E., Himes, A.R. and Frederich, M., 2016. Effects of sex and color phase on ion regulation in the invasive European green crab, Carcinus maenas. *Marine biology*, *163*, pp.1-15.

Poirier, L.A., Mohan, J., Speare, R., Davidson, J., Quijón, P.A. and St-Hilaire, S., 2016. Moulting synchrony in green crabs (Carcinus maenas) from Prince Edward Island, Canada. *Marine Biology Research*, *12*(9), pp.969-977.

Queiroga, H., 1998. Vertical migration and selective tidal stream transport in the megalopa of the crab Carcinus maenas. *Hydrobiologia*, *375*, pp.137-149.

Rathbun, M.J., 1905. Fauna of New England. Crustacea. Occ. Papers Boston Soc. Nat. Hist., vol. 7, pp. 1-117.

Réale, D., Reader, S.M., Sol, D., McDougall, P.T. and Dingemanse, N.J., 2007. Integrating animal temperament within ecology and evolution. *Biological reviews*, *82*(2), pp.291-318.

Reiber, C.L. and McGaw, I.J., 2009. A review of the "open" and "closed" circulatory systems: new terminology for complex invertebrate circulatory systems in light of current findings. *International Journal of Zoology*, *2009*.

Rice, A.L., and Ingle, R.W., 1975. The larval development of Carcinus maenus (L.) and C. mediterraneus Czerniavsky (Crustacea, Brachyura, Portunidae) reared in the laboratory. *The Bulletin of the British Museum (Natural History)*, *28*, pp.103-119.

Robertson, R.F., Meagor, J. and Taylor, E.W., 2002. Specific dynamic action in the shore crab, Carcinus maenas (L.), in relation to acclimation temperature and to the onset of the emersion response. *Physiological and Biochemical Zoology*, *75*(4), pp.350-359.

Robinson, E.M., Smee, D.L. and Trussell, G.C., 2011. Green crab (Carcinus maenas) foraging efficiency reduced by fast flows. *PloS one*, *6*(6), p.e21025.

Roman, J., 2006. Diluting the founder effect: Cryptic invasions expand a marine invader's range. *Proceedings of the Royal Society B: Biological Sciences*, 273(1600), pp. 2453–2459. doi: 10.1098/rspb.2006.3597.

Roman, J. and Palumbi, S.R., 2004. A global invader at home: population structure of the green crab, Carcinus maenas, in Europe. *Molecular Ecology*, *13*(10), pp.2891-2898.

Ropes, J., 1968. The feeding habits of the green crab, Carcinus maenus (L.). *Fisheries Bulletin*, *67*(2), pp.183-203.

Rose, R.A., Wilkens, J.L. and Walker, R.L., 1998. The effects of walking on heart rate, ventilation rate and acid-base status in the lobster Homarus americanus. *The Journal of experimental biology*, *201*(18), pp.2601-2608.

Rose, R.A., MacDougall, K., Patel, A., Wilkens, J.L. and Walker, R.L., 2001. Effects of walking on ventilatory and cardiac function in intact and cardiac-impaired lobsters. *Physiological and Biochemical Zoology*, *74*(1), pp.102-110.

Rossong, M.A., Williams, P.J., Comeau, M., Mitchell, S.C. and Apaloo, J., 2006. Agonistic interactions between the invasive green crab, Carcinus maenas (Linnaeus) and juvenile American lobster, Homarus americanus (Milne Edwards). *Journal of Experimental Marine Biology and Ecology*, *329*(2), pp.281-288.

Rossong, M.A., Quijón, P.A., Snelgrove, P.V., Barrett, T.J., McKenzie, C.H. and Locke, A., 2012. Regional differences in foraging behaviour of invasive green crab (Carcinus maenas) populations in Atlantic Canada. *Biological Invasions*, *14*(3), pp.659-669.

Ruf, T. and Geiser, F., 2015. Daily torpor and hibernation in birds and mammals. *Biological Reviews*, *90*(3), pp.891-926.

Saborowski, R., 2015. Nutrition and digestion. *The natural history of the Crustacea*, *4*, pp.285-319.

Sánchez-Paz, A., García-Carreño, F., Muhlia-Almazán, A., Peregrino-Uriarte, A.B., Hernández-López, J. and Yepiz-Plascencia, G., 2006. Usage of energy reserves in crustaceans during starvation: status and future directions. *Insect biochemistry and molecular biology*, *36*(4), pp.241-249.

Sandeman, D.C., 1967. The vascular circulation in the brain, optic lobes and thoracic ganglia of the crab Carcinus. *Proceedings of the Royal Society of London. Series B. Biological Sciences*, *168*(1010), pp.82-90.

Saver, M.A. and Wilkens, J.L., 1998. Comparison of the effects of five hormones on intact and open heart cardiac ganglionic output and myocardial contractility in the shore crab Carcinus maenas. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *120*(2), pp.301-310.

Say, T. 1817 An account of the Crustacea of the United States. J. Acad. Nat. Sei. Phila. 1, 57-63.

Scattergood, L.W., 1952. The distribution of the green crab, Carcinides maenas (L.) in the Northwestern Atlantic, 1952. *Maine Department of Sea and Shore Fisheries, Maine Department of Marine Resources, Augusta, ME. Marine Resources Documents, Paper*, *4*, pp.1-10.

Scheel, D. and Bisson, L., 2012. Movement patterns of giant Pacific octopuses, Enteroctopus dofleini (Wülker, 1910). *Journal of Experimental Marine Biology and Ecology*, *416*, pp.21-31.

Secor, S.M., 2009. Specific dynamic action: a review of the postprandial metabolic response. *Journal of Comparative Physiology B*, *179*(1), pp.1-56.

Shadwick, R.E., Pollock, C.M. and Stricker, S.A., 1990. Structure and biomechanical properties of crustacean blood vessels. *Physiological zoology*, *63*(1), pp.90-101.

Sharp, G., Semple, R., Connolly, K., Blok, R., Audet, D., Cairns, D. and Courtenay, S., 2003. Ecological assessment of the Basin Head lagoon: a proposed marine protected area. *Can. Manuscr. Rep. Fish. Aquat. Sci*, (2641), p.76.

Simonik, E. and Henry, R.P., 2014. Physiological responses to emersion in the intertidal green crab, Carcinus maenas (L.). *Marine and freshwater behaviour and physiology*, *47*(2), pp.101-115.

Sneddon, L.U., Huntingford, F.A. and Taylor, A.C., 1997. The influence of resource value on the agonistic behaviour of the shore crab, Carcinus maenas (L.). *Marine & Freshwater Behaviour & Phy*, *30*(4), pp.225-237.

Speers-Roesch, B., Norin, T. and Driedzic, W.R., 2018. The benefit of being still: energy savings during winter dormancy in fish come from inactivity and the cold, not from metabolic rate depression. *Proceedings of the Royal Society B: Biological Sciences*, *285*(1886), p.20181593.

Spicer, J.I., 2001. Development of cardiac function in crustaceans: patterns and processes. *American zoologist*, *41*(5), pp.1068-1077.

Stillman, J.H. and Somero, G.N., 1996. Adaptation to temperature stress and aerial exposure in congeneric species of intertidal porcelain crabs (genus Petrolisthes): correlation of physiology, biochemistry and morphology with vertical distribution. *Journal of experimental biology*, *199*(8), pp.1845-1855.

Styrishave, B., Rewitz, K. and Andersen, O., 2004. Frequency of moulting by shore crabs Carcinus maenas (L.) changes their colour and their success in mating and physiological performance. *Journal of Experimental Marine Biology and Ecology*, *313*(2), pp.317-336.

Symons, P.E.K., 1964. Behavioral responses of the crab Hemigrapsus oregonensis to temperature, diurnal light variation and food stimuli. *Ecology*, *45*(3), pp.580-591.

Taylor, E.W., 1981. Some effects of temperature on respiration in decapodean crustaceans. *Journal of thermal biology,* 6(4), pp. 239-248.

Taylor, G.M., 2000. Maximum force production: why are crabs so strong?. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *267*(1451), pp.1475-1480.

Taylor, E.W. and Wheatly, M.G., 1979. The behaviour and respiratory physiology of the shore crab, Carcinus maenas (L.) at moderately high temperatures. *Journal of Comparative Physiology B*, *130*(4), pp.309-316.

Taylor, E.W., Butler, P.J. and Al-Wassia, A., 1977. Some responses of the shore crab, Carcinus maenas (L.) to progressive hypoxia at different acclimation temperatures and salinities. *Journal of comparative physiology*, *122*(3), pp.391-402.

Taylor, G.M., Keyghobadi, N. and Schmidt, P.S., 2009. The geography of crushing: variation in claw performance of the invasive crab Carcinus maenas. *Journal of experimental marine biology and ecology*, *377*(1), pp.48-53.

Tepolt, C.K. and Palumbi, S.R., 2020. Rapid adaptation to temperature via a potential genomic island of divergence in the invasive green crab, Carcinus maenas. *Frontiers in Ecology and Evolution*, *8*, p.411.

Tepolt, C.K. and Somero, G.N., 2013, Cardiac thermal tolerance and acclimatory plasticity in diverse populations of the invasive green crab, Carcinus maenas. In *Integrative and Comparative Biology* (Vol. 53, pp. E213-E213). Journals Dept, 2001 Evans RD, Cary, NC 27513 USA: Oxford University Press INC.

Tepolt, C.K. and Somero, G.N., 2014. Master of all trades: thermal acclimation and adaptation of cardiac function in a broadly distributed marine invasive species, the European green crab, Carcinus maenas. *Journal of Experimental Biology*, *217*(7), pp.1129-1138.

Terwilliger, N.B., 2015. Oxygen transport proteins in Crustacea: hemocyanin and hemoglobin. *Physiology*, *4*, pp.359-390.

Thomas, N.J., Lasiak, T.A. and Naylor, E., 1981. Salinity preference behaviour in Carcinus. *Marine & Freshwater Behaviour & Phy*, *7*(4), pp.277-283.

Thresher, R., Proctor, C., Ruiz, G., Gurney, R., MacKinnon, C., Walton, W., Rodriguez, L. and Bax, N., 2003. Invasion dynamics of the European shore crab, Carcinus maenas, in Australia. *Marine Biology*, *142*(5), pp.867-876.

Torres, P.J. and González-Pisani, X., 2016. First record of green crabs, Carcinus maenas (Linnaeus, 1758) in Golfo Nuevo, Chubut Argentina: a new northern limit of distribution in the Patagonian coast. *Ecología Austral*, *26*(02), pp.134-137.

Trussell, G.C., Ewanchuk, P.J. and Bertness, M.D., 2003. Trait-mediated effects in rocky intertidal food chains: predator risk cues alter prey feeding rates. *Ecology*, *84*(3), pp.629-640.

Uzmann, J.R., Cooper, R.A. and Pecci, K.J., 1977. *Migration and dispersion of tagged American lobsters, Homarus americanus, on the southern New England continental shelf* (Vol. 705). Department of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service.

Wallace, J.C., 1973. Feeding, starvation and metabolic rate in the shore crab Carcinus maenas. *Marine Biology*, *20*(4), pp.277-281.

Walne, P.R. and Dean, G.J., 1972. Experiments on predation by the shore crab, Carcinus maenas L., on Mytilus and Mercenaria. *ICES Journal of Marine Science*, *34*(2), pp.190-199.

Wang, L.C.H., 1989. Ecological, physiological, and biochemical aspects of torpor in mammals and birds. In *Animal adaptation to cold* (pp. 361-401). Berlin, Heidelberg: Springer Berlin Heidelberg.

Wang, G. and Mcgaw, I.J., 2014. Use of serum protein concentration as an indicator of quality and physiological condition in the lobster Homarus americanus (Milne-Edwards, 1837). *Journal of Shellfish Research*, *33*(3), pp.805-813.

Wang, G. and McGaw, I.J., 2016. Potential use of Mussel farms as Multitrophic on-growth sites for American lobster, Homarus americanus (Milne Edwards). *Fish Aquac J*, *7*(161), p.2.

Waser, A.M., Dekker, R., Witte, J.I., McSweeney, N., Ens, B.J. and van der Meer, J., 2018. Quantifying tidal movements of the shore crab Carcinus maenas on to complex epibenthic bivalve habitats. *Estuaries and coasts*, *41*(2), pp.507-520.

Watling, L., 2013. Feeding and digestive system. *The natural history of Crustacea*, *1*, pp.237-61.

Welch, W.R., 1968. Changes in abundance of the green crab, Carcinus maenas (L.), in relation to recent temperature changes. *US Fish Wildl. Ser. Fish. Bull.*, *67*, pp.337-345.

Wheatly, M.G. and Taylor, E.W., 1979. Oxygen levels, acid-base status and heart rate during emersion of the shore crab Carcinus maenas (L.) into air. *Journal of comparative physiology*, *132*(4), pp.305-311.

Whiteley, N.M., Robertson, R.F., Meagor, J., El Haj, A.J. and Taylor, E.W., 2001. Protein synthesis and specific dynamic action in crustaceans: effects of temperature. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *128*(3), pp.593-604.

Whiteley, N.M., Taylor, E.W. and El Haj, A.J., 1997. Seasonal and latitudinal adaptation to temperature in crustaceans. *Journal of thermal biology*, *22*(6), pp.419-427.

Wiersma, C.A.G. and Novitski, E., 1942. The mechanism of the nervous regulation of the crayfish heart. *Journal of Experimental Biology*, *19*(3), pp.255-265.

Wieser, W., 1973. *Effects of temperature on ectothermic organisms*. Springer Berlin Heidelberg.

Wilkens, J.L., 1999. The control of cardiac rhythmicity and of blood distribution in crustaceans. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *124*(4), pp.531-538.

Wilkens, J.L., Mercier, A.J. and Evans, J., 1985. Cardiac and ventilatory responses to stress and to neurohormonal modulators by the shore crab, Carcinus maenas. *Comparative Biochemistry and Physiology Part C: Comparative Pharmacology*, *82*(2), pp.337-343.

Willmer, P., Stone, G. and Johnston, I., 2009. *Environmental physiology of animals*. John Wiley & Sons.

Wilson, C.H., Wyeth, R.C., Spicer, J.I. and McGaw, I.J., 2022. Effect of Animal Stocking Density and Habitat Enrichment on Survival and Vitality of Wild Green Shore Crabs, *Carcinus maenas*, Maintained in the Laboratory. *Animals*, *12*(21), p.2970.

Wittmann, A.C., Held, C., Pörtner, H.O. and Sartoris, F.J., 2010. Ion regulatory capacity and the biogeography of Crustacea at high southern latitudes. *Polar Biology*, *33*, pp.919-928.

Wolf, F., 1998. Red and green colour forms in the common shore crab Carcinus maenas (L.)(Crustacea: Brachyura: Portunidae): theoretical predictions and empirical data. *Journal of Natural History*, *32*(10-11), pp.1807-1812.

Wu, F., Wang, T., Cui, S., Xie, Z., Dupont, S., Zeng, J., Gu, H., Kong, H., Hu, M., Lu, W. and Wang, Y., 2017. Effects of seawater pH and temperature on foraging behavior of the Japanese stone crab Charybdis japonica. *Marine pollution bulletin*, *120*(1-2), pp.99-108.

Yamada, S.B. and Hauck, L.A.U.R.A., 2001. Field identification of the European green crab species: Carcinus maenas and Carcinus aestuarii. *Journal of Shellfish Research*, *20*(3), pp.905-912.

Young, A.M. and Elliott, J.A., 2019. Life history and population dynamics of green crabs (Carcinus maenas). *Fishes*, *5*(1), p.4.

Young, A.M., Elliott, J.A., Incatasciato, J.M. and Taylor, M.L., 2017. Seasonal catch, size, color, and assessment of trapping variables for the European green crab (Carcinus maenas) (Brachyura: Portunoidea: Carcinidae), a nonindigenous species in Massachusetts, USA. *Journal of Crustacean Biology*, *37*(5), pp.556-570.

Young, J.S., Peck, L.S. and Matheson, T., 2006. The effects of temperature on walking and righting in temperate and Antarctic crustaceans. *Polar Biology*, *29*(11), pp.978-987.

Zarrella-Smith, K.A., Woodall, J.N., Ryan, A., Furey, N.B. and Goldstein, J.S., 2022. Seasonal estuarine movements of green crabs revealed by acoustic telemetry. *Marine Ecology Progress Series*, *681*, pp.129-143.

Zeng, C. and Naylor, E., 1996. Heritability of circatidal vertical migration rhythms in zoea larvae of the crab Carcinus maenas (L.). *Journal of Experimental Marine Biology and Ecology*, *202*(2), pp.239-257.

Supplementary Tables

Table S3.1 Number of crabs caught per size category: Total number of crabs caught in all baited traps during CPUE surveys in the subtidal zone each month, divided by size category. Small = < 50 mm for males and < 40 mm for females, medium = 50 – 60 mm in males and 40 – 50 mm in females, large = 60 – 80 mm in males and 50 – 60 mm in females, and extra large = > 80 mm in males and > 60 mm in females.

Month	Large	Medium	Small	Female	Male
Sep	83	84	110	38	239
Oct	32	45	61	28	110
Nov	31	62	50	37	106
Dec	21	10	32	14	49
Jan	0	0	1	0	1
Feb	0	0	0	0	0
Mar	0	0	1	1	0
Apr	0	1	18	13	6
May	8	25	60	24	69
Jun	27	28	47	18	84
Jul	27	21	24	5	67
Aug	68	70	180	60	258

Appendix 1: An introduction to decapod physiology

Cardiovascular system

Decapod crustaceans have a complex, partially closed circulatory system (McGaw and Reiber, 2015). They have a single-chambered heart structure: the walls consist of bands of cardiac muscle forming a matrix that enables the ejection of haemolymph into the arterial system through contraction. Haemolymph is pumped through a network of fine vessels that supply tissues with gaseous and nutrient rich haemolymph. They also possess a distinct venous channel that returns haemolymph to the gills (Shadwick et al., 1990; McMahon and Burnett, 1990). Heart rate and strength of contraction are controlled by the cardiac ganglion on the surface of the heart (Wiersma and Novitiski, 1942; McMahon and Burnett, 1990; McGaw and Reiber, 2015). Decapods can regulate the haemolymph flow through their body for efficient delivery of gases and nutrients and allocation to metabolically active locations (Reiber and McGaw, 2009; McGaw and Reiber, 2015). Neural and hormonal mechanisms control cardiac function and haemolymph flow using cardioregulatory nerves, neuronal stimulation, and naturally occurring peptide and amine neurohormones (Kuramoto and Kuwasawa, 1980; Kuromot and Ebara, 1984; Saver and Wilkens, 1998; Wilkens, 1999).

Cardiac processes are affected by multiple factors such as movement, burying activity, digestion (McGaw and Reiber, 2015) and multiple environmental factors (discussed elsewhere in the Appendix). When active, heart rate and stroke volume increase, directing haemolymph flow towards walking legs and the respiratory system (Hamilton and Houlihan, 1992; De Wachter and McMahon, 1996). During burying, green crabs' heart rate becomes more erratic, with periods of bradycardia and cardiac pauses. Bursts of

tachycardia can also be caused due to short periods of increased scaphognathite beating to clear blockages of the respiratory openings (Cumberlidge and Uglow, 1977).

Decapod heart rate has been used to indicate stress and energy expenditure in response to many biotic and abiotic factors for decades (DeFur and Mangum, 1979; Aagard, 1996; Handy and Depledge, 1999). Heart rate has been used to show stress response of green crabs to many conditions such as: handling and surgery (Wilkens et al., 1985), copper exposure (Lundebye and Depledge, 1998), being restrained (McGaw and Nancollas, 2018) and body temperature (Iftikar et al., 2010). Yet, more subtle deviations to cardiovascular function not related to heart rate, often caused by stress, are missed when exclusively monitoring heart rate (Depledge et al., 1996). Additionally, in certain cases increased cardiac output has been determined to be due to increased stroke volume rather than heart rate (De Wachter and McMahon, 1996). Thus, more recently moves towards multiple measures of cardiovascular response have been made (McMahon, 1999; McGaw and Reiber; 2015).

Respiratory system

The respiratory system of decapod crustaceans is closely linked with the cardiovascular system, e.g. increases in heart rate are often paired with increases in oxygen uptake and vice versa (McGaw and Reiber, 2015). Due to their exoskeleton, crustaceans rely on the gills and epithelial lining of the branchial chambers for oxygen diffusion to take place (Terwilliger, 2015). Movement of scaphognathites is used by decapods to regulate the movement of oxygen-depleted and oxygen-rich water in and out of the gill chambers (McMahon, 2001; McGaw and Reiber, 2015). Internally controlled diffusion gradients are used to extract as much oxygen from the external water as possible through these

contact surfaces (Terwilliger, 2015). Additionally, specialised oxygen transport proteins within the haemolymph bind to oxygen molecules and transport them to areas of lower oxygen concentration (Terwilliger, 2015). These processes provide greater supply of oxygen through the body to metabolically active areas.

Oxygenated and deoxygenated haemaolymph are mostly kept separate through a complex arterial system. Oxygenated haemolymph are circulated to the heart through a series of branchio–pericardial veins, preventing mixing of oxygenated and deoxygenated haemolymph (McMahon, 2001). A pair of large infrabranchial sinuses collect deoxygenated haemolymph which is then distributed to the gills (McMahon, 2001). In green crabs' oxygen is delivered to the central nervous system through a system of distribution sinuses (Sandeman, 1967; McMahon, 2001) and a regular spread of capillary branches supplying oxygenated haemolymph to the optic lobes (Sandeman, 1967).

Oxygen consumption and retention can be adjusted in response to multiple biotic factors, for example, during starvation adult and larval green crabs have reduced oxygen uptake paired with increased oxygen capacity (Ansell, 1973; Dawirs, 1983). Exercise causes heart and ventilation rates to increase initially and plateau (Rose et al., 1998) but haemolymph oxygen content increases while carbon dioxide concentration remains stable, suggesting exercise does not impair gas exchange (Hamilton and Houlihan, 1992). Oxygen consumption declines when exposed to air by ~25 % compared to when submerged (Newell et al., 1972). Thus, internal mechanisms allow crabs to alter their oxygen requirement and uptake.

Digestive system

Decapod digestive tracts start with a chitin lined esophagus and foregut leading into a midgut and a hindgut (Watling, 2003). The foregut, equipped with teeth and ossicles, grind the food items into small digestible pieces, this is known as the gastric mill (Dall and Moriarty, 1983; Watling, 2003; McGaw and Curtis, 2013). The decapod midgut connects the foregut to the hindgut and has a large structure of thousands of tubules, called the hepatopancreas. Each of these tubules contains cells that produce digestive enzymes that are excreted into the midgut (Watling, 2013). Large particles (> 100 nm) are moved from the foregut into the midgut for digestion, while smaller particles are moved into the hepatopancreas to be absorbed or transferred to the hindgut for excretion (Hopkin and Nott, 1980). Most digestion and absorption of nutrients occurs in the midgut and hepatopancreas (Saborowski, 2015). Undigested material from the midgut is finally passed into the hindgut for excretion, although some minor digestion may also occur here (Watling, 2003).

The transit time of food items through the digestive system of crustaceans is commonly used to measure food processing rates, which is an indication of digestive efficiency (McGaw and Curtis, 2013). Slower transit times allow longer for absorption of nutrients which may be necessary due to meal size or type, animal activity levels, or other environmental factors (McGaw and Curtis, 2013). The specific dynamic action (SDA) is the term used to describe the accumulated energy expenditure occurring after a meal due to the ingestion, digestion, and absorption of the meal (Secor, 2009). An increased metabolic rate is characteristic of SDA with a slow return to pre-feeding levels after peaking (Secor, 2009). The duration of SDA in green crabs has been estimated to be between 1.44 – 3.08 hours (Wallace, 1973; Robertson et al., 2002; Secor, 2009). Due to the energy requirements of SDA, nutritional state impacts heart rate and oxygen consumption

(Depledge, 1985). Indeed, resting oxygen uptake of postprandial crabs has been shown to be two times that of starved crabs (McGaw, 2007). Furthermore, activity levels decline after feeding and it has been found that food items take twice as long to clear the foregut in active crabs than in resting crabs suggesting activity impacts digestive processing (McGaw, 2007).

Introduction to decapod behaviour: Movement

Decapod crustaceans are free moving, highly mobile benthic invertebrates: they can swim but will usually walk side-ways across the substrate (Fraser et al., 1987; Young et al., 2006). Within lab conditions, decapod movement behaviour is relatively intermittent, occurring in bursts between other behaviours, such as resting or burying (McGaw, 2007; Matheson and Gagnon, 2012b). Green crabs' maximum rate of movement has been estimated to be between 6 – 7 cm per second (Young et al., 2006). Their movement rate is affected by multiple environmental and biotic conditions including temperature, the presence of prey or competitor species and feeding. Movement rate increases with temperature, reaching a plateau at ~ 10 ° C and ceasing at ~ 0 ° C (Young et al., 2006). This complete lack of movement at low temperatures in temperate poikilotherms has been considered a torpor (Berrill, 1982; Young et al., 2006). When exposed to prey and/or competitor species, green crabs spend most of their time stationary or feeding. The percentage time spent actively moving is relatively small (< 30 %), and when coupled with decreasing temperature, continues to decline (Bélair and Miron, 2009, Matheson and Gagnon, 2012b). After feeding, green crabs' activity levels decline considerably for ~ 18 hours, presumably during digestion (McGaw, 2007).

Green crabs are known to be attracted to areas of high prey abundance, often travelling large distances to reach these areas (Fairchild et al., 2008). Within their native range, they also conduct daily migrations with the tide, returning to the subtidal zone with the retreating tide (Crothers, 1968; Naylor 1962; Edwards, 1958; Dare and Edwards, 1981). However, smaller crabs (<35 mm carapace width) do not appear to conduct this migration behaviour, instead most remain in the intertidal even during low tide (Warman et al., 1993; Hunter and Naylor, 1993; Waser et al., 2018). Indeed, consistently larger male crabs were captured in an off-shore area when compared to two on-shore areas (Edwards, 1958). Most studies investigating daily migration patterns have been conducted within their native range (primarily in the UK); such behaviours do not necessarily hold true for invasive populations.

In situ acoustic monitoring in an estuary within their invasive range found green crabs to have relatively localized movement patterns, not regularly moving between up- and down-stream zones (Zarrella-Smith et al., 2022). Previous estimates using mark recapture methods have suggested vastly different daily migration distances between 300 m - 2 km per day (Dare and Edwards, 1981; Ameyaw-Akumfi and Naylor, 1987). This recent acoustic monitoring study, however, supports the low daily movement estimates (Zarella-Smith et al., 2022). What all these studies do agree upon is a high degree of variability in movement rate between individuals. Additionally, these studies suggested avoidance of hypersalinity as a possible driver for this daily migration behaviour which may not be a factor in other environments occupied by green crabs, such as open shores (Dare and Edwards, 1981; Ameyaw-Akumfi and Naylor, 1987; Zarrella-Smith et al., 2022).

Burying

In decapod crustaceans burying is defined as when the organism has moved through the substrate to encase their body, as opposed to borrowing in which tunnels in the substrate are formed (Bellwood, 2001). Most portunid crabs (the family to which Carcinus maenas belongs) perform back-burying behaviour: they dig/push their abdomen into the sediment to submerge themselves (McLay and Osborne, 1985). Some portunid crabs have flattened pleopods to assist with digging (Bellwood, 2001). Green crabs can bury themselves very quickly (~ 5 seconds) and appear to do so in response to disturbance (Cumberlidge and Uglow, 1977). Burying behaviour is highly reduced in the presence of competitor species and this decline is more pronounced with increased prey densities (Bélair and Miron, 2009). However, time spent buried or sheltering remains the same regardless of temperature (Matheson and Gagnon, 2012a).

Burying decapods have various physiological adaptations to being submerged in the sediment. For example, while buried crabs must ensure a constant flow of water through their branchial chambers while avoiding sediment clogging their ventilatory openings, which are in direct contact with the sediment (Atkinson and Taylor, 1988; Bellwood, 2001; McGaw, 2004). This is accomplished by intermittent periods of ventilatory reversals, pushing water out of the respiratory opens along with any sediment particles (Cumberlidge and Uglow, 1977). While actively burying decapods have an increased cardiac output, this is followed by a decline once buried, which is consistent with periods of inactivity (McGaw, 2004).

Foraging

Crustaceans often use chemical cues to identify and locate prey. A mixture of compounds which are highly diffusible in seawater, including free amino acids, ammonium

compounds, nucleotides, and organic acids, create cues that attract predators (Carr, 1998; Derby and Zimmer, 2012; Derby and Weissburg, 2014). Decapods have chemosensory organs on their antennules called aesthetascs which are used to detect chemosensory cues and locate prey (Derby and Weissburg, 2014). Predator's sense excreted metabolites to find prey such as buried bivalves, while scavengers' sense chemical plumes from carcasses (Watling, 2015). Photoreception is also an important means of sensing prey mainly for crustaceans living in shallow habitats where there is good visibility (Eastman et al., 2015). Green crabs use a combination of chemo- and photoreception to locate prey.

Green crabs are opportunistic, omnivorous predators and scavengers that have an extremely varied diet, including bivalves, gastropods, crustaceans, polychaetas, fish and algae (Ropes, 1968; Baeta et al., 2006; Moore and Howarth, 1996; Morton, 2011). They have strong claws that exude great force (Taylor, 2000); when foraging they use their claws to crush bivalve shells and other shelled or tough prey (Hughes and Elner, 1979; Talyor et al., 2009). They then use their mouthparts to transfer food items to their mouth (Theil and Watling, 2015). Despite their generalist diet they show a strong preference towards bivalves (Ropes, 1968; Walne and Dean 1972; Cohen et al., 1995). As such, they have caused large declines in prey populations in newly invaded locations (Cohen et al., 1995; Grosholz et al., 2002), and continue to have highly consequential ecosystem impacts that can span multiple trophic levels (Grosholz and Ruiz, 1996; Trussel et al., 2003; Matheson et al., 2016).

Due to their generalist diet, green crabs are highly adaptable to environmental change. They can alter their diet and foraging strategy in response to con- and heterospecific competition (Griffen et al., 2008; Chakravarti and Cotton, 2014). Foraging rates decline

in response to reduced temperature (Glude, 1955; Matheson and Mckenzie, 2014) and foraging is more consistent and efficient during warmer, summer months (Elner, 1980). Foraging (measured using bait attraction) is also more common during flood tide than ebb tide (Hunter and Naylor, 1993). Prey size affects foraging strategy; handling time increases with prey size. When given the option, green crabs select small mussels that they can crush. When only presented with large mussels however, they employ boring and edge-chipping techniques (Burch and Seed, 2000). When foraging on bivalves, green crabs will reject failed attempts after a few minutes, usually if the prey item is too large (Hughes and Elner, 1979). This selective pressure on small-bodied prey can impact size structure of prey populations (Oliver and Slattery, 1985). Additionally, strong predation pressure from decapod crustaceans can also provide a natural control for invasive prey species, such as mollusks (Harding et al., 2003).

Carcinus maenas tolerance to environmental variability: Salinity

Green crabs are osmoregulators, meaning they maintain internal salinity despite environmental change. They can tolerate a wide range of salinities and are commonly found in estuarine habitats or tide pools. Therefore, they are often exposed to broad daily fluctuations in salinity. Their preferred salinity range is between 28 ppt – 42 ppt (Thomas et al., 1981), but they can endure salinities between 1.4 – 54 ppt (Zanders, 1979; Leignel et al., 2014). The initial behavioural response to both high and low salinity is avoidance through increased activity, defined as halokinesis (Thomas et al., 1981). Reduced salinities, as experienced in estuarine habitats, cause an increase in heart rate and oxygen consumption (Taylor, 1977). This is suggested to be due to the energetic demand of regulating internal salinity in hypo- and/or hyper-saline environments (McGaw and Reiber, 2015), alongside this increased movement activity (avoidance). Salinity also

appears to influence permeability of green crabs to salts (Spaargaren, 1975), which could further affect energetic cost due to increased active ion uptake. These responses have been shown to be affected by other abiotic and biotic factors (Truchot, 1986; McGaw, 2006).

Hypoxia

Green crabs commonly inhabit hypoxic environments for varying periods of time. For example, during low tide some small green crabs will remain on the shore within tide pools or beneath rocks (Crothers, 1968). Oxygen concentration within tide pools can vary between 3 – 680 μmol liter-1 (Truchot and Duhamel-Jouve, 1980), meaning animals residing within tide pools at low tide must tolerate periods of low environmental oxygen tension. The immediate response to hypoxic conditions is increased movement or emersion to escape the area of low oxygen tension (Reid and Aldrich, 1989).

If hypoxic conditions cannot be avoided green crabs are able to maintain oxygen consumption and metabolic rate during dips in environmental oxygen tension until a critical environmental oxygen level (Reid and Aldrich, 1989; McGaw and Reiber, 2015). To do this they increase the rate of scaphognathtite beating to pump more water over their gills to extract as much oxygen as possible (McMahon and Wilkens, 1983; McGaw and Reiber, 2015). The cardiac response to hypoxia is bradycardia with an increased stroke volume and alteration of blood flow to send more oxygen to the vital organs (Taylor and Wheatley, 1979; Taloyr and Wheatley, 1981; McGaw and Reiber, 2015). When hypoxia becomes severe (reaches the critical level) green crabs reduce movement and oxygen consumption and heart rate decrease to conserve oxygen (Taylor, 1976).

In a laboratory study imitating all physical tide pool conditions, haemolymph acid-base disturbances in green crabs were reduced in comparison to experiments investigating individual tide pool conditions, e.g., salinity or hypoxia (Truchot, 1986). Additionally, the ability to maintain respiration during hypoxic conditions depends on the level of movement activity by green crabs (Taylor, 1976). Thus, the ability of green crabs to cope with hypoxic conditions depends on both biotic and abiotic factors (Herried, 1980).

Emersion

Crustaceans have many different responses and mechanisms for being exposed to air depending on their environment and life history (McGaw and Reiber, 2015). Intertidal crustaceans, such as green crabs, experience air exposure regularly so are well adapted to it. When green crabs are fully exposed to air for prolonged periods of time (e.g., during low tide) they maintain a similar oxygen consumption and heart rate while maintaining haemolymph oxygen concentration (Taylor and Butler, 1978; McGaw and Reiber, 2015). This is thought to be accomplished through an increased stroke volume (Taylor and Butler, 1978). In addition, a rise in haemolymph lactate concentrations when emerged suggest increased reliance on anaerobic respiration (Simonik and Henry, 2014), although this was previously believed not to be the case (Taylor and Butler, 1978).

During hypoxic conditions green crabs may voluntarily emerge to breathe air (Taylor and Wheatly, 1979; Wheatly and Taylor, 1979). They conduct ‘bubbling’ behaviour in which they suck in air and bubble it through seawater over their gills to increase oxygen concentration. Heart rate and oxygen consumption are affected by temperature and

other abiotic factors (Truchot, 1986; Agaard, 1996; Styrishave et al., 2003; Willmer et al., 2005; McGaw, 2006), so when emerged green crabs tend to remain under rocks or seaweed, in micro-habitats, where temperature is lower and humidity is high (Simonik and Henry, 2014).

Starvation

During feeding crustaceans increase oxygen uptake, yet there is minimal effect on heart rate and other cardiovascular characteristics (McGaw, 2006). Feeding is metabolically costly due to associated increased activity, protein synthesis and mechanical processing of the meal (Carefoot, 1990; Houlihan et al., 1990; McGaw and Van Leeuwen, 2017). Retention time of food items within the gut of green crabs is estimated to be around 12 – 17.3 hours (Hopkin and Nott, 1980; McGaw and Whitely, 2012; McGaw and Curtis, 2013). Feeding is likely to be reduced in challenging abiotic conditions such as during periods of hypoxia, low salinity, or extreme temperatures, due to prioritisation of avoidance or energy conservation. Therefore, periods of starvation are likely to coincide with other abiotic stressors (Curtis et al., 2010).

Periods of starvation result in reduced oxygen consumption and a decrease in resting heart rate, in part due to reduced activity levels which are also caused by starvation (Ansell, 1973). Furthermore, starved ovigerous crabs have lower heart rates and higher ventilatory pressures than fed ovigerous crabs (Naylor and Taylor, 1999). Starvation has also been shown to impact immune parameters while not inducing oxidative stress (Matozzo et al., 2011). Additionally, digestive efficiency gradually declines with increasing temperature (measured from 5 ˚C to 25 ˚C) (McGaw and Curtis, 2013), therefore, feeding is likely to be avoided when metabolic costs are high. This is further shown

through the reduced activity and feeding behaviour seen at cold temperatures (Elner, 1980; Bélair and Miron, 2009a).