Abstract

Are All Parasitic Infections Created Equal? An Examination of Differential Infection Effects and Responses in Native and Non-Native Hosts

By

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Interactions between native and introduced species are complex, multifaceted, and can have cascading effects throughout an ecosystem. While scientists have taken great strides to understand how these dynamics change with the introduction of a novel species, less work has been done to explore how native and introduced species interactions change in the face of parasitism. I investigated these interactions using three intertidal crab species in the Western North Atlantic; the native rock crab *Cancer irroratus*, the introduced European green crab, *Carcinus maenas*, and the introduced Asian shore crab, *Hemigrapsus sanguineus* along with a parasitic trematode *Micropahllus* spp.

C. maenas and *C. irroratus* are both hosts to microphallid trematode parasites in the Western North Atlantic, but little is known about microphallid infections in *H. sanguineus* in this region. In order to properly evaluate infection in these crabs, I conducted two surveys along the New England coast to determine prevalence of infection in these three species. I utilized genetic tools to determine if the microphallids infecting these crabs are native to Western North Atlantic, potentially introduced from Europe with *C. maenas*, or a combination of both. During these surveys, I found all three crab species to be infected with one or more trematode parasites along the Western North Atlantic. Genetic analyses show that there are at least four distinct lineages of trematodes infecting crabs collected in this region, two of which matched previously identified trematodes in the region, *Microphallus similis* and *Gynaecotyla adunca*.

In conjunction with determining which parasites are infecting these crabs, I wanted to determine what effects infection had on these different host species. To understand the effects of infection I conducted both physiological assays on crabs collected during the survey, analyzing Hepatosomatic and Gonadosomatic indices (HSI and GSI) as well as infection experiments on the two introduced crabs, *C. maenas* and *H. sanguineus*, as I was unable to collect enough of the native *C. irroratus* for these experiments. I tested each species for individual behavioral responses to infection, and performed interspecific competition trials to determine how parasites may influence crab interactions. For my physiological assays, there was a trend for the HSI in both *C. irroratus* and *C. maenas* to be higher in infected crabs compared to uninfected crabs, with no difference in *H. sanguineus* crabs. There appeared to be a trend with lower GSI in infected *H. sanguineus* that was not seen in the other crab species, however a more robust sampling size would help determine if this trend exists in nature or is instead an artifact of small sample size. For my infection experiments, I saw that while my intensities of infection were low, there seems to be a trend for slower righting response in infected *C. maenas* with a similar trend seen in exposed *H. sanguineus* even though the parasite was unsuccessful in infecting *H. sanguineus*. During the competition trials I was able to detect a decreased level of exploratory by *C. maenas* exposed to the parasite when compared to control crabs, however there were very few overall differences between treatments. There were differences between species, with *H. sanguineus* conducting more exploratory behaviors overall and *C. maenas* conducting more food based behaviors.

By using this multi-layer approach to investigate parasite effects, my study provides in-depth coverage of various changes that could be induced by infection. It also provides data on how infection by microphallid trematodes may affect competitive interactions and lay the groundwork for future studies exploring how those differences could have community-wide effects throughout the ecosystem.

Are All Parasitic Infections Created Equal? An Examination of Differential Infection Effects and Responses in Native and Non-Native Hosts

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By

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1.1 Introduction

Throughout maritime history, humans have facilitated the introduction of non-indigenous species (NIS) to new and previously inaccessible locations (Carlton and Geller, 1993). These introductions can have profound effects on various enterprises in those regions such as tourism, fisheries, or even human health (Bax et al., 2003; Pejchar and Mooney, 2009). Considerable effort has been directed towards quantifying the monetary effects of various NIS in order to better direct further eradication or management practices (Pejchar and Mooney, 2009); however, these estimates are not always accurate and can vary greatly (Pejchar and Mooney, 2009). While it is important to determine the direct monetary costs introduced species may have on human industries, it is also important to understand the indirect costs of NIS through their varied effects on invaded ecosystems (Bax et al., 2003; Katsanevakis et al., 2014).

Introduced species can have especially detrimental impacts on components of marine habitats such as community structure, ecosystem services, and biodiversity (Molnar et al., 2008; Katsanevakis et al., 2014; Laverty et al., 2015). Bax et al. (2003) describes one example in which the New Zealand screwshell, *Maoricolpus roseus*, was introduced to Tasmania from New Zealand, and changed the seabed habitat by populating the soft sediment and provided hard attachment points for native and non-native species alike. This introduction is one of many that has dramatically altered the community structure from what it once was to something that may support an entirely new suite of organisms (Bax et al., 2003). In fact, the introduction of alien species to new environments has been identified as one of the leading causes of endangerment and extinction of native species (Czech and Krausman, 1997; Bellard et al., 2016). With our ever expanding economic globalization, trade networks, and connectivity of the world's ecosystems, we can expect the movement and impacts of alien species to escalate (Seebens et al., 2016).

In response to the many uncertainties surrounding the introduction of new species, a rapidly growing field of research seeks to explore why, when, and where certain species are more successful invaders than others, with a goal toward developing a more robust theoretical framework for predicting future introductions (Canning-Clode, 2015). Understanding why some species are successful or unsuccessful at invading a new area may help prevent future introductions (Carlton, 1996; Stachowicz et al., 1999; Chang et al., 2011). Studies have provided evidence that systems with strong community structure and high levels of biodiversity may better resist biological introductions (Baltz and Moyle, 1993; Stachowicz et al., 1999; Kennedy et al., 2002). For example, in Deer Creek, California, assemblages of native stream fish resisted the introduction of various NIS despite frequent attempts (Baltz and Moyle, 1993). This resistance was primarily due to the presence of appropriate predators that prevented the establishment of a large breeding population of non-indigenous fish (Baltz and Moyle, 1993). Further, both Stachowicz et al (1999) and Kennedy et al. (2002) found that increased biodiversity at local levels decreased the number of introduced species that established or settled in the area, and reduced the overall growth of the introduced species, and their survivorship.

While the biological aspects of a community are important in influencing introduction success, there are many abiotic factors that can also be important. For example, the unpredictable environment of the streams in California seemed to also favor native species success over that of an introduced species (Baltz and Moyle, 1993). However, anthropogenic disturbances like pollution, climate change, or habitat destruction may alter various abiotic components of a system too much, allowing for the successful introduction of novel organisms that can then colonize the area and reproduce (Byers, 2002; Stachowicz et al., 2002; Galil et al., 2015). In a theoretical example involving acid rain, Byers (2002) suggested that such an extreme disturbance on such a small timescale could remove native species entirely, so that a NIS would meet little to no biotic resistance when establishing in a new environment. The Suez Canal has had various documented impacts through the

introduction of novel species into the native marine communities in the Red and Mediterranean Seas, and there is potential for exacerbated impacts on those communities due to the widening of the canal (Galil et al., 2015)

Another non-mutually exclusive reason that some species may experience invasion success is the enemy-release hypothesis (ERH), wherein introduced species may leave behind some or all of their native enemies when they invade a new region (Torchin et al., 2003; Simmons, 2014). This would provide a potential competitive edge over native species that possess their full complement of predators, competitors, and parasites (Blakeslee et al., 2009; Keogh et al., 2016). A review and meta-analysis testing the ERH in native and introduced plants found that the number of herbivore species on introduced plants is lower than those on their native counterparts, herbivory is decreased on introduced plants when compared to their native counterparts (Liu and Stiling, 2006). In another study, twenty-six different host species in native and introduced regions were analyzed, and researchers found a reduction in parasites by half in the introduced regions (Torchin et al., 2003).

Parasites are a great example of organisms that can have disproportionate effects on the ecosystem they are a part of (Budria and Candolin, 2014). Parasites can have simple life cycles, in which they utilize a single host, or complex lifecycles, wherein they utilize multiple hosts (Rohde et al., 1982). They can also be host specific, where they require a single species to reproduce, or generalists, being able to utilize a suite of organisms as potential hosts (Rohde et al., 1982). These qualities of parasitic organisms dictate how much influence they have on hosts and the ecosystem as a whole. This is especially true for interactions between competing species where one or both of the interacting species can be infected by a given parasite (Thomas et al., 1995). Further, these host-parasitic interactions are often disrupted by the introduction of new species into that environment (Thieltges et al., 2009).

When an introduced species becomes established in a new region, a number of poten-

tial parasitic interactions may ensue (Figure 1). Introduced species could acquire native parasites ("parasite acquisition") which would not only increase the parasite load of the NIS, but could dilute the impact on native hosts, giving native species an advantage during competitive interactions (Goedknegt et al., 2016). However, if through infection of the introduced host the native parasite sees an increase in transmission, this could lead to increased prevalence or intensity of infection in the native host, a process called "parasite spillback" (Hoberg and Brooks, 2008).

If NIS are introduced to a region with their own parasites, we could see similar interactions as the ones noted above. These introduced parasites could host switch to use native species as competent hosts ("parasite spillover") (Goedknegt et al., 2016). Not only would this increase the parasite load in native species, which already contend with their full complement of parasites, it could also dilute the introduced parasite's impacts on its introduced host. However, like the example above, when a parasite infects a novel host, this could increase overall transmission of the parasite. As transmission, increases the NIS could have increased prevalence and intensity of infection due to the overall increased levels of parasites in the system ("parasite spillback"). While this phenomenon is not well documented, it is possible.

The above scenarios could occur if a parasite finds a new competent host. However, if a parasite (native or introduced) is unable to host switch but still attempts infection of a novel host, the original host could incur a benefit. This is because as with parasite acquisition or parasite spillover, having multiple hosts dilutes the effect of the parasite on any single host (Civitello et al., 2015).

The nature and extent of parasite loads in a native or non-native species may significantly influence a species ability to detect and escape predators or to acquire and handle prey items (Lafferty and Morris, 1996; Poulin, 2010). On the one hand, strong parasite escape may be advantageous to a non-native species, such that the new parasite-free or parasite-reduced invader could outcompete a native species for prey resources (Fofonoff

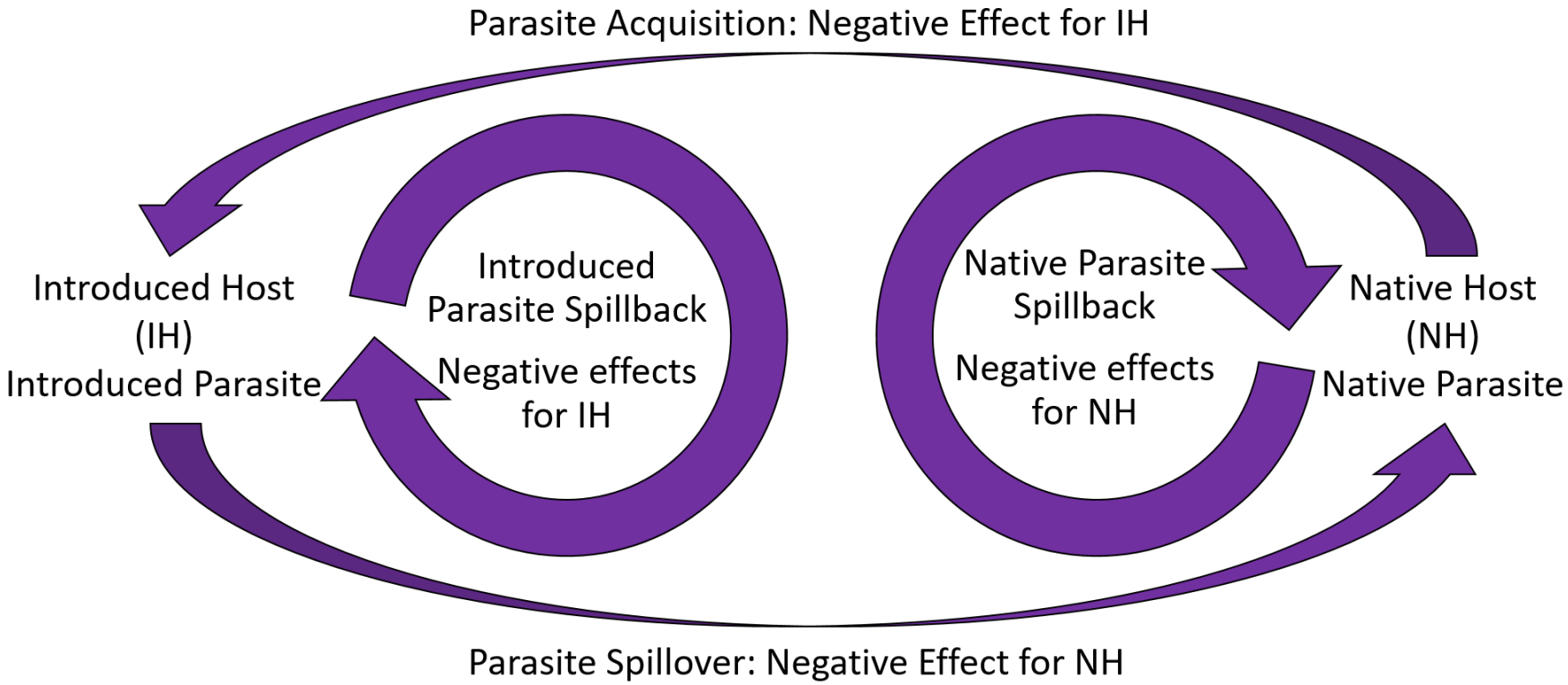

Figure 1: Potential pathways of parasitic interactions between native and introduced species. Arrows denote a parasite moving from infecting one species to another. IH= Introduced Host, NH=Native Host. Each term is defined as follows: Parasite Spillover: An introduced parasite host switches to utilize a native species as a competent host. Parasite Acquisition: A native parasite host switches to utilize an introduced species as a competent host. Introduced Parasite Spillback: After parasite spillover occurs, the transmission of the introduced parasite increases thereby increasing prevalence and intensity of infection in introduced host. Native Parasite Spillback: After parasite acquisition occurs, the transmission of the native parasite increases thereby increasing prevalence and intensity of infection in the native host.

et al., 2003; Torchin et al., 2003). On the other hand, if the invader acquires parasites from native species, the exotic species may now be at a competitive disadvantage, wherein as a novel host they may not have the evolved defenses a native host may have (Van Buskirk and Ostfeld, 1995; Kopp and Jokela, 2007).

I proposed to explore a marine system where two abundant and spreading introduced crab species; the European green crab *Carcinus maenas* (hereafter *Carcinus*) and the Asian shore crab *Hemigrapsus sanguineus* (hereafter *Hemigrapsus*), share the same habitat and co-occur with the commercially valuable native rock crab *Cancer irroratus* (hereafter *Cancer*) in the Western North Atlantic Ocean (WNA) (Williams, 1984).

Carcinus was first introduced to WNA intertidal zones from Europe, in 1817 (Carlton and Cohen, 2003). By 1871 *Carcinus* ranged from New Jersey to Cape Cod, Massachusetts, and reached southern Maine in the 1890's (Carlton and Cohen, 2003). For almost 100 years, *Carcinus* remained in the southern end of the Gulf of Maine before finally reaching Halifax, Canada in the early 1980's (Audet et al., 2003; Roman, 2006). It was not until later through the use of molecular techniques that it was determined that *Carcinus* experienced a secondary introduction to the WNA which had facilitated its colonization of colder waters in northern Nova Scotia and Newfoundland (Roman, 2006; Blakeslee et al., 2010). *Carcinus* are common in rocky shores as well as soft- sediment areas in the mid to low intertidal of the WNA and feed primarily on bivalves and crustaceans (Grosholz and Ruiz, 1996). They can reach sizes of over 80mm in carapace width, though tend to fall within 50-60mm in size (Grosholz and Ruiz, 1996).

In the southern part of its WNA range, *Carcinus* parasite load has been determined to be about 40% of its native parasite load, including a complete escape from reproductively damaging parasitic castrators like rhizocephalan barnacles (Torchin et al., 2001; Blakeslee et al., 2009). *Carcinus* has shown consistent escapes from parasites in all of its introduced ranges (Torchin et al., 2001; Zetlmeisl et al., 2011). For example, individuals collected from South Africa have been found to have no parasitic infection at all, while in Australia

they reported low prevalence and low intensity (only one individual parasite per crab) of two different parasite species (Zetlmeisl et al., 2011). Not only does *Carcinus* show an overall decrease in prevalence of all parasite taxa across introduced ranges, it also has been shown to have a larger body size, which may indicate greater survival or increased growth in those introduced regions (Torchin et al., 2001). *Carcinus* collected in Eastern Canada were also dissected for parasites and two species were identified, an acanthocephalan and trematode (Brattey et al., 1985). However, this represented collections from two sites, Halifax and St. Andrews, Canada, in 1981 and 1982 and therefore additional parasites may infect *Carcinus* at these sites.

The patterns in the WNA and other invaded regions are strongly contrasted with the parasite load in *Carcinus* crabs collected from their native European range, where crabs were infected with up to ten different species of parasite at varying prevalence and intensity levels (Torchin et al., 2001; Blakeslee et al., 2009; Zetlmeisl et al., 2011; Blakeslee et al., 2013). Due to this release from parasites, *Carcinus* may have a competitive advantage over native species in these introduced regions, where it has been shown to outcompete native species for food (Torchin et al., 2003; MacDonald et al., 2007) or even directly predate on native species, disrupting population of commercially valuable species like the American lobster *Homarus americanus* (Haarr and Rochette, 2012).

A more recent introduction, *Hemigrapsus* were first found near the mouth of Delaware Bay in 1988, however, it is hypothesized that their initial introduction via ballast water may have occurred a few years prior in the early 1980's (Epifanio, 2013), and also appears to have had multiple introductions (Blakeslee et al., 2017). These crabs quickly spread throughout intertidal zones both to the north and south; ranging from Woods Hole, Massachusetts to the mouth of the Chesapeake Bay in 1993 before expanding further to Schoodic Peninsula to the north and Cape Hatteras to the south (Epifanio, 2013). Within these regions, they are found primarily in rocky intertidal zones, and have been documented in most areas of the upper and middle intertidal (Epifanio, 2013). Their diet is

varied but overlaps with that of *Carcinus*, and will often include juvenile *Carcinus* (Epifanio, 2013). Male *Hemigrapsus* can reach over 40mm in carapace width, while female will occasionally reach sizes around 35mm (Epifanio, 2013)

Hemigrapsus has also escaped many of its parasites through its introduction to the WNA, with its load being only one-third that of its native range counterparts (Kroft and Blakeslee, 2016). One of the identified WNA parasites is a microphallid trematode believed to be *Gynaecotyla adunca*, which uses *Tritia obsoleta*, a native the eastern mudsnail, as its upstream host (Kroft and Blakeslee, 2016). In Asia, however, there are nine parasites that *Hemigrapsus* must contend with in its native range, some of which are castrating parasites which have severe impacts on population growth and fitness (Torchin et al., 2001). As of 2015, *Hemigrapsus* in the WNA have only three parasites to contend with, none of which castrate their host (Kroft and Blakeslee, 2016). This could be another example of EHR, where *Hemigrapsus*, with fewer parasites to content with, has been able to increase in abundance to push out native crabs or even other introduced species like *Carcinus* from the intertidal (Lohrer and Whitlatch, 2002; Griffen et al., 2008, 2011). The lack of parasites in the WNA could play a role in this shift seen in the WNA intertidal from a *Carcinus* dominated system to a *Hemigrapsus* dominated one.

Cancer, a native crab in the WNA, ranges mainly from Labrador to South Carolina with some seasonal differences in distribution especially in the southern portion of its range (Bigford, 1979). These crabs can grow to be over 100mm in carapace width, though smaller individuals are more typical in the shallow subtidal (Bigford, 1979). While *Cancer* adults are found in deep waters of their ranges, juveniles are often found in the intertidal zone (Bigford, 1979). Limited work has been conducted on parasites utilizing *Cancer*, however within this range they are infected by at least four different parasites and two pathogenic bacteria (MacLean and Ruddell, 1978; Bigford, 1979; Brattey et al., 1985).

In selecting a parasite to focus on for this study, I decided to use a microphallid trematode, *Microphallus* spp., which has/ have been identified in both *Cancer* and *Carcinus* crabs

(Brattey et al., 1985). In the WNA, only one species of microphallid trematode has been identified: *M. similis*. However, not all studies in the WNA have identified this species of trematode specifically, instead identifying trematodes as *Microphallus* sp. (Brattey et al., 1985; Blakeslee et al., 2009).

Microphallus similis has a complex life-cycle in which it infects multiple species to reach maturity and sexually reproduce (Combes et al., 1994; Blakeslee et al., 2015). As seen in Figure 2, *M. similis* utilizes gastropods, or more specifically *Littorina saxatilis* and *Littorina obtusata* as its primary first intermediate hosts (Galaktionov et al., 2012). These snails are castrated as *M. similis* asexually reproduces to create cercariae in the gonad region of the snails. These cercariae are shed during the tidal cycles and seek out a second intermediate host and encyst in their tissues. While these second intermediate hosts can vary from crustaceans, fish, gastropods, bivalves, and worms; in this system, both *Carcinus* and *Cancer* crabs are potential second intermediate hosts. Once they encyst in the tissue of the host (usually the hepatopancreas), they form metacercarial cysts and remain dormant until trophically transmitted from the second intermediate host (crabs) to its definitive host, usually a fish, bird, or mammal. In this system *Larus argentatus* and *Larus marinus* tend to act as the most common definitive hosts. Once within the gut, the parasite will sexually reproduce and its eggs will be released into the environment through the feces of the bird, and these eggs will be grazed upon by either *Littorina* species starting the life cycle over again.

Trematodes not only use a variety of hosts (as described above), but they also frequently elicit physiological and behavioral effects in their hosts, the strongest often felt by their intermediate hosts (Lafferty, 1999). Behavioral effects can included decreased foraging or lack of predator avoidance behaviors (Wood et al., 2007; Hansen and Poulin, 2005), and physiological effects can include damage to the host tissue or elevated immune response (Meißner and Bick, 1999; Blakeslee et al., 2015). Prior studies have also used body condition assays to determine whether trematodes can impact storage and gonad tissues

Figure 2: Life cycle of the trematode parasite *Microphallus similis* Starting at the A panel in the figure, this trematode infects the first intermediate host, *Littorina* snails, where it replicates in the gonads before cercariae are released into the environment and seek out the second intermediate host (B). Once a second intermediate host is found cercariae encyst in the tissues of *Carcinus* or *Cancer* forming metacercariae. These crabs are then preyed upon by shore birds, *Larus spps* (C), where they mature and sexually reproduce, releasing eggs through the bird's feces (D) where *Littorina* snails will accidentally ingest them, starting the cycle over again. (Figure from Blakeslee et al., 2015)

compared to uninfected crabs (e.g. Blakeslee et al., 2015). These various physiological and behavioral changes in the intermediate host may increase the hosts overall conspicuousness which could help further the parasite's life cycle by enhancing trophic transmission via consumption by shorebirds (Choisy et al., 2003). As these three crabs species (*Cancer*, *Carcinus*, *Hemigrapsus*) serve as intermediate hosts for *Microphallus* sp., utilizing this system offered a dynamic way to test how parasites alter species interactions.

It is these effects that my study focused on in these three common intertidal and subtidal crabs. Specifically, I aimed to determine how the infection may impact their individual behaviors as well as interspecific interactions. However, before testing how infection may alter their behaviors, I first needed to determine natural prevalence of parasitic trematodes along the WNA in all three species. I also then attempted to determine if the parasite(s) infecting *Cancer*, *Carcinus*, or *Hemigrapsus* crabs were the same species, multiple species, and perhaps even species that may exist outside of the WNA. Moreover, it is important to identify the natural prevalence of the microphallids in these crabs so that any experiment conducted on effects that the parasite may have on the host is done at environmentally relevant infection levels. Conducting these surveys allowed me to compare infection prevalence and intensity in a native and two non-native crab species to help understand the community dynamics of this ecosystem.

In determining which parasites are infecting these species, I used genetic techniques to identify genetically distinct trematode lineages. In previous work, identification of a parasitic trematode was only resolved to the genus level in *Cancer*, and identification in *Carcinus* have either been to the genus level or as *Microphallus similis* (Brattey et al., 1985; Torchin et al., 2001; Blakeslee et al., 2009). Identification of parasites to the species level is not always easy as the morphological features at certain life cycles may be restricted, thereby resulting in cryptic taxa (McManus and Bowles, 1996; Hung et al., 1999). Through the use of genetic tools, cryptic taxa have been distinguished as unique species, which is important when considering effects of infection or host susceptibility (Miura et al., 2005).

As discussed in Figure 1, parasites can host switch to utilize other species and there is potential for this to happen for *Carcinus* with a native parasite, especially since *Carcinus* has been on the WNA for over 200 years. It is also possibly that *Carcinus* brought with it a parasite from Europe that may have host switched to utilize *Cancer* as a competent host. Therefore, another goal was to collect samples of these trematodes and using genetic tools, identify which species were infecting each host.

Altogether, I conducted my study with three goals in mind. 1) To determine whether and where trematode parasites are found in these three host crabs in the WNA by conducting prevalence and distribution surveys (Chapter 1), 2) To determine who these trematode(s) are in the WNA utilizing standard barcoding genetic methods (Chapter 1), and 3) To determine what these trematode(s) are doing both individually and interactively in their crab hosts, through physiological assays (Chapter 1) and behavioral trials (Chapter 2).

1.2 Methods

1.2.1 Determining Prevalence of Microphallid Parasites in New England

I conducted two field surveys in 2017 ranging from Maine to Rhode Island: in mid-May and in early August, in order to determine prevalence of microphallid trematodes in *Cancer*, *Carcinus*, and *Hemigrapsus* populations in the WNA. I surveyed six sites during the first survey (Figure 3: red and green points): Camden, Maine; Orr Island, Maine; Rye, New Hampshire; Gloucester, Massachusetts; Scituate, Massachusetts; and Weekapaug Point, Rhode Island. During the second survey I sampled four sites (Figure 3: red and blue points): Camden, Maine; Rye, New Hampshire; Scituate, Massachusetts; and Providence, Rhode Island. These sites were selected to provide even coverage of the WNA.

I deployed crab traps two hours before high tide using canned tuna as bait. Cans were punctured to allow the smell of the tuna to diffuse into the water and attract crabs to the trap. The tuna cans were then placed in a bait container to prevent crabs from consuming the tuna so it would continue to attract more crabs during the entire time it was set. Most traps were collected two hours after high tide, though some traps were left overnight and collected in the morning. From the collected traps, I preferentially selected fifteen larger individuals of each species for my prevalence study. For *Cancer*, I collected all individuals as I was unable to trap more than six individuals at any one site. For *Carcinus*, I preferentially selected individuals whose carapace width was at least 30mm, with the actual size of the crabs collected ranging from 20.4mm-80mm with an average of 51.6 ± 13.03mm. For *Hemigrapsus*, I preferentially selected individuals whose carapace width was at least 20mm, with the actual size of the crabs collected ranging from 16.3mm-37.9mm with an average of 26.3 ± 4.2mm. This was done to select older individuals who would have had more time to accrue parasitic infection and therefore allowed me to determine trematode prevalence in adult populations of each crab species at each site. I transported the crabs back to ECU on ice where I then froze the crabs in a

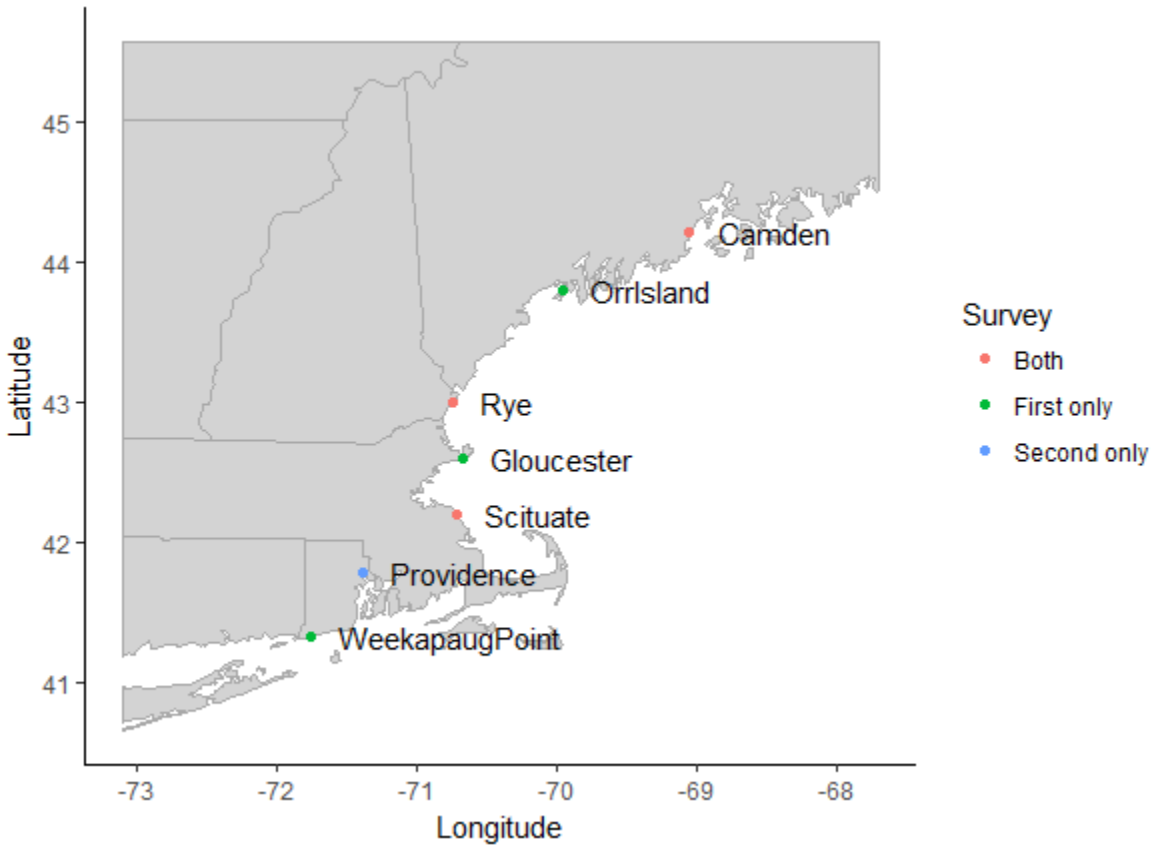

Figure 3: Site selected for New England Survey of *Cancer*, *Carcinus*, and *Hemigrapsus* crabs. Survey sites are labeled with red if they were visited on both surveys, green if visited only during the first survey, and blue if visited only during the second survey.

-20°C freezer until dissection.

I collected carapace width using calipers, measuring the widest part of the carapace of each crab. I also recorded the sex of each individual before the crabs were dissected. A razor blade was used to separate the upper and lower parts of the carapace to expose the internal tissues. In order to determine if the crabs were infected, I removed eight snips of tissue (Figure 4): six from the hepatopancreas (storage tissue), one from the gonads, and one from the thoracic ganglia (nerve center). These snips of tissue were large enough to cover the area of a 22x22mm glass cover slip. These tissue snips were placed on pre-weighed glass slides and covered with a glass cover slip.

I then took the slides and systematically scanned them for trematode cysts using a compound microscope at 4x magnification and counted total cysts per tissue snip using a handheld tally counter (Figure 5). After completing the visual inspections, I weighed each slide in order to calculate tissue snip weight. I saved all tissue snips containing trematode cysts in a 1.5mL tube and stored the tissue in the -20°C freezer for genetic analysis. Remaining hepatopancreas, gonadal tissue, and crab carcasses were dried in an incubator at 70°C for one week, and then weighed to determine the dry weight of these tissues and the remainder of the crab in order to determine the Hepatosomatic Index (HSI) and Gonadosomatic Index (GSI) for each crab species. I was interested in looking at HSI and GSI for each crab species as I wanted to see if parasitic infection would influence the mass of either tissue type across all three crab species, but especially the hepatopancreas as the metacercarial cysts tend to concentrate in that tissue. This calculation would also allow me to estimate total number of cysts per individual, which would provide a better comparison among species.

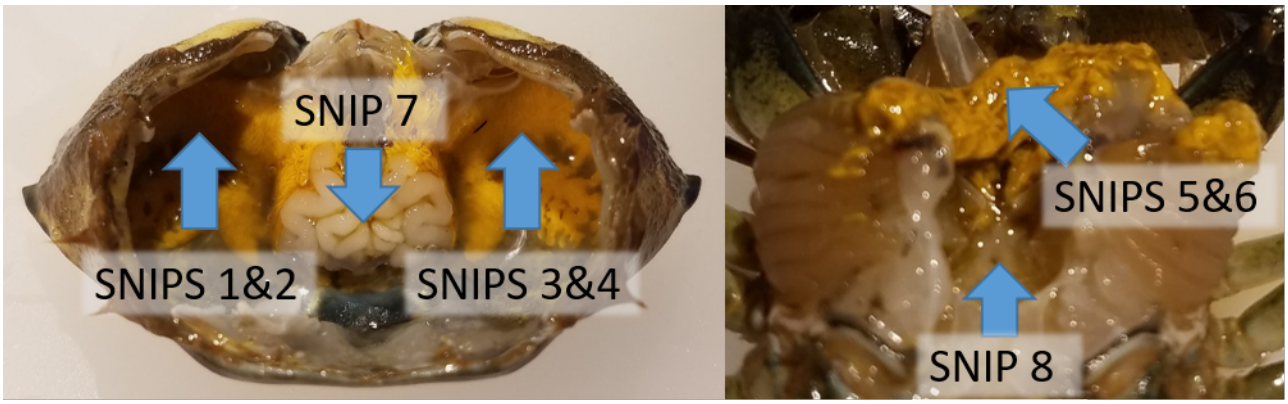

Figure 4: Dissection of each crab species consisted of removing eight different snips of tissue from different areas of the crab. Snips 1-6 came from the hepatopancreas; two from the left side of the carapace, two from the right side of the carapace, and two from above the body cavity. One snip of tissue was removed from the gonads and the final snip of tissue was of the entire thoracic ganglion.

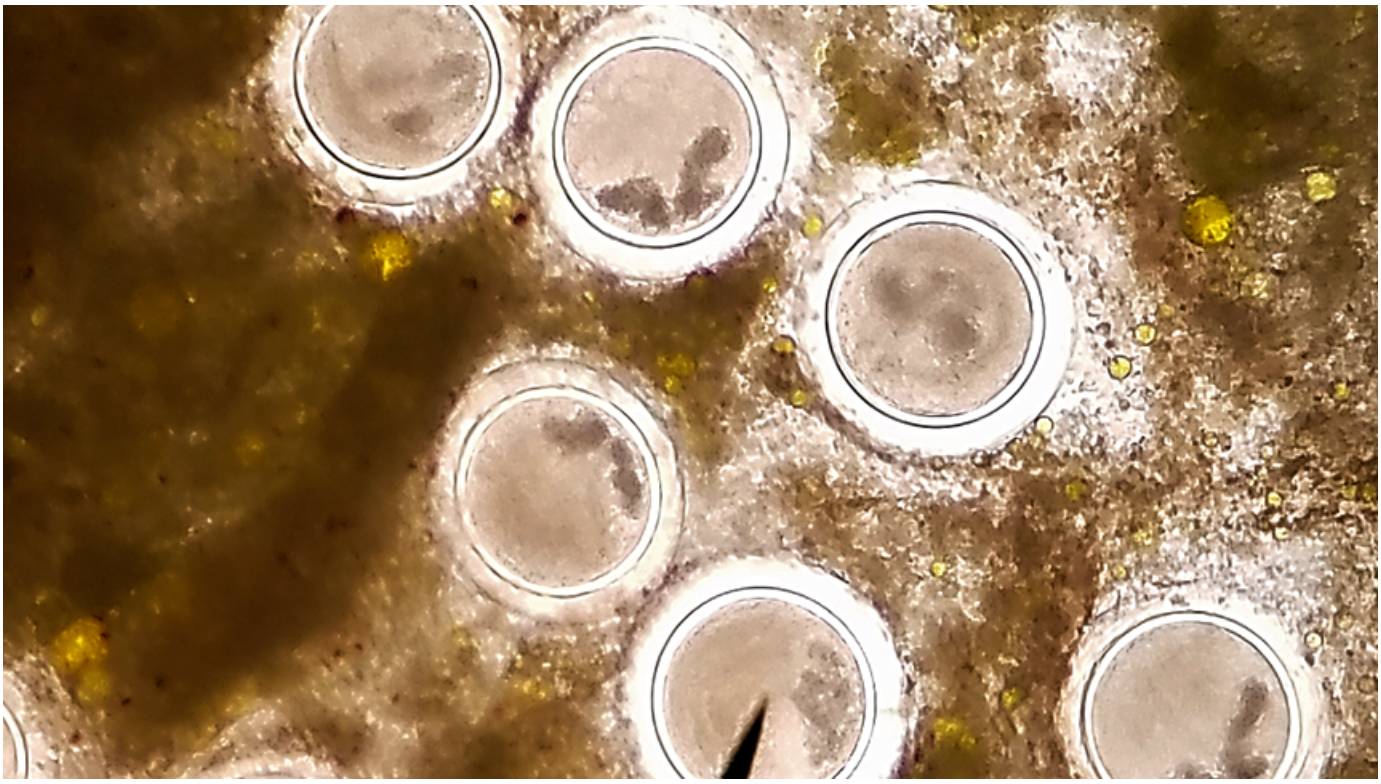

Figure 5: Metecercarial cysts of a *Microphallus* spp. trematode at 4x magnification, found in the hepatopancreas of *Carcinus maenas*.

1.2.2 Determining Trematode Identity

I performed DNA extractions using a standard cetyl trimethylammonium bromide (CTAB) protocol (France et al., 1996) on a subset of microphallid trematode metacercarial cysts dissected from infected *Carcinus* and *Cancer* crabs collected from previous field samplings in Newfoundland and the Eastern North Atlantic by the Blakeslee lab, as well as from crabs collected during the surveys conducted in New England (Figure 3). Cysts counted in one snip of tissue were pooled to increase amount of parasite DNA. I expanded the range of sites used for this study in order to better understand if there were multiple species of trematodes in the WNA and if samples from Europe could indicate if species were brought over with *Carcinus*. In Newfoundland, both *Cancer* and *Carcinus* crabs from Placentia Bay were included; however, only *Cancer* crabs from Conception Bay were included, as *Carcinus* has not yet invaded that bay. Cercariae from first intermediate hosts as well as metacercariae from *Carcinus* crabs from Europe were also included to provide DNA from microphallid parasites found in Europe where *Carcinus* in native. Extracted DNA was re-hydrated with molecular water and tested for purity and DNA concentration using a Nanodrop 8000 (ThermoFisher Scientific, Waltham, Massachusetts).

A 468-bp region of the 18S rRNA gene was amplified using primers (F: ACGGATACGGGACTCAACAG; R: TGGCATCGTTTATGGTCAGA) designed from a *Microphallus turgidus* sequence (Genbank accession #: EU825773.1). PCR was performed using a total volume of 25ul containing 7-7.5ul of water, 12.5ul of master mix, 1.5l of each primer at a concentration of 10 uM, 1-1.5ul of $MgCl_2$, 0-1uL of BSA, and 1ul of the extracted DNA product using a PCR profile of: 95 °C for 2min; 30 cycles of 95°C for 30s, 55°C for 30s, and 72°C for 60s; and 72°C for 5min (Blakeslee et al. 2010). Amplicons were purified using ExoSAP-IT (ThermoFisher Scientific, Waltham Massachusetts).

Purified samples were sequenced using the Sanger sequencing technique at Macrogen, Inc. (Rockville, Maryland, USA). I compared our sequences to Genbank sequences from other microphallids in the WNA and ENA (NCBI Resource Coordinators

2016): *M. turgidus* (accession #EU825773.1), *M. primas* (accession #AJ287541.1), and *Miratrema oocysta* (accession #AJ287534.1), and other phylogenetic studies conducted in the Blakeslee lab: *M. similis*, *Gynaecotyla adunca*, and *Miratrema arenaria* (Blakeslee et al. unpublished) using the software Genious 10.0.9 (http://www.geneious.com, (Kearse et al., 2012)). All sequences were cleaned, inspected for ambiguities, and aligned to a *M. similis* reference sequence. These sequences were analyzed for substitutions in order to determine different parasite haplotypes in each species as well as each population.

1.2.3 Statistical Methods: Prevalence and Intensity of Infection

Prevalence of infection was calculated for each species of crab at each site visited during the two New England surveys (Figure 3), by dividing the number of crabs at a given site infected with a trematode parasite by the total number of crabs collected at that site.

HSI and GSI were calculated by finding the proportion of hepatopancreas of gonad to the entire body weight of the crab. I corrected these indicies by accounting for any lost limbs and adding in the weight from those limbs to the dry crab carcass weight. This was done by obtaining dry weights of a small subset (n = 6) of *Cancer* limbs (walking legs and chelipeds) and regressing limb weight with the size of the crab carapace (walking legs: $R^2 = 0.1624$; cheliped:$R^2 = 0.8925$). For *Hemigrapsus*, I used the regression for cheliped and walking legs in Kroft & Blakeslee (2016). For *Carcinus*, all my crabs were intact and so no lost limb calculation were required. The corrected HSI was therefore the dried hepatopancreas divided by the dried crab carcass (+ any missing limb weight) and the gonad weight. Corrected GSI was calculated in a similar fashion, where the dried gonad was divided by the combined dried carcass (+ missing limb weight) and hepatopancreas weights. I then ran ANOVAs on the HSI and GSI of each crab species to compare between infected and uninfected individuals using the program R R Core Team (2013).

I also estimated total cysts in each individual crab to more accurately compare cyst intensities among crabs, since counts represented a subset of cysts in the crab's tissue. Dry weight was calculated by adding dry crab carcass weight to the dried hepatopancreas and gonad weights. This was only done with *Cancer* and *Carcinus* as *Hemigrapsus* are small enough that all of the gonad, hepatopancreas and thoracic ganglia is scanned under the microscope, so for this crab species cyst counts are exact and estimates are not required. The average hepatopancreas snip wet weight was used to estimate the number of cysts per 1 gram of wet weight of hepatopancreas. To convert the dried weight to wet weight a regression was created based on previous sampling of *Carcinus* ($R^2 = 0.8194$) (Blakeslee et al., 2015). This regression allowed for a wet to dry ratio to be determined so that dry

weight of hepatopancreas could be converted to wet weight. This wet weight was then multiplied by average cysts per gram of tissue to calculate the total hepatopancreas cyst estimates within the entire crab. These conversion and estimation methods were modeled after Blakeslee et al. (2015). I then looked at the relationship between cyst intensity and HSI or GSI in infected crabs using the package *stats* to determine (R^2) and P values R Core Team (2013). I conducted these tests on *Cancer* and *Carcinus* only due to the small sample size for *Hemigrapsus*.

1.2.4 Statistical Methods: Trematode Identity

To explore the phylogenetic relationships among trematodes found in all three species found in the survey, as well as those collected in Newfoundland, Canada, and Europe, Genious 10.0.9 (http://www.geneious.com, (Kearse et al., 2012)) was used to create Bayesian phylogenetic reconstructions (burn-in length: 100,000; total chain length: 1,100,000) using MrBayes3.2.6 (Ronquist and Huelsenbeck, 2003). PopArt 1.7 (http://popart.otago.ac.nz/index.shtml) was used to create a graphical haplotype network (using a TCS haplotype network) and haplotypes were labeled by hosts species and collection location. A Shannon-Weiner diversity test was used to analyze haplotype richness and relative abundance to compare haplotype diversity among crabs and regions.

1.3 Results/Discussion

1.3.1 Determining Prevalence of Microphallid Parasites in New England

During the survey in May 2017, I visited six different locations (Table 1): Camden, Maine, Orr Island, Maine, Rye, New Hampshire, Gloucester, Massachusetts, Scituate, Massachusetts, and Weekapaug Point, Rhode Island. Of these sites, I was only able to trap *Cancer* crabs at two sites: Orr Island and Scituate. I was assisted in our collection at Orr Island by collaborators at Bowdoin College who placed overnight traps for us. Only five *Cancer* crabs were collected from Orr Island, and four had trematode metacercariae in their tissues (prevalence = 80%). In Scituate, I was able to collect six *Cancer*, and of those, five were infected with trematode metacercarial cysts (prevalence = 83%). Through discussions with local fisherman in Maine, it was suggested that coming later in the season may increase those numbers as *Cancer* tend to be further offshore in late spring, which may help to explain why I only found *Cancer* crabs at two sites and in such low numbers in May.

I found more *Carcinus* during the first survey than I did *Cancer* crabs. At Orr Island, Maine I collected fifteen individuals, and all were infected with trematode metacercarial cysts (prevalence = 100%). However, at Sherman Cove in Camden Maine, approximately 54 miles straight line distance northeast of Orr Island, none of the fifteen *Carcinus* collected were infected (prevalence = 0%). Sherman Cove is less of a protected area than Orr Island, and it is possible that more wave action could prevent cercariae from finding hosts. A study in 2002 showed that the cercariae of *M. similis* were still able to swim towards dark conditions in turbulent water, where their second intermediate hosts would normally be found, but *Miratrema arenaria* cercariae were unable to do so, indicating that the success of cercariae in turbulent waters could be dependent on parasite species (McCarthy et al., 2002).

In Rye, New Hampshire, I hand collected nine individual *Carcinus* at Odiorne State

State	Site	Species	Caught	Infected	Prevalence
Maine	Orr Island	*Cancer*	5	4	80%
Maine	Orr Island	*Carcinus*	15	15	100%
Maine	Orr Island	*Hemigrapsus*	15	0	0%
Maine	Sherman Cove	*Cancer*	0	N/A	N/A
Maine	Sherman Cove	*Carcinus*	15	0	0%
Maine	Sherman Cove	*Hemigrapsus*	0	N/A	N/A
New Hampshire	Rye	*Cancer*	0	N/A	N/A
New Hampshire	Rye	*Carcinus*	15	12	80%
New Hampshire	Rye	*Hemigrapsus*	15	0	0%
Massachusetts	Gloucester	*Cancer*	0	N/A	N/A
Massachusetts	Gloucester	*Carcinus*	14	9	64%
Massachusetts	Gloucester	*Hemigrapsus*	15	0	0%
Massachusetts	Scituate	*Cancer*	6	5	83%
Massachusetts	Scituate	*Carcinus*	15	13	86%
Massachusetts	Scituate	*Hemigrapsus*	15	4	26%
Rhode Island	Weekapaug	*Cancer*	0	N/A	N/A
Rhode Island	Weekapaug	*Carcinus*	0	N/A	N/A
Rhode Island	Weekapaug	*Hemigrapsus*	15	0	0%

Table 1: Table of collections made during the first New England Survey in May. State indicates which New England state the site was located in. Site indicates the specific sampling area. Species indicates which of the three species was collected. Caught denotes the number of individuals collected and Infected indicates how many individuals I detected metacercarial cysts in. Prevalence is reported as percent infected

Park and used traps at Rye Harbor to collect an additional six *Carcinus*. Of the fifteen collected in total, twelve of these crabs were parasitized with metacercarial cysts (prevalence = 80%). All nine of the individuals hand collected were infected, and only three of the crabs caught in traps were parasitized.

I visited two sites in Massachusetts: Gloucester and Scituate. In Gloucester, I collected fourteen *Carcinus*, and of those collected, nine were infected (prevalence = 64%). These crabs were collected in much deeper water than the other traps due to the sampling location. No hand collections were possible in this location due to the terrain. In Scituate, I collected fifteen crabs from both traps and hand collections and thirteen were parasitized (prevalence = 86%).

Hemigrapsus crabs were collected at the following sites: Orr Island, Odiorne State Park, Gloucester, Scituate, and Weekapaug Point. All of these crabs (N = 15 per site) were hand collected. All individuals were unparasitized except for four individuals collected from Scituate (prevalence = 26%). Unlike other infections that predominately occur in the hepatopancreas, the cysts in these four crabs were only found in the thoracic ganglia. In addition, the cysts were not well encysted in the tissue and seemed to be resting on top of the thoracic ganglia rather than encysted within it.

During the second survey in August 2017, I pared down my sites and visited four locations (Table 2). I revisited three sites from the first survey: Camden, Maine; Rye Harbor, New Hampshire; Scituate, Massachusetts; and added a new site in Providence, Rhode Island. During this survey I found fewer crabs overall when compared to my first survey. I found *Cancer* crabs only at Rye Harbor and Scituate. I collected three un-parasitized crabs at Rye and seven un-parasitized crabs at Scituate. I saw a similar trend with *Carcinus*, collecting crabs from Camden, Rye, and Providence and finding no infected individuals. I was unable to collect *Carcinus* from Scituate through traps or hand collecting.

During both surveys, I was unable to trap more than seven *Cancer* crabs at any one site. I did limit my traps to the intertidal/shallow subtidal region at each site. This was done

State	Site	Species	Caught	Infected	Prevalence
Maine	Sherman Cove	*Cancer*	0	N/A	N/A
Maine	Sherman Cove	*Carcinus*	6	0	0%
Maine	Sherman Cove	*Hemigrapsus*	0	N/A	N/A
New Hampshire	Rye	*Cancer*	3	0	0%
New Hampshire	Rye	*Carcinus*	15	0	0%
New Hampshire	Rye	*Hemigrapsus*	13	0	0%
Massachusetts	Scituate	*Cancer*	7	0	0%
Massachusetts	Scituate	*Carcinus*	0	N/A	N/A
Massachusetts	Scituate	*Hemigrapsus*	14	0	0%
Rhode Island	Providence	*Cancer*	0	N/A	N/A
Rhode Island	Providence	*Carcinus*	10	0	0%
Rhode Island	Providence	*Hemigrapsus*	15	0	0%

Table 2: Table of collections made during the second New England survey in August. State indicates which New England state the site was located in. Site indicates the specific sampling area. Species indicates which of the three species was collected. Caught denotes the number of individuals collected and Infected indicates how many individuals I detected metacercarial cysts in. Prevalence is reported as percent infected

in order to collect crabs that would have been more likely to be exposed to the cercariae released by *L. saxatilis* or *L. obtusata*, the upstream hosts of *Microphallus* spp.. From prior sampling performed in Newfoundland, we know trapping *Cancer* crabs in the shallow subtidal where *Carcinus* has not yet invaded can yield high abundances (Blakeslee et al. in prep). It can be hypothesized that the presence of *Carcinus* may have affected the abundances of the native *Cancer* crabs in Newfoundland and has probably also affected abundances in New England where the crab has invaded and spread through the region in the 1800-1900s.

An explanation for the low *Carcinus* abundances at my US sites may be due to the mating season (Berrill, 1982). *Carcinus* form mating pairs in which the female is guarded by a male in their subtidal habitats, which would be far deeper than our traps would have been set (Berrill, 1982). In Maine, mating in *Carcinus* peaks in the end of August through mid-October, though occasionally mating can start in late July (Berrill, 1982). However, even if matings started in early August in 2017, we may still expect some adult individuals higher in the intertidal before the peak mating season. If we consider climate change and how that maybe shifting the mating season of *Carcinus* crabs, we might predict that the mating season would have started earlier in the summer, especially since data from Woods Hole, Massachusetts suggest that ocean water temperatures have been rising since 1970 (Nixon et al., 2004).

With these warming temperatures, it is possible that the mating season of *Carcinus* has shifted earlier in the summer months so that there are not many adults left in the intertidal or high subtidal in early August. Climate change has been implicated in temperature related behaviors in other animal systems (Van Buskirk et al., 2009; Jones et al., 2010; Jensen et al., 2018); thus it is not a stretch to suppose the same could be occurring in this system. For example, Van Buskirk el. al. saw a significant shift in the migration of song birds, with the spring migrations occurring earlier in several species (2009). Additionally the southern range contraction of the blue mussel *Mytlius edulis*, to north of Cape

Hatteras, NC due to mass mortality along with a northern expansion were both tied to high summer water temperatures (Jones et al., 2010). Rising temperatures have also been linked to the feminization of green turtles in the Great Barrier Reef (Jensen et al., 2018), in which two populations were studied and at the cooler site a slight female bias (65-69%) was observed but at the warmer site an extreme female bias was observed (99.1% in juveniles).

In my study, I found essentially no infection of *Hemigrapsus* by *Micropahllus* spp. during the first or second survey, as expected, with the exception of the crabs collected from Scituate during the first survey. I also found an overall lack of parasitized *Carcinus* and *Cancer* crabs during the second survey. This seemed counterintuitive as several known helminths and other macroparasites have been shown to have peak transmission or intensity in the summer months (Altizer et al., 2006). Based on this prior evidence, I expected to find more infected individuals at the study sites than were found in May; however, this was not the case. A possible hypothesis could be that the upstream gastropod hosts were not in high abundances, perhaps due to mortality. Fredensborg et. al. showed that infected intertidal gastropods demonstrated reduced survival at both 18°C and 25 °C when compared to their uninfected counterparts (2005). This, combined with rising temperatures in the intertidal, could induce mortality of these upstream hosts, thereby reducing transmission of the trematode to its second intermediate host.

Not only did I look at infection status of crabs collected along the coast of New England, but I also took a subset of each crab species (*Cancer*, n = 11 infected and n= 9 uninfected; *Carcinus*, n = 24 infected and n = 16 uninfected; *Hemigrapsus*, n = 4 infected and n = 76 uninfected) and calculated body indices (HSI and GSI) for each species. HSI and GSI were calculated to see if there was a physiological effect of infection on these crab hosts through alterations of the mass in these tissues when compared to uninfected crabs. After accounting for missing limbs and estimating infection within the entire crab (see methods), there is a nearly significant difference between the average HSI of infected and

uninfected *Cancer* [F(1) = 3.999, P = 0.06] and *Carcinus* [F(1) = 2.268, P=0.14] with infected individuals trending towards a higher HSI (Figure 6). This trend was not seen in *Hemigrapsus* [F(1) = 0.0657, P = 0.63). It is important to note that since I only recovered four infected *Hemigrapsus* these comparisons were completed with a very small sample size and may not be reflective of what is actually occurring in nature.

These results are interesting in terms of time spent on the WNA by each crab species and the HSI of infected vs uninfected crabs. As a native to the WNA, *Cancer* crabs have evolved with the parasites present here and therefore a native parasite could have a more targeted effect on its native host. For example, an acanthocephalan species, *Pomphorhynchus laevis*, can alter the immune defenses and behavioral traits of a native amphipod host, *Gammarus pluxes*, with far more success when compared to the introduced amphipod host, *Gammarus roeseli* (Lagrue, 2017). This co-evolutionary history could explain the trend we see with infected *Cancer* having on average a higher HSI than their uninfected counterparts. *Carcinus* crabs invaded this region in the late nineteenth- early twentieth century (Carlton and Cohen, 2003), and while native parasites may have host switched (Figure 1), they probably are not as well evolved to elicit such a specific a response from these crabs. *Hemigrapsus* has only been in New England since the late 1980's and therefore any native parasite may not be equipped to elicit any specific response from this introduced host.

I also examined GSI in all three crabs. For this body index there was no apparent difference between infected and uninfected individuals (Figure 7). *Cancer* and *Carcinus* had non-significant differences [F(1)=0.202, P=0.658 and F(1)=0.516, P=0.477] between infected and uninfected individuals. *Hemigrapsus* showed a trend indicating lower GSI in infected crabs when compared to uninfected crabs [F(1) = 3.629, P = 0.06] however as stated above this sample only includes four infected individuals so this may not be reflective of what is actually occurring in nature.

Finally, I examined how the estimated metacercarial cyst intensity of the infected in-

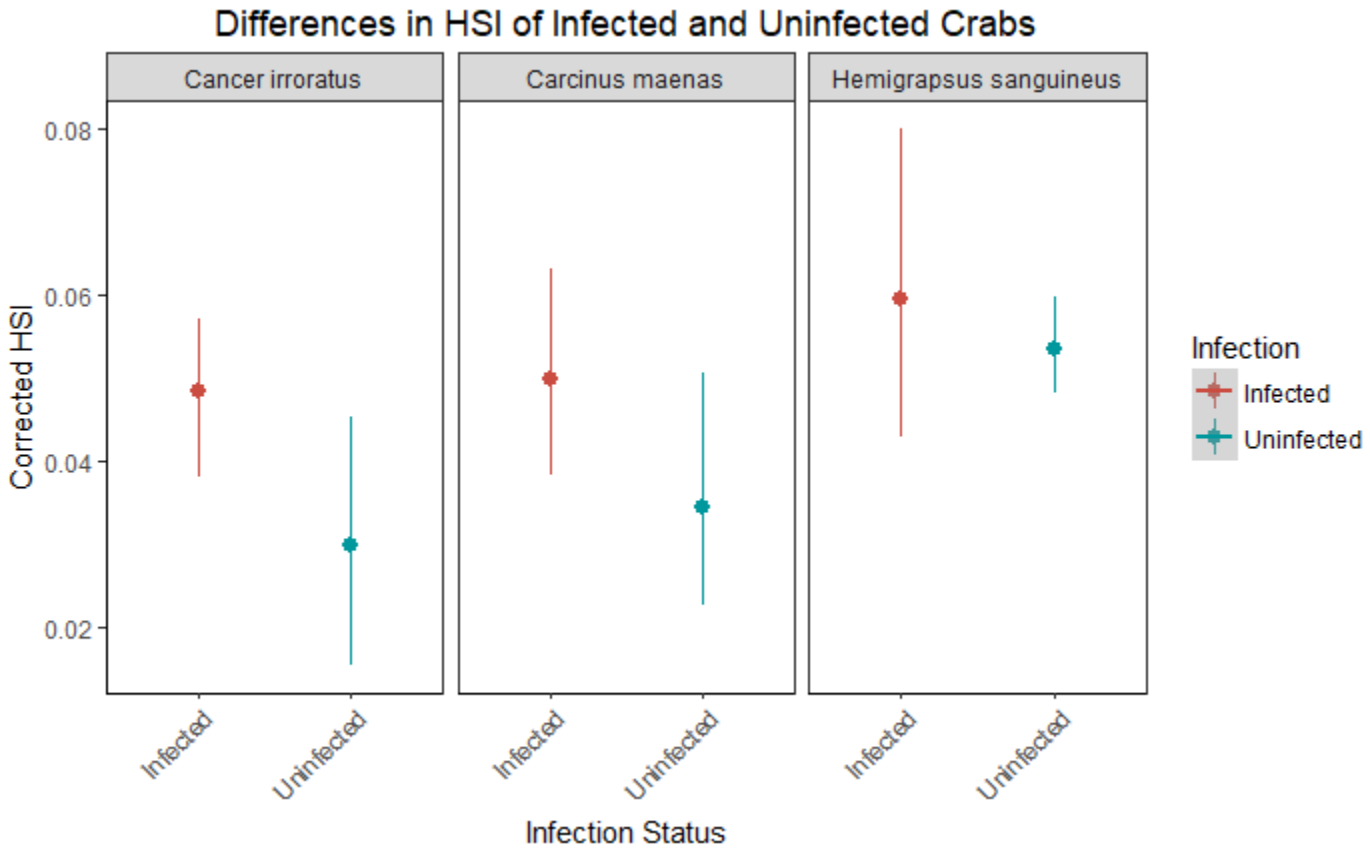

Figure 6: Plots of average corrected HSI of the three crabs collected during New England Surveys. Corrected HSI indicates the proportion of dried hepatopancreas to total dried body weight of the crab corrected for any missing walking legs or chelipeds. Lines represent 95% confidence intervals around mean corrected HSI values for each crab species. Infected crabs are shown in red while uninfected crabs are shown in blue. *Cancer* and *Carcinus* show a trend for HSI of infected crabs to be greater than uninfected crabs (P = 0.06 and P = 0.14 respectively). No difference between infected and uninfected *Hemigrapsus* (P = 0.63).

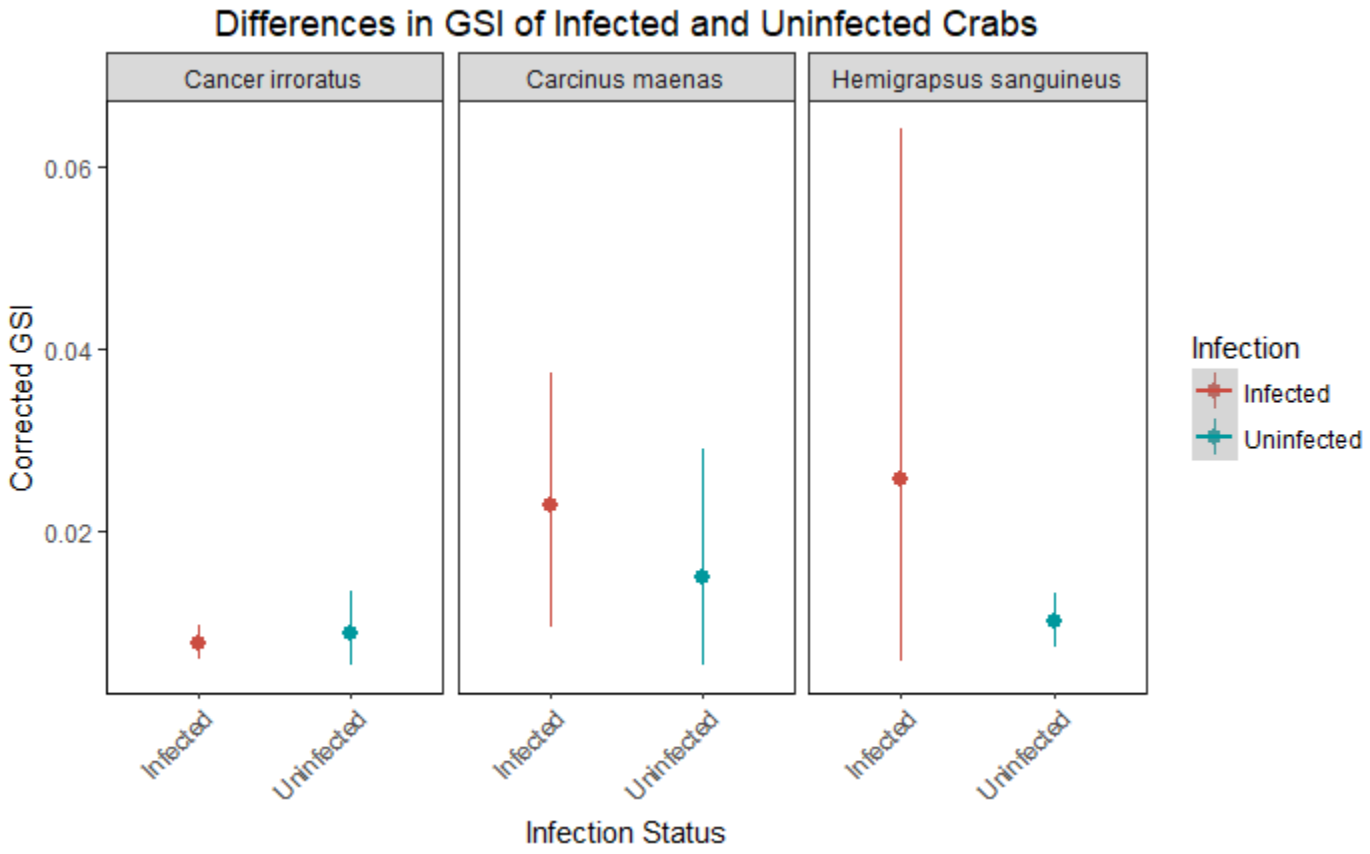

Figure 7: Plot of average corrected GSI of the three crabs collected during New England Surveys. Corrected GSI indicates the proportion of dried gonad to total dried body weight of the crab corrected for any missing walking legs or chelipeds. Lines represent 95% confidence intervals around mean corrected GSI values for each crab species. Infected crabs are shown in red while uninfected crabs are shown in blue. No significant differences seen between the GSI of infected or uninfected *Cancer* and *Carcinus* crabs (P = 0.658 and P = 0.516 respectively) while there is a trend for GSI of infected *Hemigrapsus* to be higher than uninfected crabs. This however is based off of a small sample size and more samples would be needed to support trend.

dividuals may affect HSI (Figure 8) or GSI (Figure 9) across individuals. I found no significant correlations between intensity of infection and HSI in two of the crab species (*Cancer*(n=11): adjusted $R^2 = -0.04891$, p=0.484; *Carcinus*(n=24): adjusted $R^2 = -0.045$, p=0.923). When looking at GSI, there were also no relationships between GSI and estimated cyst intensity (*Cancer*: adjusted $R^2 = -0.1013$, p=0.7832; *Carcinus*: adjusted $R^2 = -0.0333$, p=0.616). These analyses were not run on the HSI or GSI of *Hemigrapsus* as the sample size of infected individuals was too small (n=4).

These findings partially support those found by Blakeslee el. al. during their infection experiment with *Carcinus* (2015). Neither study demonstrated a relationship between cyst intensity and HSI or GSI. However, in my results, I demonstrated a trend between infection status and HSI in both *Cancer* and *Carcinus* crabs. A major difference between these two studies is that I ran these analyses on field collected individuals and the 2015 study analyzed *Carcinus* after being held at standard laboratory conditions for four weeks. Since the crabs collected during my study were immediately frozen differences between mean HSI values may be more indicative of what is seen in nature.

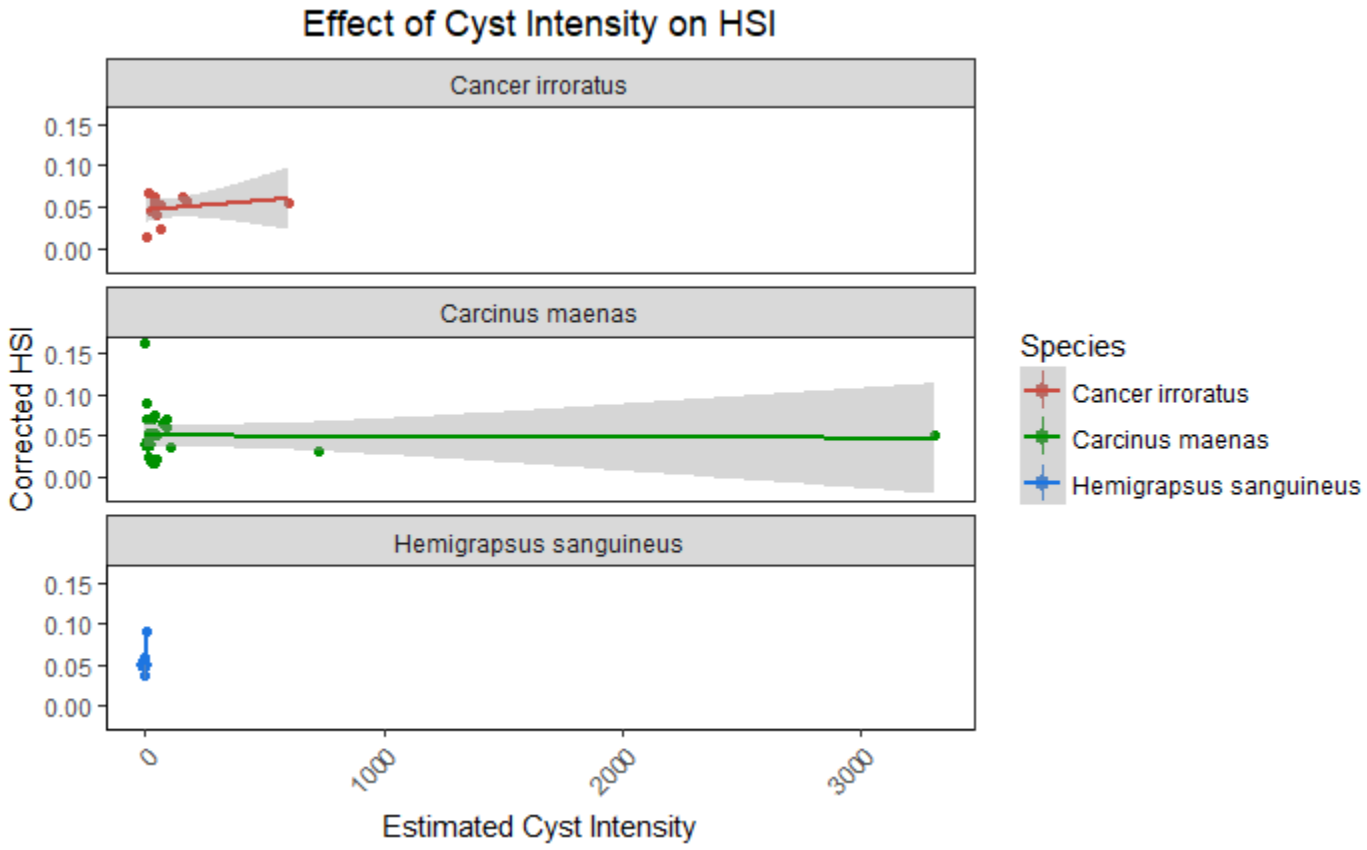

Figure 8: Scatter plots of infected individuals of each crab species plotted against their corrected HSI values. Corrected HSI indicates the proportion of dried hepatopancreas to total dried body weight of the crab corrected for any missing walking legs or chelipeds. Shading shows 95% confidence intervals. Top panel with the red line indicates infected *Cancer* individuals (n=11), middle panel with the green line indicates infected *Carcinus* individuals (n=24), and the bottom panel with the blue line indicated infected *Hemigrapsus* individuals (n=4).

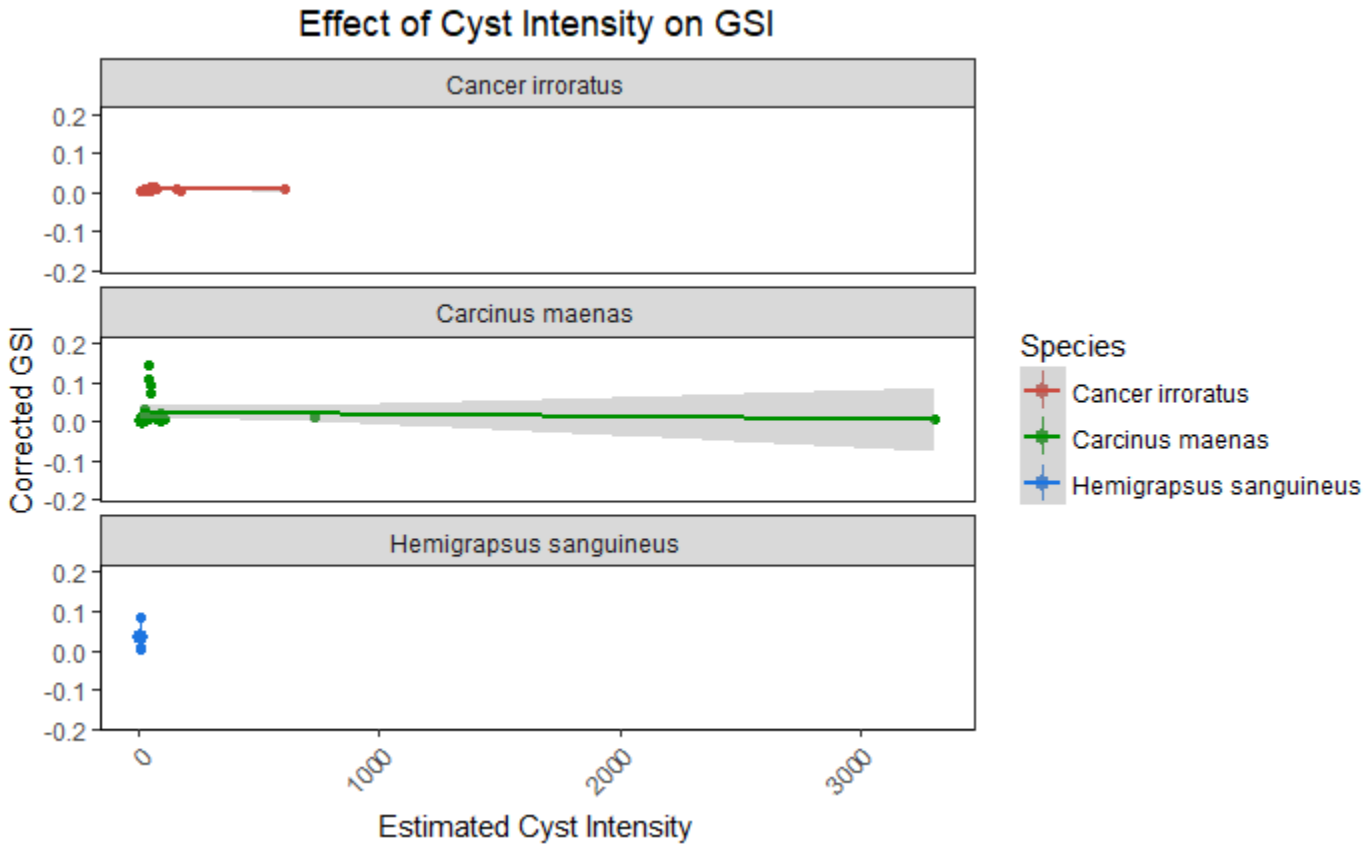

Figure 9: Scatter plots of infected individuals of each crab species plotted against their corrected GSI values. Corrected GSI indicates the proportion of dried gonad to total dried body weight of the crab corrected for any missing walking legs or chelipeds. Shading shows 95% confidence intervals.Top panel with the red line indicates infected *Cancer* individuals (n=11), middle panel with the green line indicates infected *Carcinus* individuals (n=24), and the bottom panel with the blue line indicated infected *Hemigrapsus* individuals (n=4).

1.3.2 Determining Trematode Identity

To determine trematode identities of metacercarial cysts in WNA, I utilized standard genetic techniques as it is very difficult to use morphology to identify trematodes at the metacercarial (cyst) stage. My analysis included 216 trematode cyst sequences from 126 individual crabs collected in the aforementioned New England surveys, Newfoundland (from Blakeslee et al., In Prep), Nova Scotia/ New Brunswick, and Europe (from Blakeslee et al., In Prep). I compared these samples to three Genbank sequences for *M. turgidus* (accession #EU825773.1), *M. primas* (accession #AJ287541.1), and *Maritrema oocysta* (accession #AJ287534.1), and sequences acquired from the Blakeslee lab: *M. similis*, *Gynaecotyla adunca*, and *Maritrema arenaria* (Blakeslee et al., unpublished). These treamtodes were identified using both sequence data from cercariae collected from the upstrem hosts (*Littorina* spp. and *T. obsoleta*) as morphological identifications for cercariae using published keys (James, 1968; Stunkard, 1983). For *M. similis*, sequences from cercariae from the upstream snail hosts were also combined with metacercarial cysts in crab hosts and were found to be identical (or within the same *M. similis* lineage; see below)

Using a TCS haplotype network analysis, 20 different haplotypes were identified across these samples (Table 3). Constructing a Bayesian tree allowed me to identify different lineages and to group haplotypes by lineages (Figure 10). To determine a lineage, I grouped together haplotypes that were at least 98% similar. This number was chosen as two known *Miratrema* species, *M. oocysta* and *M. arenaria*, were also mapped on our tree and they were 97% similar. When considering these results, it is important to note that I used a highly conserved marker. These analyses may therefore underestimate the differences between haplotypes, making the trematode samples appear more similar than they may actually be. Using a less conserved marker in future analyses may offer a better resolution of these parasite lineages.

Most haplotypes fouped in Lineage A (Figure 10: blue box) with 99% similarity between haplotypes and consisted of the most sequences (N = 134). Within this Lineage

Haplotype	CICB	CIPB	CMPB	CMNSNB	CMUSA	CIUSA	HSUSA	EU
1	21	12	30	4	32	5	0	14
2	1	16	0	0	2	0	0	0
3	0	0	0	0	0	0	0	14
4	0	0	0	1	13	0	0	0
5	0	0	6	0	0	0	0	0
6	0	4	1	0	0	0	0	0
7	0	0	0	4	1	0	0	0
8	0	0	4	0	0	0	0	0
9	0	0	0	2	2	0	0	0
10	0	0	0	4	0	0	0	0
11	3	0	0	0	0	0	0	0
12	0	0	3	0	0	0	0	0
13	0	0	0	0	0	0	0	2
14	0	0	2	0	0	0	0	0
15	0	0	0	0	0	2	0	0
16	0	0	0	0	2	0	0	0
17	0	0	0	0	0	0	2	0
18	0	0	0	0	0	0	0	1
19	0	0	1	0	0	0	0	0
20	0	0	0	0	0	1	0	0
Total	25	32	47	15	52	8	2	31

Table 3: All sequences generated from each region of collection. Regions are coded as follows: CB=Conception Bay Newfoundland; PB= Placentia Bay Newfoundland; NSNB= Nova Scotia/ New Brunswick, USA= United States of America and EU= Europe. Species used are coded as follows: CI= *Cancer irroratus*, CM= *Carcinus maenas*, and HS=*Hemigrapsus sanguineous*. Column headers are coded so that the first two letters represent the species so that CICB= *Cancer irroratus* in Conception Bay, with the exception of EUR as there was no separation of species done for these samples.

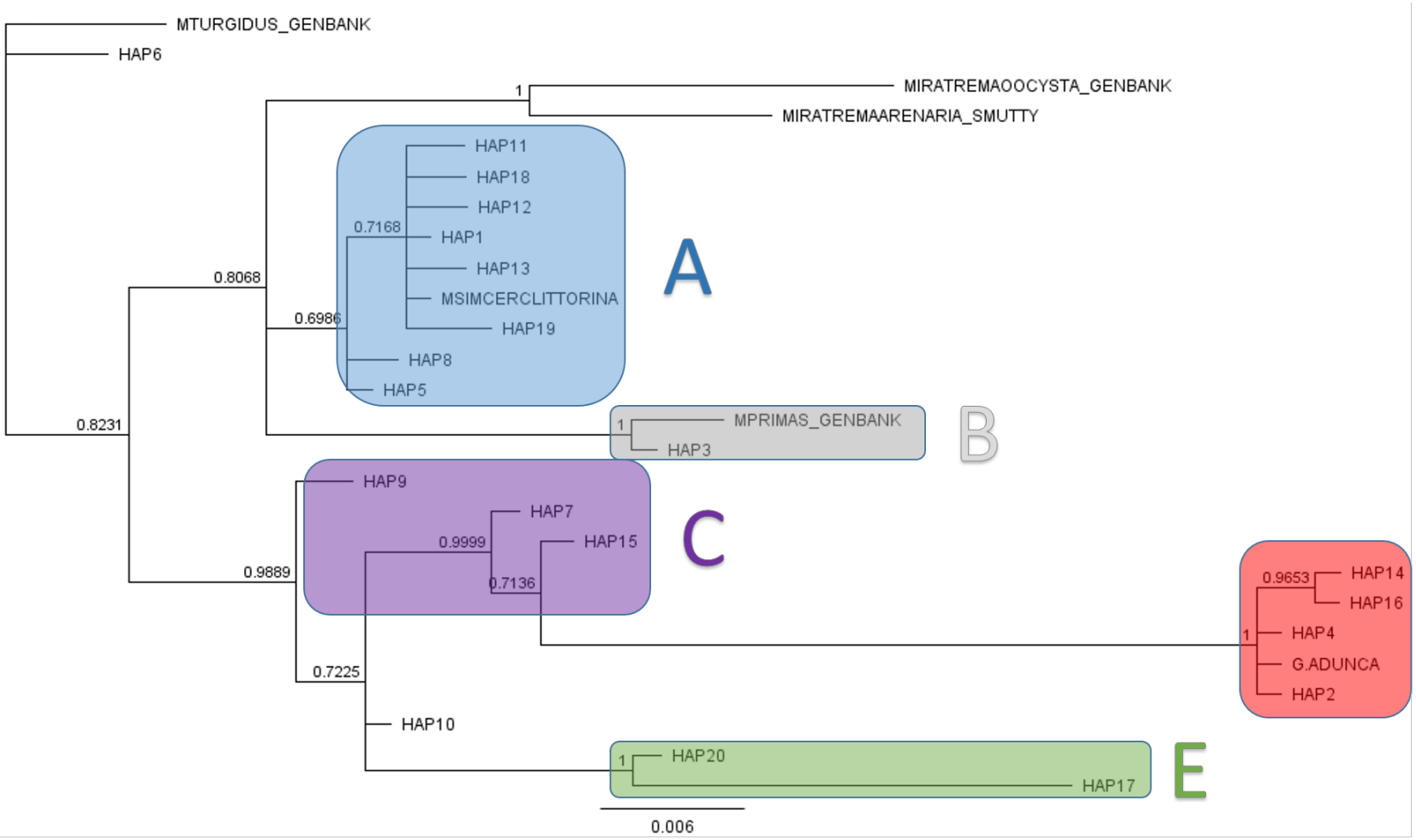

Figure 10: Phylogenetic tree created using Bayesian reconstructions with posterior probabilities shown. Lineages (haplotypes at least 98% similar) are marked with a letter (A-E) as well as color coded with a box around haplotypes in that lineage. The Genbank sequence *M. turgidus* was used as an outgroup.

was HAP1, which included the reference sequence for the microphallid trematode *M. similis*. This microphallid was also observed in upstream *Littorina* spp. snail hosts, where morphological characteristics of cercariae have been used for identification James (1968); Stunkard (1983); Galaktionov et al. (2012); Blakeslee et al. (2015). HAP1 was represented from every sampled region as well as from both *Cancer* and *Carcinus* in those regions. This haplotype was the most common in Lineage A, with 118 sequences and a frequency of 52.3%. As this haplotype was recovered on both sides of the Atlantic Ocean, it could indicate that *M. similis* is a cosmopolitan species, a possibility I will discuss further below.

The remainder of the lineages indicated in the tree contain haplotypes found either on the WNA or ENA but not both. Lineage B (Figure 10: gray box) included HAP3 and a Genbank sequence of a different microphallid species, *Microphallus primas* (100% similar). HAP3 was only found in specimens collected from Europe, which may indicate that *M. primas*, a closely related microphallid, is not in or at the very least exceedingly rare in the WNA. In Europe, *M. primas* primarily utilizes the gastropod *Hydrobia ulvae* as its upstream host before encysting in the hepatopancreas or gonads of its second intermediate host, which is often *Carcinus* (Costa et al., 2017). As with other microphallids, *M. primas* is trophically transmitted to the definitive host which is often the marine shorebird, *Larus cachinnans* (Costa et al., 2017)

Lineage C (Figure 10: purple box) contained three haplotypes that were 99% similar. This lineage was found mainly in *Carcinus* (81% of the sequences) and was found in both Nova Scotia/ New Brunswick, Canada and the United States with even frequency. While we did not recover many sequences from *Cancer* crabs, this could be an artifact of our low *Cancer* sequence numbers from the United States (n=8) or the lack of *Cancer* crabs collected in Nova Scotia/ New Brunswick. However, it could also indicate a trematode lineage that was brought over when *Carcinus* was introduced and has since host switched to infect *Cancer*. Additional sampling and more genetic markers could help to answer this question.

Lineage D (Figure 10: red box) was found in both *Cancer* and *Carcinus* crabs and was found in crabs collected from both bays sampled in Newfoundland, Canada; Nova Scotia/ New Brunswick, Canada, and the United States of America. However, it was not found in *Cancer* crabs from the United States, but this could be an artifact of our low sample size (n=8) rather than an indication that this haplotype is not present in *Cancer* found in the United States. This lineage also mapped with a sequence, *Gynaecotyla adunca* (Blakeslee et al, unpublished), in which all of these sequences were 100% similar. *G. adunca* is another microphallid that utilizes the gastropod *Tritia obsoleta* as its first intermediate host, generally followed by fiddler crabs or amphipods as the second intermediate host before completing its life cycle in shore birds or fish (Hunter and Vernberg, 1953; Blakeslee et al., 2012). Additional work could be done to determine if these are incidents of accidental infection or if *G. adunca* is using these two crabs as competent intermediate hosts.

Finally Lineage E (Figure 10: green box) consists of two haplotypes, 17 and 20 which were 98% similar. Even though these two haplotypes are within my similarity cut-off, they are clearly more divergent from one another than the other haplotypes within my lineage groupings for Lineages A-D. In addition, these haplotypes were also very rare. Interestingly, one of the two haplotypes, HAP17, was only found in *Hemigrapsus* collected from the United States and HAP20 was only found in *Cancer* collected from the United States. While these two haplotypes were grouped within the same lineage, we do not know what microphallid parasite could be infecting both *Hemigrapsus* and *Cancer* in the WNA. What is interesting is that the cysts in these haplotypes were all recovered from the thoracic ganglia, not the hepatopancreas. Due to the small number of individual sequences for these haplotypes (N=3), as well as the low level infection intensity (maximum of four cysts in one crab), this could potentially indicate an accidental infection by this mystery microphallid; however, more evidence is needed to determine this.

I also created a haplotype network to better observe how these lineages were related

to one another (Figure 11). Based on this network, HAP 10 appears to group with lineage C; however I referred to the Bayesian tree while assigning lineages. This could indicate that there are additional haplotypes that we did not recover, and the addition of those haplotypes may better resolve the relationships of this lineage.

The haplotype network also allows for better visualization of geographic groupings. Lineage B, which consists only of European samples, is most closely related to lineage A, the only other lineage to contain European samples. Lineage C contains mostly samples from Nova Scotia/ New Brunswick, Canada, though there are a few samples from the United States also represented in this lineage. These US samples within this lineage are from Maine and New Hampshire which, at most, are 238 miles away from the Canadian sites with suitable habitat for *Cancer* and *Carcinus* crabs between these points. Therefore the sharing of this lineage between these geographic region makes sense.

The haplotype map allows for a better examination of Lineage E as well. There are several changes in the nucleotide sequence between haplotypes 17 and 20. While perhaps this could be expected as they were found infecting two different species of crab, this pattern does not occur in similar situations between *Cancer* and *Carcinus* haplotypes. Moreover, *Cancer* and *Carcinus* actually share the same haplotypes in some lineage groups. However, *Cancer* and *Carcinus* are far more closely related to each other than either one is to *Hemigrapsus* (Tsang et al., 2014).

What can be seen by both the phylogenetic tree and the network is that this system is far more complicated than originally thought. Through the use of genetic tools, I was able to determine that there are four different lineages in my survey sites alone (five including Europe). Adding in additional samples from Nova Scotia/ New Brunswick, Newfoundland, and Europe only strengthened the different lineage groupings. Many of the samples collected from Europe are not the microphallid *M. similis* but instead are far more similar to the microphallid *M. primas*. Additional samples from Europe would be needed in order to better determine if the other lineages (C or D) found in the WNA are native to the

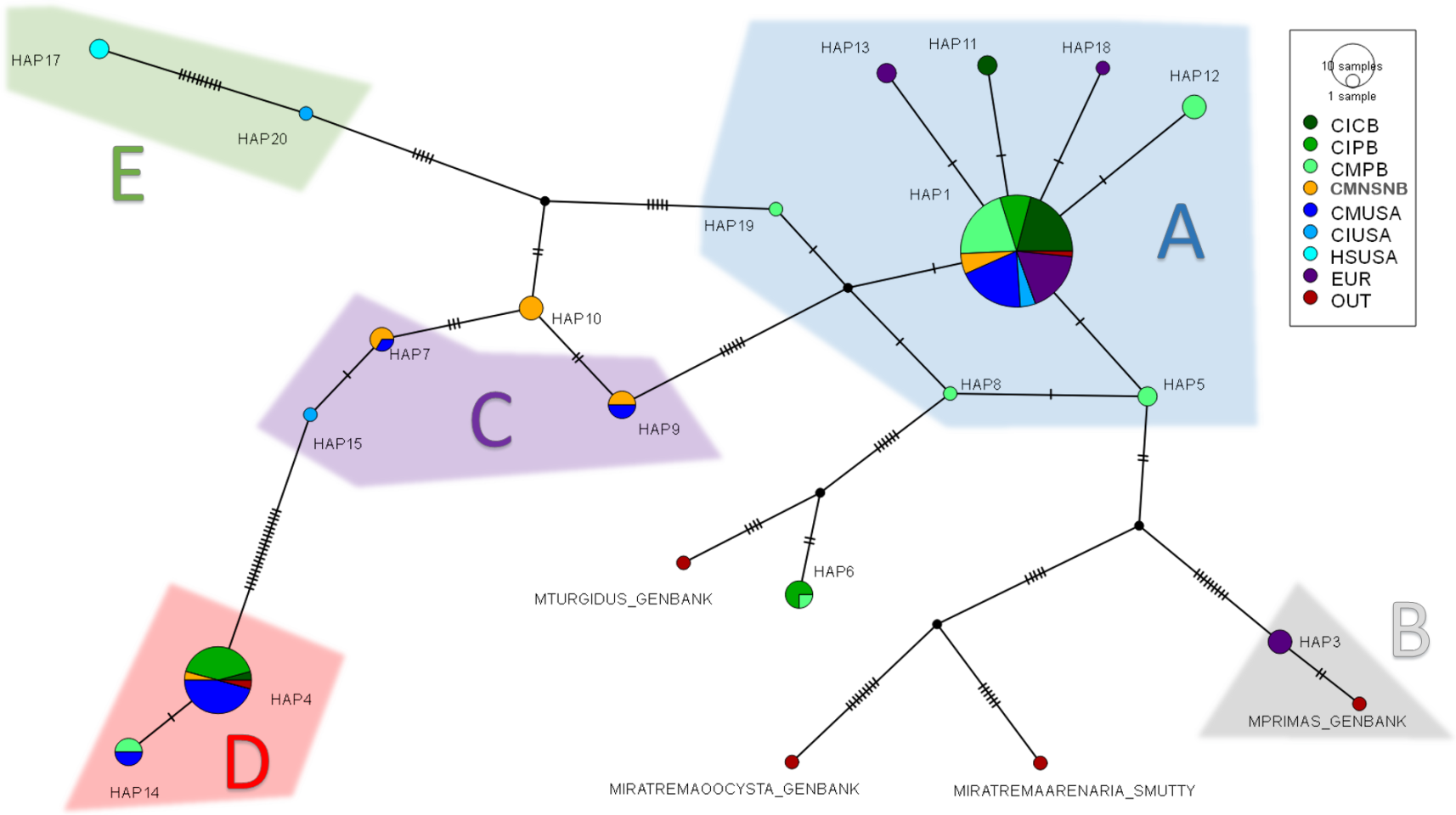

Figure 11: Haplotype network created using a TCS network. Colored boxes indicate different lineages (distance values of at least 98%). Size of circle indicate number of unique individuals that matched to that haplotype. Colors of the circles indicate species collected (First two letters) and area of collection (remaining letters) with Europe being the only exception where all samples were pooled and not divided by species. CI= *Cancer*, CM=*Carcinus*, HS= *Hemigrapsus*, CB= Conception Bay, PB= Placentia Bay, C= Canada, USA= United States of America, EUR= Europe, OUT=Outgroup. Outgroup in this network is defined by all sequences acquired from GenBank. Black circles indicate a missing haplotype

WNA, or were brought over during the introduction of *Carcinus*. As I used a highly conserved marker, using a less conserved marker in the future could not only help determine the origins of lineages C or D but also help tease apart any differences between the WNA and European haplotypes of lineage A. In addition, including data from trematodes infecting *Hemigrapsus* in their native range could help to better understand lineage E and if that parasite is native to the WNA or Asia.

Finally, using the final count of unique individuals per haplotype (Table 4), I calculated a Shannon-Weiner diversity index pooled by region (Conception Bay, Placentia Bay, Canada, United States of America, and Europe). I found Conception Bay to be the least diverse (H=0.58), followed by Europe (H=0.74), Nova Scotia/ New Brunswick (H=1.52), and Placentia Bay (H=1.56), with USA being the most diverse of the sampled areas (H=1.65). It is clear that the last three regions had very similar diversity values despite the differences in sample sizes among these regions. Interestingly, when I compared Placentia Bay, Newfoundland and USA by species I noted higher diversity in *Carcinus* when compared to *Cancer* and, in the case of the USA, *Hemigrapsus*. In both cases there was a greater sample size of *Carcinus* used in the genetic sequencing, especially when looking at the USA samples (*Carcinus*: n=29; *Cancer*: n=5; *Hemigrapsus*: n=2), and so sample size has likely influenced the results in these comparisons.

It is possible that definitive hosts of these parasites could be increasing gene flow between the regions I explored. As Lineage A (the *M. similis* lineage) was recovered on both sides of the Atlantic, it may represent a cosmopolitan species and it has been identified in upstream snail hosts in the WNA and ENA (James, 1968; Stunkard, 1983). The final host in *M. similis* are shore birds, specifically *Larus argenetatus* and *Larus marinus* serve as the definite hosts and have impressive ranges on both sides of the Atlantic (Good, 1999; Nisbet et al., 1999), however, these populations do not frequently mix. *L. argenetatus* is often divided further into sub species; the WNA sub species, *L. argenetatus smithsonianus*, and two European sub species, *L. argenetatus argentatus* and *L. argenetatus argenteus* (Nis-

Haplotype	Conception Bay	Placentia Bay	Nova Scotia/ New Brunswick	United States	Europe
1	14	20	4	16	12
2	1	10	0	2	0
3	0	0	0	0	3
4	0	0	1	9	0
5	0	2	0	0	0
6	0	4	0	0	0
7	0	0	4	1	0
8	0	1	0	0	0
9	0	0	2	2	0
10	0	0	3	0	0
11	2	0	0	0	0
12	0	3	0	0	0
13	0	0	0	0	2
14	0	2	0	0	0
15	0	0	0	1	0
16	0	0	0	2	0
17	0	0	0	2	0
18	0	0	0	0	1
19	0	1	0	0	0
20	0	0	0	1	0
Total	17	43	12	36	18

Table 4: Summary of unique individual hosts per the twenty unique haplotypes identified. Regions are pooled, combining sites from Conception, Placentia, and the United States, and species collected in that region, to better categorize each location for Shannon-Weiner Diversity Index.

bet et al., 1999). This is similar in *L. marinus* where populations in the WNA remain there for migration and breeding (Good, 1999). However, this species has been known to also breed in Europe, which could drive a cosmopolitan distribution of *M. similis* around the Atlantic Ocean, or potentially in a stepping stone fashion across the North Atlantic islands. Sampling adult trematodes from gulls throughout the North Atlantic could help to support this hypothesis.

1.4 Conclusion

I had three main research questions that drove this study: 1) to determine if trematode parasites were infecting the selected crab species, 2) to determine natural prevalence, intensities, and body indices in each infected crab species, and 3) to determine if the trematodes found in all three host crabs were all the same species. To answer these questions I conducted two surveys in New England, dissected hundreds of crabs, performed HSI and GSI assays, and collected genetic data on the parasite cysts found in the crab tissues.

Focusing on my first goal, I found microphallid metacercarial cysts infecting all three crab species, *Cancer*, *Carcinus*, and *Hemigrapsus*, during my surveys. It is important to note that I was only able to collect infected crabs during my first survey in May, as well as that there were only four infected *Hemigrapsus* collected from one site. More surveys in this area can help us to understand why parasite prevalence dropped in the second survey, as well as see if we can collect additional infected *Hemigrapsus* crabs from the WNA.

Examining prevalence and intensities showed that trematode prevalence is high overall, and prevalence tends to be higher in *Carcinus* when compared to *Cancer*. I also saw trends to indicate that HSI varied between infected and uninfected *Cancer* and *Carcinus* crabs, with infected crabs having higher HSIs while there were no difference in the HSIs of *Hemigrapsus*. GSIs also showed no difference between infected and uninfected crabs *Caner* and *Carcinus*, with a slight trend in different GSI of infected and uninfected *Hemigrapsus*, however the small sample size gives this result little statistical power. I also determined that cyst intensity had no effect on the difference in HSI values between infected and uninfected crabs. I believe that these data can help link infection by a trematode to the effects felt by a host, especially behavioral effects that are explored in my next chapter.

Finally, I was able to determine that within these three crab species, there are five distinct lineages around the Atlantic Ocean, four of which are found in the WNA. I was able to identify three of the five lineages as *M. similis*, *M. primas*, and *G. adunca*. In the WNA we have *M. similis* and *G. adunca*, however *M. primas* was only recovered in the samples ac-

quired from Europe. There are two additional lineages collected from the WNA, however we are uncertain of their identity at this time. Lineage C was collected from *Cancer* and *Carcinus* crabs while Lineage E was collected from *Cancer* and *Hemigrapsus* crabs. These data demonstrate the there is more than just *M. similis* infecting these crabs in the WNA. This is extremely important when considering the effects parasitism may have on a host as different parasites may induce different effects.

2 Chapter 2

2.1 Introduction

The previous chapter focused on where infected populations of crabs are found in the WNA, as well determining identities of microphallids found infecting *Cancer*, *Carcinus*, and *Hemigrapsus*. However, knowing where and which trematodes are infecting these crabs is only part of the story. It is also important to understand what effects these parasites are having on their various hosts and how those effects may alter interspecific competition between these hosts. In my previous chapter, I noted that there may be some physiological effects of trematode parasitism on the body condition of *Cancer* and *Carcinus* crabs, specifically related to storage tissue (Hepatosomatic index, or HSI). Thus parasitism could play a role in influencing the physiological health of infected crabs compared to uninfected crabs. Parasites have also been show to have significant impacts on the behaviors of their hosts, which may also have a role in the interactions that these hosts have in their natural environments. I will focus on these potential behavioral effects in this chapter. From my New England surveys, I found both *Carcinus* and *Hemigrapsus* in high abundance in the intertidal zones, so I focused to these two NIS. As *Carcinus* and *Hemigrapsus* are commonly found together, understanding how trematode parasites may alter host behavior can help us understand their competitive interactions.

There have been numerous studies published that have tested and analyzed interactions between these two species for space, food, or even predation of one crab by the other (Jensen et al., 2002; Lohrer and Whitlatch, 2002; DeGraaf and Tyrrell, 2004; MacDonald et al., 2007; Griffen et al., 2008; Peterson et al., 2014). Here, I will summarize the results of these studies, which will provide the necessary background information for understanding whether parasites could influence factor in their interactions.

Several studies have found *Hemigrapsus* to outcompete *Carcinus*, whether it is in the acquisition of preferred shelter or choice prey items. Lohrer and Whitlatch conduced

field surveys which not only saw a steep decline in *Carcinus* abundance coupled with a steady increase in *Hemigrapsus* abundance, but also saw a decrease in young of the year recruitment and survival of *Carcinus* when exposed to *Hemigrapsus* adults (2002). This shift in abundances was also seen along the North Sea where *Carcinus* is native and *Hemigrapsus* has recently been introduced (Jungblut et al., 2017).

Jensen et. al. reported similar displays of dominance by *Hemigrapsus* both in securing preferred shelter as well as feeding (2002). When *Hemigrapsus* were present, *Carcinus* were rarely found beneath rocks, which is their preferred habitat and where they were found in the absence of *Hemigrapsus* (Jensen et al., 2002). In competitive displays, *Hemigrapsus* were able to reduce feeding times and take food from *Carcinus* with a higher success rate than *Carcinus* crabs were able to take food from *Hemigrapsus* competitors (Jensen et al., 2002).

Further investigations have found feeding rates to be influenced by competitive interactions between the two species. In one study, as size of the prey item increased, the biomass of prey items consumed by *Hemigrapsus* was greater than the biomass consumed by *Carcinus* (DeGraaf and Tyrrell, 2004). In addition, Griffen et. al. reported that feeding rates of *Carcinus* were negatively impacted by the presence of *Hemigrapsus* but that *Hemigrapsus* were unaffected by the presence of *Carcinus* (Griffen et al., 2008). Not only do *Hemigrapsus* seem to eat more and decrease the feeding rates of *Carcinus* crabs when present, but Lohrer and Whitlatch also saw that *Hemigrapsus* crabs will often (50-80%) eat a *Carcinus* crab at least 10mm smaller than itself (2002). This behavior was not observed at similar rates when *Carcinus* crabs were paired with *Hemigrapsus* crabs 10mm smaller than them. In those trials predation only occurred 10-40% of the time (Lohrer and Whitlatch, 2002).

In contrast to these competition and feeding studies, MacDonald et. al. found that *Carcinus* found prey items faster and consumed more food when compared to *Hemigrapsus* in individual trials (2007). This trend continued in their interspecific competitions

between both species, where *Carcinus* outcompeted *Hemigrapsus* when it came to finding and consuming food (MacDonald et al., 2007). They also found that while *Hemigrapsus* were more likely to instigate fights compared to *Carcinus*, there was no significant difference in which species won those fights (MacDonald et al., 2007).

While these studies seek to understand competition between these two introduced species, none of them have addressed how parasites may alter their competitive interactions. Parasites are an important part of the community ecology of any system and can impact processes including interspecific interactions, host ecology, ans food web structure (Hudson and Greenman, 1998; Lefèvre et al., 2009). One particularly important concept is that of apparent competition, wherein a parasite impairs the fitness of one species at a higher degree than another (Hatcher et al., 2006; Lefèvre et al., 2009). This uneven effect of infection can also lead to an increase in biodiversity, as species which may normally be extirpated from an area through competition may persist through apparent competition (Lefèvre et al., 2009). In 1948, this idea was demonstrated through the use of two species of flour beetles, *Tribolium castaneum* and *Tribolium confusum* (Park, 1948). Park showed that when together *T. castaneum* would outcompete *T. confusum* to the point to extinction (1948). However, this trend was flipped when a parasite, *Adelina tribolii*, was introduced to the two beetles leading to *T. confusum* outcompeting *T. castaneum* (Park, 1948).

Understanding how parasites may mediate interactions between native species in an ecosystem is necessary to understanding community dynamics. Equally important is understanding how parasites affect native versus introduced species interactions in the face of increased globalization (Seebens et al., 2016). Several studies have been conducted on how parasites mediate the success of introductions, either through the introduced species escaping their native parasites (Torchin et al., 2001; Blakeslee et al., 2009; Simmons, 2014), the co-introduction of host and parasite (Lymbery et al., 2014; Goedknegt et al., 2016), or the acquisition of native parasites by a non-native species (Prenter et al., 2004; Goedknegt et al., 2016).

When studying parasites and their effects on competition in the context of the enemy release hypothesis, we could predict that newer introductions would have fewer parasites and therefore potentially an advantage over previously introduced species (Torchin et al., 2001; Simmons, 2014). This is not always the case, as was seen in a 2002 study which observed no difference in infection prevalence of the trematode, *Cercaria batillariae* in the introduced gastropod, *Batillaria cumingi* or the native gastropod *Cerithidea californica*, but a difference in the number of infecting trematode species was found between the two host species (*B. cumingi*:n=1, *C. californica*: n=10) (Torchin et al., 2005). This difference in parasite pressure is also seen when comparing parasite load in the crab species I have chosen for my study, with *Carcinus* escaping sixty percent of parasites found in its native range and *Hemigrapsus* escaping sixty-seven percent (Blakeslee et al., 2009; Kroft and Blakeslee, 2016).

If we consider competition in the context of a NIS bringing along a parasite from its native range, there could be two main outcomes: either the co-introduced parasite successfully host switches to use a native species as a host (parasite spillover), or the parasite does not switch to use a native host (Goedknegt et al., 2016). If the introduced parasite does switch to use a native species as a host, it can lead to devastating effects on naïve hosts (Bauer et al., 2002; Prenter et al., 2004). For example, the rapid decline of sturgeon in the Aral sea was linked to the introduction of a monogenean gill fluke after another sturgeon species was introduced in hopes of recovering the population (Bauer et al., 2002). There are numerous studies showing that introductions of hosts that can lead to the co-introduction of various diseases that native species have no prior immunity to and can lead to devastating effects (Anderson and May, 1986; Holdich and Reeve, 1991; Kohler and Wiley, 1997; Harvell et al., 1999; Daszak et al., 2000). However, if the parasite does not host switch, the native species could incur a competitive advantage over the NIS (Goedknegt et al., 2016).

We can also examine how native parasites host switching to introduced species may

alter competitive interactions. By gaining a new parasite (parasite acquisition) the introduced species could be at a disadvantage, in that being a naïve host without any co-evolutionary history with the native parasite could lead to increased effects of infection (MacNeil et al., 2003). For example, MacNeil et. al. found that the acanthocephalan parasite *Echinorynchus truttae* had higher prevalence in and greater influence on an introduced amphipod than on a native amphipod, altering competitive outcomes (MacNeil et al., 2003). However, host switching could ultimately lead to increased prevalence in native populations (parasite spillback), with the introduced hosts acting as a reservoir for the native parasites (Goedknegt et al., 2016).

In my study, I wanted to determine which (if any) of these varied responses to infection may be occurring in the WNA between these two common intertidal crabs (*Carcinus* and *Hemigrapsus*). In particular, I first wanted to test how a microphallid trematode may alter an individual crab's behavior in the two species. Previous research demonstrated that infection initially delayed the righting response (Figure 12), or the ability for a crab to flip right side up after being flipped onto its back, of parasitized *Carcinus* (Blakeslee et al., 2015). Thus far, this particular trematode (*M. similis*) has not been found infecting *Hemigrapsus* but I wanted to test if there was a behavioral response to being exposed to these parasites (even if not ultimately successfully infected by it). As these two crabs are commonly found in the intertidal together, I also wanted to test how exposure to a microphallid trematode would influence the interspecific competition between them.

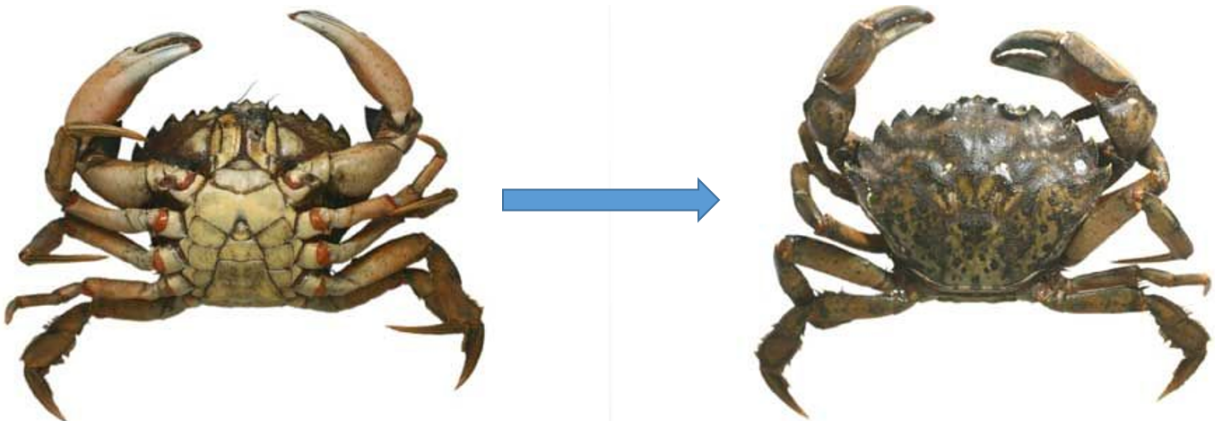

Figure 12: Crab species are placed ventral side up (left side of image) and a stopwatch is used to see how long it takes each individual to flip back ventral side down (right side of image). This was done three times to calculate average righting response time

2.2 Methods

2.2.1 Exposure Experiment: Individual Responses

In order to test how infection by a microphallid trematode may alter host behaviors, I used crabs collected from areas of low microphallid trematode prevalence and high crab abundance. *Carcinus* were collected from Cape Cod, Massachusetts by a collaborator (Dr. Carolyn Tepolt) while I collected the *Hemigrapsus* from Jackson Estuarine Marine Lab in Durham, New Hampshire. Using the methods described in section 1.2.1, fifteen *Carcinus* crabs received from Cape Cod were dissected and found to have no microphallids present (prevalence=0%). For the *Hemigrapsus* crabs I collected specifically at Adam's Point where crab abundances were high and parasite prevalences were known to be low based on prior field research there by Dr. Blakeslee (Blakeslee et al., 2009, Blakeslee unpublished).

Forty-eight *Carcinus* and forty-eight *Hemigrapsus* crabs were collected from their respective sites. Using established protocols (Blakeslee et al., 2015), I divided each species of crab into 2 treatment groups (n= 24 crabs each), and exposed (see methodology below) one of the groups to microphallid trematodes for 72 hours to induce infection. Control groups were composed of unexposed crabs. All groups contained equal numbers of similarly sized adult males and females kept at standard conditions throughout the experimental duration.

For the exposure treatment, I exposed crabs to 800 *Littorina obtusata* and *Littorina saxatilis* snails, which serve as the upstream host for these microphallid trematodes (Blakeslee et al., 2015). These snails were collected from York, Maine, and 100 individuals of each species were dissected to determine prevalence of *M. similis* at this site(prevalence =5%). Snails were then desiccated for 12 hours before being placed in a small strainer (to prevent predation by the crabs) and re-hydrated for 12 hours in the exposure container (Figure 13). This was done to simulate a tidal cycle and induce shedding of the cercariae These strainers were tied below the water line at the top of the exposure tanks, which

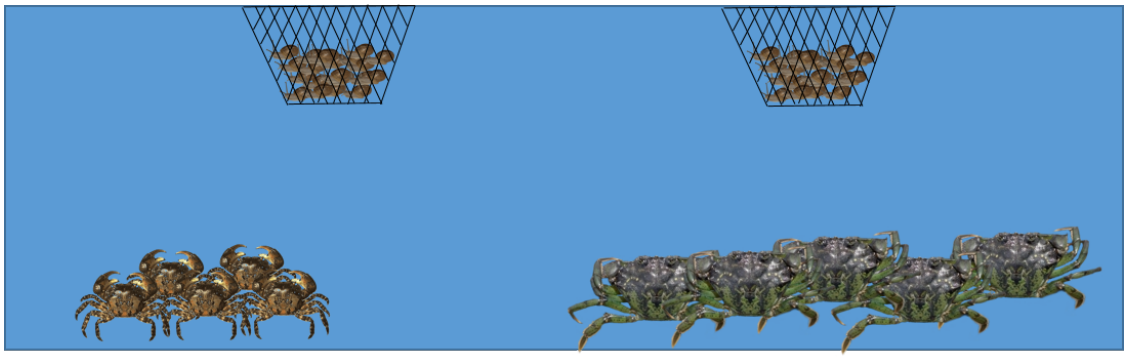

Figure 13: Diagram of exposure tank used in infection experiment. Crabs were placed at the bottom of container with *Littornia* spp. contained in strainers to prevent predation. These snails act as the upstream host and released cercariae into the water column. The snails were switched out with a new set of snails every 12 hours until the end of the 72 hour exposure period. Control tanks were identical to exposure tanks however, strainers did not contain snails.

allowed for the release of trematode cercariae into the water and for the cercariae to enter the crabs in the exposure tanks through the gills. These steps follow the successful methodologies of induced infection in Blakeslee et al. (2015). In addition to changing the snails every 12 hours I also removed 5mL of water from the four corners of the exposure tanks as well as from the center and looked at the water under a dissecting scope. This was done to confirm the presence of *M. similis* cercariae in the water using morphology. After the 72 hours in the exposure or control tanks, crabs were moved to individual aquaria and housed in climate controlled incubators for the duration of the experiment and kept at the following standard conditions: 20 °C air temperature, a 12 hour day/ night cycle, 32 ppt salinity and fed every two days.

I conducted behavioral trials at two time intervals: Immediately post infection (T0) and three and a half weeks (T3.5) post-infection. At each interval, I conducted a behavioral trial testing righting response (Figure 12) on all *Carcinus* and *Hemigrapsus* crabs. This behavioral response was found to differ in infected/uninfected treatments in prior investigations (Blakeslee et al., 2015)

For the behavioral trial, crabs of each species were placed in a tray dorsal side down and timed for how long it took them to flip back over, dorsal side up (Figure 12). Number of walking legs and claws missing were noted for each individual. To account for variation in righting response times, each crab was tested three times to acquire an average righting time. Trials were stopped if after five minutes a crab had still not righted itself. Each crab was measured and given an identification number and a tag placed on the outside of the aquaria it was placed in so all individuals could be followed throughout the experiment.

Following the behavioral trials after time point 3.5 weeks I dissected all crabs in the experiment and counted newly formed microphallid cysts. Newly formed cysts were easily distinguished from naturally acquired cysts based on the presence of a thick cyst wall in older cysts (Figure 5) as opposed to the extremely thin cyst wall in newly formed

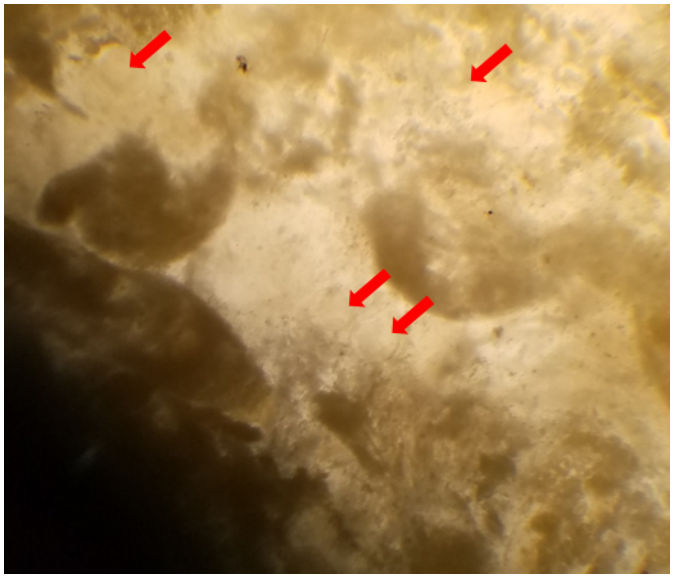

Figure 14: Picture of newly developed cysts in the hepatopancreas of *Carcinus* at 4x magnification. Red arrows indicate where these newly formed cysts are located in the tissue.

cysts (Figure 14). All cysts found were saved in 1.5ml micro centrifuge tubes at -20 °C for later genetic analysis.

2.2.2 Exposure Experiment: Competition Trials

Interspecific competition trials were performed one week post-infection. As with the individual and immune response trials (section 2.2.1). I used *Carcinus* crabs collected from Cape Cod, Massachusetts and *Hemigrapsus* crabs collected from Jackson Estuarine Marine lab in Durham, New Hampshire. Sixteen *Carcinus* and sixteen *Hemigrapsus* crabs were collected from their respective sites and I divided *Carcinus* crabs into 2 treatment groups, exposed and unexposed. In this particular study, I did not divide *Hemigrapsus* into these two treatments due to sampling and timing constraints, so all *Hemigrapsus* crabs were unexposed (Figure 5).

Crabs were exposed using the same methodology described in section 2.2.1, using the same snails. Infections were staggered over seven days to give the snails one full day to recover from any stress. After 72 hours in the exposure or control tank crabs were moved to individual aquaria and housed in climate controlled incubators for the following week and kept at standard conditions (described above). During that week crabs were only fed flake food and no crab was fed for 24 hours leading up to their competition trial. Crabs were visually paired together based on chelae size rather than carapace width as *Carcinus* are known to grow up to 90mm in carapace width (Grosholz and Ruiz, 1996) whereas *Hemigrapsus* males can reach a maximum of 40mm and females rarely exceed 35mm (Epifanio, 2013).

For these competition trials, we used both males and females of both species. Average carapace width of male *Hemigrapsus* used in these trials was 29.6 ± 3.3mm while average carapace width of *Carcinus* was 55.5 ± 3.9mm. Average sizes for females of both species were similar to their male counterparts, with average carapace width of female *Hemigrapsus* being 28.2 ± 2.9mm and average carapace width of female *Carcinus* being 55.9 ± 2.5mm. In addition to size matching chelae of each crab to their competitor, all crabs were checked to ensure all limbs and chelae were intact before the start of the trials so that neither crab started at a disadvantage.

Treatment	*Carcinus*	*Hemigrapsus*	Sex	Replicates
1	Unexposed	Unexposed	Males	3
2	Unexposed	Unexposed	Females	4
3	Exposed	Unexposed	Males	4
4	Exposed	Unexposed	Females	3

Table 5: Treatments for Interspecific Competition Trials. There were four different treatments types with different combinations of treatments and sexes. Under each species name, treatment type is indicated: either exposed or unexposed. Sex of both of the crabs used in the trial indicated, and sexes were matched so there was never a male: female pairing. Replicates indicates how many times that treatment type was replicated.

Competition trials were run two at a time in a low light setting (Figure 15). Tanks were set up with a single rock with *Fucus* spp. attached to it using fishing line. Each trial used a different rock and a different sample of *Fucus* spp. attached. *Fucus* spp. was weighed prior to being attached to the rocks to ensure the same amount of cover was allotted to each trial pair (80-85g of *Fucus* spp. per rock). In order to look at prey capture and consumption, two small *Mytilus edulis* (blue mussel, a very common prey item in natural settings) were added to the tanks. These mussels were cracked with a hammer before being placed across from the shelter in the tanks, so that the crabs could scent the prey more easily.

Prior to filming, I placed one *Carcinus* and one *Hemigrapsus* crab on either side of the tank in individual containers to allow them to acclimate to the environment for 10 minutes. After the 10 minutes, I removed each crab from their cage and placed them on either side of the tank with the shelter and prey items between them. Video cameras were affixed above the tanks; recording started before the crabs were released from their individual cages and stopped 30 minutes after both crabs had been released. Due to mortality and to ensure trials were even between treatments, 7 trials per treatment were recorded for 30 minutes. Videos were watched after the trials so as not to disturb the crabs. Behaviors related to aggression, shelter-use, foraging time and consumption were analyzed (Table 6) and an ethogram was filled out to catalog all behaviors within the 30 minutes period at 30 second intervals. Figure 16 shows a sample of the ethogram filled out for the first 12 minutes of a trial.

After the competitions, crabs were placed back in individual aquaria for three weeks to allow for the cysts to fully develop. After that time I dissected all crabs in the experiment and counted newly formed microphallid cysts. Newly formed cysts were easily distinguished from naturally acquired cysts based on the presence of a thick cyst wall (Figure 5) as opposed to the extremely thin cyst wall in newly formed cysts (Figure 14). All cysts found were saved in 1.5ml micro centrifuge tubes at -20 °C for later genetic analysis.

Figure 15: Tanks were set up with a single rock with *Fucus* spp. attached to it using fishing line. Two small *Mytilus edulis* were used as prey items and were broken with a hammer and placed across from the shelter. Crabs were dropped in individual containers acclimate to the tank for 10 minutes before they were released and filmed for 30 minutes in a low light environment.

Category	Behavior	Description
Neutral Behaviors	Walking	Movement around tank
Neutral Behaviors	Climbing Walls	Movement or attempts to move up the walls of the tank
Neutral Behaviors	Standing Still	Crab is stationary in tank, chelae may move but walking legs do not
Neutral Behaviors	Walking under/ over Other Crab	Movement on or under other crab without apparent notice of other crab being present. Action not accompanied by grabbing, fighting, or other antagonistic displays
Shelter Behaviors	Beside	Standing or walking adjacent to the shelter provided
Shelter Behaviors	On top of	Standing or walking on top of the shelter provided
Shelter Behaviors	Underneath	Standing or walking beneath the shelter provided
Feeding Behaviors	Handling	Holding, manipulating, or breaking prey item without eating it
Feeding Behaviors	Eating	Consuming prey item
Feeding Behaviors	Monopolizing	Hording food items by standing on top of both or dragging them away from other crab without engaging with other crab
Interactive Behaviors	Antagonistic displays	Charging or moving towards other crab, sometimes accompanied by chelae raised
Interactive Behaviors	Fighting	Grappling with other crab; sometimes accompanied by pulling at prey items, pulling of chela, or climbing on other crab
Interactive Behaviors	Grab carapace	Using chela to grab or pull at carapace
Interactive Behaviors	Grab walking legs	Using chela to grab or pull at walking legs
Interactive Behaviors	Flee	Escaping from other crab, usually through quick movements away

Table 6: Table of behaviors and definitions used in ethogram during experimental trials. Behaviors were marked so that multiple actions could be tallied at the same time point. This allowed me to capture information on a crab that was both under the shelter and consuming a prey item, for example

	Neutral Behaviors				Shelter Behaviors			Feeding Behaviors			Interactive Behaviors					
Time	Walking	Climbing walls of tank	Standing still	Walkover/under crab	Beside	On top	Under	Handling	Eating	Monopolizing	Agonistic display/ charging	Fighting	Grab Carapace	Steal Prey Item	Pull legs	Flee
00:30									1							
01:00									1							
01:30			1													
02:00			1													
02:30									1							
03:00									1							
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10:30		1														
11:00	1				1											
11:30		1														
12:00	1															
12:30		1														
13:00		1														

Figure 16: Example of filled in ethogram to minute 13. Blank cells indicate that action was not seen at the 30 second mark and a '1' indicates that was the action the crab was performing at the 30 second mark. Behaviors were marked so that multiple actions could be tallied at the same time point. This allowed for me to capture information on a crab that was both under the shelter and consuming a prey item.

2.2.3 Statistical Methods: Exposure Experiments

I used the package *stats* in the program R to determine the mean flip times for each species in each treatment as well as to compare between average flip times by species and treatment using a regression to allow me to look at how righting response changes by treatment type. (R Core Team, 2013).

Boxplots were made to compare between all behaviors of crabs under different treatments as well as behaviors pooled by category (neutral, shelter, food, or aggressive). A model was made using the *lme4* package in R using a Poisson distribution to determine if treatment affected the frequency at which any given behavior was documented (R Core Team, 2013). I examined the frequency of a behavior by the treatment type (ie: Control *Hemigrapsus*, Control *Carcinus*, Exposed *Carcinus*, *Hemigrapsus* competing with exposed *Carcinus*) to determine differences between treatments and species.

2.3 Results/Discussion

2.3.1 Exposure Experiment: Individual Responses

After calculating the average righting response of each species per treatment, I created a graph showing mean flip time with a 95% confidence interval around the mean (Figure 17). Interestingly, there is a trend to suggest that exposure to parasite does slightly slow the righting response in *Hemigrapsus* at T0 (T0: P = 0.184, T3.5: P = 0.362). This was surprising since after all the trials there were no infections found in the crabs. However, could indicate an attempt of infection that was ultimately unsuccessful.

I did not see a significant difference in the righting response of infected or uninfected *Carcinus*, contrary to previous work Blakeslee et al. (2015). While not significant, I saw a weak trend suggesting that righting response increased with infection (T0: P=0.345; T3.5: P=0.594). When I accounted for crabs with missing chela and walking legs, I saw a strengthening in support of this trend (T0: P=0.225, T3.5: P=0.586). While I hypothesized a difference in righting response at the initial time point for these crabs I also hypothesized too that this would disappear over time given prior work demonstrating such an effect (Blakeslee et al., 2015). In my study I did see some weakening of that response.

While I observed a trend but not a significant difference in righting response, it is important to note that the crabs in my study had very low levels of infection intensity compared to the aforementioned 2015 study. While I was not able to calculate an estimate of total cysts in the crabs for these trials because I did not weight hepatopancreas tissue as I did for the survey crabs (see above), I was able to compare the number of cysts found in the six hepatopancreas tissue snips between both studies. In the six hepatopancreas snips of tissue that were collected in this study, I found a maximum 28 new cysts (Table 7). For comparison, the greatest number of new cysts found in the hepatopancreas snips during the 2015 study at the 72hr exposure time point was over 2200 (Blakeslee et al., 2015). That study also reported crabs with natural infection (Appledore Island, Maine)

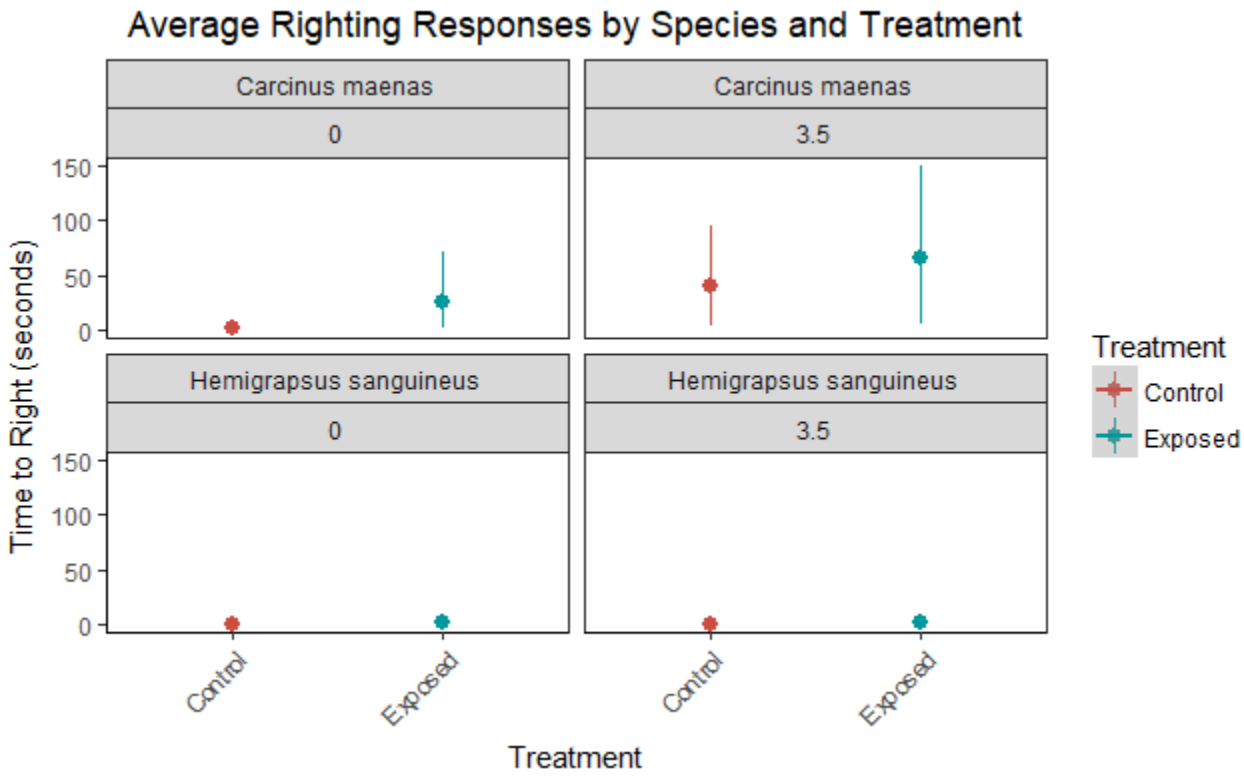

Figure 17: Average Righting Response of both crab species post 72hr incubation period either in control set-up, or exposure set-up. Averages are of the three replicate flip trials per individual. Bars represent 95% confidence intervals around the mean. Crabs in exposure treatment are indicated in blue, while crabs in control treatment are indicated in red

having a maximum infection level of 784 cysts in the hepatopancreas snips, almost thirty times more than I saw in the crabs in my study.

There is a remarkable difference between the infection levels I was able to obtain in my study crabs and those seen by Blakeslee et.al in 2015. One likely reason for this difference is the drop in prevalence in the upstream gastropod hosts, *L. saxatilis* and *L. obtusata*. During the 2015 study, prevalence levels of *M. similis* were found to be at 9% (Blakeslee et al., 2015), while during my experiment prevalence levels were around 4%. I did try to control for this by using smaller exposure tanks to increase the likelihood of *M. similis* encountering a crab host. While this likely had some effect, increasing the number of parasites would of course increase likelihood of infection as well as intensity of that infection.

However, what is noteworthy about my study is that even with low intensity levels, I still observed a trend that newly infected crabs have a slower righting response. It also seems likely that infection intensity is a contributing factor influencing righting response in infected crabs (i.e., I noted a trend with lower infection intensity and Blakeslee et al. (2015) had a significant effect). Overall, these results could indicate that this parasite exerts some amount of stress on its host upon infection (slower response time), which could lead to increased trophic transmission of this parasite to the definitive host, especially if this makes the crab more conspicuous and vulnerable to predation. If left with its ventral side exposed, a crab could be at greater risk of predation by various shore birds like *L. argentatus* or *L. marinus*, thereby continuing the parasites life cycle. In fact, research performed by a summer intern at the Shoals Marine Lab (Appledore Island, Maine) advised by Dr. Blakeslee in 2017 explored this question and found more vulnerability to predation by gulls with time since infection (Love & Blakeslee, in prep).

It is important to note, however, that my lab experiments detected an artifact of lab confinement in *Carcinus* crabs during the course of my experiments (Figure 17). It is likely the increase in response time is linked with the aquaria the crabs were being housed in. Every *Carcinus* had been housed in its own aquarium but perhaps these were too small to

Species	Crab ID	Treatment	Total Pre-Existing Cysts	Total New Cysts
Carcinus	CMCC02	Control	0	0
Carcinus	CMCC04	Control	0	0
Carcinus	CMCC06	Control	0	0
Carcinus	CMCC08	Control	1	0
Carcinus	CMCC11	Control	3	0
Carcinus	CMCC15	Control	0	0
Carcinus	CMCC21	Control	0	0
Carcinus	CMCC22	Control	1	0
Carcinus	CMCC25	Control	0	0
Carcinus	CMJEL03	Control	3	0
Carcinus	CMCC05	Exposed	0	7
Carcinus	CMCC07	Exposed	0	8
Carcinus	CMCC09	Exposed	0	0
Carcinus	CMCC10	Exposed	0	0
Carcinus	CMCC12	Exposed	1	11
Carcinus	CMCC13	Exposed	0	28
Carcinus	CMCC17	Exposed	0	0
Carcinus	CMCC19	Exposed	0	2
Carcinus	CMCC26	Exposed	0	0
Carcinus	CMJEL02	Exposed	0	23

Table 7:]
Infection Intensities of microphallid trematode counted in *Carcinus* after exposure experiment. Pre-existing are indicated to show background level of infection from site. New Cysts are indicated to show induced infection induced during experiment

allow for freedom of movement. This lack of moving over the course of three and a half weeks could be why we are seeing an overall increase in righting response in all *Carcinus* crabs. Since *Hemigrapsus* are smaller crabs, being housed in a similar sized aquaria probably allowed for sufficient space to move, and therefore they were not affected by confinement like *Carcinus* seemed to be. However since the increase in righting response time affected both exposed and control *Carcinus* crabs, it does not affect the comparisons within time points.

2.3.2 Exposure Experiment: Competition Trials

I graphed the average occurrence of each behavior by species and treatment resulting in fifteen plots. However, discussions within the body of my thesis will focus on pooled behaviors by category (Table 6) and comparisons of infected and uninfected individuals within each category. All of the specific behavioral plots not discussed in detail here can be viewed in the thesis Appendix. The first category of behaviors was neutral behaviors (Figure 18). This consisted of walking around the tank, climbing on the walls, standing still, or moving over or under the other crab without any interaction with that crab. There is a clear difference between species and this is statistically significant (P=2.78e-05), where *Hemigrapsus* engaged in neutral behaviors at a much higher frequency than *Carcinus*, doing so for most of the 30 minute trial. *Carcinus* rarely engaged in these neutral behaviors and instead tended to interact with objects (food or another crab).

Hemigrapsus were more frequently seen walking around the tank, climbing the walls of the tank, and remaining stationary when compared to their *Carcinus* counterparts regardless of treatment (See Appendix, Figures: 26, 27, 28). Neither species engaged in "walking over or under the other without an accompanying aggressive interaction". We do see that unexposed *Carcinus* conduct neutral behaviors more frequently that exposed *Carcinus* and this observation is very close to significant (P=0.0549). This may indicate that unexposed *Carcinus* are more active, or less likely to interact with other objects as these neutral behaviors were not paired with feeding behaviors or aggressive interactions. These neutral behaviors were often paired with Shelter based behaviors which I will address next.

The next category of behaviors was shelter based behaviors (Figure 19), which consisted of remaining beside, under, or on top of the shelter. Unlike neutral behaviors there were no significant differences between species or treatments when it came to use of shelter. This is interesting since previous work had noted that *Hemigrapsus* outcompeted *Carcinus* when it came to shelters (Jensen et al., 2002), but I did not see that here. Both

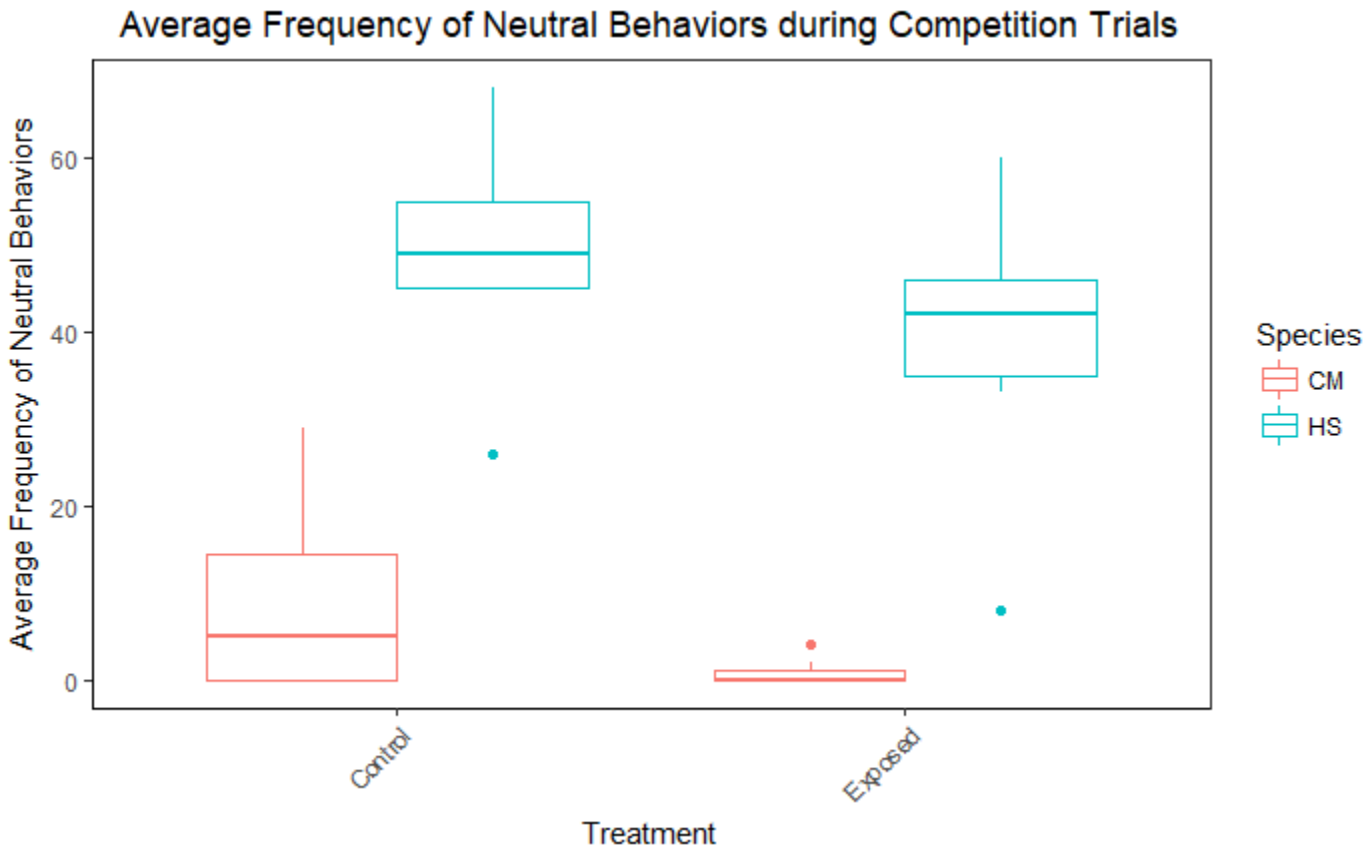

Figure 18: Frequency of Neutral behaviors shown by treatment. Treatments are indicated on the x-axis with the frequency of the behavior on the y-axis. *Carcinus* are shown in red (CM) and *Hemigrapsus* are shown in blue (HS).

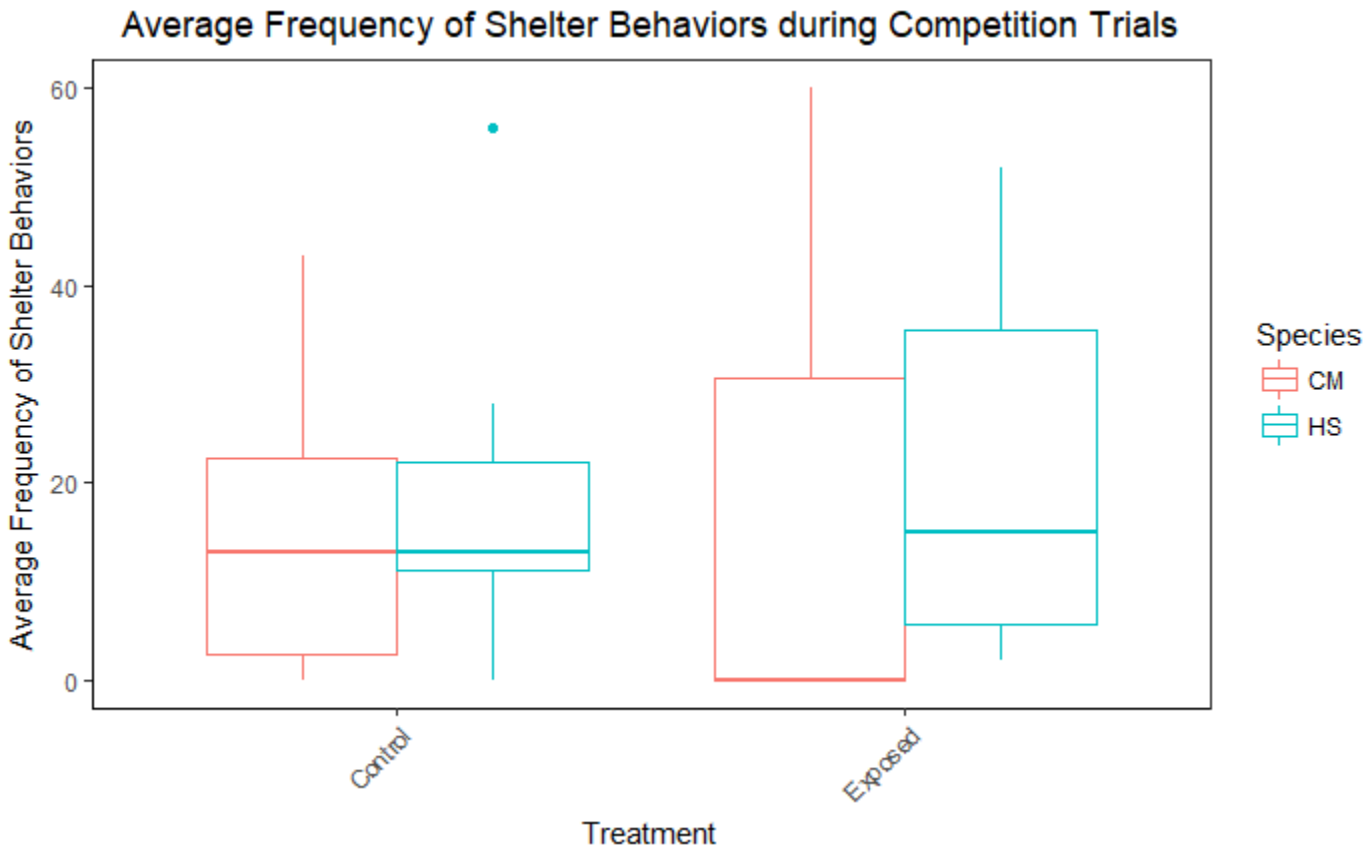

Figure 19: Frequency of Shelter behaviors shown by treatment. Treatments are indicated on the x-axis with the frequency of the behavior on the y-axis. *Carcinus* are shown in red (CM) and *Hemigrapsus* are shown in blue (HS).

species tended to use the shelter for no more than a third of the trial, though there is a lot of variation in these data. Interestingly, exposed *Carcinus* would hide under the shelter more frequently than control *Carcinus* (Figure 20). While not significant (P=0.1343) there is a trend that suggests exposed crabs appear to be less conspicuous than control crabs. This is a little counterintuitive since we may expect exposed crabs to be more conspicuous. However, early on in the infection process, being less conspicuous may in fact be in the parasite's best interest. This is because the parasite needs time to develop within the host.

I also examined the behaviors associated with feeding between species and treatment (Figure 21), which consisted of prey handling, feeding, and monopolizing prey items. While there are no differences with exposure, there are striking and significant differences between species (P=5.54e-07). These results are very interesting, since I hypothesized that *Hemigrapsus* would outcompete *Carcinus*, especially when *Carcinus* was exposed to *M. similis*. Previous experiments had found that *Hemigrapsus* was better at acquiring food when compared to *Carcinus* (Jensen et al., 2002; Griffen et al., 2011), however during these competition trials *Hemigrapsus* spent no more than a third of the time handling, eating, or monopolizing the food. In contrast *Carcinus* spent nearly the entire trial engaged in feeding based behaviors.

Looking at the specific breakdown of behaviors that go into feeding, the behavior that seems to be driving this difference is the actual eating itself (Figure 22). This behavior alone is also highly significant between species (P= 4.37e-07) which, as stated previously, is in contrast with what was found by many of the previous studies. This finding does agree with a 2007 study that found *Carcinus* to be more likely to first find prey items and consume some amount of those prey items than *Hemigrapsus* (MacDonald et al., 2007). This study also noted that *Carcinus* required very little time, often no more than two minutes, to find the prey item in their experimental set ups (MacDonald et al., 2007). While I did not record 'first to food', anecdotally, I repeatedly observed *Carcinus* find the

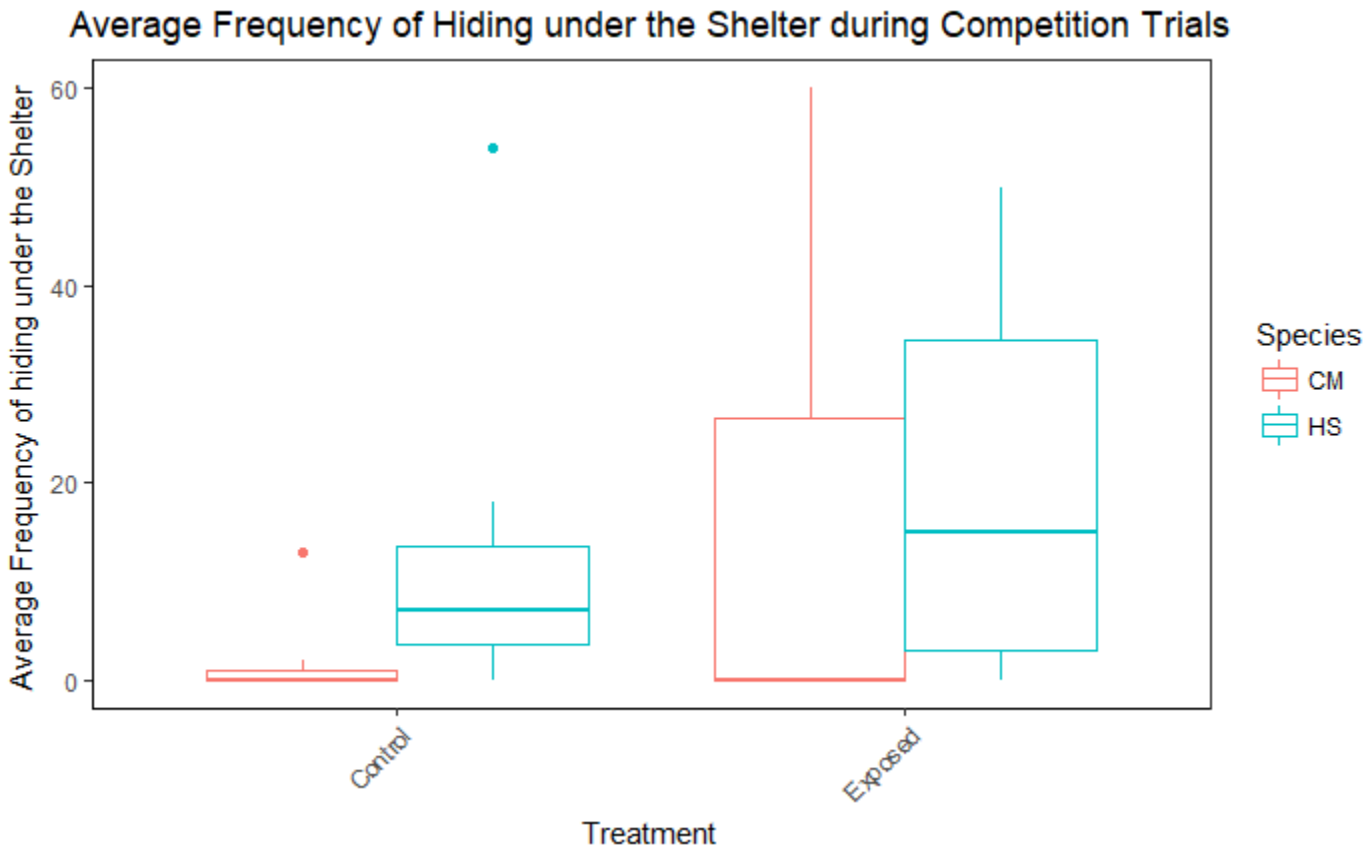

Figure 20: Frequency of hiding under the shelter by a crab shown by treatment. Treatments are indicated on the x-axis with the frequency of the behavior on the y-axis. *Carcinus* are shown in red (CM) and *Hemigrapsus* are shown in blue (HS).

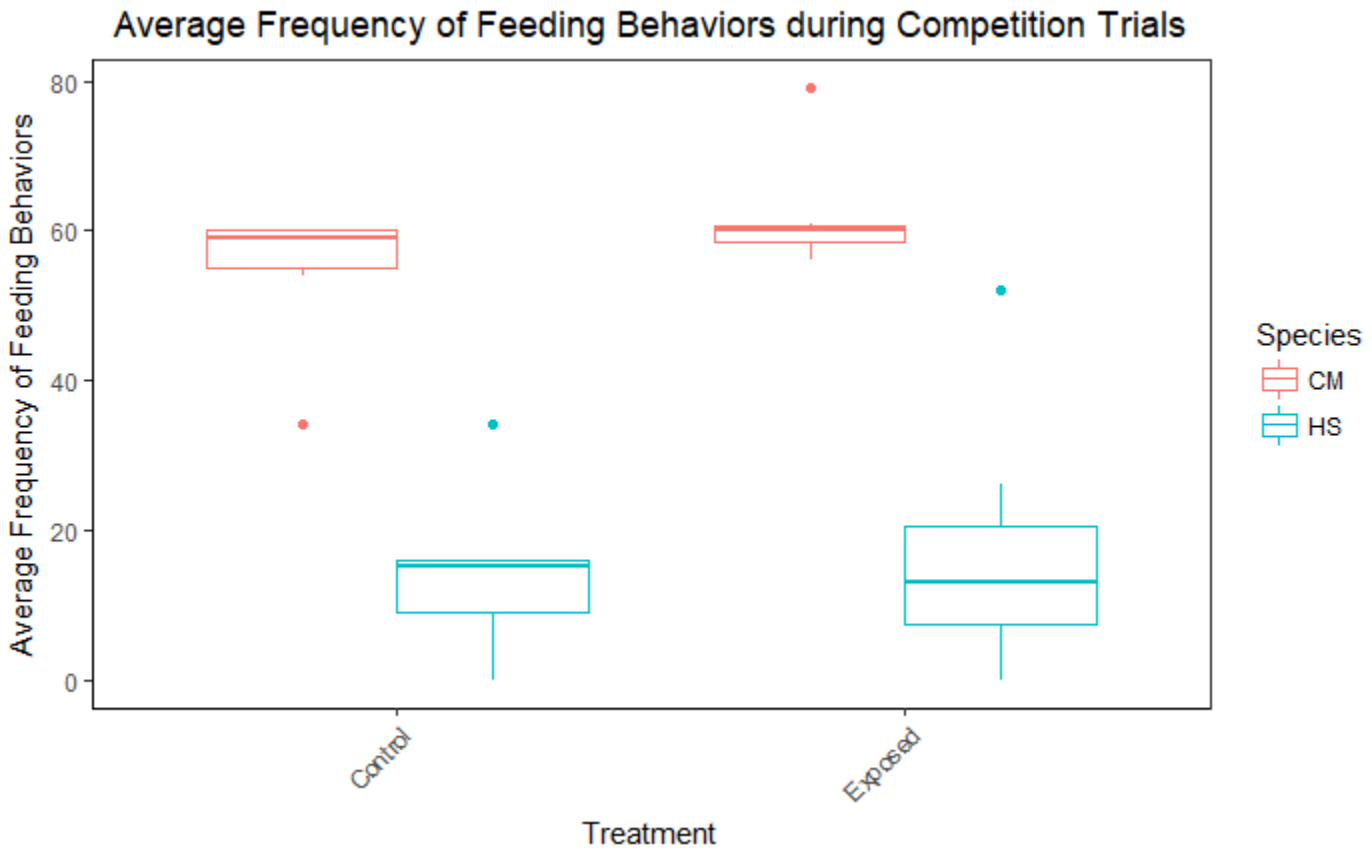

Figure 21: Frequency of Feeding behaviors shown by treatment. Treatments are indicated on the x-axis with the frequency of the behavior on the y-axis. *Carcinus* are shown in red (CM) and *Hemigrapsus* are shown in blue (HS).

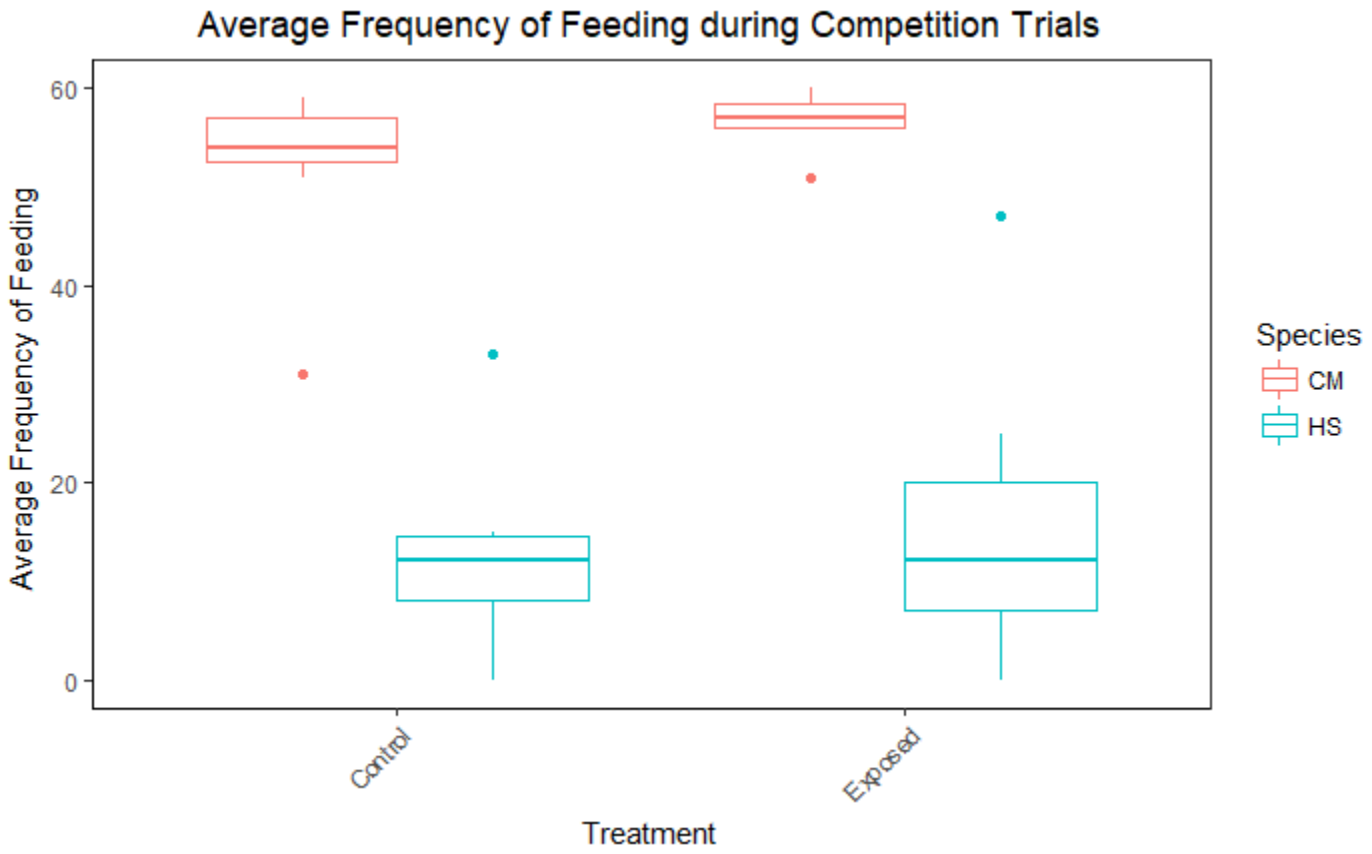

Figure 22: Frequency of eating prey items shown by treatment. Treatments are indicated on the x-axis with the frequency of the behavior on the y-axis. *Carcinus* are shown in red (CM) and *Hemigrapsus* are shown in blue (HS).

food in the tank quickly, and usually before *Hemigrapsus*.

The last group of behaviors I examined was interactive/ aggressive behaviors (Figure 23), which consist of aggressive displays, fighting, grabbing at the carapace of the other crab, grabbing the walking legs of the other crab, or fleeing from the other crab. While there were no strictly statistically significant effects of species or treatment, there was one interaction that was extremely interesting. When paired with exposed *Carcinus*, *Hemigrapsus* were more likely to engage in interactive/ aggressive behaviors (P=0.115). While not statistically significant, this trend is very interesting. Looking at the break down of the behaviors that comprise interactive/aggressive behaviors we can see that the specific behavior driving this difference is fighting (Figure 24).

This behavior alone is also nearly significant (P=0.168) and could point to a change in behavior of these crabs due to some cue or change in the exposed *Carcinus* that the *Hemigrapsus* competitors were able to pick up on. While *Hemigrapsus* did this behavior more frequently than *Carcinus*, it was still not a common behavior. What was not captured by this ethogram is that during these fighting interactions *Carcinus* was always feeding (n=3) and *Hemigrapsus* was never able to steal the prey item from *Carcinus*. These findings differ from a previous experiment that reported that *Hemigrapsus* took food from *Carcinus* with more success than *Carcinus* could remove a prey item from *Hemigrapsus* (Jensen et al., 2002). However, it is in agreement with a more recent study that found that while *Hemigrapsus* were more likely to instigate conflict between their competitor, they were no more likely to win said conflict than *Carcinus* (MacDonald et al., 2007).

As I alluded to earlier, my experimental design was different than others conducted. Many experiments tethered their prey items down to prevent the crabs from moving away with the items, but I did not. I wanted to allow the crabs to be able to flee with prey items, and see if the other individual would pursue or give up. I also size matched these crabs based on chelae size rather than body mass or carapace width. I did this so each crab would have similar offensive and defensive capabilities since the chelae to

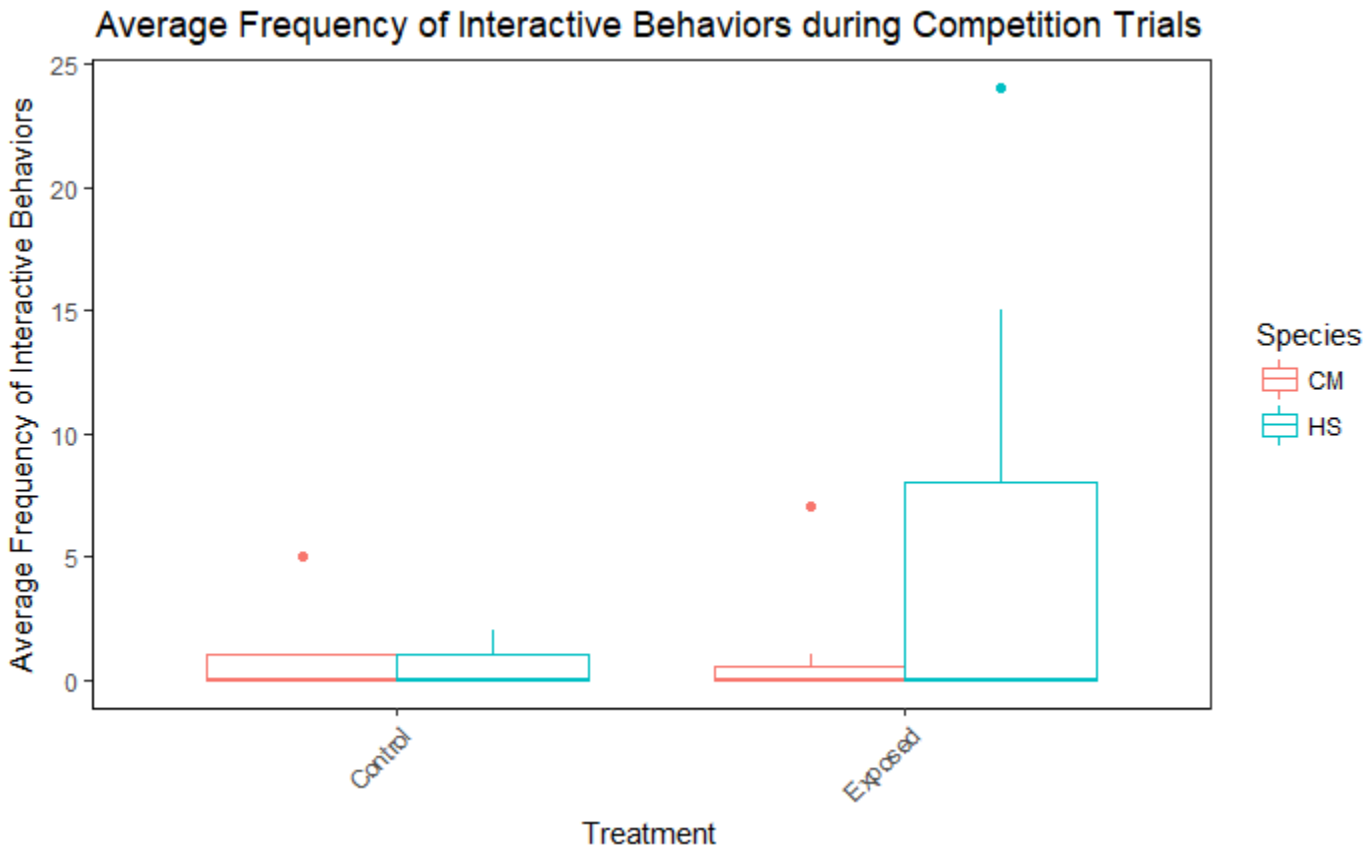

Figure 23: Frequency of interactive behaviors shown by treatment. Treatments are indicated on the x-axis with the frequency of the behavior on the y-axis. *Carcinus* are shown in red (CM) and *Hemigrapsus* are shown in blue (HS).

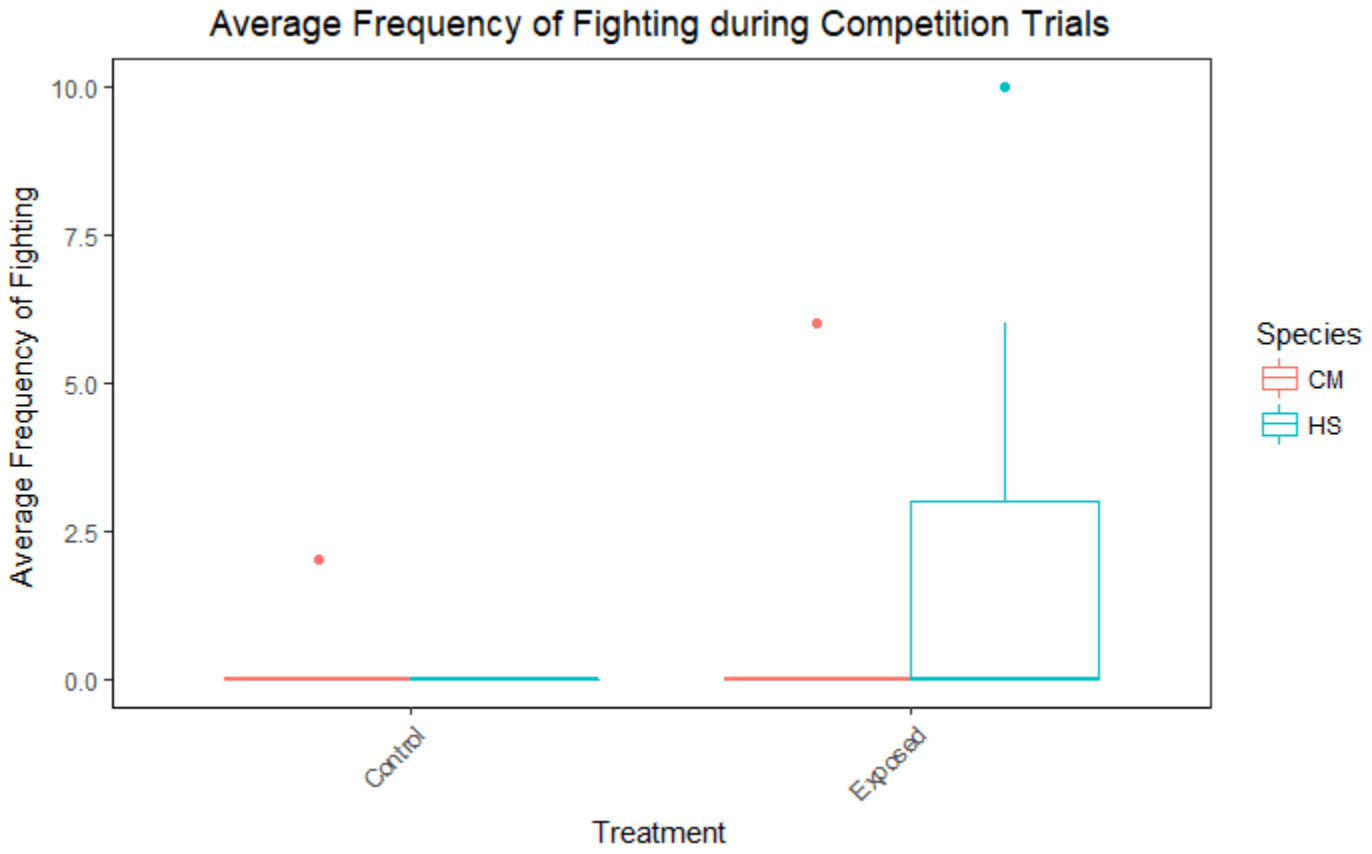

Figure 24: Frequency of Fighting shown by treatment. *Carcinus* are shown in red (CM) and *Hemigrapsus* are shown in blue (HS).

body size ratio of *Hemigrapsus* is larger than that of other crabs of a similar size. Like the 2007 study, we also completed our competition trials in low light conditions instead of complete darkness (MacDonald et al., 2007) due to constraints of the equipment.

Matching crabs by chelae may have unintentionally given *Carcinus* an advantage during these competitive trials. Since I matched by chelae, the actual size of *Carcinus* was larger than the size of the *Hemigrapsus* they were competing against. This size difference could have made it easier for these crabs to win the fights for food, as well as monopolize the prey items. As stated, I wanted to put each crab in similar offensive standings which is why I decided to pair by chelae and not body size. While there were not many aggressive interactions, perhaps if the crabs had been more similar in body size the number of aggressive interactions would have increased. Moreover, *Caricnus* may have been able to better monopolize prey items by using its larger body size to block *Hemigrapsus* from the prey.

Even with these differences in experimental design I do see clear differences with *Carcinus* regardless of parasite exposure. When pitted against *Hemigrapsus* one on one, *Carcinus* appear better able to find and consume the prey items. While *Hemigrapsus* instigates aggressive interactions, these conflicts do not results in *Hemigrapsus* gaining the prey item from *Carcinus*. These findings are in agreement with the 2007 study which saw similar behaviors (MacDonald et al., 2007).

One caveat to conducting one on one competition experiments with these crab species is that it may not be environmentally relevant. *Hemigrapsus* shows strong aggregation behaviors (Epifanio, 2013; Fofonoff et al., 2003), and when hand collecting these crabs during the surveys I could often find ten or more individuals under a rock (personal observations). Therefore it is unlikely that a *Carcinus* would ever be in competition with a single *Hemigrapsus* at any given time. Peterson et al. (2014) conducted an experiment looking at how density of *Carcinus* and *Hemigrapsus* altered proportion of prey items consumed and found that *Carcinus* did seem to consume more prey items than *Hemigrapsus*.

This experiment, like mine, had an even number of individuals of each species and to be more environmentally relevant, it would be good to conduct a competition trial in which there are more *Hemigrapsus* than *Carcinus* to better simulate the WNA intertidal zones.

Another potential experimental design change I could make in future studies would be to count out a set number of cercariae per crab and expose each crab individually. This may have prevented my low levels of infection intensity and could also potentially result in more similar infection intensities in exposed crabs. However, this would be unrealistic in natural settings where crabs are never exposed to cercariae in such a controlled way. I wanted to maintain an infection process that would have been similar to how these crabs would be exposed in nature. The aim was to make these results more environmentally relevant and not overinflate our intensities. We also would have no guarantee that altering our exposure process would increase infection intensities as there is a lot about the infection process that we do not understand. While having a large number of cercariae in the water is important, it is not possible at this point to replicate the behavioral aspect of the infection process in such a controlled way and also ensure infection.

2.4 Conclusion

My previous chapter explored the distribution, prevalence, and identities of trematode parasites in three host crabs along the WNA. In that chapter, I also noted the influence the trematodes had on body condition in the crab. For this chapter I tested what behavioral effects trematode parasites have on a host. I conducted these experiments with two primary research questions: 1) does this trematode parasite induce any behavioral changes to an individual host and 2) does infection by this parasite alter competitive interactions between two species?

I saw a trend indicating that there may be an increase in righting response of exposed *Hemigrapsus* when compared to control crabs at the initial time point. This may indicate that the crabs are experiencing some stress when placed in tanks with this trematode parasite that may relate to the attempt of infection. Unexpectedly, I did not see a significant difference between control and exposed *Carcinus*. However in the context of a prior study (Blakeslee et al., 2015) demonstrating a significant difference in righting response, it seems likely that the limited response was due to much lower infection intensity. In fact, the maximum number of new cysts recovered during my infection trials was 28, whereas in the 2015 study the maximum number of new cysts they recovered was 2200 (Blakeslee et al., 2015). This could indicate that it is not only infection that is important when considering the effects a parasite has on its host but also the intensity of that infection.

When testing how infection may alter competitive interactions between two species I found very little differences between exposed and unexposed treatments. There was a nearly significant trend that indicated exposed *Carcinus* engaged in less exploratory behaviors compared to their unexposed counterparts. This could allow the crab to engage more with its environment, such as a shelter, prey items, or other competitors. Most of the differences I saw was between species, were *Hemigrapsus* spent more time overall conducting exploratory behaviors and *Carcinus* spent nearly the entirety of a trial consuming prey items compared to the less than a third of a trial *Hemigrapsus* spent consuming a prey

item.

While I did not find significant differences in the behaviors of exposed crabs, I was able to demonstrate that there are trends in how this trematode parasite may be altering its hosts behaviors. I hypothesize that my lack of significant results is due to the low intensities of infection that were achieved during my individual and competition trials. Despite low intensities, observed trends in my study could help motivate more research the determines the threshold in intensity that results in a strong response.

3 Conclusion and Future Directions

3.1 Conclusions

Altogether, this thesis had three main objectives: 1) to look at natural distribution, prevalence, and intensity of trematodes in three species of crab, *Cancer irroratus*, *Carcinus maenas*, and *Hemigrapsus sanguineus* along the WNA, 2) to determine the identities of microphallid trematodes infecting those crabs, and 3) to understand how infection may affect the host, through change in body condition or behavior. Understanding how parasites may affect the interactions between native and introduced species is extremely important since parasites have a disproportional effect on the ecosystem (Budria and Candolin, 2014) and parasites have been linked to the success or failure of new introduced species (Torchin et al., 2003; Blakeslee et al., 2009; Simmons, 2014; Keogh et al., 2016).

To address the first objective, during my two surveys, I found a wide range of prevalences of a trematode parasite. In the first survey in May, I found prevalence in *Cancer* to be at least 80% (Table 1), with prevalence dropping to 0% during the second survey in August (Table 2). This drop could be due to mortality in the upstream gastropod hosts (Fredensborg et al., 2005), or even mortality of infected *Cancer*. Of note is that in general I had a difficult time collecting sufficient numbers of *Cancer* during these surveys. It could be that this represents the influence of introduced crabs in the region, as seen in the recent introduction of *Carcinus* to Newfoundland where its presence has already impacted abundance of the native *Cancer* (Blakeslee et al., prep).

Carcinus had infection levels ranging from 0-100%, though when we found parasites at a site infection ranged from 64-100%. There was only one site, Sherman Cove, Maine, in which we collected *Carcinus* but did not find any individuals infected by a trematode. This was likely due to the lack of definitive host (personal observations) as without the definite host, the parasite can not sexually reproduce (figure 2). As with *Cancer* I was unable to collect any infected crabs during our second survey (Table 2) which I hypothesize

has to do with a shift in their peak mating season earlier in the summer than previously documented (Berrill, 1982). This shift may have to do with warming waters, driving individuals to seek their deep subtidal habitats where our traps would have been unable to capture them.

I only recovered four *Hemigrapsus* during the first survey infected with a trematode parasite. What is especially interesting about those infections is that the metacercarial cysts were not found in the hepatopancreas, as was the case in infections of both *Cancer* and *Carcinus*, but was instead found in the thoracic ganglia. This seems to indicate, along with my genetic data (objective 2), that this probably represents a different species of trematode, and *Hemigrapsus* may not be its most suitable host, given the low prevalence found (26%) and the fact that we only found infected crabs at one site during our survey.

Addressing the second objective, I was able to use genetic tools to compare trematode sequences collected during my surveys with those collected from crabs in Noa Scotia/ New Brunswick, Newfoundland, and Europe (Table 3). From these sequences I was able to create a phylogenetic tree that identifies five different lineages, four of which were found in crabs collected during my surveys (Figure 10). This demonstrates that the trematode parasite we have been collecting on the WNA and identifying as *M. similis* may not just be *M. similis*. Lineage A does contain the reference sequence of *M. similis* but that leaves three other lineages in the WNA. One, lineage D, grouped with a GenBank sequence of *G. adunca* but what species the other two lineages are is still unknown.

For my third objective was further broken down into three parts, analyzing body conditions of infected and uninfected crabs, conducting individual behavioral trials, and conducting interspecifc competition trials. To address the first part, I compared body condition between crabs collected during the surveys as well as conducted behavioral trials in the lab. There was a trend in both *Cancer* and *Carcinus* that showed elevated HSI in infected crabs compared to uninfected crabs. I saw no difference in GSI for these crabs though these was a slight trend in *Hemigrapsus* having a lower GSI in infected crabs when

compared to uninfected crabs. However, due to the small *Hemigrapsus* sample size more infected crabs would need to be collected to determine the strength of this trend.

When comparing behavior of exposed *Carcinus* and *Hemigrapsus* I did see a slight trends of an increased righting response in both crabs species. In *Carcinus*, while this was only a trend, I do think this is meaningful, as the experimental infection levels in the crabs was very low when compared to the previous study looking at effect of infection on *Carcinus* (Blakeslee et al., 2015). To see any difference between these two groups with a low level of infection is really interesting, and speaks to the stress the crabs may be under during infection. With *Hemigrapsus*, this slower righting response could indicate that the parasite is attempting infection and that there is a cost of infection on these crabs.

Finally, during the competition experiments I was only able to find an effect of infection on the exploratory behaviors of *Carcinus* with infected crabs engaging in these behaviors less than control crabs. The differences I saw where mainly through comparisons between species. These differences included *Carcinus* being more successful at finding and consuming the prey items when compared to *Hemigrapsus*, as well as *Hemigrapsus* instigating more aggressive interactions with exposed *Carcinus*. While these results were not in line with several previous studies (Jensen et al., 2002; DeGraaf and Tyrrell, 2004; Griffen et al., 2008), my findings were similar to those in a 2007 competition study (MacDonald et al., 2007). While I was able to look at differences across species, the objective of these trials was to see how infection affected competition between these introduced crabs. I was unable to see any differences between infected and uninfected crabs, possibly because my experimental infection levels were very low.

Introduced species have and will continue to have detrimental effects on various components of marine habitats (Molnar et al., 2008; Katsanevakis et al., 2014; Laverty et al., 2015). While the field has focused on understanding the success of introduced species in order to prevent future introductions (Canning-Clode, 2015), some key components of a community such as parasites are often overlooked. Parasites, or perhaps more ap-

propriately the lack of parasites, has aided in the success of various non-native species (Lafferty and Morris, 1996; Torchin et al., 2003; Poulin, 2010; Simmons, 2014), but when evaluating the competitive advantages of introduced species the parasites they may or may not be infected with are rarely considered. By understanding which parasites are infecting native and non-native species, the interactions between those species and the advantages or disadvantages parasites might impose on the individuals of a community can be identified.

3.2 Future Directions

In the future I would like to survey sites along the WNA on a more continuous basis during the summer months. This would enable greater detection of any changes in parasite abundance over the course of the summer. I would be able to document the shift in *Carcinus'* mating season if that is what led to the reduced numbers collected during my second survey. I would also like to combine these crab surveys with a survey of upstream gastropod hosts (*L. saxatilis* and *L. obtusata*) at each collection time. This would allow me to identify if the parasite is in the upstream hosts in that area and how prevalence may change over the course of the summer months.

In combination with a more thorough survey, I would like to supplement my current HSI and GSI analyses with additional samples so that we would have close to equal numbers of infected and uninfected individuals. Especially when it comes to *Hemigrapsus*, these numbers are not even and having equal and larger sample sizes could help to determine what trends actually exist between HSI/ GSI and infection / intensity of infection.

To combine genetic techniques with morphological identifications, in the future I would want to perform digestion of these metacercarial cysts to simulate entry into the definitive hosts' stomach. I could then identify the species of trematode through their adult morphologies, which have more distinguishing characteristics than the metacercarial cysts and has been done in previous work (Srisawangwong et al., 1997). If we did this work in addition to the surveys of upstream hosts, we would also be able to identify possible trematodes in this area by their cercariae morphology.

To strengthen this genetic work I would also like to add addition samples from trematodes collected from *Carcinus* in its native European region. I was able to use some samples but only recovered two haplotypes. I suspect if I was able to collect more samples throughout Europe I might have been able to find evidence for whether certain lineages are native to the WNA or were also introduced to the WNA from Europe. In addition

to samples from Europe, samples of trematodes collected from *Hemigrapsus* throughout Asia may help in to understand if Lineage E includes a trematode that was introduced from Asia.

I think conducting these competition and behavioral trials again with either smaller exposure tanks or using snails collected from an area where parasite prevalence is high is necessary. While there were significant differences in the behaviors of the two competing crabs, these behaviors did not seem to be related to exposure to parasites. It is possible that parasites may not alter the interactions between *Carcinus* and *Hemigrapsus*, but achieving higher intensities of infection would allow for a more thorough study of this interaction.

As the goal of my behavioral and competition trials was to see how a trematode parasite may alter the behaviors of its host, the next step would be to look at the gene expression of these crabs post infection to determine if there are genes being differentially expressed between exposed and unexposed crabs. My advisor and I are currently conducting this experiment with a set of crabs that were exposed for 72hrs using the same protocol outlined above. To see if the parasite is altering behaviors we removed the thoracic ganglia from the crabs as this serves as the nerve center and is where I hypothesize differentially expressed genes to be concentrated. We collected tissues from a total of 48 individuals (Table 8) The thoracic ganglia was stored in RNAlater (Sigma Aldrich; Darmstadt, Germany) in a -20 °C freezer.

The RNA was extracted from the tissues using TRIzol (Invitrogen, Carlsbad, CA, USA) following the manufacturers suggested protocol (Simms et al., 1993). At the end of the extraction the DNA pellets were resuspended in 50uL of RNase-free water and stored in a -20 °C freezer overnight. Total RNA was quantified using the RNA BR assay on a Qubit 4.0 fluorometer (Invitrogen, Carlsbad, California) and no more than 4ug of RNA was used to construct a cDNA library for each sample. We removed one sequence from each treatment due to low concentrations of cDNA. To construct these libraries, I used the TruSeq

Treatment	***Species***	***Time Point***	**Replicates**
Unexposed	*Carcinus*	0	8
Exposed	*Carcinus*	0	8
Unexposed	*Hemigrapsus*	0	8
Exposed	*Hemigrapsus*	0	8
Unexposed	*Carcinus*	5 weeks	8
Exposed	*Carcinus*	5 weeks	8

Table 8: Outline of treatments for individuals used in the RNA experiment. Treatment indicates if crab was exposed to parasites or not. Species indicates species of crab used. Time point indicates time since the end of the 72hr the exposure/ control treatments. Replicates indicate how many crabs were used in each treatment

RNA Sample Prep Kit from Illumina (San Diego, California). No in-line control reagents were used and sampled were tagged with one of 20 different adapters for multiplexing.

Libraries were then sent to University of California at Berkley where they were quantified using a Bioanalyzer (Agilent, Santa Clara, California). We decided to remove two samples due to low cDNA concentrations and ran two lanes, with nineteen samples each. The samples were sequenced using an Illumina HiSeq 4000 sequencer. We are currently waiting to receive our data to determine any differences in gene expression due to infection of *Carcinus* and any genomic-level effects of exposure on *Hemigrapsus*. To complete this study exploring how trematodes may alter the gene expression of its host, I think that adding in *Cancer* would help to determine the roles parasites have played in dominance shifts and perhaps will continue to play, especially with the constant movement of species to novel environments (Carlton and Geller, 1993).

Bibliography

Altizer, S., A. Dobson, P. Hosseini, P. Hudson, M. Pascual, and P. Rohani
2006. Seasonality and the dynamics of infectious diseases. *Ecology Letters*, 9(4):467–484.

Anderson, R. M. and R. M. May
1986. The invasion, persistence and spread of infectious diseases within animal and plant communities. *Phil. Trans. R. Soc. Lond. B*, 314(1167):533–570.

Audet, D., D. S. Davis, G. Miron, M. Moriyasu, K. Benhalima, and R. Campbell
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):255–262.

Baltz, D. M. and P. B. Moyle
1993. Invasion resistance to introduced species by a native assemblage of California stream fishes. *Ecological Applications*, 3(2):246–255.

Bauer, O., O. Pugachev, and V. Voronin
2002. Study of parasites and diseases of sturgeons in Russia: a review. *Journal of Applied Ichthyology*, 18(4-6):420–429.

Bax, N., A. Williamson, M. Aguero, E. Gonzalez, and W. Geeves
2003. Marine invasive alien species: a threat to global biodiversity. *Marine policy*, 27(4):313–323.

Bellard, C., P. Cassey, and T. M. Blackburn
2016. Alien species as a driver of recent extinctions. *Biology Letters*, 12(2):20150623.

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):31–39.

Bigford, T. E.
1979. Synopsis of biological data on the rock crab, *Cancer irroratus* (Say).

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

Blakeslee, A. M., I. Altman, A. W. Miller, J. E. Byers, C. E. Hamer, and G. M. Ruiz
2012. Parasites and invasions: a biogeographic examination of parasites and hosts in native and introduced ranges. *Journal of Biogeography*, 39(3):609–622.

Blakeslee, A. M., R. B. Barnard, K. Matheson, and C. McKenzie
in prep. Crabs without borders: the spread of an invasive crab (*Carcinus maenas*) in Newfoundland results in parasite host-switching in a native crab species (*Cancer irroratus*).

Blakeslee, A. M., A. E. Fowler, and C. L. Keogh
2013. Marine invasions and parasite escape: updates and new perspectives. In *Advances in Marine Biology*, volume 66, Pp. 87–169. Elsevier.

Blakeslee, A. M., Y. Kamakura, J. Onufrey, W. Makino, J. Urabe, S. Park, C. L. Keogh, A. W. Miller, M. S. Minton, J. T. Carlton, and O. Miura
2017. Reconstructing the invasion history of the asian shorecrab, *Hemigrapsus sanguineus* (De Haan 1835) in the western Atlantic. *Marine Biology*, 164(3):47.

Blakeslee, A. M., C. L. Keogh, J. E. Byers, A. M. Kuris, K. D. Lafferty, and M. E. Torchin
2009. Differential escape from parasites by two competing introduced crabs. *Marine Ecology Progress Series*, 393:83–96.

Blakeslee, A. M., C. L. Keogh, A. E. Fowler, and B. D. Griffen
2015. Assessing the effects of trematode infection on invasive green crabs in eastern North America. *PloS one*, 10(6):e0128674.

Brattey, J., R. W. Elner, L. S. Uhazy, and A. E. Bagnall
1985. Metazoan parasites and commensals of five crab (brachyura) species from eastern Canada. *Canadian Journal of Zoology*, 63(9):2224–2229.

Budria, A. and U. Candolin
2014. How does human-induced environmental change influence host-parasite interactions? *Parasitology*, 141(4):462–474.

Byers, J. E.
2002. Impact of non-indigenous species on natives enhanced by anthropogenic alteration of selection regimes. *Oikos*, 97(3):449–458.

Canning-Clode, J.
2015. *Biological Invasions in Changing Ecosystems: Vectors, Ecological Impacts, Management and Predictions*. Walter de Gruyter GmbH & Co KG.

Carlton, J. T.
1996. Pattern, process, and prediction in marine invasion ecology. *Biological conservation*, 78(1):97–106.

Carlton, J. T. and A. N. Cohen
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.

Carlton, J. T. and J. B. Geller
1993. Ecological roulette: the global transport of nonindigenous marine organisms. *Science*, 261(5117):78–82.

Chang, A. L., A. M. Blakeslee, A. W. Miller, and G. M. Ruiz
2011. Establishment failure in biological invasions: a case history of *Littorina littorea* in California, USA. *PLoS One*, 6(1):e16035.

Choisy, M., S. P. Brown, K. D. Lafferty, and F. Thomas
2003. Evolution of trophic transmission in parasites: why add intermediate hosts? *The American Naturalist*, 162(2):172–181.

Civitello, D. J., J. Cohen, H. Fatima, N. T. Halstead, J. Liriano, T. A. McMahon, C. N. Ortega, E. L. Sauer, T. Sehgal, S. Young, et al.
2015. Biodiversity inhibits parasites: broad evidence for the dilution effect. *Proceedings of the National Academy of Sciences*, 112(28):8667–8671.

Combes, C., A. Fournier, H. Moné, and A. Théron
1994. Behaviours in trematode cercariae that enhance parasite transmission: patterns and processes. *Parasitology*, 109(S1):S3–S13.

Costa, G., S. Soares, F. Carvalho, and E. Melo-Moreira
2017. A checklist of digenean parasites (Platyhelminthes: Digenea) infecting molluscs and fishes in Portuguese waters (northeast Atlantic). *Museu De Historia Natural do Funchal*, 67(348):25–45.

Czech, B. and P. R. Krausman
1997. Distribution and causation of species endangerment in the United States. *Science*, 277(5329):1116–1117.

Daszak, P., A. A. Cunningham, and A. D. Hyatt
2000. Emerging infectious diseases of wildlife–threats to biodiversity and human health. *Science*, 287(5452):443–449.

DeGraaf, J. D. and M. C. Tyrrell
2004. Comparison of the feeding rates of two introduced crab species, *Carcinus maenas* and *Hemigrapsus sanguineus*, on the blue mussel, *Mytilus edulis*. *Northeastern Naturalist*, 11(2):163–167.

Epifanio, C. E.
2013. Invasion biology of the Asian shore crab *Hemigrapsus sanguineus*: a review. *Journal of Experimental Marine Biology and Ecology*, 441:33–49.

Fofonoff, P., G. Ruiz, B. Steves, A. Hines, and J. Carlton
2003. National exotic marine and estuarine species information system. *Web publication: http://invasions. si. edu/nemesis. Accessed June 2018*.

France, S. C., P. E. Rosel, and J. Ewann
1996. DNA sequence variation of mitochondrial large-subunit rRNA. *Molecular Marine Biology and Biotechnology*, 5(1):15–28.

Fredensborg, B. L., K. N. Mouritsen, and R. Poulin
2005. Impact of trematodes on host survival and population density in the intertidal gastropod *Zeacumantus subcarinatus*. *Marine Ecology Progress Series*, 290:109–117.

Galaktionov, K. V., I. Blasco-Costa, and P. D. Olson
2012. Life cycles, molecular phylogeny and historical biogeography of the Pygmaeus microphallids (Digenea: Microphallidae): widespread parasites of marine and coastal birds in the holarctic. *Parasitology*, 139(10):1346–1360.

Galil, B. S., F. Boero, M. L. Campbell, J. T. Carlton, E. Cook, S. Fraschetti, S. Gollasch, C. L. Hewitt, A. Jelmert, E. Macpherson, et al.
2015. Double trouble: the expansion of the Suez Canal and marine bioinvasions in the Mediterranean Sea. *Biological Invasions*, 17(4):973–976.

Goedknegt, M. A., M. E. Feis, K. M. Wegner, P. C. Luttikhuizen, C. Buschbaum, K. C. Camphuysen, J. Van der Meer, and D. W. Thieltges
2016. Parasites and marine invasions: ecological and evolutionary perspectives. *Journal of Sea Research*, 113:11–27.

Good, T. .
1999. Great Black-Backed Gull (*Larus marinus*) version 2.0. in the Birds of North America (A.F. Poole and F.B. Gill, editors). cornell lab of ornithology.

Griffen, B. D., I. Altman, J. Hurley, and H. Mosblack
2011. Reduced fecundity by one invader in the presence of another: a potential mechanism leading to species replacement. *Journal of Experimental Marine Biology and Ecology*, 406(1-2):6–13.

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

Grosholz, E. D. and G. M. Ruiz
1996. Predicting the impact of introduced marine species: lessons from the multiple invasions of the European green crab *Carcinus maenas*.

Haarr, M. L. and R. Rochette
2012. The effect of geographic origin on interactions between adult invasive green crabs *Carcinus maenas* and juvenile American lobsters *Homarus americanus* in Atlantic Canada. *Journal of Experimental Marine Biology and Ecology*, 422:88–100.

Hansen, E. K. and R. Poulin
2005. Impact of a microphallid trematode on the behaviour and survival of its isopod intermediate host: phylogenetic inheritance? *Parasitology Research*, 97(3):242–246.

Harvell, C., K. Kim, J. Burkholder, R. Colwell, P. R. Epstein, D. Grimes, E. Hofmann, E. Lipp, A. Osterhaus, R. M. Overstreet, et al.
1999. Emerging marine diseases–climate links and anthropogenic factors. *Science*, 285(5433):1505–1510.

Hatcher, M. J., J. T. Dick, and A. M. Dunn
2006. How parasites affect interactions between competitors and predators. *Ecology Letters*, 9(11):1253–1271.

Hoberg, E. P. and D. R. Brooks
2008. A macroevolutionary mosaic: episodic host-switching, geographical colonization and diversification in complex host–parasite systems. *Journal of Biogeography*, 35(9):1533–1550.

Holdich, D. M. and I. D. Reeve
1991. Distribution of freshwater crayfish in the British Isles, with particular reference to crayfish plague, alien introductions and water quality. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 1(2):139–158.

Hudson, P. and J. Greenman
1998. Competition mediated by parasites: biological and theoretical progress. *Trends in Ecology & Evolution*, 13(10):387–390.

Hung, G.-C., N. Chilton, I. Beveridge, X. Zhu, J. Lichtenfels, and R. Gasser
1999. Molecular evidence for cryptic species within *Cylicostephanus minutus* (Nematoda: Strongylidae). *International Journal for Parasitology*, 29(2):285–291.

Hunter, W. S. and W. B. Vernberg
1953. Early stages in the life cycle of the trematode, *Gynaecotyla adunca* (linton, 1905). *Transactions of the American Microscopical Society*, 72(2):163–170.

James, B.
1968. The distribution and keys of species in the family Littorinidae and of their digenean parasites, in the region of Dale, Pembrokeshire. *Field Studies*, 2(5):615–650.

Jensen, G. C., P. S. McDonald, and D. A. Armstrong
2002. East meets west: competitive interactions between green crab *Carcinus maenas*, and native and introduced shore crab *Hemigrapsus* spp. *Marine Ecology Progress Series*, 225:251–262.

Jensen, M. P., C. D. Allen, T. Eguchi, I. P. Bell, E. L. LaCasella, W. A. Hilton, C. A. Hof, and P. H. Dutton
2018. Environmental warming and feminization of one of the largest sea turtle populations in the world. *Current Biology*, 28(1):154–159.

Jones, S. J., F. P. Lima, and D. S. Wethey
2010. Rising environmental temperatures and biogeography: poleward range contraction of the blue mussel, *Mytilus edulis* l., in the western Atlantic. *Journal of Biogeography*, 37(12):2243–2259.

Jungblut, S., J. Beermann, K. Boos, R. Saborowski, and W. Hagen
2017. Population development of the invasive crab *Hemigrapsus sanguineus* (De Haan, 1853) and its potential native competitor *Carcinus maenas* (Linnaeus, 1758) at Helgoland (North Sea) between 2009 and 2014. *Aquatic Invasions*, 12(1):85–96.

Katsanevakis, S., I. Wallentinus, A. Zenetos, E. Leppäkoski, M. E. Çinar, B. Oztürk, M. Grabowski, D. Golani, A. C. Cardoso, et al.
2014. Impacts of invasive alien marine species on ecosystem services and biodiversity: a pan-European review. *Aquatic Invasions*, 9(4):391–423.

Kearse, M., R. Moir, A. Wilson, S. Stones-Havas, M. Cheung, S. Sturrock, S. Buxton, A. Cooper, S. Markowitz, C. Duran, et al.
2012. Geneious basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics*, 28(12):1647–1649.

Kennedy, T. A., S. Naeem, K. M. Howe, J. M. Knops, D. Tilman, and P. Reich
2002. Biodiversity as a barrier to ecological invasion. *Nature*, 417(6889):636.

Keogh, C. L., M. E. Sanderson, and J. E. Byers
2016. Local adaptation to parasite selective pressure: comparing three congeneric co-occurring hosts. *Oecologia*, 180(1):137–147.

Kohler, S. L. and M. J. Wiley
1997. Pathogen outbreaks reveal large-scale effects of competition in stream communities. *Ecology*, 78(7):2164–2176.

Kopp, K. and J. Jokela
2007. Resistant invaders can convey benefits to native species. *Oikos*, 116(2):295–301.

Kroft, K. L. and A. M. Blakeslee
2016. Comparison of parasite diversity in native Panopeid mud crabs and the invasive Asian shore crab in estuaries of northeast North America. *Aquatic Invasions*, 11(3).

Lafferty, K. D.
1999. The evolution of trophic transmission. *Parasitology Today*, 15(3):111–115.

Lafferty, K. D. and A. K. Morris
1996. Altered behavior of parasitized killifish increases susceptibility to predation by bird final hosts. *Ecology*, 77(5):1390–1397.

Lagrue, C.
2017. Impacts of crustacean invasions on parasite dynamics in aquatic ecosystems: a plea for parasite-focused studies. *International Journal for Parasitology: Parasites and Wildlife*.

Laverty, C., W. Nentwig, J. T. Dick, and F. Lucy
2015. Alien aquatics in Europe: assessing the relative environmental and socioeconomic impacts of invasive aquatic macroinvertebrates and other taxa. *Management of Biological Invasions*, 6(4):341–350.

Lefèvre, T., C. Lebarbenchon, M. Gauthier-Clerc, D. Missé, R. Poulin, and F. Thomas
2009. The ecological significance of manipulative parasites. *Trends in Ecology & Evolution*, 24(1):41–48.

Liu, H. and P. Stiling
2006. Testing the enemy release hypothesis: a review and meta-analysis. *Biological invasions*, 8(7):1535–1545.

Lohrer, A. M. and R. B. Whitlatch
2002. Interactions among aliens: apparent replacement of one exotic species by another. *Ecology*, 83(3):719–732.

Lymbery, A. J., M. Morine, H. G. Kanani, S. J. Beatty, and D. L. Morgan
2014. Co-invaders: the effects of alien parasites on native hosts. *International Journal for Parasitology: Parasites and Wildlife*, 3(2):171–177.

MacDonald, J. A., R. Roudez, T. Glover, and J. S. Weis
2007. The invasive green crab and Japanese shore crab: behavioral interactions with a native crab species, the blue crab. *Biological Invasions*, 9(7):837–848.

MacLean, S. A. and C. L. Ruddell
1978. Three new crustacean hosts for the parasitic dinoflagellate *Hematodinium perezi* (Dinoflagellata: Syndinidae). *The Journal of Parasitology*, 64(1):158–160.

MacNeil, C., N. J. Fielding, J. T. Dick, M. Briffa, J. Prenter, M. J. Hatcher, and A. M. Dunn
2003. An acanthocephalan parasite mediates intraguild predation between invasive and native freshwater amphipods (Crustacea). *Freshwater Biology*, 48(12):2085–2093.

McCarthy, H. O., S. Fitzpatrick, and S. W. B. Irwin
2002. Life history and life cycles: production and behavior of trematode cercariae in relation to host exploitation and next-host characteristics. *Journal of Parasitology*, 88(5):910–918.

McManus, D. and J. Bowles
1996. Molecular genetic approaches to parasite identification: their value in diagnostic parasitology and systematics. *International Journal for Parasitology*, 26(7):687–704.

Meißner, K. and A. Bick
1999. Mortality of *Corophium volutator* (Amphipoda) caused by infestation with *Maritrema subdolum* (Digenea, Microphallidae)–laboratory studies. *Diseases of Aquatic Organisms*, 35(1):47–52.

Miura, O., A. M. Kuris, M. E. Torchin, R. F. Hechinger, E. J. Dunham, and S. Chiba
2005. Molecular-genetic analyses reveal cryptic species of trematodes in the intertidal gastropod, *Batillaria cumingi* (Crosse). *International Journal for Parasitology*, 35(7):793–801.

Molnar, J. L., R. L. Gamboa, C. Revenga, and M. D. Spalding
2008. Assessing the global threat of invasive species to marine biodiversity. *Frontiers in Ecology and the Environment*, 6(9):485–492.

Nisbet, I. C. T., D. V. Weseloh, C. E. H. Herbert, M. L. M. Mallory, A. F. P. Poole, J. C. Ellis, P. P. Pyle, and M. A. Pattern
1999. Herring Gull (*Larus argenentatus*) version 3.0. in the Birds of North America 9p.g. rodewald, editor). Cornell Lab of Ornithology.

Nixon, S. W., S. Granger, B. A. Buckley, M. Lamont, and B. Rowell
2004. A one hundred and seventeen year coastal water temperature record from Woods Hole, Massachusetts. *Estuaries*, 27(3):397–404.

Park, T.
1948. Interspecies competition in populations of *Trilobium confusum* Duval and *Trilobium castaneum* Herbst. *Ecological Monographs*, 18(2):265–307.

Pejchar, L. and H. A. Mooney
2009. Invasive species, ecosystem services and human well-being. *Trends in Ecology & Evolution*, 24(9):497–504.

Peterson, B. J., A. M. Fournier, B. T. Furman, and J. M. Carroll
2014. *Hemigrapsus sanguineus* in Long Island salt marshes: experimental evaluation of the interactions between an invasive crab and resident ecosystem engineers. *PeerJ*, 2:e472.

Poulin, R.
2010. Parasite manipulation of host behavior: an update and frequently asked questions. In *Advances in the Study of Behavior*, volume 41, Pp. 151–186. Elsevier.

Prenter, J., C. MacNeil, J. T. Dick, and A. M. Dunn
2004. Roles of parasites in animal invasions. *Trends in Ecology & Evolution*, 19(7):385–390.

R Core Team
2013. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.

Rohde, K. et al.
1982. *Ecology of Marine Parasites.* University of Queensland Press.

Roman, J.
2006. Diluting the founder effect: cryptic invasions expand a marine invader's range. *Proceedings of the Royal Society of London B: Biological Sciences*, 273(1600):2453–2459.

Ronquist, F. and J. P. Huelsenbeck
2003. Mrbayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, 19(12):1572–1574.

Seebens, H., N. Schwartz, P. J. Schupp, and B. Blasius
2016. Predicting the spread of marine species introduced by global shipping. *Proceedings of the National Academy of Sciences*, 113(20):5646–5651.

Simmons, K. R.
2014. Evidence of the enemy release hypothesis: parasites of the lionfish complex (*Pterios volitans* and *P. miles*) in the western North Atlantic, Gulf of Mexico, and Caribbean Sea.

Simms, D., P. E. Cizdziel, and P. Chomczynski
1993. Trizol: A new reagent for optimal single-step isolation of RNA. *Focus*, 15(4):532–535.

Srisawangwong, T., P. Sithithaworn, and S. Tesana
1997. Metacercariae isolated from cyprinoid fishes in Khon Kaen district by digestion technique. *Southeast Asian journal of tropical medicine and public health*, 28:224–226.

Stachowicz, J. J., H. Fried, R. W. Osman, and R. B. Whitlatch
2002. Biodiversity, invasion resistance, and marine ecosystem function: reconciling pattern and process. *Ecology*, 83(9):2575–2590.

Stachowicz, J. J., R. B. Whitlatch, and R. W. Osman
1999. Species diversity and invasion resistance in a marine ecosystem. *Science*, 286(5444):1577–1579.

Stunkard, H. W.
1983. The Marine Cercariae of the Woods Hole, Massachusetts region, a review and a revision. *The Biological Bulletin*, 164(2):143–162.

Thieltges, D. W., K. Reise, K. Prinz, and K. T. Jensen
2009. Invaders interfere with native parasite–host interactions. *Biological Invasions*, 11(6):1421.

Thomas, F., F. Renaud, F. Rousset, F. Cézilly, and T. De Meeus
1995. Differential mortality of two closely related host species induced by one parasite. In *Proceedings from the Royal Society*, volume 260, Pp. 349–352. The Royal Society.

Torchin, M. E., J. E. Byers, and T. C. Huspeni
2005. Differential parasitism of native and introduced snails: replacement of a parasite fauna. *Biological Invasions*, 7(6):885–894.

Torchin, M. E., K. D. Lafferty, A. P. Dobson, V. J. McKenzie, and A. M. Kuris
2003. Introduced species and their missing parasites. *Nature*, 421(6923):628.

Torchin, M. E., K. D. Lafferty, and A. M. Kuris
2001. Release from parasites as natural enemies: increased performance of a globally introduced marine crab. *Biological Invasions*, 3(4):333–345.

Tsang, L. M., C. D. Schubart, S. T. Ahyong, J. C. Lai, E. Y. Au, T.-Y. Chan, P. K. Ng, and K. H. Chu
2014. Evolutionary history of true crabs (Crustacea: Decapoda: Brachyura) and the origin of freshwater crabs. *Molecular Biology and Evolution*, 31(5):1173–1187.

Van Buskirk, J., R. S. Mulvihill, and R. C. Leberman
2009. Variable shifts in spring and autumn migration phenology in North American songbirds associated with climate change. *Global Change Biology*, 15(3):760–771.

Van Buskirk, J. and R. S. Ostfeld
1995. Controlling Lyme disease by modifying the density and species composition of tick hosts. *Ecological Applications*, 5(4):1133–1140.

Williams, A. B.
1984. Shrimps, lobsters, and crabs of the atlantic coast of the eastern United States, Maine to Florida.

Wood, C. L., J. E. Byers, K. L. Cottingham, I. Altman, M. J. Donahue, and A. M. Blakeslee
2007. Parasites alter community structure. *Proceedings of the National Academy of Sciences*, 104(22):9335–9339.

Zetlmeisl, C., J. Hermann, T. Petney, H. Glenner, C. Griffiths, and H. Taraschewski
2011. Parasites of the shore crab *Carcinus maenas* (L.): implications for reproductive potential and invasion success. *Parasitology*, 138(3):394–401.

4 Appendix

	Newfoundland										Canada				United States																				Europe		
	Placentia Bay							Conception Bay			STA	GUN	YRM	CMB	OIME			SCME			JEL			OSPNH			RHNH			SLMA			GMA			CC			NOIZ	Total	Haplotype
Haplotype	FH(CI)	FH(CM)	FOX(CI)	FOX(CM)	NH(CI)	NH(CM)	NH(LO)	HM(CI)	HLY(CI)	FT(CI)	CM	CM	CM	CM	CM	CI	HS	CM	CI	HS	CM	CI	HS	CM	CI	HS	CM	CI	HS	CM	CI	HS	CM	CI	HS	CM	CI	HS	CM/Snails	Individuals	Frequency
1	2	1	2	8	2	5	2	5	4	5			4		1			3			2	2									2	1				5			12	68	0.531
2	8				2			1							1																	1							13	0.102
3																																							3	3	0.023
4														1	2						2									1			4							10	0.078
5						2																																		2	0.016
6	3	1																																						4	0.031
7												1	1		1																									3	0.023
8		1																																						1	0.008
9											1		1		1												1													4	0.031
10											3																													3	0.023
11									2																															2	0.016
12						3																																		3	0.023
13																																							2	2	0.016
14				2																																				2	0.016
15																						1																			1	0.008
16																					1									1											2	0.016
17																																2									2	0.016
18																																								1	1	0.008
19				1																																				1	0.008
20																						1																			1	0.008
Total	13	3	2	11	4	10	2	6	6	5	4	1	6	1	6	0	0	3	0	0	5	4	0	0	0	0	1	0	0	4	1	2	5	0	0	5	0	0	18		

Figure 25: Haplotypes identified in each collection area and color coded by identified lineage: Blue= Lineage A, Gray= Lineage B, Purple=Lineage C,Red= Lineage D, Green= Lineage E. Numbers indicate unique individuals from that area. Species are abbreviated as follows: CI= *Cancer irroratus*, CM= *Carcinus maenas*, HS=*Hemigrapsus sanguineus*. Site labels are as follows: FH=Fair Haven, FOX=Fox Harbour, NH= North Harbour, HM= Harbor Main, HLY=Holyrood, FT=FoxTrap, STA= St. Andrew's, GUN= Gunning Cove, YRM= Yarmouth, CMB= Two Rivers, OIME= Orr Island, Maine, SCME= Sherman Cove, Maine, JEL= Jackson Estuarine Laboratory, Durham, New Hampshire, OSPNH= Odiorne State Park, New Hampshire, RHNH= Rye Harbor, New Hampshire, SLMA= Scituate Lighthouse, Massachusetts, GMA= Gloucester, Massachusetts, CC= Woods Hole, Massachusetts, NOIZ= Texel, Netherlands.

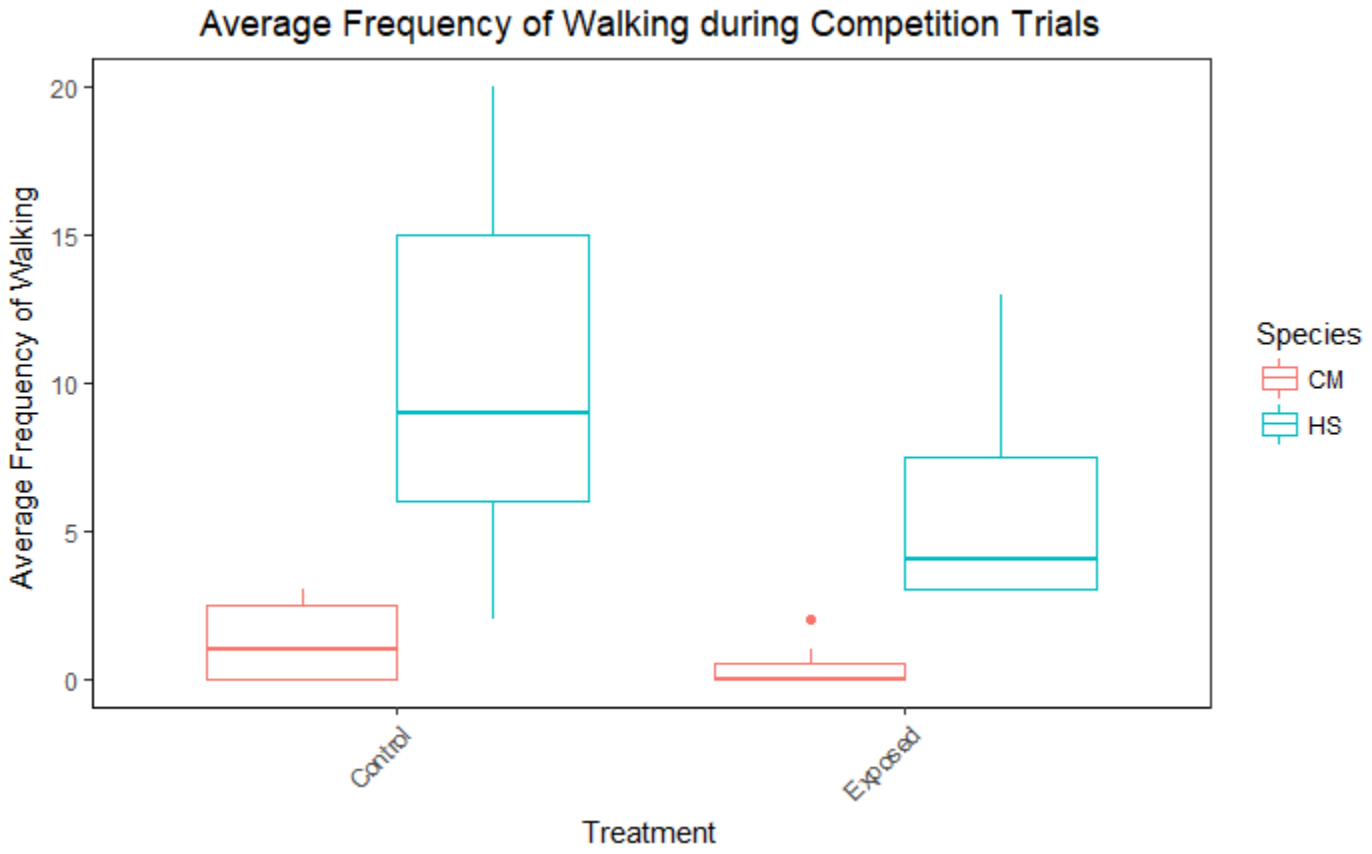

Figure 26: Frequency of a given crab walking around the tank shown by treatment. *Carcinus* are shown in red (CM) and *Hemigrapsus* are shown in blue (HS).

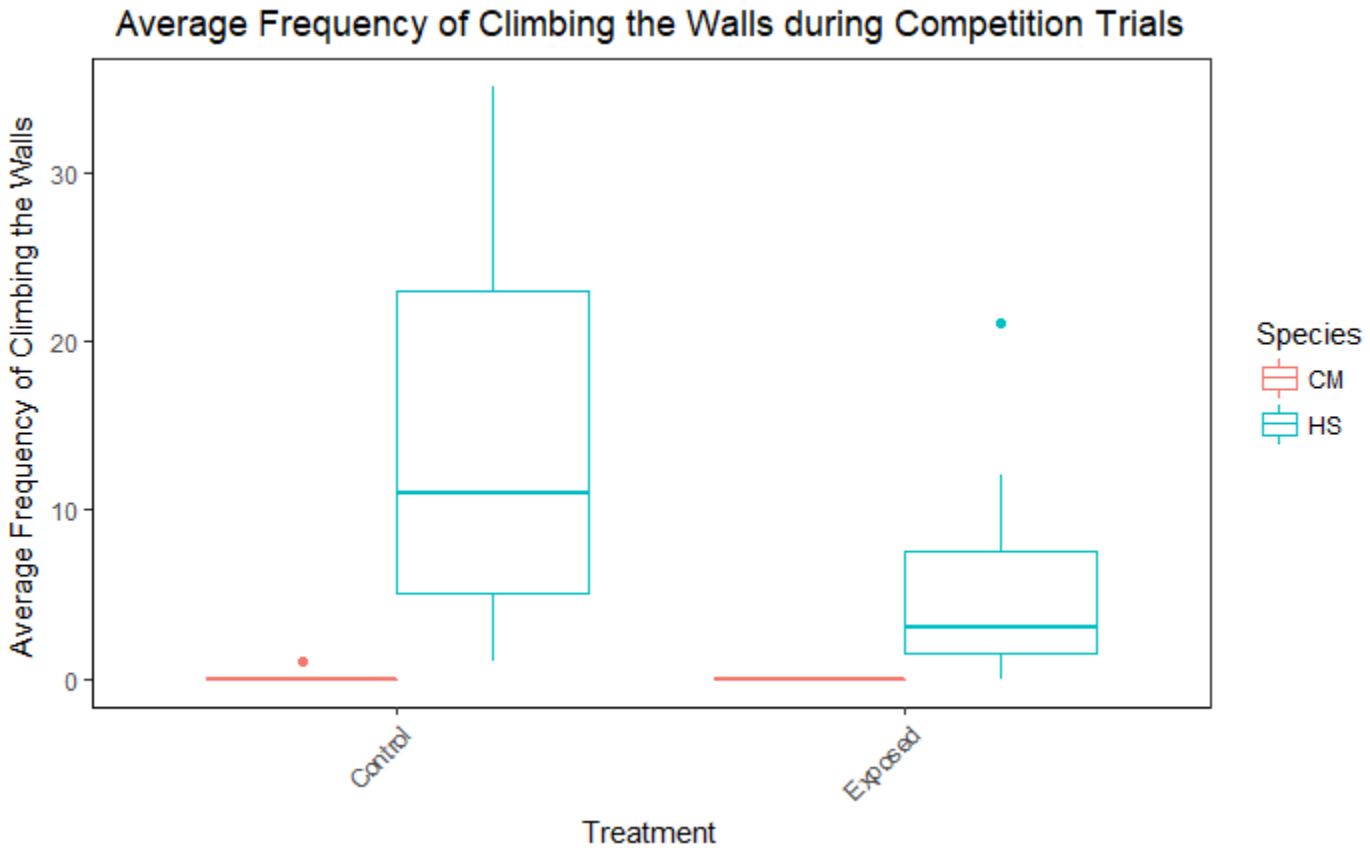

Figure 27: Frequency of a crab climbing the walls of the trial tank behaviors shown by treatment. *Carcinus* are shown in red (CM) and *Hemigrapsus* are shown in blue (HS).

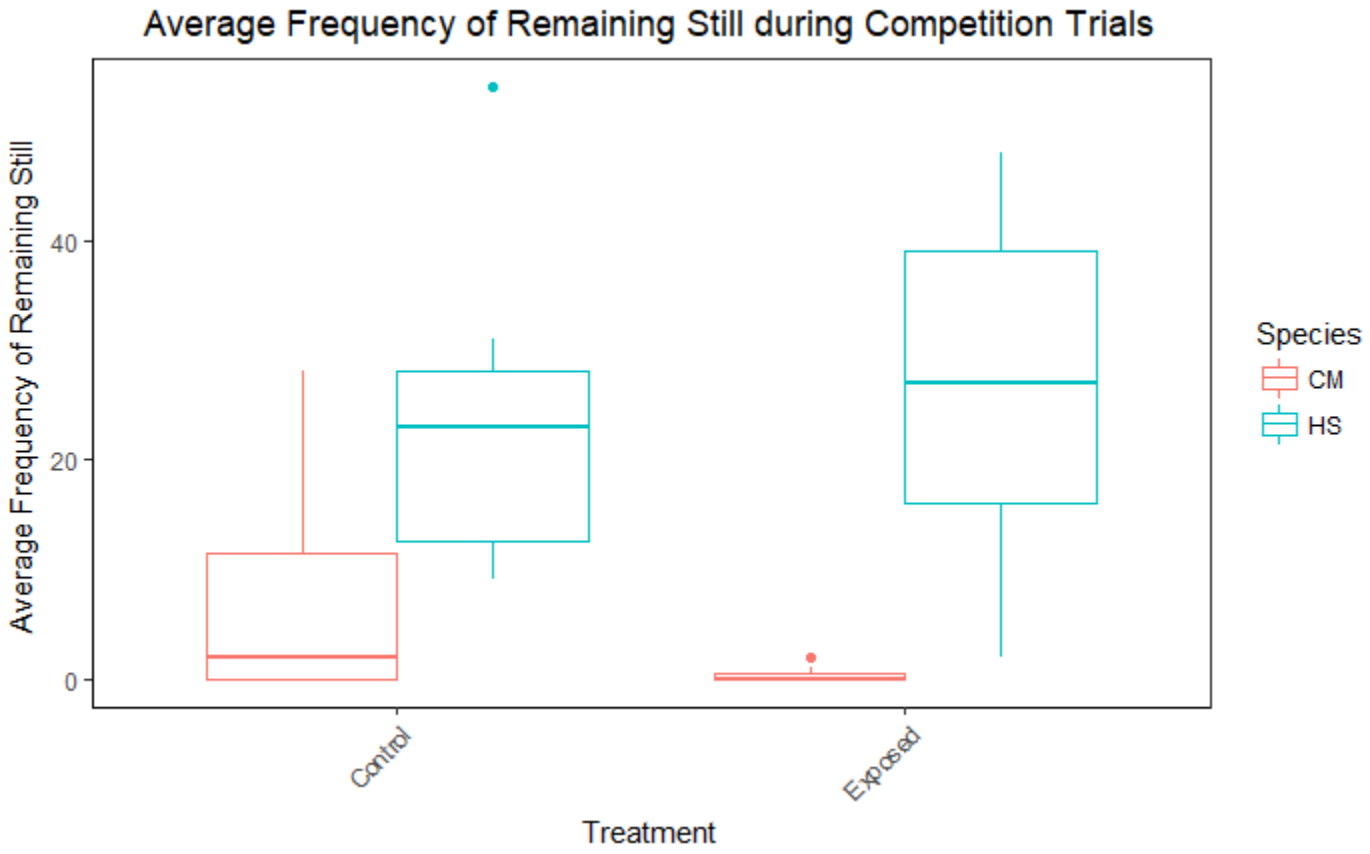

Figure 28: Frequency of a crab remaining still during a trial shown by treatment. *Carcinus* are shown in red (CM) and *Hemigrapsus* are shown in blue (HS).

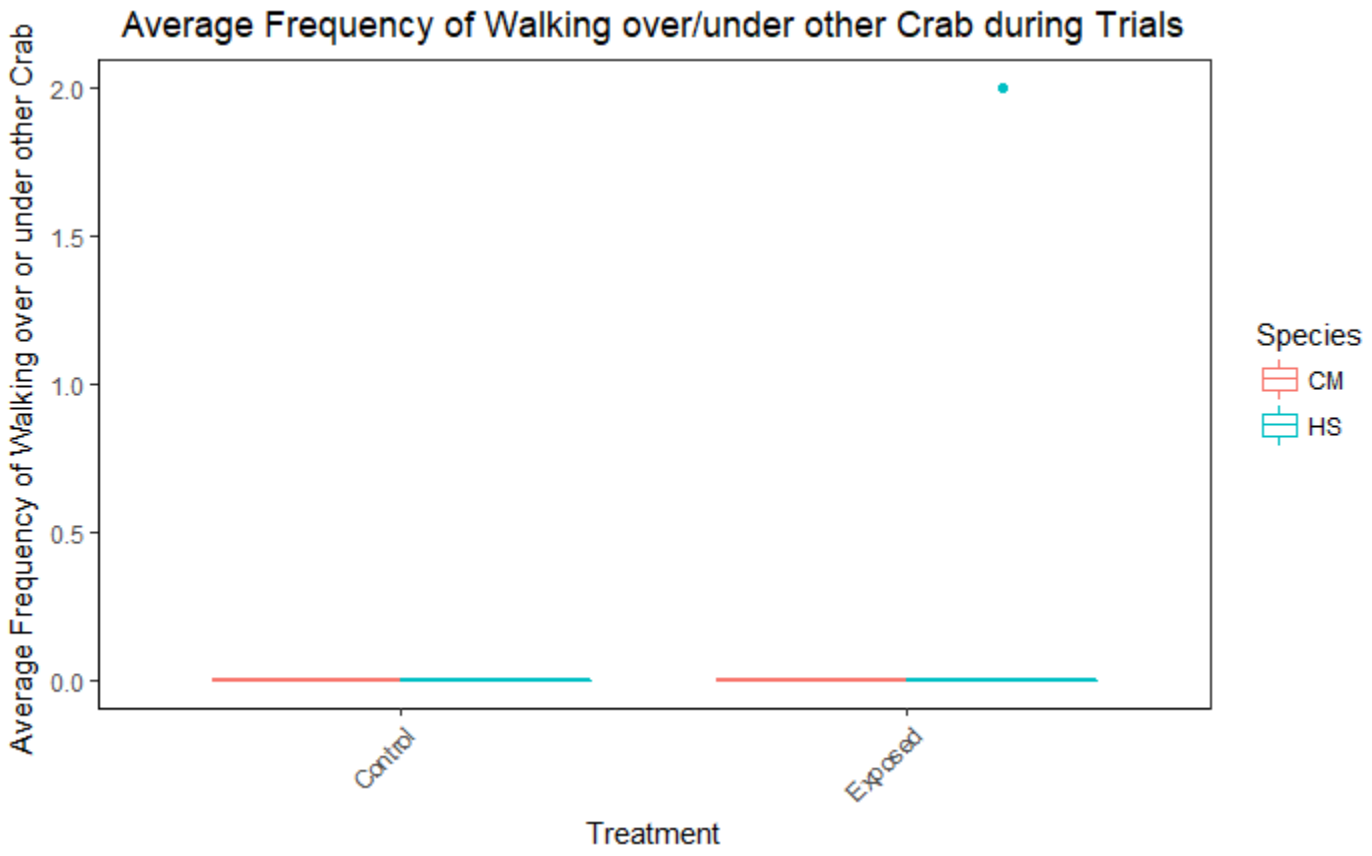

Figure 29: Frequency of one crab walking under or over the other crab without any accompanying display of aggression shown by treatment. *Carcinus* are shown in red (CM) and *Hemigrapsus* are shown in blue (HS).

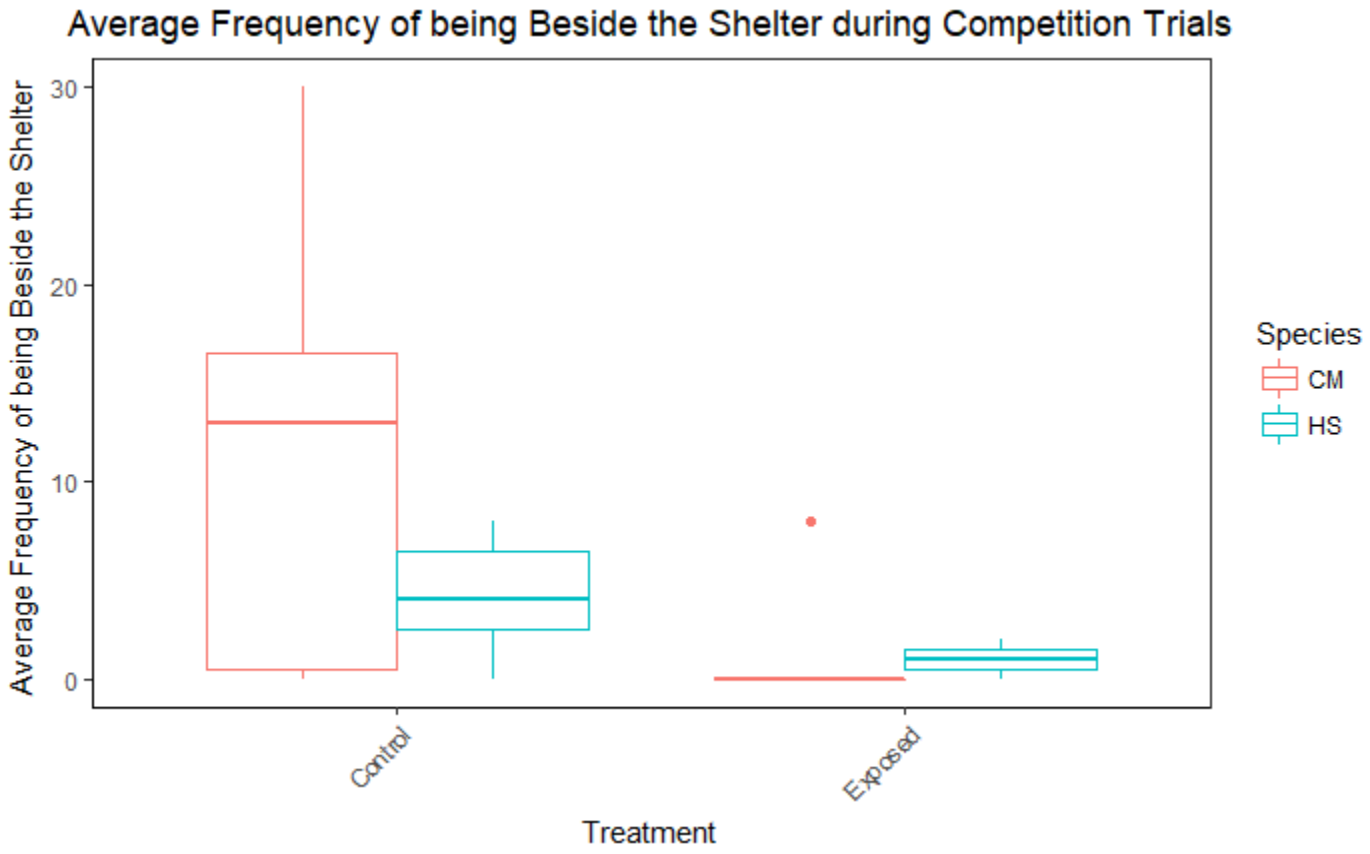

Figure 30: Frequency of a crab remaining beside the shelter during a trial, shown by treatment. *Carcinus* are shown in red (CM) and *Hemigrapsus* are shown in blue (HS).

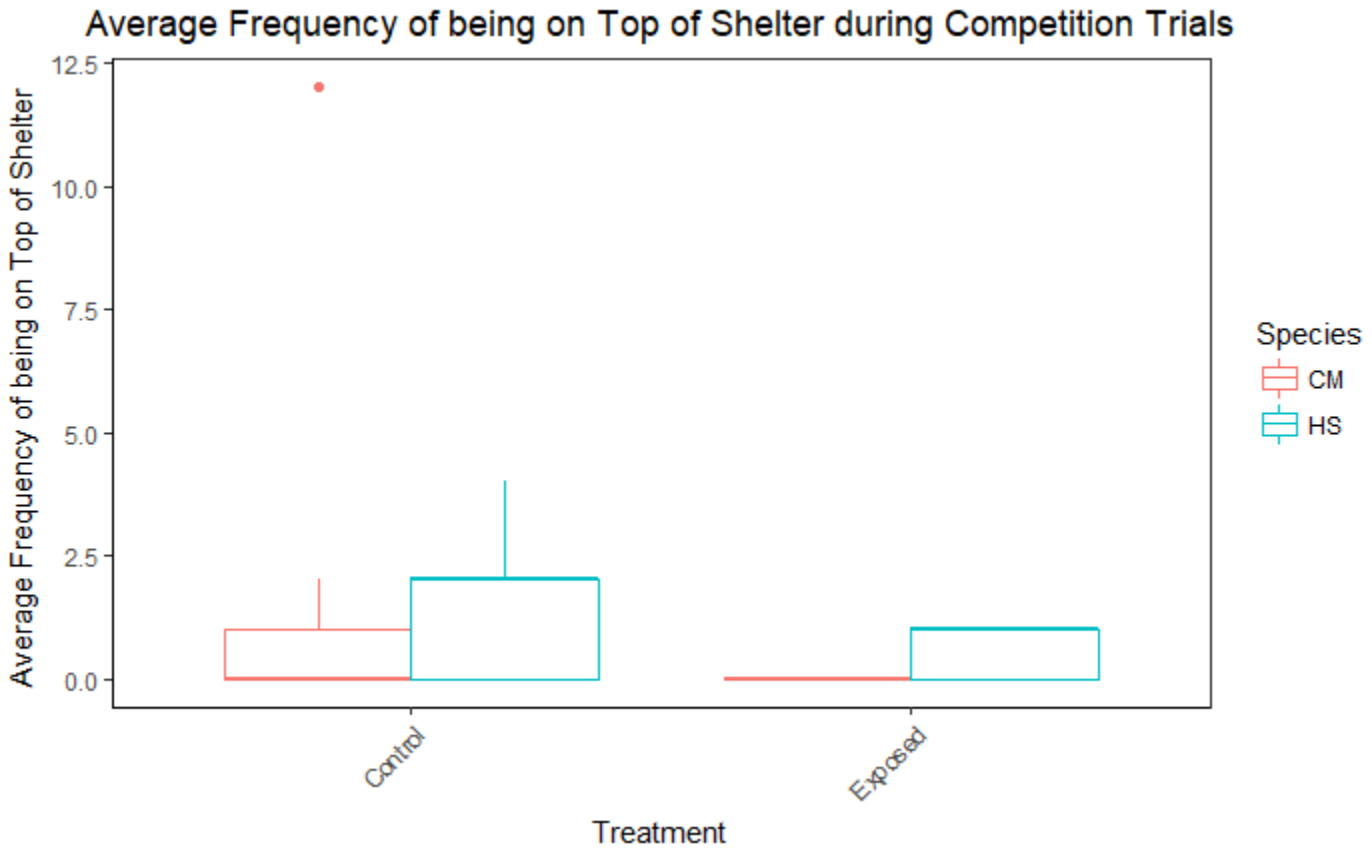

Figure 31: Frequency of a crab remaining on top of the shelter during a trial, shown by treatment. *Carcinus* are shown in red (CM) and *Hemigrapsus* are shown in blue (HS).

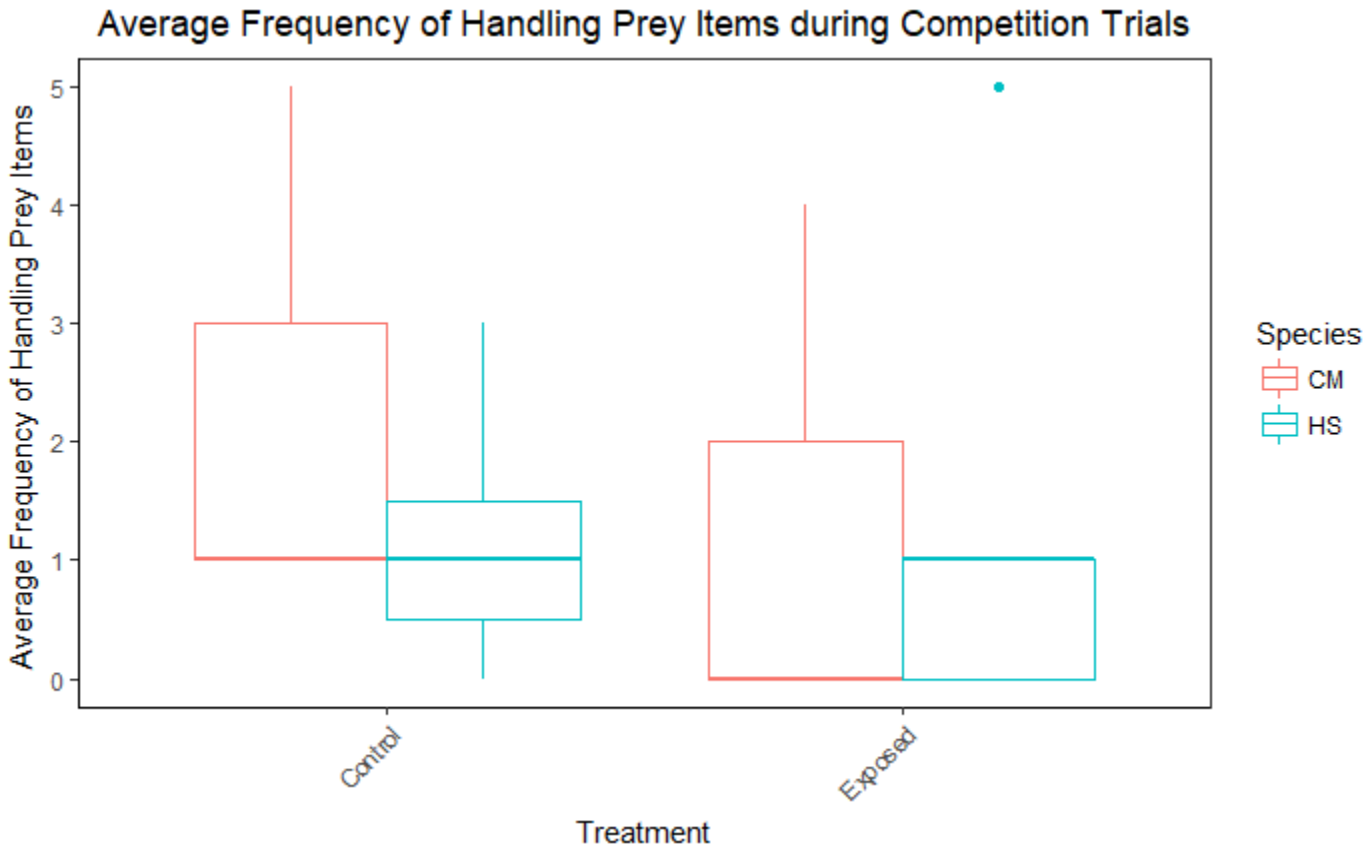

Figure 32: Frequency of a crab handling a prey items during a trial, shown by treatment. *Carcinus* are shown in red (CM) and *Hemigrapsus* are shown in blue (HS).

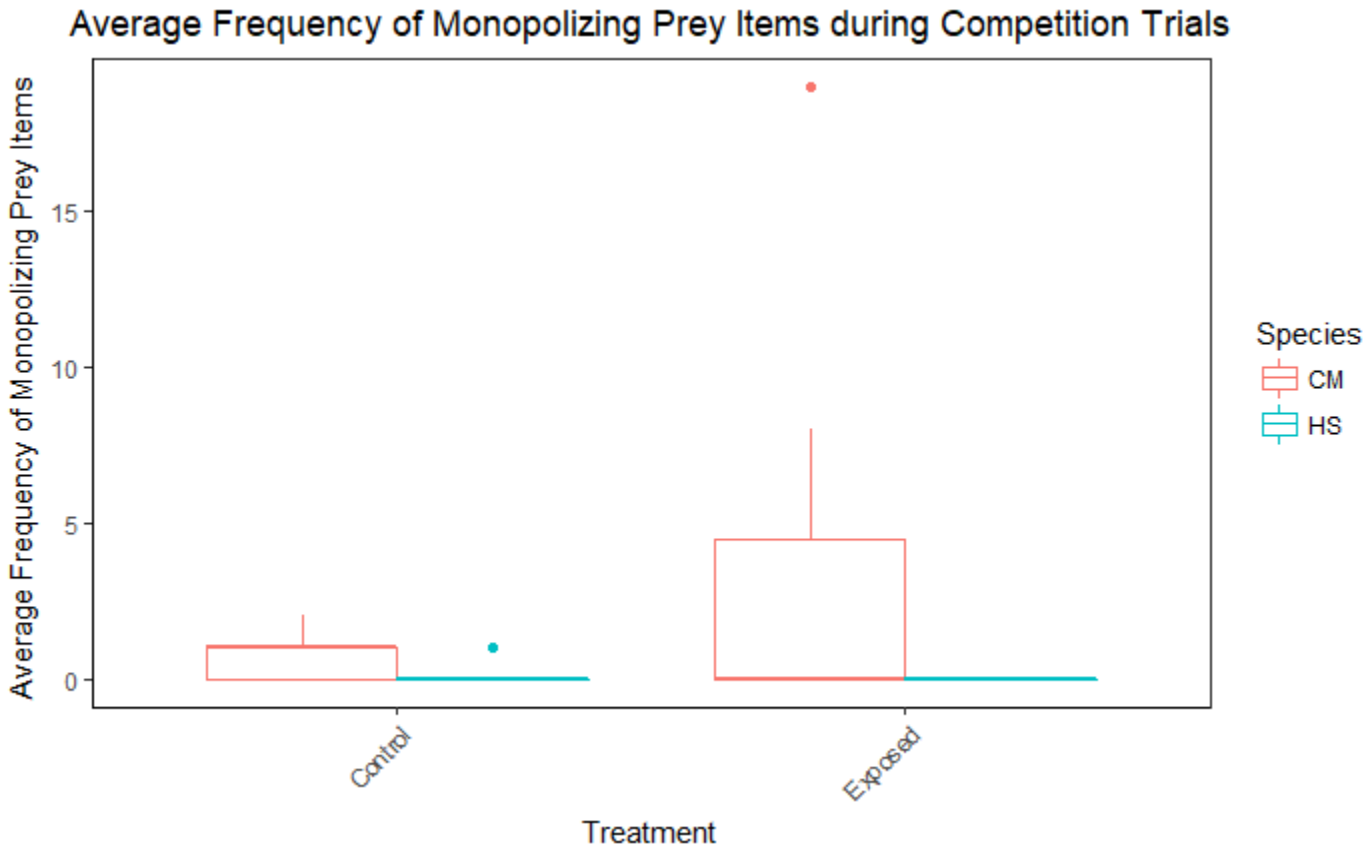

Figure 33: Frequency of a crab monopolizing a prey item during a trial, shown by treatment. *Carcinus* are shown in red (CM) and *Hemigrapsus* are shown in blue (HS).

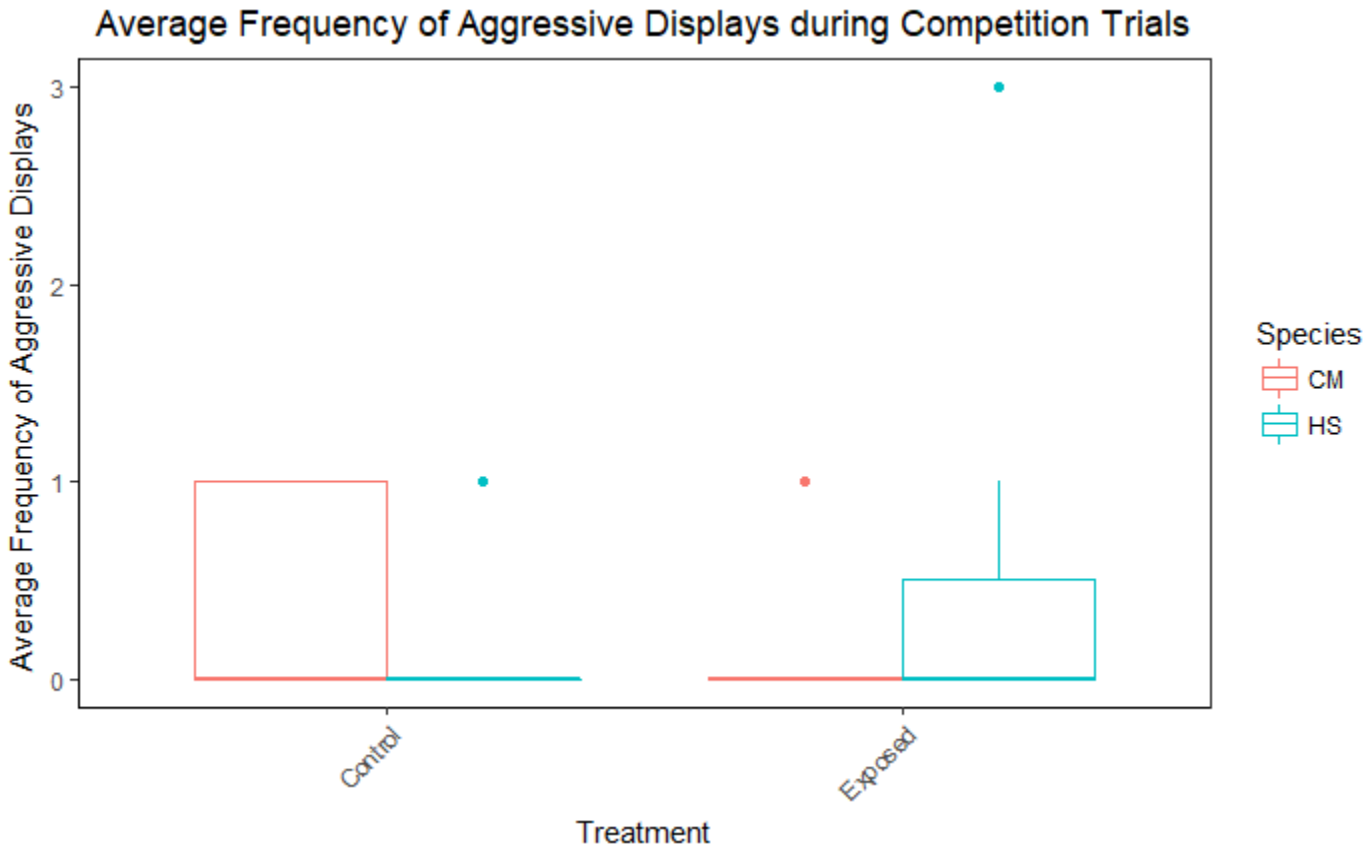

Figure 34: Frequency of a crab engaging in aggressive displays during a trial, shown by treatment. *Carcinus* are shown in red (CM) and *Hemigrapsus* are shown in blue (HS).

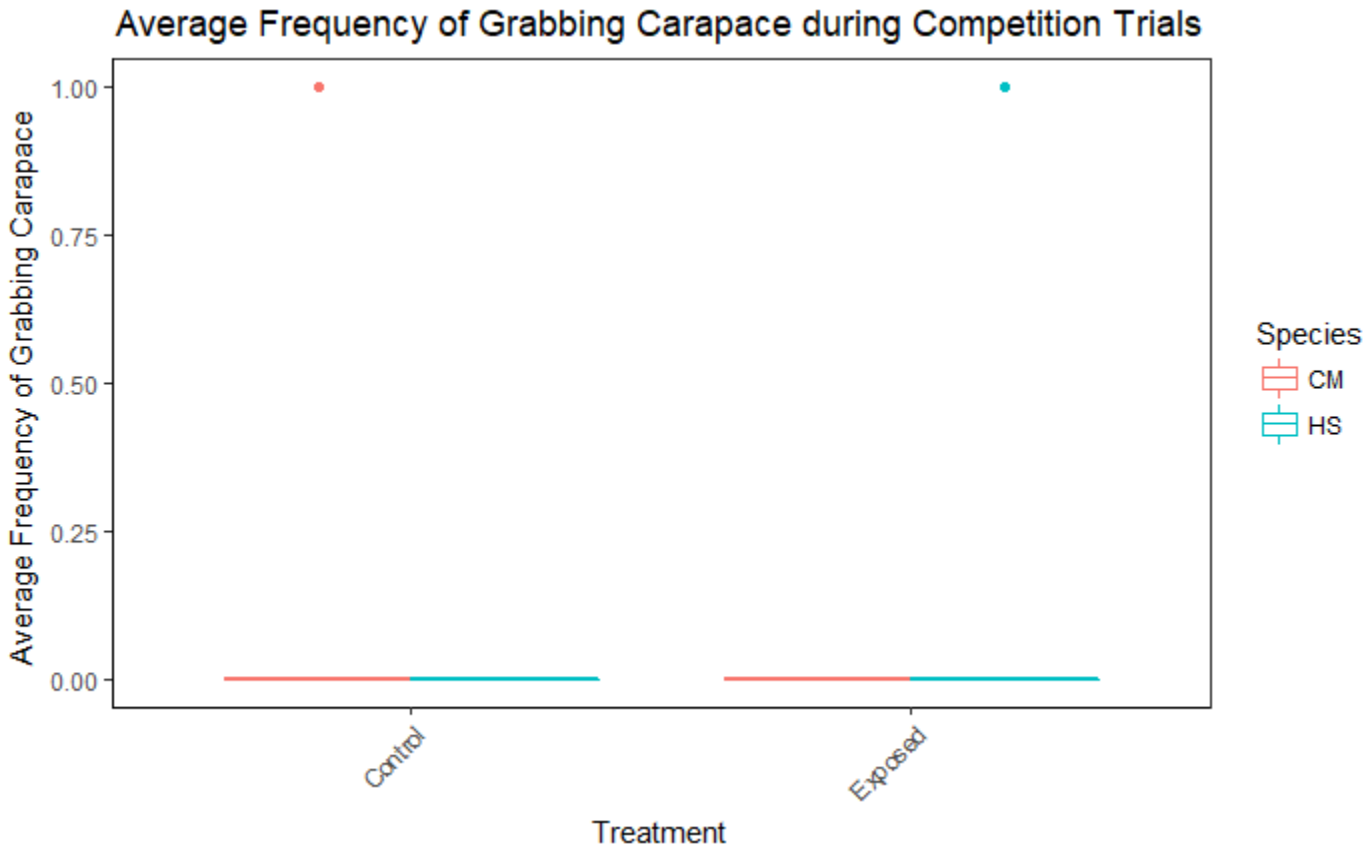

Figure 35: Frequency of a crab grabbing at the other crab's carapace during a trial, shown by treatment. *Carcinus* are shown in red (CM) and *Hemigrapsus* are shown in blue (HS).

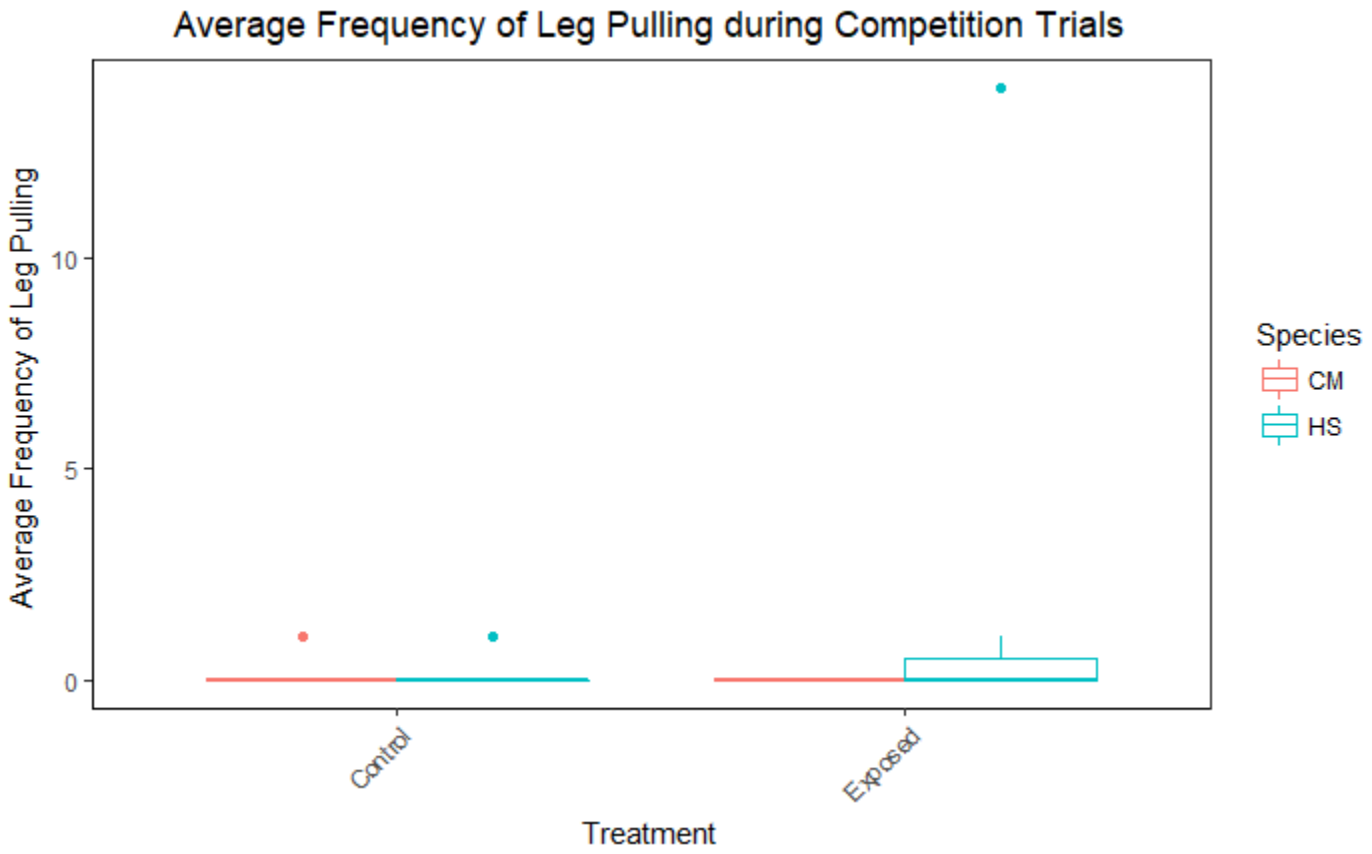

Figure 36: Frequency of a crab grabbing at the other crab's walking legs during a trial, shown by treatment. *Carcinus* are shown in red (CM) and *Hemigrapsus* are shown in blue (HS).

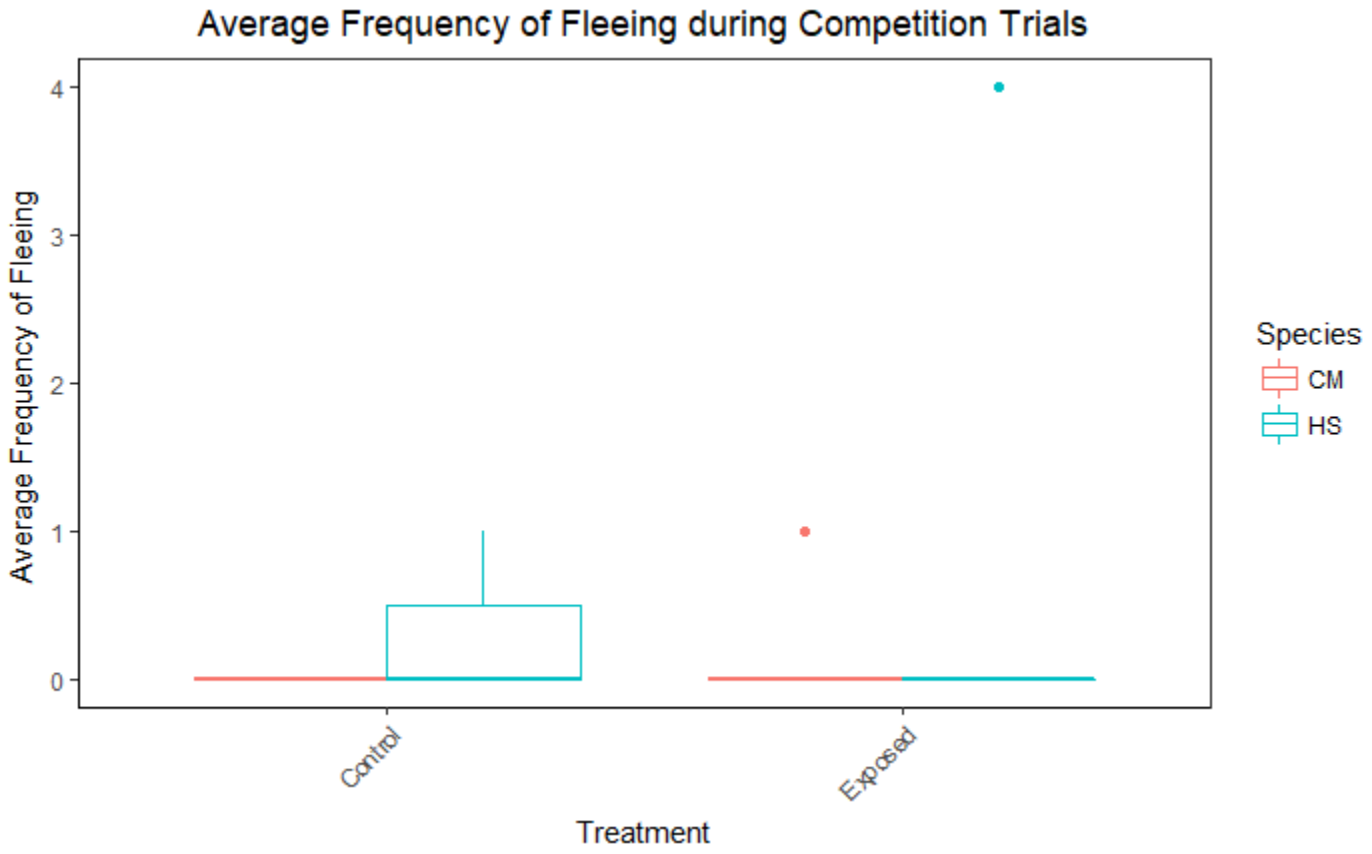

Figure 37: Frequency of a crab fleeing from the other crab during a trial, shown by treatment. *Carcinus* are shown in red (CM) and *Hemigrapsus* are shown in blue (HS).