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The effect of parasitism and predation on phenotypically plastic traits of the marine gastropod *Tritia obsoleta*

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Mica McCarty-Glenn

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Abstract of the Dissertation

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Interactions among species have important influences on the structure and function of the communities in which they reside. Much is known about interactions involving two species, but little is known about the potential synergistic or antagonistic effects when a species is confronted with multiple types of interactions. Organisms may respond to species interactions through phenotypic plasticity, where the same genotype can produce different phenotypes depending on the environment. Many aquatic gastropods are known to have phenotypically plastic behavior and shell morphology in response to two common interactions, parasitism and predation. However, few studies have examined the synergistic effects of both predation and parasitism on gastropod plasticity. This dissertation uses the marine snail *Tritia (Ilyanassa) obsoleta* to answer the following questions: Do predators and parasites alter the feeding behavior of *T. obsoleta*? Do parasites alter the antipredator behavior of their gastropod host? Do parasites and predators alter the shell morphology of *T. obsoleta*? Do juvenile and adult *T. obsoleta* respond similarly to risk of predation? I found that neither predators nor parasites altered the feeding rates of juvenile or adult *T. obsoleta*. Adult snails did exhibit antipredator behaviors when exposed to risk of predation, but juvenile snails did not. Generally, parasitized snails exhibited the same antipredator behaviors as unparasitized individuals, but snails infected with certain species of parasites altered their behavior in both the laboratory and in the field. Although snails from different sites had different shell morphologies, long-term exposure to risk of predation did not alter shell morphology, but gastropods infected with certain parasite species did have different shell morphologies than unparasitized snails. There appeared to be no interaction between parasitism and predation with regards to feeding behavior, antipredator behavior, or shell morphology, which was counter to my predictions. The lack of synergism is probably due to few impacts of either predation or parasitism separately on *T. obsoleta* phenotypes, which is counter to results in other gastropods. *Tritia obsoleta* exhibits both thick shells and high density population, which both decrease predation risk and may explain lack of its responses to predators.

I dedicate this to my parents, Mark and Tricia.

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Chapter 1

Introduction

Biotic interactions have large impacts on community structure, species coexistence, and biodiversity (e.g., Stachowicz 2001, Wardle 2006, Gross et al. 2009). The impact of each interaction depends on the distribution of the species, various abiotic factors and, of course, the type of interaction. Increasing the number of interactions that a species is involved in increases the number of possible outcomes for any ecological process of interest (Hatcher et al. 2006), and multiple biotic interactions may have synergistic, or even antagonistic, rather than simply additive effects. Synergistic and antagonistic effects of species interactions have been observed in systems that involve predation and competition (e.g., Holbrook & Schmitt 2002, Hixon & Jones 2005, Calsbeek & Cox 2010), predation and parasitism (e.g., Johnson et al. 2006, Hesse et al. 2012), and competition and parasitism (e.g., Chapman et al. 2006, Kolluru et al. 2008).

Phenotypic plasticity, defined in this dissertation as "the environmentally contingent expression of phenotypes" (Bourdeau et al. 2015), is one way organisms are able to cope with variability in species interactions (Agrawal 2001, Miner et al. 2005). Many organisms are known to have plastic phenotypes when exposed to competition (e.g., Kurashige & Agrawal 2005, Allen et al. 2008, Stomp et al. 2008), predation (e.g., Van Buskirk & Schmidt 2000, Peluc et al. 2008, Scoville & Pfrender 2010), and parasitism (e.g., Chadwick & Little 2005, Vizoso & Ebert 2005, Singer et al. 2009). Moreover, many empirical and theoretical studies have shown that the effects of predation and parasitism are nonadditive. For example, juvenile long-toed salamanders subjected to both intraspecific predation and trematode infection have four times the

amount of limb malformation than those only exposed to parasites (Johnson et al. 2006). Also, *Daphnia* (Cladocera) exposed to both parasites and to cues from predatory fish are smaller and produce smaller offspring than when exposed to either separately (Hesse et al. 2012).

Many aquatic gastropods are known to be phenotypically plastic, and can alter their behavior and shell morphology in response to both predation (e.g., Richardson & Brown 1992, Trussell et al. 2003, Bourdeau 2009) and parasitism (e.g., Levri et al. 2005, Kamiya & Poulin 2012, O'Dwyer et al. 2014). However, little is known about how snails respond when exposed to both types of species interactions simultaneously (Table 1-1). In order to examine the effects of parasitism and predation on phenotypic plasticity, hereafter referred to as plasticity, I used an aquatic gastropod, *Tritia obsoleta*, as a study system. *Tritia obsoleta* (Say, 1822), formerly *Ilyanassa obsoleta* (Galindo et al. 2016), is a marine gastropod that is common in the intertidal zone on the East Coast of North America from Nova Scotia to northern Florida (Abbot & Morris 1995). They are usually found on mudflats or sandy/ cobble beaches where they are scavengers as well as consuming algae and detritus (Curtis & Hurd 1979, Feller 1984). These snails are often found in dense populations (often greater than 500 snails per square meter), and can have a large impact on their community by altering the abundance of algae in sediments, and affecting the distribution of other benthic invertebrates (Connor et. al 1982, Kelaher et. al 2003). *Tritia obsoleta* is the first intermediate host of nine trematode species: *Austrobilharzia variglandis* (Miller & Northup 1926), *Diplostomum nassa* (Martin 1945), *Gynaecotyla adunca* (Linton 1905), *Himasthla quissetensis* (Miller & Northup 1926), *Lepocreadium setiferoides* (Miller & Northup 1926), *Pleurogonius malaclemys* (Hunter 1961), *Stephanostomum dentatum* (Linton 1900), *Stephanostomum tenue* (Linton 1898), and *Zoogonus lasius* (Leidy 1891, reviewed in Blakeslee et al. 2012, Phelan et al. 2016), and is preyed on by crabs, seastars, and terrapins

(Stenzler & Atema 1977, Peterson 1979, Tucker et al. 1997). Little is known about how predation and parasitism can influence the behavior of *T. obsoleta*, and no studies have examined how predation or parasitism can alter their shell morphology.

Table 1-1. Interactions between both predation and parasitism in gastropods.

Gastropod Prey Species	**Predator Species**	**Parasite Species**	**Interaction**	**Citation**
Nucella lapillus	Crab *Carcinus maenas*	Polychaete *Polydora* sp.	Parasitized snails more susceptible to predation, move slower, have decreased survival	Fisher 2010
Zeacumantus subcarinatus	Crab *Hemigrapsus sexdentatus*	Trematode *Maritrema novaezealandensis* and *Philophthalmus* sp.	Decreased time to display antipredator behavior Significantly delayed antipredator behavior	Kamiya & Poulin 2012
Physa integra	Fish *Semotilus atromaculatus* Crayfish *Orconectes nais*	Trematode *Paramphistomidae* sp. *Cathaemasiidae* sp.	Infected snails less likely to engage in refuge seeking behavior	Bernot 2003
Potamopyrgus antipodarum	Fish *Gobiomorphus coidianus*	Trematode *Microphallus* sp.	Infected Snails less likely to engage in refuge seeking behavior	Levri 1998b
			Parasite-mediated behavior makes snails (and parasites) less likely to be eaten by unsuitable hosts	Levri 1998a

Although little is known about the influence of predation and parasitism on *T. obsoleta* morphology, these species interactions are generally known to affect shell morphology in many other snail species. Snails often produce an altered shell shape in the presence of predators

(Appleton & Palmer 1988, Krist 2002, Lakowitz et al. 2008, Bourdeau 2009). These shell shapes usually differ depending on the predator that the snail was exposed to, and help to protect the gastropod from being consumed by specific predators (Johannesson 1986, Bourdeau 2009). Parasitism can also result in altered snail shell morphology in various ways (McCarthy et al. 2004, Levri et al. 2005, Hay et al. 2005, Żbikowska & Żbikowski 2005, Thieltges et al. 2009), including an increased volume of the apical whorls where the parasites reside (Thieltges et al. 2009).

Gastropods can also alter their behavior in response to increased risk of predation by decreasing their feeding rates (Levri & Lively 1996, Trussell et al. 2003, Bourdeau 2009, Hooks & Padilla 2014), altering their spatial distribution (Vermeij 1972, McQuaid 1982), or by exhibiting antipredator behaviors, such as crawling out of water (Alexander & Covich 1991, Turner et al. 2000, Dalesman et al. 2006, Klose 2011) or burrowing into sediment (Phillips 1977, McCarthy & Fisher 2000). Parasitism can also affect host feeding rates (Bernot & Lamberti 2008, Wood et al. 2007) and alter host spatial distributions (Curtis 1987, Miller & Poulin 2001, McCarthy et al. 2000, Miura et al. 2006, Wesolowska & Wesolowska 2014), which usually results in parasitized snails high on the shore and out of the water (known as crawl-out behavior).

Phenotypic plasticity in response to species interactions can change through ontogeny because individuals are typically more vulnerable to different types of interactions at different life stages (Dangles et al. 2007, Landberg & Azizi 2010, Hopkins et al. 2011). Juvenile snails have smaller, thinner shells and are thought to be more vulnerable to predation than adults (Vermeij 1972, Alexander & Covich 1991). Also, juvenile *T. obsoleta* do not have mature gonads, so they are not infected by trematode parasites, but once they reach sexual maturity, they become susceptible to parasitism (Scheltema 1964). Adult gastropods can be infected by

parasite trematodes and can be consumed by predators. Although predation risk depends on size and parasitism risk depends on maturity, they are often linked in gastropods.

Based on studies of phenotypic plasticity of other gastropod species in response to predation and parasitism mentioned above, I generated predictions about how predation and parasitism would impact adult and juvenile *T. obsoleta*. I predicted that both parasitism and predation would alter the morphology of *T. obsoleta*, and that different species of parasites would affect morphology differently. I also hypothesized that *T. obsoleta* would decrease feeding rates and exhibit antipredator behaviors when exposed to chemical cues of predators, and that parasites would alter feeding rates, antipredator behavior, and crawl-out movement of *T. obsoleta*, which would also likely differ by parasite species. Finally, I predicted that both adults and juveniles would exhibit behavioral and morphological responses to predation, but that the magnitude of the responses would be stronger in juveniles. Since juveniles do not have parasites, only adults would be affected by parasitism (Fig. 1-1).

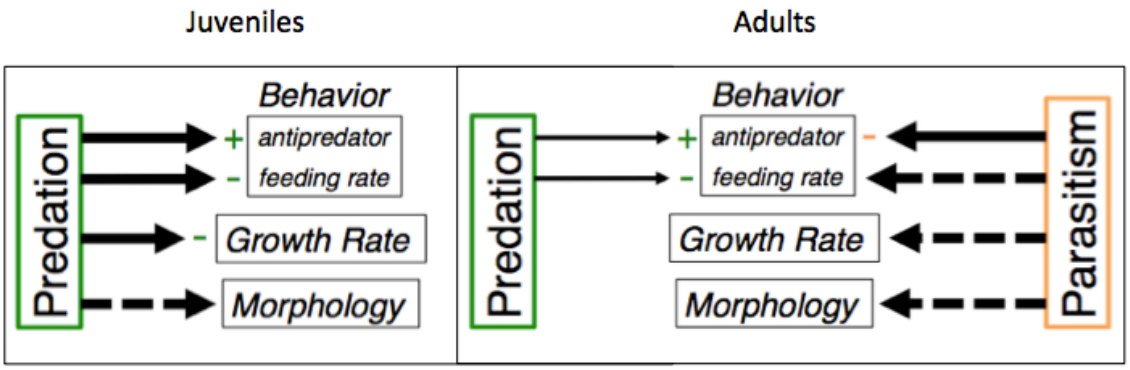

Figure 1-1. Hypotheses about how predation and parasitism may affect the behavior and morphology of aquatic gastropods based on studies of other gastropods. Arrows indicate where predation and parasitism are expected to alter behaviors, growth rate, or morphology. The direction of the effect is indicated by the plus (increase) and minus (decrease) signs, while

dashed lines signify uncertainty. Line thickness indicates predicted impact on traits, with thicker lines representing stronger impacts. Predation is also expected to have a small affect on the growth rates and morphology of adult gastropods, but that was not examined in this dissertation.

The goal of this dissertation was to examine how both parasitism and predation can impact phenotypically plastic traits, behavior and morphology, of the marine snail, *Tritia obsoleta* (Say, 1822).

I asked the following questions:

Do parasitism and risk of predation affect the feeding rates of *T. obsoleta*? And, do juvenile and adult snails respond differently to the threat of predation? – **Chapter 2**

Does *T. obsoleta* exhibit refuge-seeking behaviors when exposed to threat of predation? And, are these behaviors seen in both juveniles and adults? – **Chapter 3**

Do parasites alter *T. obsoleta* behavior? And, are these behaviors consistent in the field and the laboratory? – **Chapter 4**

Does the threat of predation affect snail shell morphology and growth? – **Chapter 5**

Does parasitism alter snail shell morphology? And, could parasitism influence size in *T. obsoleta*? – **Chapter 6**

Chapter 2

The effect of parasitism, predation and ontogeny on the feeding rates of a marine snail

Abstract

Phenotypic plasticity is one mechanism species can use in response to antagonistic interactions, such as predation, competition and parasitism, by altering their behavior, morphology or physiology. However, the effects of antagonistic interactions, such as parasite infections and vulnerability to predators, can also vary over ontogeny, making individuals more or less vulnerable at different life stages. As a consequence, phenotypic changes are expected to correlate with these different interactions through ontogeny. For example, juvenile mud snails, *Tritia obsoleta* (formerly known as *Ilyanassa obsoleta*), are vulnerable to predation, but not parasitism by trematodes, while adults are less vulnerable to predators, but can become parasitized. Therefore, I examined plasticity in feeding behaviors of juvenile, and unparasitized and parasitized adult mud snails when exposed to chemical cues from a predator, *Carcinus maenas*. When exposed to predators, gastropods commonly reduce their feeding rates, and some parasites can also increase or decrease feeding rates of their snail hosts. However, I found that the presence of chemical cues from a predator did not have a significant effect on feeding rates of juvenile or adult *T. obsoleta*. Also, snails parasitized by either of two trematode species, *L. setiferoides* or *S. dentatum*, consumed similar amounts of food as the unparasitized snails. There are a few possible explanations for this lack of change in feeding rates with increased risk of predation. *Tritia obsoleta* exhibit trail following behaviors, which lead to large local aggregations of conspecifics, which help protect individuals from predation via a safety-in-numbers effect, rendering other antipredator behaviors unnecessary. Another possibility is a potential tradeoff between antipredator behaviors and feeding. In juveniles, although antipredator behavior would provide short-term decrease in predation risk, it would result in slower growth. Feeding instead of displaying antipredator behavior would lead to increased growth, and a large shell size, which would provide long term protection from predators. More work is needed to determine how *T. obsoleta* responds to the threat of predation, and how parasitism might affect other aspects of host behavior.

Introduction

Phenotypic plasticity is a common response of organisms faced with antagonistic species interactions especially when the strength or presence of these interactions is variable in time or space (Agrawal 2001, Callaway et al. 2003, Miner et al. 2005). For many species, individuals can alter their morphology, behavior and/or physiology when exposed to interactions with other species or other environmental cues (West-Eberhard 1989). Such phenotypic changes, or the thresholds that trigger phenotypic responses, can vary across ontogeny, particularly when different life stages of a species are more vulnerable to antagonistic interactions (Dangles et al. 2007, Landberg & Azizi 2010, Hopkins et al. 2011). For example, salamanders are most vulnerable to predation when they are larvae, and larval salamanders have faster escape responses than adults and metamorphosing individuals (Landberg & Azizi 2010). Intraguild predation (in which one species preys upon a species they compete against) presents another example whereby a species interacts with another species as both a competitor and as a predator. Juveniles are frequently more susceptible to predation by intraguild predators, while adults are affected more by competition (reviewed in Polis et al. 1989).

Another example of varying antagonistic interactions across ontogeny is seen in gastropods, where small juveniles are often more vulnerable to crushing predators (Vermeij 1972, Alexander & Covich 1991), but adult snails are more vulnerable to parasitism by trematode flatworm parasites that consume the host's gonads, and often the digestive gland as well (Cheng et al. 1973, Hoskin 1975, Sullivan et al. 1985). Trematodes do not infect juvenile snails since they do not yet have gonads. Both predation risk and parasitism are known to alter gastropod behavior. When snails are exposed to the threat of predation, they may hide in refugia (Turner & Montgomery 2003, Bourdeau 2009, 2010) and decrease their feeding rate (Bourdeau

2010, Hooks & Padilla 2014). Trematode parasites can alter gastropod behavior in various ways, and are known to alter snail movement (McCarthy et al. 2000, Miura et al. 2006, O'Dwyer et al. 2014), foraging behavior (Levri 1998b, Levri & Lively 1996), and antipredator behavior (Bernot 2003, Voutilainen 2010, Kamiya & Poulin 2012). Thus, gastropods are an interesting system in which to examine how both predation and parasitism can affect foraging behavior over ontogeny.

Gastropod size is important when interacting with both predators and parasites. Juvenile snails are thought to be more susceptible to predation because they are smaller and have thinner shells than adults (Vermeij 1972). However, a decrease in feeding rate, while avoiding predation in the short-term, would reduce growth rate (Ireland 1991) and leave snails in a smaller, more vulnerable size class for a longer period of time. Adult growth rate is also thought to be important for parasitized snails because larger snails are more likely to outlive the infection (Esch & Fernandez 1994, Genner et al. 2007), although, larger snails might also benefit the parasites by providing more space inside the shell to live and reproduce (McCarthy et al. 2004, Levri et al. 2005, Genner et al. 2007).

This study examined the feeding rates of juvenile, unparasitized adult, and trematode parasitized adult *Tritia obsoleta* when exposed to chemical cues from a common predator, the green crab, *Carcinus maenas*. I hypothesized that juveniles and unparasitized adult snails would decrease their feeding rates when exposed to predator chemical cues, with a larger decrease seen in juvenile snails, which are thought to be more vulnerable to predation (Levri 1998a, Soomdat et al. 2014, Appendix 1). Previous work (Liebman 1991) found that two trematode parasites, *Himasthla quissetensis* (Miller & Northup 1926) and *Zoogonus lasius* (Leidy 1891), did not alter *T. obsoleta* foraging behavior, but different parasite species can have different effects on host behavior in other snail species (Belgrad & Smith 2014). Therefore, I examined *T. obsoleta*

parasitized by two species of trematodes, *Lepocreadium setiferoides* (Miller & Northup 1926) and *Stephanostomum dentatum* (Linton 1900), to determine if these parasites alter gastropod feeding rates, and to determine if feeding rates of parasitized and unparasitized snails differed in the presence of chemical cues from a predator.

Methods

Study System

Tritia obsoleta (Say, 1822), formerly *Ilyanassa obsoleta* (Galindo et al. 2016), is a marine gastropod that is common in the intertidal zone of salt marshes, mudflats and beaches along the east coast of North America from Nova Scotia to northern Florida. These gastropods consume detritus and algae and are also scavengers (Curtis & Hurd 1979, Feller 1984). Important predators for *T. obsoleta* include the green crab, *Carcinus maenas* (Linnaeus, 1758) (Stenzler & Atema 1977), terrapins (Tucker et al. 1997), and sea stars, including the Forbes seastar, *Asterias forbesi* (Desor, 1848) (Peterson 1979). Prior work found that *T. obsoleta* respond to chemical cues from *C. maenas* by climbing out of the water (Atema & Stenzler 1977).

Trematodes are parasitic flatworms with complex life cycles with two to six different larval stages (miracidium, sporocyst, redia, cercaria, metacercaria, mesocercaria), which inhabit two to four different host species (Galaktionov & Dobrovolskil 2013). The first intermediate host is usually a gastropod, and the final host, where sexual reproduction occurs, is usually a vertebrate (fish, bird, or terrapin, reviewed in Blakeslee et al. 2012, Phelan et al. 2016). Snails are infected by consuming trematode eggs or after becoming infected by a newly hatched miracidium larva. The miracidia feed on and reproduce within the gonads and the digestive gland of the host snail. The miracidium produces sporocyst larvae, which can then produce more

sporocysts or rediae larvae. Cercaria larvae are then produced asexually by either sporocysts or rediae, which leave the snail to parasitize the next host (Stunkard 1938, 1983, Moore 2002). Because these parasites inhabit and consume the gonads, the snail host is fully or partially castrated (Cheng et al. 1973, Sullivan et al. 1985).

Tritia obsoleta is the first intermediate host to eight species of trematode parasites along the shores of Long Island, NY, and 7-40% of *T. obsoleta* adults in a population can be parasitized by one of these species (infection by multiple species of parasites is rare, Appendix 2). Snails infected by *L. setiferoides* were chosen for this experiment because their effect on feeding rate had not been determined before, and because they were the most abundant parasite. Snails infected by *S. dentatum* were also examined after many snails that were categorized as unparasitized prior to the experiment were found to be infected with this trematode species upon dissection.

Snail and Crab Collection

During June 2015, adult snails were collected on Long Island, NY near Shinnecock Canal (SC, 40.8835, -72.4848), and juveniles were collected from Crab Meadow Beach (CM, 40.9293, -73.3281; Appendix 3). I collected juveniles smaller than 12 mm in length and adult snails larger than 14 mm, since snails reach maturity at about 12-14 mm (Scheltema 1964, personal observation). To determine infection status of adults prior to the experiment, each snail was placed in a 17.7 ml well of a six well plate filled with 1 μm filtered seawater (~10 ml). The well plates were placed under fluorescent lights for approximately 16 hours, after which the water was inspected for presence of parasites. Snails were then separated by the parasite species found in the well plate, and were placed in screened containers labeled with the name of the parasite,

and were kept in a recirculating seawater aquarium (~18˚C) and fed algae (*Ulva lactuca*, Giannotti & McGlathery 2001) until the experiment was performed.

Green crabs were used as predators, and were collected from Stony Brook Harbor (40.9023, -73.1748) by using a net and chicken as bait. Crabs were then transported to the laboratory where they were kept in a recirculating saltwater tank (separate from all experimental snails) and fed raw tilapia until used in the experiments.

Agar-Tetramin® Disks

To determine the amount of food consumed by each snail, I made agar disks that contained Tetramin® fish flakes. Agar disks were made by mixing finely powdered Tetramin® fish flakes (25 g) into a hot agar mixture (200 ml water and 4.6 g agar powder). The agar-Tetramin® mixture was then poured into a glass baking dish and allowed to cool. Once the agar had set, a hollow glass tube (inside diameter 20.52 mm) was used to cut out small circular agar disks. Each disk was weighed prior to use in the experiment. At the end of the experiment, the disks were blotted dry with a paper towel and weighed again.

Chemical Cues from Predators

Chemical cues associated with predators have been used in many studies to test the indirect effects of predation, such as antipredator behaviors (e.g., tadpoles, Kiesecker et al. 1996; spiders, Persons & Rypstra 2001; snails, McCarthy & Fisher 2000, Dalesman et al. 2006, Bourdeau 2009, 2010), instead of the direct effect of predation where predators handle and consume the prey. To prepare the water with chemical cues from predators, two experimental tanks (33.8 cm long x 19.6 cm wide x 21.2 cm high) were each filled with 10 L of 1 μm filtered seawater (~ 29 ppt) that was drawn from a saltwater well at the Flax Pond Marine Laboratory

(40.9613, -73.1387). One green crab (~ 45 g) was placed into a container with mesh that enabled water to flow between the crab container and the rest of the tank. The crab was placed into one of the tanks for approximately 16 hours prior to the experiment, and remained in the tank during the experiment. An empty mesh container was placed in the control treatment tank. Both tanks had aeration from an airstone, and were maintained at 20˚C in a temperature-controlled room with a 16:8 light: dark schedule.

Experimental Design

A block design was used to test the effects of chemical cues from predators on different life stages and parasite infected snails. Each block consisted of two tanks, one containing predator chemical cues and one control tank without predator chemical cues. Four groups of snails were tested: juveniles, unparasitized adults, adults parasitized by *L. setiferoides*, and adults parasitized by *S. dentatum*. Blocks were run for 24 hours on consecutive days where each block began immediately after another block was taken down. Blocks were run until there were at least 40 replicates of each type of snail in each water treatment.

Prior to experimental trials, gastropods (eight unparasitized adult snails, eight parasitized adult snails and eight juvenile snails) were placed in well plates (17.7 ml well plates with ~ 10 ml seawater) without food for 24 hours. After the 24-hour starvation period, each snail was placed into a small mesh container (cylinder: height = 5.75 cm, diameter = 4.32 cm) with a pre-weighed agar disk and haphazardly assigned to the crab chemical cue treatment or the control treatment. This was done so that four snails from each category (juvenile, adult, parasitized adult) were placed in each treatment in each block. An agar disk, but with no snail present, was placed in an additional 8 mesh containers, 4 for each treatment. Thus, each block included 16

containers, 12 with snails from each of the three aforementioned categories and 4 with just agar disks, for each treatment. After 24 hours, the mesh containers were removed from the tanks. The agar disks were patted dry with a paper towel and reweighed. The shell length of each snail was measured and snails were dissected to confirm their infection status and, if infected, the species of parasite. Dissections were performed by gently tapping a hammer on a snail shell that was covered by paper towels. This method cracked the shell and left the body of the snail intact so that it could be removed from the shell and examined for parasites.

The method for detecting parasitized snails prior to the experiment was not always accurate (as seen by Curtis & Hubbard 1990), thus many snails that were originally identified as unparasitized were actually infected with parasites, particularly with *S. dentatum.* Therefore, trials were run until at least 40 replicate snails in each of the four new groups: unparasitized adult, juvenile, snails infected with *L. setiferoides*, and snails infected with *S. dentatum*, had been tested in both chemical cue treatments (Table 2-1).

Data Analysis

The food disks in the containers without snails (control disks) were weighed before and after each block to determine the change in disk weight without snail feeding. The average change in control disk mass was calculated for each tank in each block, which was then subtracted from the final disk weight for each snail within that treatment in that particular block. This was done to account for any disintegration or water absorption that may have occurred during the experiment.

To obtain the mass of food consumed by each snail, the final mass of the agar disk (after subtracting the weight loss in control disks) was subtracted from the initial mass. Negative

values found after subtracting final mass from initial mass were deemed to represent zero mass consumed.

Since juvenile snails did not overlap in size with adults, two analyses, using permutation tests for linear models, were used to compare the feeding rates based on 1) life stage (juvenile, unparasitized adult), and 2) infection category (unparasitized adult, adult infected by *L. setiferoides*, adult infected by *S. dentatum*).

For comparisons between adult and juvenile snails, shell length could not be used as a covariate because there was no size overlap in the two groups of snails. Therefore, in order to take shell length into account when examining the effect of life stage on feeding rates, the mass of food consumed was divided by the shell length, and the resulting coefficient was used as the response variable. A permutation test was used to evaluate differences in feeding rates for juvenile and adult snails in each of the two predator chemical cue treatments, while taking the blocks into account. To test for the effects of parasitism on feeding rates, a permutation test was used to assess differences in feeding rate among adult snail groups (unparasitized adult, infected by *L. setiferoides*, infected by *S. dentatum*) in the two treatments (predator chemical cues, control seawater) using shell length as a covariate, and taking blocks into account. Thus, the unparasitized adult snails were used in both analyses. Both permutations were performed using the lmPerm package in R (Version 3.2.1, R Core Team 2014).

Results

For the feeding rates of adult and juvenile *T. obsoleta* (controlling for shell length and taking the blocks into account), there was no significant effect of chemical cue treatment (permutation, $p = 0.194$, Fig. 2-1, Table 2-2) or of life stage (permutation, $p = 0.941$, Fig. 2-1,

Table 2-2), and there was no interaction between life stage and chemical cue treatment (permutation, $p = 0.249$, Table 2-2). Although there was no significant difference between the amount of food consumed by juveniles and adults when controlling for body size, there was a trend for juveniles to consume more than adults (Fig. 2-1).

I found no significant effect of parasite infection (permutation, $p = 0.902$, Table 2-3) or chemical cue treatment (permutation, $p = 1.00$, Table 3) on feeding rates of adult snails (Fig. 2-2). There was also no interaction between the chemical cue treatment and parasite infection on feeding rate (permutation, $p = 0.372$, Table 2-3). Parasitized snails were, on average, larger than unparasitized snails (t test; *L. setiferoides*: $t = 2.12$, $df = 174.1$, $p = 0.035$; *S. dentatum*: $t = 7.62$, $df = 187$, $p < 0.0001$; Table 2-1), but this did not have an effect on feeding rates (permutation, $p = 0.174$, Fig. 2-3, Table 2-3).

Discussion

Phenotypic plasticity induced by antagonistic species interactions may vary over ontogeny, especially if the organism's vulnerability to these interactions changes throughout its life history. Often, juvenile gastropods are more vulnerable to predation than adults because they are smaller and have thinner shells than adult snails (Levri 1998a, Soomdat et al. 2014, Appendix 1). Juveniles are not affected by trematode parasites, but once they reach maturity they can become infected. Only adults have fully developed gonads, which the trematodes consume (Cheng et al. 1973, Sullivan et al. 1985). Since *T. obsoleta* are vulnerable to different species interactions throughout ontogeny, I predicted that juveniles would display plasticity in their behavioral response risk of predation, and that parasites would affect adult behavior. Feeding rates were examined because they are plastic in gastropods, and are often reduced when snails

are exposed to risk of predation (Bourdeau 2010, Hooks & Padilla 2014). However, neither predator chemical cues nor parasitism were found to alter feeding behaviors of either juvenile or adult *T. obsoleta*.

There are many possible explanations as to why these snails responded differently than most species of gastropods that have been tested. For example, there may be a tradeoff between growth and reproduction, and exhibiting antipredator behaviors like reduced feeding, especially for juvenile *T. obsoleta*. Decreased feeding rates would slow growth rates and result in smaller snails; but if juveniles continued to forage, this would lead to continued growth and eventually to a larger size class. Being a member of a large size class decreases predation risk (Rochette & Himmelman 1996, Perez et al. 2009), and would provide long-term protection from predation compared to the short-term benefits of antipredator behaviors.

Another possibility for lack of typical antipredator behaviors is that *T. obsoleta* already have multiple traits that help protect them from predation. Both juveniles and adults have thick shells, relative to other gastropod species. Thick shells are harder to break than thin shells (Palmer 1985, Rochette et al. 2007), and they provide protection from predators that need to break the shell to consume their prey, such as *C. maenas*. *Tritia obsoleta* also exhibits trail following behaviors (Trott & Dimock 1978), which lead to high-density populations and is known to decrease risk of predation (Ng et al. 2013). Since *T. obsoleta* already display features that deter predation, it is possible that other antipredator behaviors, such as decreasing feeding rates, provide no additional advantage.

An alternative explanation as to why *T. obsoleta* did not alter feeding rates in response to predation risk is lack of predator recognition. *Carcinus maenas* is a non-native predator of *T.*

obsoleta that was introduced to the east coast of North America around 200 years ago (Carlton & Cohen 2003). Often, gastropods exhibit phenotypic plasticity in response to native predators but not in response to non-native predators (reviewed in Hollander & Bourdeau 2016). However, *T. obsoleta*, along with other species in North America (e.g., *Nucella lapillus*: Trussell et al. 2003, *Mytilus edulis*: Freeman & Byers 2006, *Littorina obtusata*: Rochette et al. 2007), have been shown to sense and respond to chemical cues from *C. maenas* (Atema & Stenzler 1977).

Parasitism did not have an effect on the feeding rates of adult *T. obsoleta*; there was no difference in the amount of food consumed among unparasitized snails and snails parasitized by either of the two trematode species. These results are similar to other work that found no difference in feeding rates of *T. obsoleta* infected with *H. quissetensis* or with *Z. lasius* or that of unparasitized snails (Liebman 1991). Not altering feeding rates could be beneficial for the parasites, because the host snail would continue to grow, which would enable them to complete their life cycle. In addition, because *L. setiferoides* and *S. dentatum* are not trophically transmitted to their next host (reviewed in Blakeslee et al. 2012, Phelan et al. 2016), predation of their snail host would negatively impact the parasites. Parasitized snails may also be relying on large, thick shells and high snail population densities in order to avoid predation.

In this experiment, I found high variation in feeding rates among snails, particularly for adults. I focused on the infection status of adult snails, and did not test for differences between male and female snails. Levri and Lively (1996) found that non-brooding female snails were more likely to forage during times of high predation risk than brooding female snails. Since trematode parasites partially or fully castrate their gastropod hosts (reviewed in Lafferty & Kuris 2009), it would be interesting to compare the behavior of parasitized snails to that of

unparasitized females with ripe gonads versus those without ripe gonads when exposed to predator chemical cues.

Exposure to chemical cues from predators did not alter the feeding rates of juvenile or adult *T. obsoleta*. There are a few possible explanations for these results, such as a tradeoff between growth and antipredator behaviors for juvenile snails, or the fact that all *T. obsoleta* are protected from predation by their thick shells and high population densities. Trematode parasites did not alter the feeding rates of infected *T. obsoleta*, and there was no interaction between predator chemical cues and the species of parasite on feeding rates. By not reducing feeding rates, parasitized snails will continue to grow, producing more tissue for the parasites to consume allowing them to reproduce and complete their life cycle. More work is needed to determine the consequences of predation threat for both juvenile and adult *T. obsoleta*, and how each species of parasite that infects *T. obsoleta* might affect other aspects of host behavior, as well as the cause of the large difference in feeding rates among individuals.

Table 2-1. The total number of *T. obsoleta* from each snail category that was tested in each experimental treatment. The shell length reported represents the mean shell length of snails in each category plus or minus the standard error.

	Treatment	n	Shell Length (mm)
Juveniles	Control	40	11.06 ± 0.053
	Green Crab	40	
Unparasitized Adults	Control	48	18.78 ± 0.144
	Green Crab	48	
Adults parasitized by *L. setiferoides*	Control	42	19.22 ± 0.152
	Green Crab	41	
Adults parasitized by *S. dentatum*	Control	47	20.32 ± 0.142
	Green Crab	46	

Table 2-2. Permutation test results for the effect of treatment (predator chemical cues, control) and life history stage (juvenile, adult) on the feeding coefficients (mass consumed/ snail shell length) of *T. obsoleta*.

	df	SS	MS	p-value
Treatment	1	15.43	15.4331	0.1942
Life Stage	1	4.46	4.4564	0.9412
Treatment*Life Stage	1	4.61	4.6149	0.2492
Block	1	10.774	10.774	1.0000
Residuals	171	930.21	5.4398	

Table 2-3. Permutation test results for the effect of treatment (predator chemical cues or control) and infection category (unparasitized, *L. setiferoides* infected, or *S. dentatum* infected) on the feeding rate of adult *T. obsoleta*.

	df	SS	MS	p-value
Treatment	1	151	151.3	1.00
Infection Category	2	21122	10561.1	0.9020
Shell Length	1	12110	12110.2	0.1736
Treatment*Category	2	6351	3175.7	0.3718
Block	1	28063	28063	1.00
Residuals	264	745924	2825.5	

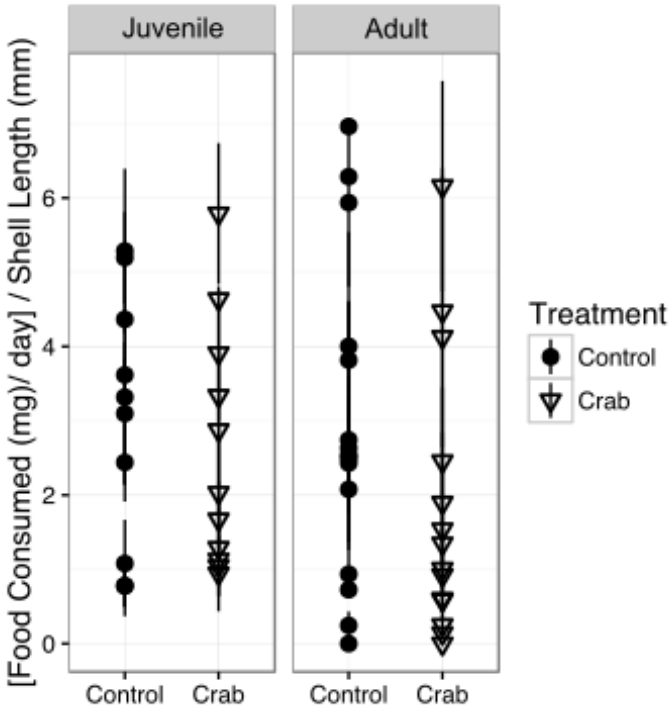

Figure 2-1. Feeding rate of *T. obsoleta* juveniles and adults when controlling for shell length. The amount of food consumed per day was divided by the shell length of the individual snail for adult and juvenile snails in the two chemical cue treatments (control and crab predator). The points represent the mean from each block in each treatment, and the error bars represent the standard error around each block mean. When controlling for shell length, there was no difference in feeding rates between adult and juvenile snails, and there was no difference in feeding rates between treatments.

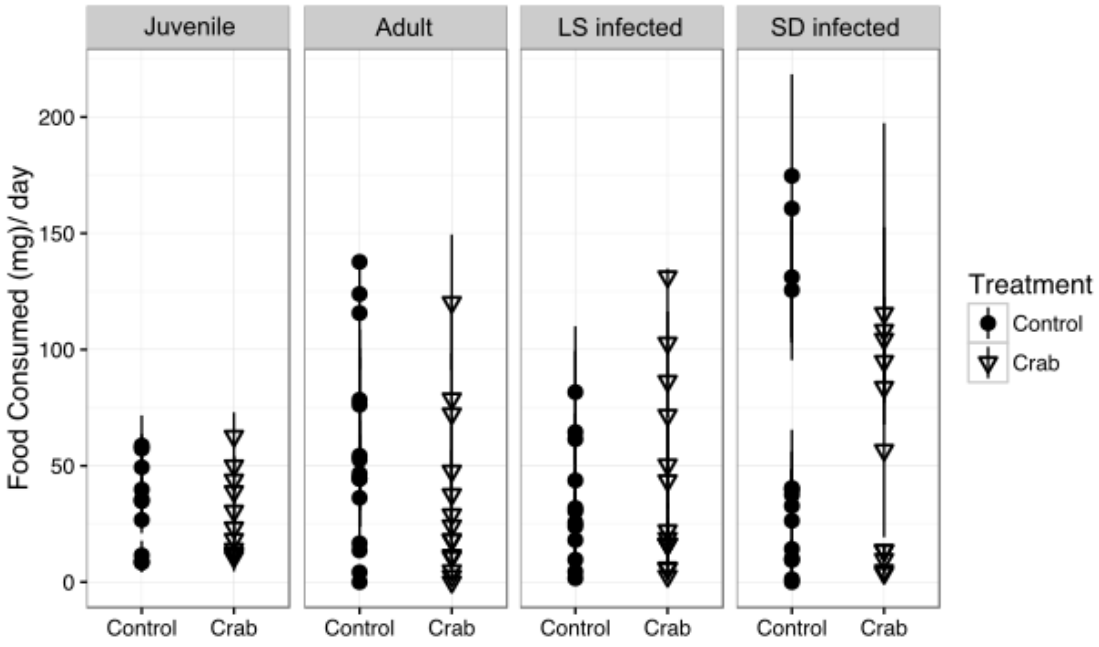

Figure 2-2. Feeding rate of juvenile and adult *T. obsoleta*, and *T. obsoleta* infected with either *L. setiferoides* (LS) or *S. dentatum* (SD) in the predator chemical cue and in the control treatments. The points represent block means for each treatment, and the error bars represent the standard error around each block mean.

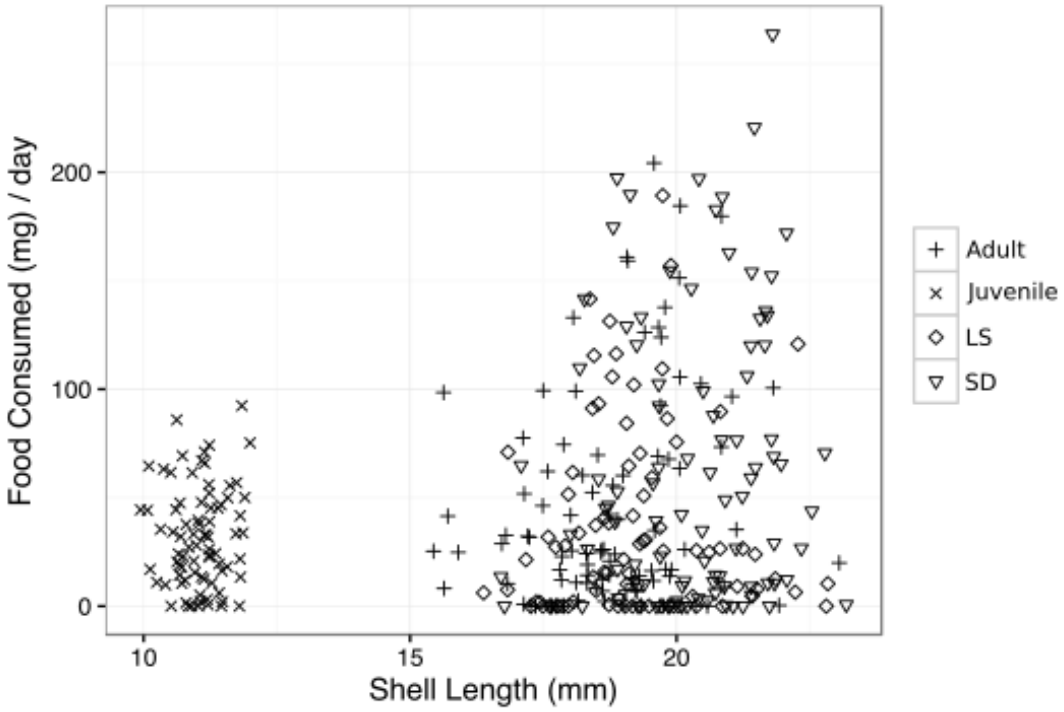

Figure 2-3. The amount of food consumed (mg) per day versus the shell length (mm) of each snail in the experiment. Each point represents an individual snail, and shapes denote whether that snail is a juvenile, an unparasitized adult, parasitized by *L. setiferoides* (LS), or parasitized by *S. dentatum* (SD).

Chapter 3

Phenotypic plasticity of antipredator behavior across ontogeny

Abstract

Risk of predation is one of the biggest threats to survival and fitness for many organisms. As a consequence, individuals frequently employ strategies to escape or avoid predation, including antipredator behavior. However, if susceptibility to predation changes through ontogeny, antipredator behaviors would also be expected to change across various life stages. Aquatic gastropods often exhibit behaviors such as crawling out of the water or burrowing into sediment in order to avoid predation. I examined whether chemical cues from two common predators, green crabs (*Carcinus maenas*) and Forbes seastars (*Asterias forbesi*), affected the burrowing behavior or the propensity of juvenile and adult eastern mud snails (*Tritia obsoleta,* formerly *Ilyanassa obsoleta*) to crawl into or out of the water. In the laboratory, snails were exposed to either water containing chemical cues from a predator or to water with no predator chemical cues. When exposed to chemical cues from either predator, adult snails were more likely to burrow into the sediment or crawl out of the water than those in the control treatments. However, juveniles did not change their propensity to crawl out of the water and they burrowed less when exposed to predator chemical cues. These results suggest that there may be a tradeoff between behaviors that reduce predation risk and those that facilitate feeding, and therefore growth rate, in juvenile snails. Although reducing predation risk may be beneficial in the short-term, increasing feeding may help snails attain a larger size faster, which would provide a long-term defense against predation. This study demonstrates the importance of examining how phenotypically plastic behavior may change over ontogeny. More work is needed to determine how the behavioral differences of *T. obsoleta* across ontogeny affect susceptibility to and interactions with predators.

Introduction

Predation is one of the largest threats to survival for many organisms. As a consequence, individuals frequently display phenotypic traits, including behaviors, which allow them to escape predators or reduce predation risk (reviewed in Lima & Dill 1990, Apfelbach et al. 2005). In many systems, size plays an important role in affecting vulnerability to predators, and juveniles are typically more susceptible to predation than adults (e.g., fish: Sogard 1997, crabs: Moksnes et al. 1998, snails: Rochette & Himmelman 1996). As a result, different antipredator behaviors might be most effective or important at different life stages (e.g., snakes: Creer 2005, crickets: Dangles et al. 2007, dragonfly larvae: Hopkins et al. 2011, geckos: Landova et al. 2013). For example, in the racer snake, *Coluber constrictor*, juveniles have a blotched color pattern while adults are a solid color. When approached by a stuffed raccoon predator, juvenile racers are more aggressive and adults are more likely to flee (Creer 2005). These behaviors follow the observed antipredator behaviors of other snakes, where snakes with disruptive patterning (blotches or spots) are more aggressive towards predators, while snakes with striped or uniform patterns tend to flee because of the relationship between movement, patterning and camouflage (Brodie 1992, Allen et al. 2013). In the case of the racers, the ontogenetic shift in color pattern corresponds to a similar ontogenetic shift in behavior.

Aquatic gastropods commonly display shifts in behavior in response to predators. When exposed to predators, or to chemical cues associated with predators, snails often seek refuge or display avoidance behavior (Alexander & Covich 1991, McCarthy & Fisher 2000, Turner et al. 2000, Dalesman et al. 2006), decrease their feeding rate (Levri & Lively 1996), or do both (Richardson & Brown 1992, Trussell et al. 2003). To escape predation, aquatic gastropods exhibit two common avoidance behaviors: climbing out of the water (Alexander & Covich 1991,

McCarthy & Fisher 2000, Dalesman et al. 2006, Klose 2011), or burrowing into the sediment (Phillips 1977, McCarthy & Fisher 2000). Gastropods are also known to have differing feeding behaviors (Hughes et al. 1992, Montiel et al. 2005, Morton et al. 2007) and antipredator behaviors (Berg 1972, Rochette et al. 1996) through ontogeny, and these behavioral differences are often attributed to size differences between juveniles and adults (Berg 1972, Hughes et al. 1992, Rochette et al. 1996, Morton et al. 2007).

Tritia obsoleta (Say, 1822), formerly *Ilyanassa obsoleta* (Galindo et al. 2016), is a common intertidal zone snail found in salt marshes, mudflats and beaches along the east coast of the United States. These gastropods consume detritus and algae and are also scavengers (Curtis & Hurd 1979, Feller 1984). Important predators for *T. obsoleta* include the green crab, *Carcinus maenas* (Linnaeus, 1758) (Stenzler & Atema 1977), terrapins (Tucker et al. 1997), as well as sea stars, including the Forbes seastar, *Asterias forbesi* (Desor, 1848) (Peterson 1979).

Many studies use chemical cues associated with predators to test for antipredator responses (e.g., tadpoles, Kiesecker et al. 1996; spiders, Persons & Rypstra 2001; snails, McCarthy & Fisher 2000, Dalesman et al. 2006, Bourdeau 2009). Chemical cues from predators allow an experimental test of the non-consumptive effects (such as changes in feeding or movement behaivors) that predators have on their prey, instead of the direct effect of the predator handling and consuming the prey. Prior work has found that *T. obsoleta* respond to chemical cues from *C. maenas* by crawling out of the water rather than burrowing into sand (Atema & Stenzler 1977).

In this study, I used laboratory experiments to examine whether *T. obsoleta* exhibits antipredator behavior in the presence of chemical cues associated with predators, and whether

these behaviors varied at different life stages. I tested whether adult (experiment 1) or juvenile snails (experiment 2) crawled out of or into the water when they were exposed to chemical cues from predators. In two additional experiments I examined if the burrowing behavior of adult snails (experiment 3) or juvenile snails (experiment 4) was altered in response to predator chemical cues. I predicted that both adult and juvenile *T. obsoleta* would exhibit typical snail antipredator behaviors by crawling out of the water and by burrowing into the sand when exposed to predator chemical cues. Since juveniles are smaller and have thinner shells than adults, I expected juveniles to display a stronger response to the threat of predation than adult snails.

Materials and Methods

Snail collection

I collected 1,920 adult *T. obsoleta* from four beach sites on Long Island, NY during the summer of 2013 (Crab Meadow Beach (CM) 40.9293, -73.3281, Old Ponquogue Bridge Marine Park (PB) 40.8433, -72.4985, near Shinnecock Canal (SC) 40.8835, -72.4848, and West Meadow Beach (WM) 40.9443, -73.1466; 480 snails per site; Appendix 3), and collected 480 juveniles from three sites during the summer of 2015 (CM, PB, and WM; 160 snails per site). Because *T. obsoleta* reach sexual maturity at around 12-14 mm in length (Scheltema 1964, personal observation), all adult snails collected were larger than 13 mm in length, and juvenile snails were less than 12 mm in length. During collection, I placed the snails in containers marked with the date and site of collection, returned them to the lab, and housed them in a recirculating seawater tank (~20˚C) with a 16:8 light/dark cycle. Snails were fed the alga *Ulva lactuca* (Giannotti et al.

2001) and flaked fish food (TetraMin®) *ad libitum* until used in an experiment, which was one day to about three weeks after collection.

Predator Collection

During the summer of 2013, baited minnow traps were used at all four sites (CM, PB, SC, and WM) to collect *C. maenas*. Green crabs were only caught at CM, so additional crabs were caught at Stony Brook Harbor (40.9023, -73.1748) by using a net and chicken as bait. In 2015, I used minnow traps at CM to collect green crabs for the experiments. In 2013, I collected *A. forbesi* from Stony Brook Harbor using a net. During the summers of 2014 and 2015 I was unable to find sea stars at any of my sites or at Stony Brook Harbor during the summers of 2014 or 2015, likely due to the spread of wasting disease (as seen in California: Dungan et al. 1982). Because of the lack of sea stars, I was not able to examine the behavior of juvenile snails when exposed to sea star predators.

After each collection trip, the predators were transported to the laboratory where they were kept in a recirculating saltwater tank (separate from all experimental snails) and fed raw tilapia until used in the experiments.

Experimental Design

For each of the 4 experiments, twelve 10 L tanks (30.5 cm long x 19.1 cm wide x 20.3 cm high) were set up in a randomized design such that no replicate of any treatment was next to another replicate of that same treatment. During each set of twelve replicates, snails from each site where tested at the same time. Each snail was used once to ensure independence in the resulting behaviors. Successive sets of replicates were run until the target sample size of at least 120 for each treatment was reached.

Crawl out behavior experiments

Each test tank had a sloped bottom made of Plexiglas® sheeting (~ 26.6 cm long) that mimicked the slope of a shoreline (~10˚), and was filled with approximately 1.5 liters of the appropriate treatment water such that half of the slope was covered with water and half was left dry. I placed one snail in the middle of the Plexiglas® slope at the air-water interface. After one hour, whether the snail moved up the slope and out of the water or down the slope and into the water was recorded. All snails moved either up- or down-slope from their original position. Some snails crawled down into the water and then up the side of the tank and completely out of the water; these snails were counted as crawling out of the water.

Experiment 1 included only adult snails (400 replicates of each treatment) from each of the 4 populations (CM, PB, SC, and WM). There were three chemical cue treatments (see preparation below): chemical cues from the green crab, chemical cues from the seastar, and a control with no chemical cues. Experiment 2 included only juvenile snails (120 replicates per treatment) from 3 populations (CM, PB, and WM). This experiment only had two chemical cue treatments, chemical cues from the green crab, and a control with no chemical cues.

For the experiment with adults (Exp. 1), three 19 L containers were filled with filtered seawater, one for each of the three treatments. I placed one large *A. forbesi* (~ 90 g) in the first container and three small *C. maenas* (total of ~100 g) into the second container. No predator was placed into the last container, which served as the control water. Due to scarcity of *A. forbesi*, similar methods were used for the juvenile experiment (Exp. 2), but only tested two treatments, green crab predator and control, which were set up as for Experiment 1.

Prior to each experiment, seawater was conditioned with live predators so that chemicals cues from each predator would be found in the water (hereafter referred to as chemical cue water or predator cues). First, I drew seawater from a saltwater well at the Flax Pond Marine Laboratory (40.9613, -73.1387), which is at least 5 km from the four snail collection sites. Since the well is far from the collection sites, any environmental cues in the well seawater should not be similar to the cues in the water where the snails were collected. The seawater was filtered (1 μm) and aerated with an airstone for approximately 16 hours prior to experimentation. I then placed predators into the water (as described below) to condition water with the chemical cues for each predator tested.

Predators were in their respective containers with aeration overnight (~16 hours) and then removed from the water for the crawl out experiments the following morning before any replicate tanks were set up. By removing the predators at the beginning of the day, I prevented the water from becoming more concentrated with predator chemical cues through time, as water was periodically removed for each experimental replicate. All cue water was used within six hours of removing the predators. Each replicate had freshly replaced water from the stock container for each treatment. Each respective 19 L batch of chemical cue water was used for 16 replicates of each experiment. New chemical cue and control water was prepared as described above, and replicates were continued on successive days until the replicates were completed.

Burrowing behavior experiments

For these experiments, rather than using seawater conditioned with chemical cues of predators, small mesh containers were attached inside each test tank containing either a predator or nothing to control for the presence of the mesh container. The mesh container allowed for

water and chemical cue exchange between the tank and the container that housed the predator. For adults (Exp. 3), the experimental tanks contained either one *A. forbesi* (~30 g), one *C. maenas* (~20 g), or no predator (control treatment) in the mesh container of each tank, and snails from all 4 populations were tested (240 replicates per treatment). I placed the predators in the tank approximately 16 hours before the experiment began. Predators remained in the tanks in their mesh container during the experiment, but they could not directly interact with *T. obsoleta*. I repeated the same procedure for the juveniles (Exp. 4) from 3 populations (CM, PB, and WM), except without the seastar treatment (120 replicates per treatment). Each tank was filled with cleaned beach sand (at least 3 cm deep) and with about 4 L of seawater.

To examine burrowing behavior, I placed one snail on the sand surface in the middle of the tank. After one hour, I recorded whether or not the snail was buried (more than half of the shell was covered) in the sand.

Data Collection

After each replicate of each of the four experiments, I removed snails from every tank, and measured the length of the shell. The adult snails were then dissected (Chapter 2) and viewed under a dissecting microscope to determine if they were parasitized with trematode parasites. Only unparasitized snails were used in data analyses. For the crawl out experiments, water was removed from each tank and all tanks were wiped down to remove any mucus trails before the next replicate. The water in the tank was then replaced with water from the source bucket. For the burrowing experiment, the side of each tank was wiped down and the sand was stirred to break up any mucus trails between replicates, but the water was not exchanged. Tanks

were left without aeration for about 20 minutes so that the sand could settle before the next replicate, during which the air stones were replaced.

Data Analysis

I used contingency table analyses (G-tests) to determine if the frequencies of behavioral responses differed in each chemical cue treatment, and also to determine if the frequency of snail behavior in the control treatment was different across collection sites. I used separate G-tests for each of the four experiments since the adult and juvenile experiments differed in both treatment and site number. Since adult and juvenile *T. obsoleta* differed in shell length, I used G-tests to determine if the frequencies of behavioral responses differed by predator chemical cue treatment and by size class. To examine the effect of size on behavior, snails were placed into size bins (e.g., snails between 15.5-16.49 mm were in size bin 16). Size bins containing more than 30 animals were used in the analyses (juveniles: 7-11 mm, adults: 16-24 mm). I also used G-tests to examine whether the behavioral responses changed over the course of the day (whether the replicate was conducted right after the predators were removed, or hours afterwards). Analyses were performed on the crawl out experimental results, and the results from the burrowing experiments to test whether antipredator behavior changed throughout the day. When needed, a stepwise Bonferroni correction was used to adjust the critical alpha when testing for significance to correct for multiple comparisons with the same data.

Results

In the two adult snail experiments, approximately 20% of snails were parasitized (368 out of 1920 snails), and were not used in data analyses (although antipredator behaviors did not differ between parasitized and unparasitized snails, Appendix 4), resulting in unequal sample

sizes of snails in each treatment and from each collection site (Table 3-1). Snails smaller than 12 mm were not parasitized.

The shell lengths of adult snails varied by site (CM: 13.4 - 22.34 mm, PB: 14.27 - 32.19 mm, SC: 14.89 - 22.29 mm, WM: 13.42 - 28.78 mm), but the mean size was within 1.70 mm across all sites (mean ± SE, CM: 18.22 ± 0.06 mm, PB: 19.97 ± 0.10 mm, SC: 18.40 ± 0.07 mm, WM: 19.33 ± 0.10 mm). The shell lengths of juvenile snails also varied across sites (CM: 7.99 - 11.97 mm, PB: 6.99 - 10.10 mm, WM: 6.08 - 9.08 mm), with the largest juveniles from CM and the smallest from WM (mean ± SE, CM: 9.87 ± 0.05 mm, PB: 8.10 ± 0.05 mm, WM: 7.07 ± 0.04 mm). Shell length did not impact the behavioral responses in the different predator chemical cue treatments in any experiment (3-way G tests, Exp. 1: $df = 9$, $G = 7.75$, $p > 0.25$; Exp. 2: $df = 5$, $G = 6.40$, $p > 0.25$; Exp. 3: $df = 7$, $G = 5.08$, $p > 0.50$; Exp. 4: $df = 5$, $G = 5.61$, $p > 0.25$). There were also no behavioral differences between replicates run early or late in the day (Exp. 1: G test, $df = 3$, $G = 1.14$, $p > 0.50$, Exp. 2: G test, $df = 5$, $G = 3.81$, $p > 0.50$, Exp. 3: G test, $df = 5$, $G = 5.11$, $p > 0.25$, Exp. 4: G test, $df = 5$, $G = 4.19$, $p > 0.25$).

When the critical alpha for significance was adjusted for multiple testing, I found a significant effect of the predator treatment on the crawl out movement of adult *T. obsoleta* (G test, $df = 2$, $G = 7.06$, $p < 0.01$, Figure 3-1A). Significantly more snails in the control treatment moved into the water than those exposed to the green crab treatment or the sea star treatment. I found no difference between the behaviors exhibited by the snails in the two predator treatments (G test, $df = 1$, $G = 0.30$, $p > 0.60$). On the other hand, juveniles in the predator chemical cue treatment and the control were equally likely to crawl out of the water (G test, $df = 1$, $G = 0.34$, $p > 0.90$, Fig. 3-1B).

I also found a significant effect of predator treatment on the burrowing behavior of adult (G test, df = 2, G = 16.12, $p < 0.005$, Fig. 3-1C) and juvenile *T. obsoleta* (G test, df = 1, G = 10.09, $p < 0.005$, Fig. 3-1D). Adult snails did not respond differently to the two different predator treatments with regard to burrowing behavior (G test, df = 1, G = 0.01, $p > 0.90$). When exposed to either of the predator chemical cues, adult snails were more likely to burrow, while juvenile snails were less likely to burrow than those in the control treatment (Fig. 3-1C, 3-1D).

To test for effects of source site, I analyzed the behavior of snails in the control treatments. I found no effect of collection site on the crawl out behavior of adult (G test, df = 3, G = 2.50, $p < 0.75$, Fig. 3-2A) or juvenile snails (G test, df = 2, G = 1.83, $p < 0.25$, Fig. 3-2B). Similarly, burrowing behavior did not differ among adult snails from different collection sites (G test, df = 3, G = 3.86, $p < 0.75$, Fig. 3-2C), but there was an effect of collection site on juvenile burrowing behavior in the control treatment (G test, df = 2, G = 7.49, $p < 0.025$, Fig. 3-2D). Juvenile snails from PB and WM exhibited different burrowing behaviors (G test, df = 1, G = 7.49, $p < 0.025$; PB vs. SC: df = 1, G = 1.99, $p > 0.10$; SC vs. WM df = 1, G = 1.81, $p > 0.10$; Fig. 3-2D). I then examined all snails in the juvenile burrowing experiment to test whether both predator treatment and collection site influenced juvenile burrowing behavior, and used a stepwise Bonferroni correction to adjust the critical alpha for multiple testing. I found that the difference in behaviors of juveniles exposed to different predator treatments remained (3-way G test, df = 1, G = 10.26, $p < 0.010$), but there was no longer a difference in behavior based on collection site (3-way G test, df = 2, G = 4.01, $p > 0.95$). Thus, although juveniles from different sites behaved differently in the control treatment, behavior was similar across sites in the predator chemical cue treatment.

Discussion

Susceptibility to predation can vary across ontogeny (e.g., Sogard 1997, Moksnes et al. 1998, Rochette & Himmelman 1996), and often this change in susceptibility can be paired with alterations in the organism's habitat, resource use, or individual size (reviewed in Werner & Gilliam 1984), as well as with behavioral changes (Creer 2005, Dangles et al. 2007, Hopkins et al. 2011, Landova et al. 2013). Climbing out of the water and burrowing behaviors are commonly seen in other aquatic gastropods (Phillips 1977, Alexander & Covich 1991, McCarthy & Fisher 2000, Dalesman et al. 2006, Klose 2011), which allows snails to move away from predator chemical cues and seek refuge. I performed four experiments to determine if *T. obsoleta* display these two common antipredator behaviors and if these behaviors differed between juveniles and adults. I found that there was a significant increase in the proportion of adult snails that displayed both of these behaviors when exposed to cues from both seastar and crab predator cues. In contrast, the juvenile snails did not display either of the expected antipredator behaviors when exposed to chemical cues from predators, even though they are thought to be more vulnerable to predation than adults (Appendix 1). Exposure to chemical cues from the crab predator did not alter the crawl out behavior of juvenile snails, and made juveniles less likely to burrow than conspecifics in the control treatment. Moreover, these behavioral differences between juvenile and adult snails do not seem to be driven by a change in body size since there were no behavioral differences seen between small and large adults.

There are many possible reasons why juvenile *T. obsoleta* may not display the common antipredator behaviors of aquatic gastropods. In other snail species, juveniles tend to be less tolerant to desiccation and to heat stress than adults (Gosselin & Chia 1995, Arad & Avivi 1998, Diederich et al. 2015). Intolerance of heat and desiccation could contribute to the lack of antipredator response by juveniles when exposed to predator chemical cues, and may explain

why they were more likely to move into the water. Also, these snails are highly gregarious and follow the mucus trails of conspecifics (Trott & Dimock 1978), resulting in extremely dense populations with greater than 500 snails per square meter (Kelaher et al. 2003). These high densities and trail following behaviors may provide a safety-in-numbers effect for *T. obsoleta*, where the chance of any one snail being consumed by a predator is very small (Ng et al. 2013). Juvenile snails are easier for predators to consume (Rochette & Himmelman 1996, Perez et al. 2009, Appendix 1) and high population densities can protect against predation. Also, there may be a high risk of desiccation for juveniles, which combined with high population densities could explain the lack of juvenile antipredator behavior.

Antipredator behaviors often result in reduced feeding rates as animals that are buried or move out of the water will spend less time feeding. This is commonly seen in other gastropods exposed to predators (Appleton & Palmer 1988, Trussell et al. 2003, Bourdeau 2010), and buried *T. obsoleta* have less food in their guts than unburied snails (Levinton et al. 1994). Juvenile *T. obsoleta* have high individual feeding rates, which are similar to those of adults when controlling for shell size (Chapter 2). For the juveniles, there may be a tradeoff between continuing to forage (which would affect growth rate and enable them to achieve a larger, less vulnerable size) and exhibiting antipredator behavior (Levri & Lively 1996, Hamilton & Heithaus 2001). Therefore, instead of moving out of the water or burrowing, juvenile snails might benefit most by living in very dense assemblages and remaining in the water feeding or searching for food. Fast growth may then allow juvenile snails to reach a larger size more quickly where it is harder for predators to consume them.

It is interesting that the proportion of both adults and juveniles that burrowed into the sand when exposed to predator chemical cues was similar (~ 20%). But, in the control

treatments, adults were much less likely to burrow (~7%) than juveniles (~40%). So although juveniles and adults had similar responses when exposed to predator chemical cues, the control animals exhibited opposite behaviors. This suggests that their burrowing behavior of *T. obsoleta* in general differs over ontogeny, and increased predator risk resulted in lower rates of burial in juveniles, opposite to predictions.

Although there was an increase in the observed antipredator behaviors of adult snails, the behavioral response was weak. Just over half of the snails crawled out of water (compared to about 40% in the control treatment), and only 20% of snails burrowed when exposed to predator chemical cues compared to < 10% in the control treatment. Similar to the juvenile snails, adults exhibit trail following behaviors and are often found in large groups, which may also protect adult snails from predation (Ng et al. 2013). Also, the large size and robust shells of adult *T. obsoleta* make it difficult for crushing predators to consume them (Appendix 1).

A previous study by Atema & Stenzler (1977) found that *T. obsoleta* exposed to the chemical cues of the predator *C. maenas* crawl out of the water rather than bury into the sand. I used different experiments to examine the burrowing and crawl out behaviors, so these two behaviors were not examined in the same experiment. During the burrowing experiment, I did find that some adult snails would crawl out of the water instead of burrowing into the sand, but a lack of sediment in the crawl-out tanks did not allow a direct test of these previous findings. And, although some adult *T. obsoleta* exhibited both antipredator behaviors in my separate experiments, a greater number of adult snails crawled out of the water (~50%) compared to the number that burrowed into the sand (~20%) when exposed to predator chemical cues. Therefore, my results are consistent with those of Atema and Stenzler (1977).

Green crabs were introduced to the East Coast of the United States around 1817 (Carlton & Cohen 2003), and as a consequence, *C. maenas* is not a native predator of *T. obsoleta*. Gastropods do not always exhibit phenotypically plastic behaviors in response to non-native predators (reviewed in Hollander & Bourdeau 2016), which likely indicates a lack of long-term coevolutionary history between them. However, *C. maenas* has resided in the native habitat of *T. obsoleta* for almost 200 years. A study conducted almost 40 years ago (Atema & Stenzler 1977) found that *T. obsoleta* were shown to respond to *C. maenas* chemical cues. In addition, several other molluscan species have also shown phenotypically plastic responses to non-native green crabs predators (e.g., *Nucella lapillus*: Trussell et al. 2003, *Mytilus edulis*: Freeman & Byers 2006, *Littorina obtusata*: Rochette et al. 2007, *Haustrum vinosum*: Freeman et al. 2013). Moreover, there was no difference in the response of adult *T. obsoleta* to chemical cues of *C. maenas* and the native predator *A. forbesi*. Similar data were not available for juvenile *T. obsoleta*, so further experimentation is needed to determine if they respond differently to increased risk from a native predator relative to their response to *C. maenas.* In addition, it is important to determine how juveniles respond to predator chemical cues from predators that use different feeding methods than *C. maenas*, such as *A. forbesi*, which does not have to break the shell to consume the snail. Although adult *T. obsoleta* have similar behavioral responses to both *C. maenas* and *A. forbesi*, that may not be the case with juvenile snails, and the different feeding methods might elicit different phenotypically plastic responses, as seen in other molluscs (Smith & Jennings 2000, Bourdeau 2009).

Although I found an effect of collection site on snail behavior in the juvenile burrowing experiment, juveniles from all sites responded similarly to the threat of predation. The behavioral variability among sites is unlikely to be due to genetic differences because this

species has a long lived veliger larva (Scheltema 1964) that results in long range dispersal, as seen in other gastropods with similar dispersal ability that are genetically similar at these spatial scales (Berger 1973, Riquet et al. 2013). The differences found may be due to other environmental differences at each site, such as differences in substrate, water flow/ waves, and presence of other predators, which are known to affect phenotypically plastic traits in other gastropod species (e.g., Saura et al. 2012, Marquez et al. 2015, Gustafson & Bolek 2016). Small waves can easily displace adult *T. obsoleta* from the surface of the substrate (personal observation), and increased burrowing would help prevent snails (especially the smaller juveniles) from being washed away by the waves, which might explain the differences in burrowing behavior of juveniles among sites. Further experiments are needed to determine the possible causes of these population level differences in propensity to burrow. But, the overall similarity in behavior for snails from the different sites suggests that these antipredator responses are common for *T. obsoleta* in this region.

For the experiments reported here, each snail was only tested once to maintain independence of responses among replicates. Repeated testing of the same individuals would determine the consistency of the behaviors among individuals, which could indicate genetic differences in the propensity to respond to predators. In addition, because different snails were used in the different experiments, it is not possible to determine if there is a correlation between burrowing behavior and the likelihood of crawling out of water. Further studies are needed to determine if the observed antipredator behaviors are correlated or if there are genetic differences among snails that displayed different responses.

Adult *T. obsoleta* displayed antipredator behaviors when exposed to chemical cues from a predator, although in smaller numbers than expected. However, juvenile snails did not alter

their behavior by crawling out of the water when exposed to cues from predators and behaved contrary to what was expected by burrowing less frequently when exposed to cues from predators. Juveniles might not express antipredator behaviors due to potential lower heat and desiccation tolerance relative to adults, or due to potential differences in the consequences of tradeoffs between feeding rate, and growth rate. Both adult and juvenile *T. obsoleta* might be benefitting from gregarious behavior and large population size, resulting in safety-in-numbers from predation. More work is needed to determine how these behaviors affect individual snail survivorship, predator consumption rates, and how these antipredator behaviors might alter other species interactions, and subsequent effects on the whole community.

Table 3-1. Number of *T. obsoleta* examined in each experiment by treatment and collection site. These numbers do not include the parasitized adult snails, which were excluded from the analyses.

Experiment	*Treatment*	Number of snails			
		Crab Meadow	**Ponquogue Bridge**	**Shinnecock Canal**	**West Meadow**
1 Adult Slope	*Control*	89	82	74	76
	Crab	96	74	78	73
	Seastar	93	79	79	72
2 Juvenile Slope	*Control*	40	40		40
	Crab	40	40		40
	Seastar				
3 Adult Burrowing	*Control*	56	51	51	44
	Crab	58	51	48	38
	Seastar	54	46	48	42
4 Juvenile Burrowing	*Control*	40	40		40
	Crab	40	40		40
	Seastar				

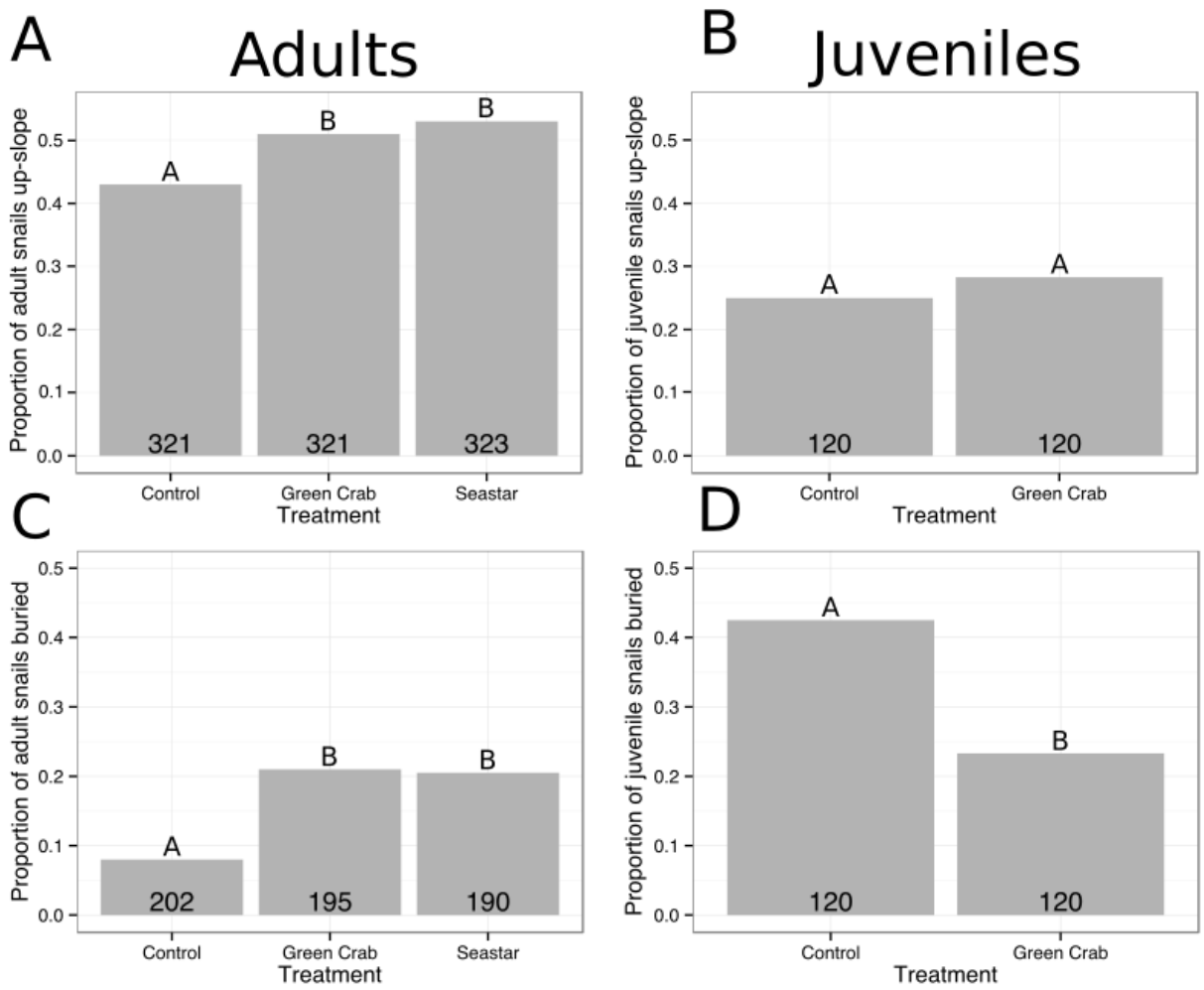

Figure 3-1. Antipredator behavior of *T. obsoleta* by predator chemical cue treatment. The proportion of (A) adult or (B) juvenile snails that crawled out of the water in each treatment (predator chemical cue treatments; *C. maenas* or *A. forbesi*, and control treatment), and the proportion of (C) adult or (D) juvenile snails that burrowed in each treatment. Sample sizes for each group are listed at the bottom of each bar, and bars with the same letter were not statistically significantly different (G-test).

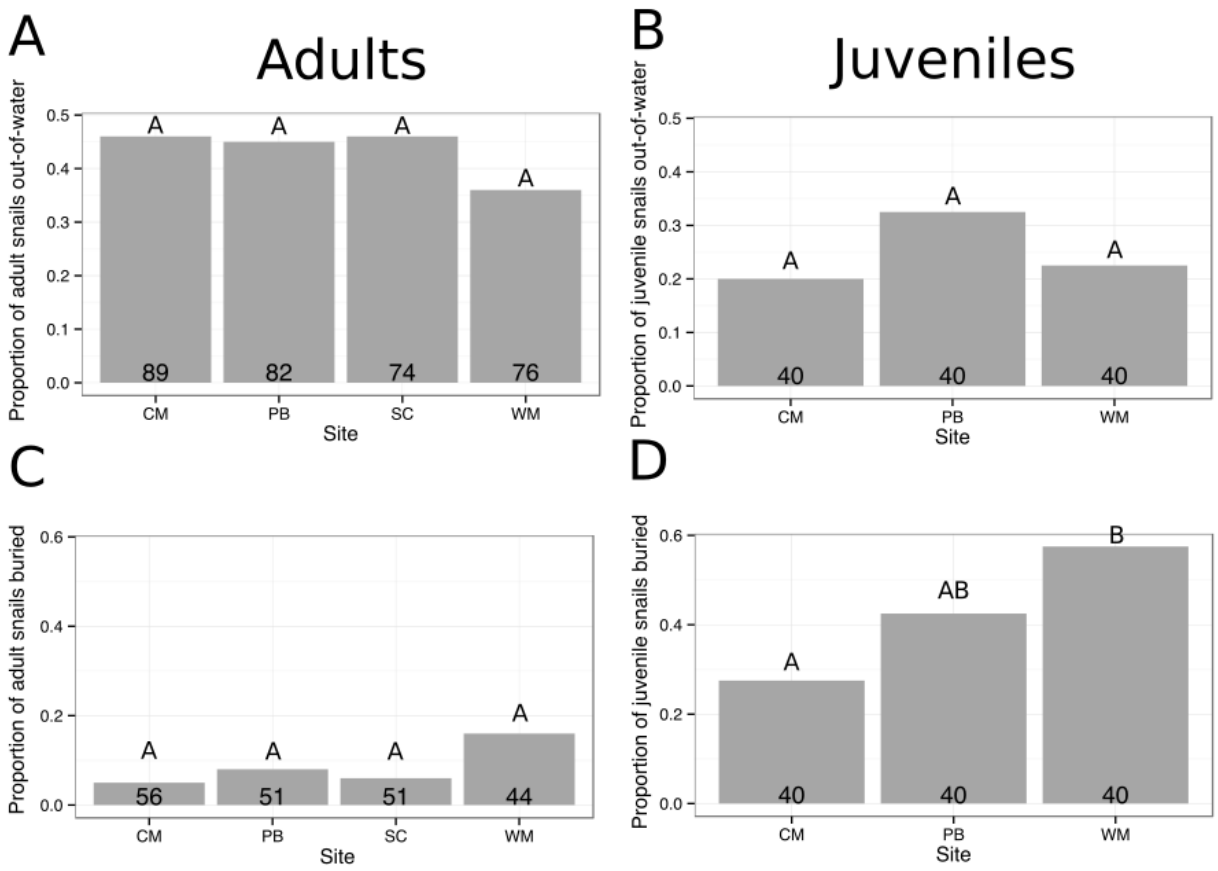

Figure 3-2. Behavior of *T. obsoleta* from different collection sites in the control treatment of each experiment. Control treatment behavior was used to determine whether snails from different sites behave differently in the absence of any environmental cues. The proportion of (A) adult or (B) juvenile snails that crawled out of the water for each site in the control treatment, and the proportion of (C) adult or (D) juvenile snails that burrowed by each site in the control treatment. Sample sizes for each group are listed at the bottom of each bar, and bars with the same letter were not statistically significantly different (G-test). Collection site codes: Crab Meadow Beach (CM), Old Ponquogue Bridge Marine Park (PB), Shinnecock Canal (SC), and West Meadow Beach (WM).

Chapter 4

The effect of parasitism on the movement of marine snail *Tritia obsoleta* in the laboratory and field

Abstract

Parasites with complex life cycles can alter host behavior in order to increase the probability of transmission between hosts. One of the first studies on gastropods and parasite manipulation of host behavior found that mudsnails, *Tritia obsoleta*, infected by the trematode parasite *Gynaecotyla adunca* are more likely to be found high on the shoreline (Curtis 1987). A later study found conflicting evidence that *G. adunca* infected snails were more likely to be lower along the shoreline (McCurdy et al. 2000). This study examined the up- and down-slope movement of *T. obsoleta* to determine if parasitism by different trematode parasites, including *G. adunca*, affected behavior in the laboratory. The results of these experiments were then compared to behaviors observed in a field experiment. In general, parasitized snails were more likely to move downslope after an hour in the laboratory, but not in the field. Snails infected by *G. adunca* were more likely to move down-slope in the laboratory and were more likely to move either up- or down-slope in the field than unparasitized snails. These results suggest that downslope movement might be a manipulation by *G. adunca* parasites or just a by-product of parasitism, but that this behavior is likely to be affected by other environmental conditions as well.

Introduction

Parasites with complex, multi-host life cycles face many challenges completing their life cycle. They must locate and infect intermediate hosts and be able to reach their final host where they can reproduce. Many parasites with complex life histories have been found to influence host phenotype in ways that facilitate completion of the life cycle (reviewed in Moore 2002). Such host manipulation, or modification of host behavior, is hypothesized to have a selective advantage when the behavior facilitates completion of the parasite life cycle (Moore 2002, Poulin 1994, 2010). The infected host can exhibit behaviors that increase consumption by a predator that is the parasite's next host, or hosts can move to a habitat that increases the spread of parasites to the next host (reviewed in Lafferty & Shaw 2013, Moore 2002, Poulin 1994, 2010).

Parasite manipulation of host behavior often increases interactions among host species, such as enhancing trophic transmission through conspicuous behaviors (Koella et al. 2002, Rogers & Bates 2007, Stafford et al. 2011), or through host movements that bring them closer to a location or habitat suitable for the parasite's next life stage (Andersen et al. 2009, Curtis 1987, Thomas et al. 2002). For example, when infected with juvenile nematomorphs, insects behave erratically and eventually jump into streams or lakes where the adult nematomorphs live and reproduce (Sanchez et al. 2008, Thomas et al. 2002). Ants with fungal parasites bite into the bottom of leaves where the humidity and temperature is ideal for parasite growth and reproduction (Andersen et al. 2009).

Behavioral changes of hosts have been found for viruses, fungi, bacteria, and protozoans, as well as nematode, nematomorph, trematode, cestode, and acanthocephalan parasites (Lafferty & Shaw 2013, Lefèvre et al. 2009, Poulin 2007). Since gastropods are the primary first

intermediate hosts of trematode parasites (Poulin & Mouritsen 2003, Faltýnková et al. 2007), they have been the focus of many studies on parasite manipulation. One of the first studies examining the effect of parasitism on gastropod host behavior focused on the marine snail *Tritia obsoleta* (Say, 1822), formerly *Ilyanassa obsoleta*. Snails infected with the trematode species *Gynaecotyla adunca* were more likely to be found high on the shoreline than unparasitized snails (Curtis 1987). Later, another study was conducted which found that *T. obsoleta* infected with that same parasite species were more likely to be found lower along the shore (McCurdy et al. 2000). There are three possible explanations for the different results seen in these two studies: (1) Differences in snail behavior are due to the next intermediate host of *G. adunca* resides at different locations at the different study sites (Curtis 1987: Cape Henlopen, Delaware Bay; McCurdy et al. 2000: Minas Basin, Bay of Fundy), (2) Different species of crustaceans act as a second intermediate host at these different sites, and these host species may have different distributions along the shore at the different sandflat and mudflat sites (as hypothesized by McCurdy et al. 2000), and (3) Other environmental factors are affecting snail behaviors at the different study sites.

In this study, I examined *T. obsoleta* in the laboratory to determine if different species of parasite that infect this snail alter host up- or down-slope movement, hereafter referred to as crawl-out behavior. I then examined snail behavior in a field experiment to determine if behaviors seen in the laboratory were similar to those seen in nature. I also tested whether there were behavioral differences between parasitized and unparasitized snails. If parasitized snails all behaved similarly independent of the species of parasite, it would indicate that altered behavior may be a by-product of parasitism rather than the result of parasite manipulation (Minchella 1985, Sorensen & Minchella 2001, Thomas et al. 2005).

Materials and Methods

Study System

Tritia obsoleta is an intertidal zone gastropod found in salt marshes, mudflats, and beaches along the Atlantic coast of North America. They are extremely abundant, often found at densities greater than 500 snails per square meter (Kelaher et al. 2003). The abundance of these snails and the fact that they are non-selective consumers (scavengers that also consume algae and detritus) indicates that they can have a large impact on their community by, for example, altering the abundance of algae in sediments, and affecting the distribution of other benthic invertebrates (Connor et. al 1982, Curtis & Hurd 1981, Feller 1984, Kelaher et. al 2003). *Tritia obsoleta* is the first intermediate host for nine species of trematode parasites (reviewed in Blakeslee et al. 2012, Phelan et al. 2016). Approximately 7-40% of adult *T. obsoleta* within a population are infected by trematode parasites along the shores of Long Island, NY (Appendix 2).

Parasite Life Cycle

Trematodes are flatworm parasites with complex life cycles. They can have between two and six different larval stages (miracidium, sporocyst, redia, cercaria, metacercaria, mesocercaria) that can infect between two and four different host species. The first intermediate host is typically a species of gastropod (in this case, *T. obsoleta*), and the final host, where sexual reproduction occurs, is usually a vertebrate (fish, bird, terrapin, reviewed in Blakeslee et al. 2012, Phelan et al. 2016). Snails can become infected by consuming trematode eggs or by a newly hatched miracidium larva. The miracidia reproduce asexually within the gonads of the snail, producing sporocysts, which go on to produce more sporocysts or rediae larvae. These sporocysts or rediae then asexually produce cercariae larvae, which leave the gastropod host in

search of their next host (Stunkard 1938, 1983, Moore 2002). Since these parasites inhabit and consume the gonads, and often the digestive glands, of their gastropod host, the snail is usually fully or partially castrated (Cheng et al. 1973, Hoskin 1975, Sullivan et al. 1985).

Snail Collection

Tritia obsoleta were haphazardly collected from three beach sites (n = 1402 per site) on Long Island, NY during the summer of 2014 (Old Ponquogue Bridge Marine Park (PB) 40.8433, -72.4985, near Shinnecock Canal (SC) 40.8835, -72.4848, and West Meadow Beach (WM) 40.9443, -73.1466; Appendix 3). Snails were collected from each site six times between May and August. Snails 14 mm length and larger were collected because parasite infection occurs in snails larger than 12-14 mm in shell length, as this is the size when snails reach sexual maturity (Scheltema 1964). The snails were placed in containers marked with the date and site of collection, returned to the lab, and were kept in a recirculating seawater tank at ~ 20 ˚C and fed *Ulva* alga (Giannotti & McGlathery 2001) *ad libitum* until used in the experiment (lab exp: 0-12 days, field exp: 4-30 days).

Experimental Design

Laboratory Experimental Design

For the laboratory experiment, eighteen 10 L tanks (30.5 cm long x 19.1 cm wide x 20.3 cm high) were set up with a sloped bottom made of Plexiglas® sheeting (~ 26.6 cm long) that mimicked the slope of a shoreline (~10˚) and filled with 1.5 liters of seawater to cover half of the slope, which was approximately 4 cm at the deepest point. No natural substrate was placed on top of the Plexiglas®, because I wanted to examine the crawl-out movement alone and not burrowing behaviors of the snails. Experiments were conducted in a laboratory lit with overhead

fluorescent lights (no windows), and there was no light or temperature gradients among the tanks. The seawater (salinity 29) was drawn from a saltwater well at the Flax Pond Marine Laboratory (40.9613, -73.1387), which is over 5 km from the nearest collection site. The water was aerated with an airstone for approximately 16 hours prior to use. For each replicate, water was freshly replaced from the stock container.

For each replicate (3 populations, 6 snails per population), one snail was placed facing the water in the middle of the Plexiglas® slope at the air-water interface. After one hour, the location (up-slope or down-slope) of the snail was recorded. All snails moved either up- or down-slope from their original position. Some snails (47, 2.2% of all snails in laboratory experiment) crawled down into the water and then up the side of the tank and out of the water; these snails were counted as crawling up-slope. The tanks were then wiped down to remove any mucus trails, the water was replaced, and the next replicate was started with each tank containing a snail from a different site than the previous replicate. Replicates were run until 700 snails from each site were tested.

At the end of both experiments, the length of each shell was measured with digital calipers (± 0.01 mm), and snails were dissected (Chapter 2) and viewed with a dissecting microscope to determine infection status (parasitized or unparasitized). If the snails were parasitized, parasites were identified to species by examining the morphologically distinct cercaria (McDermott 1951, Stunkard 1983). If the cercaria life stage of the parasite was not present, the species of parasite could not be identified.

Field Experimental Design

For the field experiments, snails were separated by collection date and site. Three experimental trials were conducted over the summer with snails collected on two different dates for each trial (702 snails per site, 234 snails per site per trial, 117 snails per site per collection date). Snail shells were dried with snails gently prodded into their shell, and one of six colors of spray paint was applied to the shell of each snail, with a different color for each site and collection date in order to keep track of when and where the snails were collected, as well as to tell them apart from snails in the field. The snails were then kept separate by collection date and placed in a recirculating seawater tank until the following day. On the next day, snails were removed from the tank and taken to a new site, Crab Meadow Beach (CM, 40.9293, -73.3281, greater than 5 km from all source population sites, shore slope ~1.5˚), for the early morning tide (low tide earlier than 9 am). A transect was laid out at least ten meters up-shore from tidal datum (mean low tide). Snails painted the same color (117 snails of each color) were laid out evenly along 1 m intervals of transect with two meters left between snails of each paint color. Snails were placed along the transect (±1 cm from the transect), and were doused with seawater to encourage movement. The snails were then left for one hour, after which they were recollected. They were categorized as moving up-shore (> 3 cm above the transect line), down-shore (> 3 cm below the transect line), or they did not move (snails either did not move, or moved along the transect line). The search area was 3 meters on either side of the transect line although most snails were within 0.5 meters of their starting location. Not all snails were found at the end of each trial (recovery for trial 1: 602/702, trial 2: 655/702, trial 3: 512/702). Snail recovery was likely not affected by predation or by the snails moving underwater because snails were placed at least ten meters up-shore from the water.

At the end of the experiment, snails were measured and dissected to determine the infection status, and when found parasites were identified to species as described in the laboratory experiment.

Data Analysis

A two-tailed t-test was used to determine if the mean shell length of parasitized snails differed from the mean length of unparasitized snails for each source population of snails for the experiment.

I used G-tests to determine whether the frequency of snails that exhibited each behavior (laboratory: up-/down-slope, field: up-slope, down-slope, no movement) differed by infection status (unparasitized, parasitized) or by collection site for both the laboratory and field experiment. I then tested whether snails infected with different parasite species differed in behavior. Odds ratios were also calculated to test for any interaction between infection status and collection site on snail behavior. I also used G-tests to examine whether the frequency of unparasitized snails exhibiting each behavior differed by collection site in the laboratory and field experiment. When needed, stepwise Bonferroni corrections were used to correct for multiple comparisons with the same data.

I also used permutation tests to determine if the behavior of snails infected with each species of parasite differed from unparasitized snail behavior in the laboratory and also in the field. Using the observed distribution of unparasitized snail behaviors (laboratory: up- or down-slope; field: up-slope, down-slope, no movement), a behavioral response was randomly assigned to each parasitized snail, and this was repeated 10,000 times to create a new distribution of behavioral responses for snails infected with each parasite species. This expected distribution

was then compared to the observed data, and a p-value was determined. Similar permutations were conducted for each parasite species where there were greater than 25 snails infected with that species in the given experiment (snails infected by *A. variglandis*, *D. nassa*, and *S. tenue* were not tested). P-values less than or equal to 0.05 were regarded as significant. All permutations were performed using R (Version 3.2.1, R Core Team 2014).

Results

Eight species of parasites were found in snails collected from the field: *Austrobilharzia variglandis* (Miller & Northup 1926), *Diplostomum nassa* (Martin 1945), *Gynaecotyla adunca* (Linton 1905), *Himasthla quissetensis* (Miller & Northup 1926), *Lepocreadium setiferoides* (Miller & Northup 1926), *Stephanostomum dentatum* (Linton 1900), *Stephanostomum tenue* (Linton 1898), and *Zoogonus lasius* (Leidy 1891; Table 4-1). The prevalence of parasites in snails collected for experiments varied among collection sites (PB: 21.0%, SC: 36.1%, WM: 37.1%). Most parasitized snails (1130) were infected with a single identifiable parasite species (Table 4-2). Seventeen snails were infected with two parasite species, 58 snails were infected with parasites that were unidentifiable because no cercariae were present, and 2648 snails were not parasitized (68.7% of all snails).

The shell lengths of snails varied by site and by infection status. The largest snails were from PB and the smallest were from SC (Table 4-3). The size ranges of parasitized and unparasitized snails largely overlapped, but parasitized snails were significantly larger than unparasitized snails at each collection site ($p < 0.0001$ for each comparison, Table 4-3). Since snails were collected from the field, the size differences between parasitized and unparasitized snails may also be due to differences in snail age or site-specific growth rate.

In the laboratory, there was a significant effect of infection status on the up- and down-slope behavior of *T. obsoleta* (G test, df = 1, G = 12.46, $p < 0.001$, Figure 4-1A). Parasitized snails were more likely to move down into the water than unparasitized snails. Overall, the species of parasite that a snail was infected with did not impact behavior in the laboratory (G test, df = 2, G = 1.96, $p < 0.20$, Fig. 4-1B), however the permutation tests showed that snails infected with *G. adunca* were more likely to move down-slope than unparasitized snails (permutation, $p = 0.013$, Table 4-1).

In the field experiment, infection status did not alter snail behavior (G test, df = 2, G = 1.96, $p > 0.20$, Fig. 4-1C), but the parasite species that infected the snail did (G test, df = 8, G = 26.35, $p < 0.001$, Fig. 4-1D). Snails infected with *G. adunca* were more likely to move either up- or down-slope (permutation, $p = 0.0056$), while snails with *L. setiferoides* (permutation, $p = 0.0032$) or *S. dentatum* (permutation, $p = 0.0074$) were less likely to move (either up or down) than unparasitized snails. Snails parasitized by *H. quissetensis* were less likely to move up-slope than unparasitized snails (permutation, $p = 0.001$, Table 4-1, Fig. 4-1D).

Source population affected the up-slope/down-slope behavior of unparasitized snails in both the laboratory (G test, df = 2, G = 31.64, $p < 0.001$, Fig. 4-2A) and field experiment (G test, df = 4, G = 74.81, $p < 0.001$, Fig. 4-2B). Since there were behavioral differences among sites in unparasitized snails, the behavior of all snails was examined by site and infection status. When examining all snails (parasitized and unparasitized), the site that the snails were collected had an effect on behavior in the laboratory (3-way G test, df = 2, G = 22.82, $p < 0.005$), and in the field (3-way G test, df = 4, G = 94.50, $p < 0.005$, Fig. 4-2). In the laboratory, there was no difference among the behavior of parasitized snails collected from different sites, however, uninfected snails from site SC were more likely to move up-slope than uninfected snails from sites SB and

WM (Odds ratio: Table 4-4, Fig. 4-2A). In the field experiment, parasitized snails from sites SC and WM had similar behaviors while SB snails were more likely to up-slope (Odds ratio: Table 4-3, Fig. 4-2B). Unparasitized snails from each site differed in behavior in the field experiment (Table 4-4, Fig. 4-2B).

Discussion

Parasitism often affects host behavior to facilitate the completion of the parasite's life cycle (Lafferty & Shaw 2013, Moore 2002, Poulin 2010). *Gynaecotyla adunca* is thought to manipulate the up-slope or down-slope behavior of its host, *T. obsoleta*, to help this parasite reach its next host. Two previous observational field studies found that *G. adunca* altered behavior in opposite ways (Curtis 1987, McCurdy et al. 2000), each claiming that this behavior was likely to facilitate transmission of the parasite to its next host. This study examined snail behavior in the laboratory to determine if the different parasite species are manipulating behavior, and these results were then compared to short-term field experiments examining the same behavior. I found that snails infected by *G. adunca* were more likely to move down-slope than unparasitized snails in the laboratory consistent with the findings of McCurdy et al. (2000). In the field, however, *G. adunca* infected snails were more likely to move (either up- or down-slope) than uninfected snails, with no consistent up- or down-slope movement. Snails infected with *H. quissetensis* were less likely to move up-slope, and snails parasitized by *L. setiferoides* and *S. dentatum* were less likely to move than unparasitized snails in field. Overall, parasitized snails were more likely to move down-slope in the laboratory, but in the field snails infected by different species of parasites behaved differently. Furthermore, the site which snails were collected from also significantly affected behavior in both the laboratory and the field experiments.

Snails parasitized by *G. adunca* were the only snails that behaved differently than unparasitized snails in the laboratory, which suggests that there may be some parasite manipulation of behavior occurring. But the field experiment found no clear indication of movement either up- or down-slope (more likely to move either up- or down-slope than unparasitized snails, which could match either Curtis' (1987) or McCurdy's (2000) study). Snails infected by *Z. lasius* behaved the same as unparasitized snails in both the laboratory and the field experiment, which suggests that this parasite species does not manipulate or alter host movement behavior.

Infection status had a large effect on behavior in the laboratory experiment, where parasitized snails were more likely to move down-slope than unparasitized snails. This movement into water may be a by-product of parasitism. In other gastropod species, parasitized snails are more susceptible to desiccation than unparasitized individuals (Jensen et al. 1996); if this is occurring with *T. obsoleta*, it may explain the down-slope movement seen in the laboratory, especially since the up-slope movement left them on dry Plexiglas®. However, once in the field, there was no difference between the behavior of the parasitized and unparasitized snails. This indicates that although up-/ down-slope behaviors may be a by-product of parasitism, environmental cues seem to have a larger impact on host movement.

The behaviors of *T. obsoleta* seen in the field varied depending on the species of parasite; only snails infected by *H. quissetensis*, *L. setiferoides*, and *S. dentatum* behaved differently than unparasitized snails. Those infected with *H. quissetensis* were more likely to move down-slope or more likely to not move, and snails infected with *L. setiferoides* and *S. dentatum* were more likely to not move either up- or down-slope. Differences between the behaviors of snails infected by these three species in the field and the laboratory may be the result of the 3 possible

response variables in the field experiment (compared to the 2 possible responses in the laboratory, up- or downslope), or due to environmental factors present during the field experiment.

Many environmental cues are known to alter gastropod behaviors; food availability (Kelaher et al. 2003), water flow (Levinton et al. 1995), and conspecific trails (Trott & Dimock 1978) are all known to alter distributions of *T. obsoleta* and where parasitized individuals reside (Rossiter & Sukhdeo 2012). In the laboratory, tanks were free of food, did not have flowing water, and were cleaned between replicates to remove mucus trails. However, all of these environmental signals could affect the response of snails in the field. In addition, the snails in the laboratory were tested individually while multiple snails were placed next to each other in the field. Field experiments were conducted up-shore from low tide, so there was no direct effect of water flow, but various types of food were readily available, and previous mucus trails were likely to be present, both of which could have altered the behavior of snails in the field experiment. Another difference between the laboratory and field experiments is that natural substrate was used in the field, while snails in the laboratory had Plexiglas® as substrate. Since Plexiglas® has properties unlikely any natural substrate that snails would encounter in the field, it could alter their behavior. These factors may not only be important for differences in behavior between the laboratory and field experiments, but may also contribute to the behavioral differences seen when comparing snail behavior across snails collected from different source populations.

Behavioral differences for snails collected from different sites could result from various biotic and environmental factors that may differ among sites; natal habitats can influence behavior, and have a continued impact throughout the organism's lifetime (reviewed in Benard

& McCauley 2008). While there might be microhabitat differences between sites PB and SC, they are found on opposite sides of Shinnecock Bay (Appendix 3), and share similar overall habitats and any chemical cues in the water are likely similar. Predator abundance at each site would alter chemical cues that snails from each site are exposed to, but predator abundance is similar among sites (personal observation). It is also possible that there are genetic differences among the snails from different sites, and differences in behaviors are genetically based rather than due to phenotypic plasticity. However, *T. obsoleta* have planktonic larvae that remain in the water column for up to 30 days (Scheltema 1964), which should allow mixing of larvae among sites. Other gastropods in the region with planktonic development have not been found to have genetically distinct populations over distances further than that between sites PB and SC (Berger 1973, Riquet et al. 2013).

Host behavior was affected by an interaction between collection site and infection status in both laboratory and field experiments. These interactions could be driven by the dominant parasite species found at each site. For both the laboratory and field experiments the most prevalent parasite species (40- 70% of infected animals, depending on site and experiment) was different for each site (PB: *H. quissetensis*, SC: *G. adunca*, WM: *L. setiferoides*). Therefore, the interaction seen between infection status and source site may be driven by the different species found at each site, rather than infection status per se. However, due to small sample sizes for many of the parasites, and because site and parasite species were confounded, a much greater sample size of parasitized snails infected with each of the parasite species from each site would be required to test this hypothesis.

Snails that were infected by multiple species of parasites (0.4%) or that were infected by parasites that could not be identified (1.5%) were included in the group of parasitized snails, but

were not examined separately. It is interesting to note that there were very few snails that were infected with more than one parasite species, especially since other studies have found much higher rates of multiple infections of *T. obsoleta* (Curtis 1997: 12.57% double infections, Hendrickson & Curtis 2002: 3.7-33.7% double infections).

In this study, individual snails were used in experiments only once to maintain independence among replicates. Parasitism may not only influence the behavior of the hosts, but it might also influence the consistency of a certain behavior (Poulin 2013). Parasitized organisms may exhibit more erratic behavior than unparasitized individuals, contrary to the behavioral changes seen when parasites manipulate host behavior. Further studies are needed to determine the consistency of the behaviors exhibited by *T. obsoleta* infected with each species of parasite to determine if parasites are manipulating behavior in these snails.

In the laboratory, snails parasitized by *G. adunca* were more likely to move down-slope, which aligns with McCurdy's previous observations (2000). But when examined in the field, there was no clear pattern of movement. These results suggest that the down-slope behavior may be a by-product of parasitism, but that this behavior can be overridden by other environmental cues in the field. More work is needed to determine which biotic or abiotic cues can affect snail behavior, how natal environmental cues affect behavior when animals are moved to a novel environment, and how environmental cues interact with parasite infections to alter behavior of their hosts. Further work is also needed to determine if different behaviors of hosts affect parasite transmission for each species, as well as the consequences of these behaviors on the hosts, other species interactions, and the whole community.

Table 4-1. The behavior of *T. obsoleta* seen in the laboratory and field experiments by parasite species, and the next hosts for these parasites. Bold arrows indicate how behavior differed from unparasitized control snails. Equal signs indicate that behavior was similar to control snails. Arrows pointing to the left and right indicate that snails were more likely to not move than to move up or down. Untested indicates that there were insufficient sample sizes to examine behavior.

Parasite Species	Behavior Observed		Next Intermediate Host	Definitive Host
	Laboratory Exp.	Field Exp.		
Austrobilharzia variglandis (Miller & Northup 1926)	(untested)		(rock, shell)	bird
Diplostomum nassa (Martin 1945)	(untested)		fish	bird ?
Gynaecotyla adunca (Linton 1905)	↓	↑ ↓	crustaceans	bird
Himasthla quissetensis (Miller & Northup 1926)	↓	↔ ↓	molluscs	bird
Lepocreadium setiferoides (Miller & Northup 1926)	=	↔	polychaetes	fish
Stephanostomum dentatum (Linton 1900)	=	↔	fish	fish
Stephanostomum tenue (Linton 1898)	(untested)		fish	fish
Zoogonus lasius (Leidy 1891)	=		polychaetes	fish

Table 4-2. Number of *T. obsoleta* infected with each species of parasite at each site. (Collection site abbreviations: PB = Old Ponquogue Bridge Marine Park, SC = near Shinnecock Canal, WM = West Meadow Beach). The most prevalent species at each site is in bold.

Parasite Species	Source Site		
	PB	SC	WM
A. variglandis	8	7	7
D. nassa	2	4	0
G. adunca	2	**235**	12
H. quissetensis	**161**	14	38
L. setiferoides	24	58	**316**
S. dentatum	57	89	30
S. tenue	1	3	7
Z. lasius	14	34	7
TOTAL	269	444	417

Table 4-3. The average shell lengths (± standard error) of parasitized and unparasitized *T. obsoleta* collected from each collection site. The snails used in the laboratory and field experiments were pooled together. P-values result from t tests used to compare the shell lengths between the unparasitized and parasitized snails from each site, and significant differences are in bold.

Collection Site	Infection Status	n	Average Shell Length (mm)	Size Range (mm)	p-value
Ponquogue Bridge	Parasitized	278	24.17 ± 0.146	18.06- 29.87	**< 0.0001**
	Unparasitized	1040	21.36 ± 0.060	15.56- 29.30	
Shinnecock Canal	Parasitized	463	19.88 ± 0.696	15.56- 25.05	**< 0.0001**
	Unparasitized	821	18.71 ± 0.046	14.87- 23.38	
West Meadow	Parasitized	464	20.64 ± 0.072	15.82- 26.18	**< 0.0001**
	Unparasitized	787	19.92 ± 0.052	14.26- 25.21	
All Sites	Parasitized	1205	21.16 ± 0.070	15.56- 29.87	**< 0.0001**
	Unparasitized	2648	20.10 ± 0.039	14.26- 29.30	

Table 4-4. Odds ratios were used to test for interactions between collection site and infection status on *T. obsoleta* behavior in the laboratory and field experiments. Significant differences are in bold. (Collection site abbreviations: PB = Old Ponquogue Bridge Marine Park, SC = near Shinnecock Canal, WM = West Meadow Beach)

	Laboratory experiment		Field experiment	
	Odds ratio	95% Confidence Interval	Odds ratio	95% Confidence Interval
Parasitized Snails				
PB versus SC	1.114	0.695 – 1.786	**3.801**	**1.899 – 7.607**
PB versus WM	1.147	0.7123 – 1.848	**5.693**	**2.813 – 11.52**
SC versus WM	1.03	0.6876 – 1.542	1.498	0.8399 – 2.671
Unparasitized Snails				
PB versus SC	**1.888**	**1.452 – 2.456**	**2.931**	**1.942 – 4.422**
PB versus WM	0.9346	0.7099 – 1.231	**4.803**	**3.131– 7.368**
SC versus WM	**0.495**	**0.3752 - 0.6531**	**1.639**	**1.017 – 2.641**

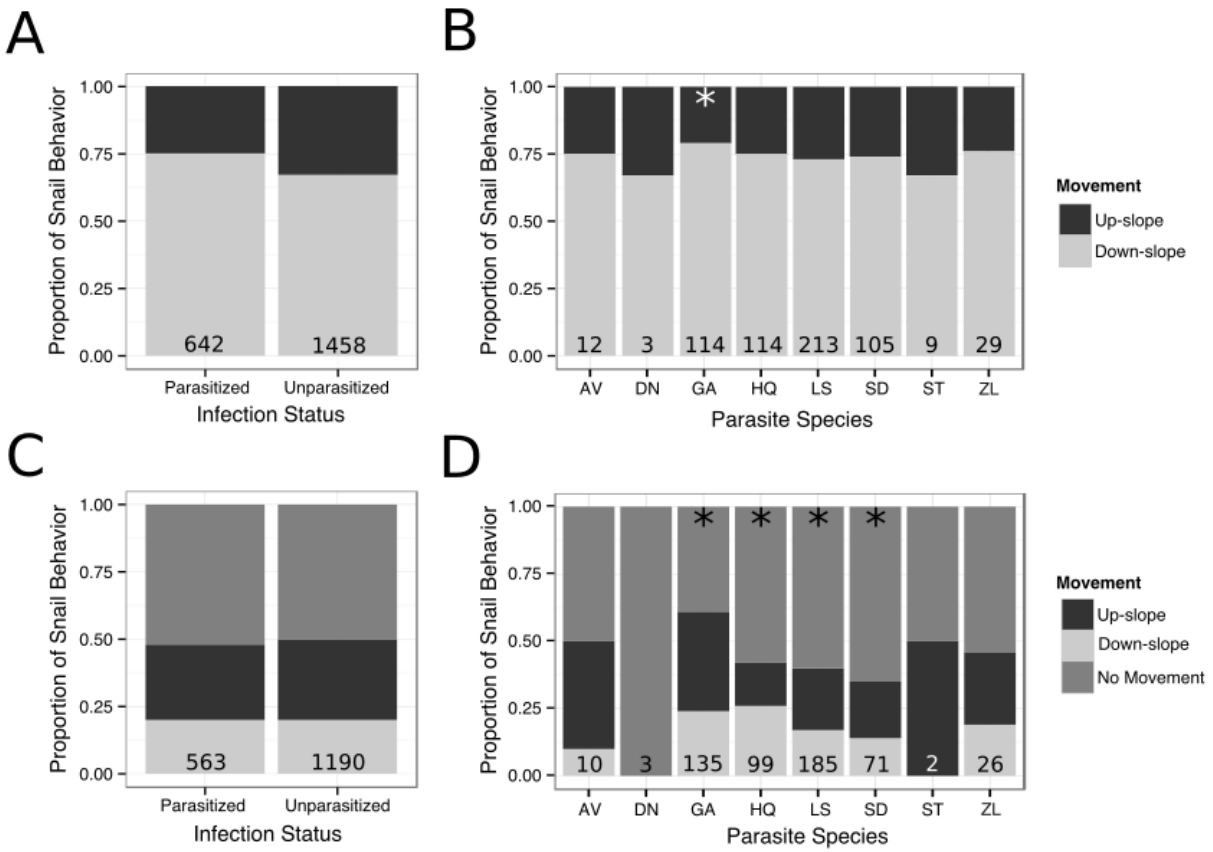

Figure 4-1. Behavior of *T. obsoleta* by infection status and by parasite species for the laboratory and field experiments. The proportion of parasitized and unparasitized snails that moved up-slope (black) or down-slope (light gray) in the laboratory (A) and up-slope (black), down-slope (light gray) or did not move (gray) in the field experiment (C). The proportion of snails infected with each species of parasite that moved up-slope in the laboratory (B) and up-slope, down-slope, or did not move in the field experiment (D). Sample sizes are listed at the bottom of each bar for each graph, and significance determined by permutation test is denoted with an asterisk. Only parasite species found in 26 or more snails were used in the permutation tests. The number of parasitized snails in each experiment includes snails that had double infections (lab: 14, field: 3) or were parasitized by an unknown species (lab: 29, field: 29). (Parasite species code: AV = *A. variglandis*, DN = *D. nassa*, GA = *G. adunca*, HQ = *H. quissetensis*, LS = *L. setiferoides*, SD = *S. dentatum*, ST = *S. tenue*, ZL = *Z. lasius*).

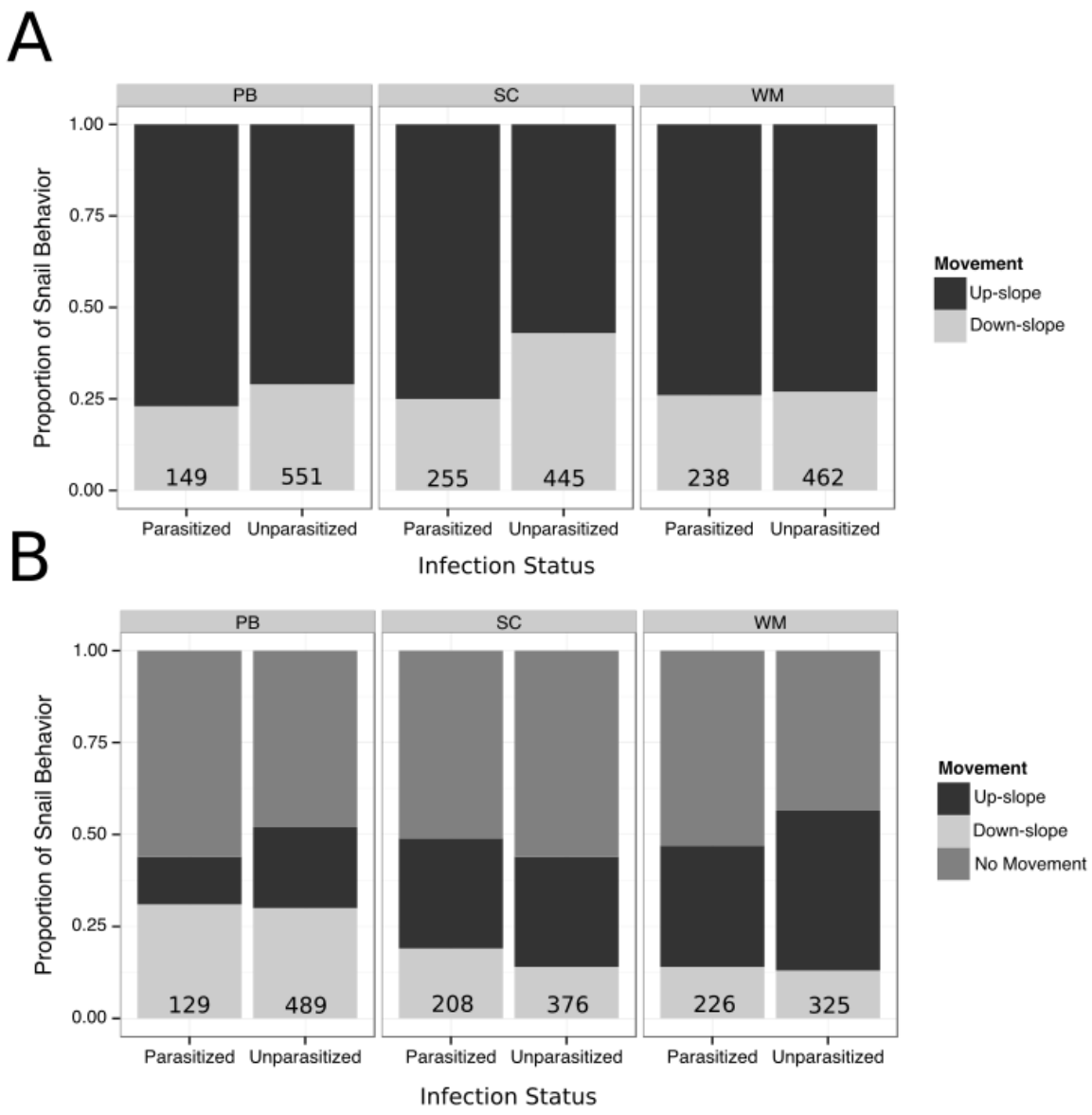

Figure 4-2. Behavior of *T. obsoleta* by infection status and collection site for the laboratory and field experiments. The proportion parasitized and unparasitized snails from each site that moved up-slope (black) or down-slope (light gray) in the laboratory (A) and up-slope (black), down-slope (light gray) or did not move (gray) in the field experiment (B). Sample sizes are listed at the bottom of the bars. (Collection site abbreviations: PB = Old Ponquogue Bridge Marine Park, SC = near Shinnecock Canal, WM = West Meadow Beach)

Chapter 5

Morphological plasticity in response to the environment, but not in response to predators

Abstract

Phenotypic plasticity is used by many organisms to reduce the risk of predation in variable environments. Plasticity is common in snails, which frequently display increased shell mass and altered shell shape when exposed to predators, typically due to reduced growth as a consequence of reduced feeding rates. However, some species of gastropods, such as *Tritia obsoleta*, formerly known as *Ilyanassa obsoleta*, are relatively invulnerable to predators, especially as adults. Hence, I conducted experiments to explore potential plasticity in juvenile *T. obsoleta* when exposed to chemical cues from a crab predator, *Carcinus maenas*. Juvenile snails were collected from three sites and exposed to chemical cues from predators or a control treatment. After 12 weeks, growth of *T. obsoleta* was measured and geometric morphometrics analyses were used to quantify differences in shell shape. Surprisingly, there was no evidence of induced defenses in *T. obsoleta*. Juvenile snails exposed to predator chemical cues had similar growth and morphologies as conspecifics in the control treatment. Rather than having plastic growth and morphology in response to predation, like most species of snails, they appear to feed more and grow as large as possible, which likely allows them to reach a size refuge from predation more quickly. However, other environmental factors do appear to impact shell morphology. Snails collected from different sites had different initial shapes, but converged on a similar morphology over the course of the experiment, regardless of experimental treatment.

Introduction

Environmental conditions in many habitats can change through time. Phenotypic plasticity is one way organisms respond to variable environments. The environmental conditions that induce plasticity can be abiotic (Silim et al. 2001, Pan et al. 2006, Chown et al. 2007, Guevara et al. 2010) or biotic, including intraspecific density dependence and interspecific interactions (Agrawal 2001, Callaway et al. 2003, Miner et al. 2005, Berg & Ellers 2010).

Phenotypic plasticity is often seen when species are exposed to risk of predation, and such defensive plasticity can be seen in prey behavior, life history traits or morphology. For example, when exposed to predators, the gastropod *Helisoma trivolvis* alters habitat preference, time to reproduction, and shell shape (Hoverman et al. 2005). Often, the morphology of prey will change in a way that deters predation or extends handling time (e.g., cladocerans: Black 1993, Miehls et al. 2014; tadpoles: Relyea 2001, Touchon & Warkentin 2008, Nunes et al. 2014; gastropods: Cotton et al. 2004, Hoverman et al. 2005). For example, species of *Daphnia* undergo various morphological changes, (e.g., production of helmets, neck teeth, dorsal spines) when exposed to predators, which reduce predation success (Sell 2000, Riessen & Trevett-Smith 2009, Rabus & Laforsch 2011).

Tritia obsoleta (Say), formerly *Ilyanassa obsoleta*, is a marine snail commonly found in the intertidal zone along the east coast of North America in salt marshes, mud flats and beaches. Important predators for *T. obsoleta* include crabs, such as the green crab, *Carcinus maenas*, and terrapins. Many species of snails exhibit shape changes when exposed to predators (Appleton & Palmer 1988, Krist 2002, Lakowitz et al. 2008, Bourdeau 2009). However, adult *T. obsoleta* have large, thick shells that are thought to protect them from many predators, including terrapins

(Tucker et al. 1997). Given the thickness of their shells, it is likely difficult for any predator to crush the shell of an adult, no matter the shape. As these snails grow, there may be a size refuge at which they are safe from crushing predators such as *C. maenas* and terrapins.

I examined the effects of chemical cues from the predator *C. maenas* on the growth and shell morphology of juvenile *T. obsoleta*. If shell morphology affects how crabs handle and crush shells, I expected snails exposed to predator chemical cues to have different shell shapes than snails in a control treatment without chemical cues from predators. Typically, snails exposed to a higher risk of predation by crushing predators have smaller apertures and a thicker aperture lip (Appleton & Palmer 1988, DeWitt et al. 2000, Bourdeau 2009). Both of these shape changes deter predation and lengthen handling time (DeWitt et al. 2000, Hoverman & Relyea 2009). Alternatively, defensive morphology might be less important than reaching a size refuge from predation. If *T. obsoleta* relies primarily on a size refuge to escape predation, I would expect continuous fast growth rather than a shift in morphology.

Materials and Methods

Snail Collection

The environment can have large impacts on snail morphology and growth (Boulding & Hay 1993, Trussell 2000a, Gustafson & Bolek 2016), so *T. obsoleta* were collected from multiple sites to test whether there were population differences and if local environment or population differences affected the responses of snails to increased predation risk. Juvenile *T. obsoleta* were collected from three beach sites on Long Island, NY during June 2014 (Crab Meadow Beach (CM) 40.9293, -73.3281, Old Ponquogue Bridge Marine Park (PB) 40.8433, -72.4985, and West Meadow Beach (WM) 40.9443, -73.1466; Appendix 3). From each site, 200

juvenile snails (6-12 mm shell length) were collected. Snails were placed in containers that were marked with the collection site, returned to lab, and kept in a 10˚C recirculating seawater tank until used in the experiment. *Carcinus maenas* were collected from CM and Stony Brook Harbor (40.9023, -73.1748) and kept in a separate recirculating seawater tank. Prior to the experiment, digital images were taken of each snail with the aperture facing towards the camera and with the camera lens parallel to the axis of coiling. The initial shell length of each snail was also measured using digital calipers (precision ± 0.01 mm). Each snail was then marked on its shell with colored nail polish to designate which site the snail was collected from.

Experimental Design

Many studies examine the non-consumptive effects of predators on prey species by using predator chemical cues instead of the direct effect in which the predator consumes the prey (e.g., tadpoles, Kiesecker et al. 1996; spiders, Persons & Rypstra 2001; snails, McCarthy & Fisher 2000, Dalesman et al. 2006, Bourdeau 2009). This experiment had two chemical cue treatments: presence of a predator, *C. maenas*, and absence of predators (control). There were six replicate tanks (30.5 cm long x 19.1 cm wide x 20.3 cm high) for each treatment, for a total of 12 tanks. Each tank contained approximately 4.5 L of seawater that was drawn from a saltwater well at the Flax Pond Marine Laboratory (40.9613, -73.1387). Within each tank there were six screened cylindrical cages (interior height = 57.47 m, diameter = 43.23 mm, 1 mm mesh), each with one snail from each site, for a total of 3 snails per cage, 18 snails per tank, 108 snails in each treatment, and 216 experimental snails overall.

Since the snails collected at each site differed in size, the 72 largest snails from the CM and PB sites were chosen for the experiment and the 72 smallest snails from the WM site were

chosen to reduce size effects among sites. Replicate tanks were randomly assigned to a treatment, and within each treatment snails were haphazardly assigned a tank and a cage within that tank.

Each tank had a permeated barrier that separated the crabs from the snail cages so that there was water flow but no physical contact between the predator and prey. Each crab (30-35 mm carapace width) resided in one third of the tank while the snail cages were in the other two thirds of the tank. Tanks in the control treatment also had the same barrier, with snail cages on one side, but the other side remained empty. All tanks had aeration from an airstone, and were maintained at 20˚C in a temperature-controlled room with a 16:8 light: dark schedule.

Snails were fed flaked fish food (Tetramin®) twice a week and had continuous access to the green alga, *Ulva lactuca* (Giannotti & McGlathery 2001). Tanks and cages were cleaned and seawater was replaced weekly. Crabs were fed tilapia twice a week. Tilapia was also placed into the control tanks for approximately 4 hours twice a week to control for any effect the tilapia may have had. If a crab molted during the experiment, the whole tank was cleaned and filled with fresh seawater, and the crab was removed and replaced with a similarly sized crab.

The experiment ran for 12 weeks, after which shell length, total damp mass and wet shell mass were measured for each snail. Shell length was measured using digital calipers as stated above. Snails were weighed when suspended in seawater to estimate shell mass, then removed from the water and dried with a paper towel and reweighed in air to determine total damp mass. The estimated shell mass was subtracted from the total damp mass to give an estimate of soft body mass (Palmer 1982). Two digital images were taken of each shell. The first image was taken with the shell arranged in the same orientation as the beginning photographs and was used

to quantify shell shape differences. The second image was taken with the siphonal canal down and the apex pointing towards the camera so that the axis of coiling was perpendicular to the camera lens. The second image was used to determine whorl growth by measuring from the aperture to the growth mark in the shell formed at the beginning of the experiment (ImagePro Premier, v. 9.0, Media Cybernetics). Snails were all kept at low temperatures prior to the experiment to slow growth, which left growth marks on the shell where growth rate slowed prior to the experiment. This thin groove on the shell indicated where shell was added during the experiment and whorl growth was measured from these growth marks to the end of the aperture of the shell.

Data Analysis

Geometric Morphometric Analyses

Ten landmarks were digitized onto the before and after treatment images for each snail using TpsDig2 (Rohlf 2006, Fig. 1A). Two landmarks (1, 2) were placed on either side of the opening of the siphonal canal, with the third placed across from 1 and 2 to represent the longest axis of the aperture. Two landmarks (4, 5) were placed to represent the widest part of the aperture, and were placed perpendicular to landmarks 1-3. Landmark 6 was placed on the same axis as 4 and 5 to indicate the width of the apertural lip. Two landmarks were placed at the widest point of the body axis above (7) and below the aperture (8). The final two landmarks were placed on the widest point on the apical whorl next to the body whorl (9, 10). Landmarks 7-10 were all placed parallel to the axis of coiling. The apex, which is a common landmark location for morphometric analysis of gastropods, was not used because the apex of *T. obsoleta* is typically very worn, often with few apical whorls remaining (Fig. 5-1A).

The landmarks were used in a generalized Procrustes analysis to remove size and orientation from the images. A MANOVA was performed to test for initial shape differences between snails collected at the different sites, and another MANOVA was performed on the initial and final images to determine if shapes of snails, independent of size, in the control treatment differed after the experiment. A third MANOVA was performed to determine whether treatment and site had a significant effect on shell shape after the experiment was concluded. Thin-plate splines were then produced to visualize the shape differences (average thin-plate spline of initial and final pictures, Fig. 5-1B). All analyses of landmark data were performed using the package geomorph (Adams & Otarola-Castillo 2013) in R (R version 3.1.1). Canonical Variate Analyses (CVAs) were used to distill the multivariate morphological variance into two-dimensions to help visualize shell shape differences (Klingenberg 2011). CVAs are an ordination method similar to Principal Components Analyses except that CVAs maximize differences among predetermined groups (in this case, grouped by collection site or by chemical cue treatment) in order to help visualize morphological difference when taking group into account.

Growth Data Analyses

An ANOVA was performed to determine whether there was a difference among the initial shell lengths of snails that were collected from the three sites. Four separate two-way ANOVAs were then used to examine differences in final shell length and whorl growth, as well as estimated body and shell mass between the treatments and across collection sites. For each ANOVA, treatment was crossed by site, and cage was nested within tank, which was nested within treatment. Both treatment and collection site were treated as fixed factors while the

nested factors (cage within tank within treatment) were treated as random factors. All analyses of growth data were performed using the lme4 package (Bates et al. 2015) in R (R version 3.1.1).

Results

Due to high mortality in two tanks (one tank from each treatment) and in order to have a balanced design, 5 replicate tanks for each treatment (180 snails total, with 90 in each treatment, and 30 snails from each site in each treatment) were used for analyses. No mortality was seen in any of the other experimental tanks.

Geometric Morphometric Analyses

There was a significant difference in the initial morphology of snails collected from different sites (MANOVA, $F_{2, 179} = 16.73$, $p = 0.001$, Table 5-1, Fig. 5-2). At the end of the experiment, source site still had a significant impact on shell shape (MANOVA, $F_{2, 179} = 14.69$, $p = 0.002$, Table 5-2, Fig. 5-2). However, predator chemical cue treatment did not have a statistically significant effect on final shell shape (MANOVA, $F_{1, 179} = 1.56$, $p = 0.073$, Table 5-2, Fig. 5-3), and there was no interaction between treatment and site for shell morphology (MANOVA, $F_{2, 179} = 1.22$, $p = 0.275$, Table 5-2). When testing initial and final shell morphologies, there was a significant shape difference among sites (MANOVA, $F_{2, 359} = 23.83$, $p = 0.001$, Table 5-3, Fig. 5-2), a difference between the initial and final shell shapes (MANOVA, $F_{1, 359} = 50.04$, $p = 0.001$, Table 5-3, Fig. 5-2), and there was an interaction between site and change in shape from the beginning to the end of the experiment (MANOVA, $F_{2, 359} = 6.00$, $p = 0.001$, Table 5-3). The results of the CVA showed that final shell morphologies from each site overlapped, but there was very little difference between the shell shapes of snails from the two treatments (Fig. 5-4). Also, the initial morphologies of snails from different sites

differed from the final shell morphologies, and the final morphologies of snails from each site converged on a similar shape (Fig. 5-4C).

Throughout the experiment, snails from CM tended to have morphologies similar to that of the average snail, while snails from PB and WM contributed to the extremes. Before the experiment, the largest difference in shape amongst sites was in the apical whorls (Fig. 5-1A). Snails from CM had very narrow apical whorls; PB snails had more stout apical whorls, while snails from WM had broader and more elongate apical whorls. Snails from PB also had a wider aperture, and WM snails had a wider siphonal canal (Fig. 5-2). After the experiment, snails from all sites were starting to converge on a similar morphology (Fig. 5-4C). Snails from CM and WM had very similar shell morphologies with a few differences: WM shells were narrower, with a larger apertural lip and larger apical whorls. Snails from PB had the most distinct final shape, with very globose shells compared to conspecifics at CM and WM, and a wider aperture with very stout, narrow apical whorls (Fig. 5-2). When comparing snails from all sites, the initial shell shapes were more globose with a broader aperture, while at the end of the experiment, the shells were more elongate with narrower apertures in both treatments (Fig. 5-2).

Growth Data Analyses

Snails collected from different sites had different shell lengths before (ANOVA, $F_{2, 170} = 152.71$, $p < 0.0001$, Table 5-4, Fig. 5-5A), and after the experiment was conducted (ANOVA, $F_{2, 169} = 24.49$, $p < 0.0001$, Table 5-4, Fig. 5-5A). However, there was no effect of chemical cue treatment on the final shell length (ANOVA, $F_{1, 169} = 0.032$, $p = 0.862$, Table 5-4, Fig. 5-5A). Pairwise comparisons (Tukey's HSD) indicated that the initial shell lengths differed among all sites, but that only snails from WM had different final shell lengths (Table 5-5).

For axial growth, there was an effect of collection site (ANOVA, $F_{2, 169} = 18.97$, $p < 0.0001$, Table 5-4, Fig. 5-5B), but not an effect of chemical cue treatment (ANOVA, $F_{2, 169} = 0.0791$, $p = 0.7857$, Table 5-4, Fig. 5-5B). The same was seen for whorl growth, which was effected by collection site (ANOVA, $F_{2, 169} = 20.49$, $p < 0.0001$, Table 5-4, Fig. 5-5B), but not treatment (ANOVA, $F_{1, 169} = 0.164$, $p = 0.696$, Table 5-4, Fig. 5-5B). Pairwise comparisons (Tukey's HSD) indicate that there were some similarities in growth, with snails from CM and PB displaying similar amounts of axial and whorl growth, while snails from WM had significantly different amounts of axial and whorl growth than CM and PB snails (Table 5-5).

There was an effect of collection site on both final body mass (ANOVA, $F_{2, 169} = 70.96$, $p < 0.0001$, Table 5-4, Fig. 5-5C) and final wet shell mass (ANOVA, $F_{2, 169} = 115.742$, $p < 0.0001$, Table 5-4, Fig. 5-5C), but no effect of predator chemical cue treatment on final body mass (ANOVA, $F_{1, 169} = 0.078$, $p = 0.787$, Table 5-4, Fig. 5-5C) or final shell mass (ANOVA, $F_{1, 169} = 0.056$, $p = 0.819$, Table 5-4, Fig. 5-5C). Tukey's HSD tests indicate that there were significant differences among all sites for both final body mass and final shell wet mass (Table 5-5).

Discussion

A variety of shell attributes can protect gastropods from predation, including larger shells, thicker shells, and altered shell shapes (reviewed in Vermeij 1993), all of which can be affected by snail growth rate. A decrease in growth rate usually leads to an increase in shell thickness, where an increase in growth rate leads to thinner shells (Trussell 2000b, Edgell & Neufeld 2008, Pascoal et al. 2012, Manriquez et al. 2013). Growth rate also impacts shell morphology in species specific ways, with faster growth rates leading to more elongate, narrower shells in some species (Boulding & Hay 1993), and to stouter, more globose shells in others

(Kemp & Bertness 1984). Many species of gastropods exposed to the threat of predation decrease their growth rates, which in turn increases shell thickness and alters shell morphology (Trussell 2000b, Bourdeau 2010, Hooks & Padilla 2014). These growth rate driven changes in shell size, thickness, and shape make it harder for predators to consume the snails (Palmer 1990, Bourdeau 2009). However, this response was not seen in *T. obsoleta*; snails did not alter their growth or morphology when exposed to chemical cues from a major predator, the crab *C. maenas*. This lack of response would allow these snails to reach a larger size more quickly, which would provide protection from predation in the long term. *Tritia obsoleta* have very thick shells, and the combination of a relatively large size and a thick shell likely provides sufficient protection from most predators. *Tritia obsoleta* are gregarious and are often found at high densities (Kelaher et al. 2003), which is thought to provide safety-in-numbers, with reduced risk of predation, on average, to individuals. Also, *T. obsoleta* are known to exhibit trailing following behaviors (Trott & Dimock 1978), which can reduce predation and desiccation (Ng et al. 2013). Therefore, large, thick shells combined with the high population density and gregarious behaviors probably provide enough protection from predation reducing any additional benefit to be gained through altered shell morphology.

Although there was no significant morphological difference between the snails in the two chemical cue treatments, there were shape differences among snails collected from different sites at the beginning of the experiment that persisted, but began to diminish by the end of the experiment. *Tritia obsoleta* have planktonic larvae that remain in the water column for about 30 days (Scheltema 1964). Gastropod species with such long larval stages would disperse over large distances, resulting in little to no genetic differences among populations at the spatial scale at which these snails were collected (Berger 1973, Riquet et al. 2013). Differences in shell

morphology across sites at the beginning of the experiment was likely due to differing environmental factors at each site. This is supported by the fact that by the end of the experiment, and residing in a similar habitat for 12 weeks, the shell morphology of *T. obsoleta* from the different sites was converging. Although site differences remained at the end of the experiment, particularly for snails from PB when compared to snails from CM and WM, the experimental environment seemed to have affected morphology as well.

A variety of environmental factors are known to impact snail shell morphology. For example, snails in areas of high water flow or large waves tend to have larger apertures, which can prevent snails from being dislodged (Haase 2003, Marquez et al. 2015, Gustafson & Bolek 2016). *Tritia obsoleta* in this experiment were collected from two sites on the north shore of Long Island NY along Long Island Sound (CM and WM), and one, Old Ponquogue Bridge Marine Park (PB), on the south shore of the island. Because these populations are exposed to different bodies of water, they may experience different temperatures, which is known to impact growth and morphology in other species of gastropods (Trussell 2000a, Dunithan et al. 2012). The substrate snails live on can also alter the shape of their shells (Rueda et al. 2011, Dunithan et al. 2012), as can food availability (Bourdeau 2010, Hooks & Padilla 2014). Since *T. obsoleta* has many different food sources, it is unlikely that food availability caused the observed morphological differences, but the food sources at each site may vary, which could affect growth rate and morphology (Boulding & Hay 1993, Rueda et al. 2011, Saura et al. 2012). More work is needed to determine which of these environmental factors may be influencing shell morphology for snails at these sites.

Over the course of this experiment, snails from different sites converged on a similar shell morphology. This convergence could be due to being grown in a common laboratory

environment, or it could be the natural progression of shell shape as these animals age. When comparing shell shape between juveniles and adults from two sites (PB and WM), I found that there was an interaction between site and the life stage of the snail (Appendix 5). Adults and juveniles have different shell morphologies, and juveniles from different sites have more distinct shape differences than the adults from those same sites.

Although this experiment found no effect of chemical cues from a shell crushing predator (*C. maenas*) on shell growth or morphology, *T. obsoleta* may respond to chemical cues from a predator, such as the seastar *Asterias forbesi,* that does not crush the snail's shell. Larger shell size is unlikely to provide a defense from these predators, and larger snails may be preferred prey because there is more to consume. Bourdeau (2009b) found that another snail, *Nucella lamellosa,* produced a narrower aperture when exposed to seastar predators compared to the shell shape observed when exposed to a crushing crab predator.

When exposed to chemical cues from *C. maenas*, growth and morphology of *T. obsoleta* did not differ from those of the control treatment snails. These results differ from those seen in most gastropod species where exposure to predation results in decreased growth and a change in shell morphology. These results suggest that, for this species, shell morphology may not be as important for deterring predation, and that there may be a size refuge at which *T. obsoleta* is too large to be consumed by this crushing predator. It is also possible that *T. obsoleta* has high metabolic requirements and must spend more time consuming food than in refugia, which could explain the lack of change in growth between treatments. Future work is needed to determine whether shell morphology is altered by non-crushing predators, how shell shape is affected by the environment, and how the shell shape differences for snails from each site impacts survivorship and performance of *T. obsoleta* in each environment.

Table 5-1. MANOVA table for analysis of initial shell morphologies of juvenile *T. obsoleta* from three sites. Ten landmarks were placed on images of each snail, and were used to analyze shape differences among sites independent of shell size. P-values < 0.05 are considered significant, and are in bold.

	Df	Sum Sq	MS	Rsq	F value	Z	Pr(>F)
Site	2	0.0706	0.03530	0.1590	16.73	12.30	**0.001**
Residuals	177	0.3735	0.00211				
Total	179	0.4441					

Table 5-2. MANOVA table from analysis of the effects of treatment and site on shell morphology of *T. obsoleta*. Ten landmarks were placed on images of each snail, and were used to analyze shape differences independent of size. Treatment (control, green crab chemical cues) and site (CM, PB, WM) were treated as fixed effects, while the nested factors (cage within tank within treatment) were treated as random effects. P-values < 0.05 are considered significant, and are in bold.

	Df	Sum Sq	MS	Rsq	F value	Z	Pr(>F)
Site	2	0.0568	0.02839	0.1293	14.69	9.877	**0.0020**
Treatment	1	0.0045	0.00449	0.0102	2.324	1.563	0.0739
Treatment*Site	2	0.0047	0.00235	0.0107	1.215	1.088	0.2755
Treatment: Tank	8	0.0406	0.00507	0.0924	2.623	2.371	**0.0020**
Treatment: Tank: Cage	50	0.1083	0.00217	0.2466	1.120	1.164	**0.0060**
Residuals	116	0.2242	0.00193				
Total	179	0.4390					

Table 5-3. MANOVA table for the analysis of shell morphology of juvenile *T. obsoleta* at the beginning and the end of the experiment. Initial and final morphologies, as well as shapes among sites (CM, PB, WM) were analyzed. This analysis was done only using individuals in the control treatment. P-values < 0.05 are considered significant, and are in bold.

	Df	Sum Sq	MS	Rsq	F value	Z	Pr(>F)
Initial/Final Shape	1	0.1068	0.1068	0.1079	50.04	23.57	**0.001**
Site	2	0.1017	0.0509	0.1028	23.83	16.59	**0.001**
Site*Initial/Final	2	0.0256	0.0128	0.0259	6.003	5.603	**0.001**
Residuals	354	0.7558	0.0021				
Total	359	0.9900					

Table 5-4. ANOVA tables of the growth data of juvenile *T. obsoleta* before (initial shell length) and after the experiment (all other dependent variables). Collection site and chemical cue treatment were treated as fixed factors, while the nested factors (cage within tank within treatment) were random factors that were taken into account. P-values less than 0.05 were considered significant are in bold.

Dependent Variable	Factor	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Initial Shell Length	Site	2	105.5	52.76	152.7	< **0.001**
Final Shell Length	Treatment	1	0.025	0.0253	0.0321	0.8622
	Site	2	38.61	19.30	24.49	< **0.001**
	Treatment*Site	2	1.604	0.8021	1.018	0.3637
Axial Growth	Treatment	1	0.0527	0.0527	0.0791	0.7857
	Site	2	25.30	12.65	18.97	< **0.001**
	Treatment*Site	2	0.4914	0.2457	0.3685	0.6923
Whorl Growth	Treatment	1	2.57	2.57	0.1643	0.6958
	Site	2	640.7	320.3	20.50	< **0.001**
	Treatment*Site	2	0.31	0.16	0.010	0.9900
Wet Shell Mass	Treatment	1	0.0000	0.0000	0.056	0.8191
	Site	2	0.0445	0.0222	115.7	< **0.001**
	Treatment*Site	2	0.0004	0.0002	1.082	0.3422
Soft Body Mass	Treatment	1	0.0002	0.0002	0.078	0.7868
	Site	2	0.3400	0.1700	70.96	< **0.001**
	Treatment*Site	2	0.0071	0.0036	1.488	0.2301

Table 5-5. Pairwise comparisons (Tukey's HSD) to determine differences in initial shell lengths and final growth measurements that were collected at the end of the experiment among sites. P-values < 0.05 are considered significant, and are in bold. (Collection site abbreviations: CM = Crab Meadow Beach, PB = Old Ponquogue Bridge Marine Park, WM = West Meadow Beach).

Pairwise Comparison	Initial Shell Length	Final Shell Length	Axial Growth	Whorl Growth	Soft Body Mass	Wet Shell Mass
	p-value					
PB – CM	**0.0088**	0.1296	0.6991	0.5127	**0.0067**	**0.0006**
WM – CM	**< 0.001**	**0.00218**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
WM – PB	**< 0.001**	**< 0.001**	**0.0113**	**0.0043**	**< 0.001**	**< 0.001**

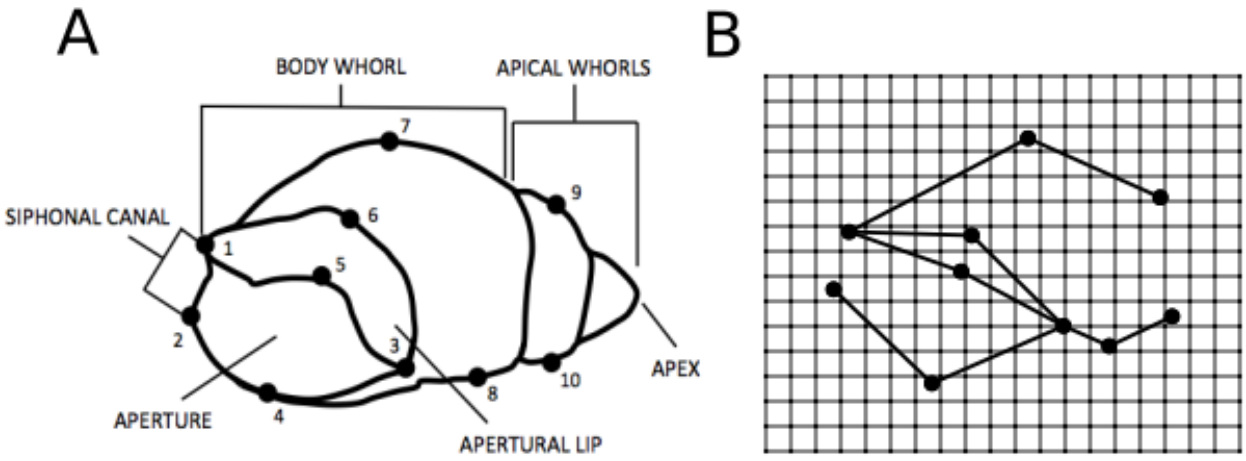

Figure 5-1. Diagram of *T. obsoleta* with the location of all 10 landmarks used for the morphological analyses, and labeled with shell terminology. (A). The landmarks are displayed in a thin plate spline, which is a grid that represents the average of both the initial and final shell shapes (B). The links between landmarks are used to represent the shell shape.

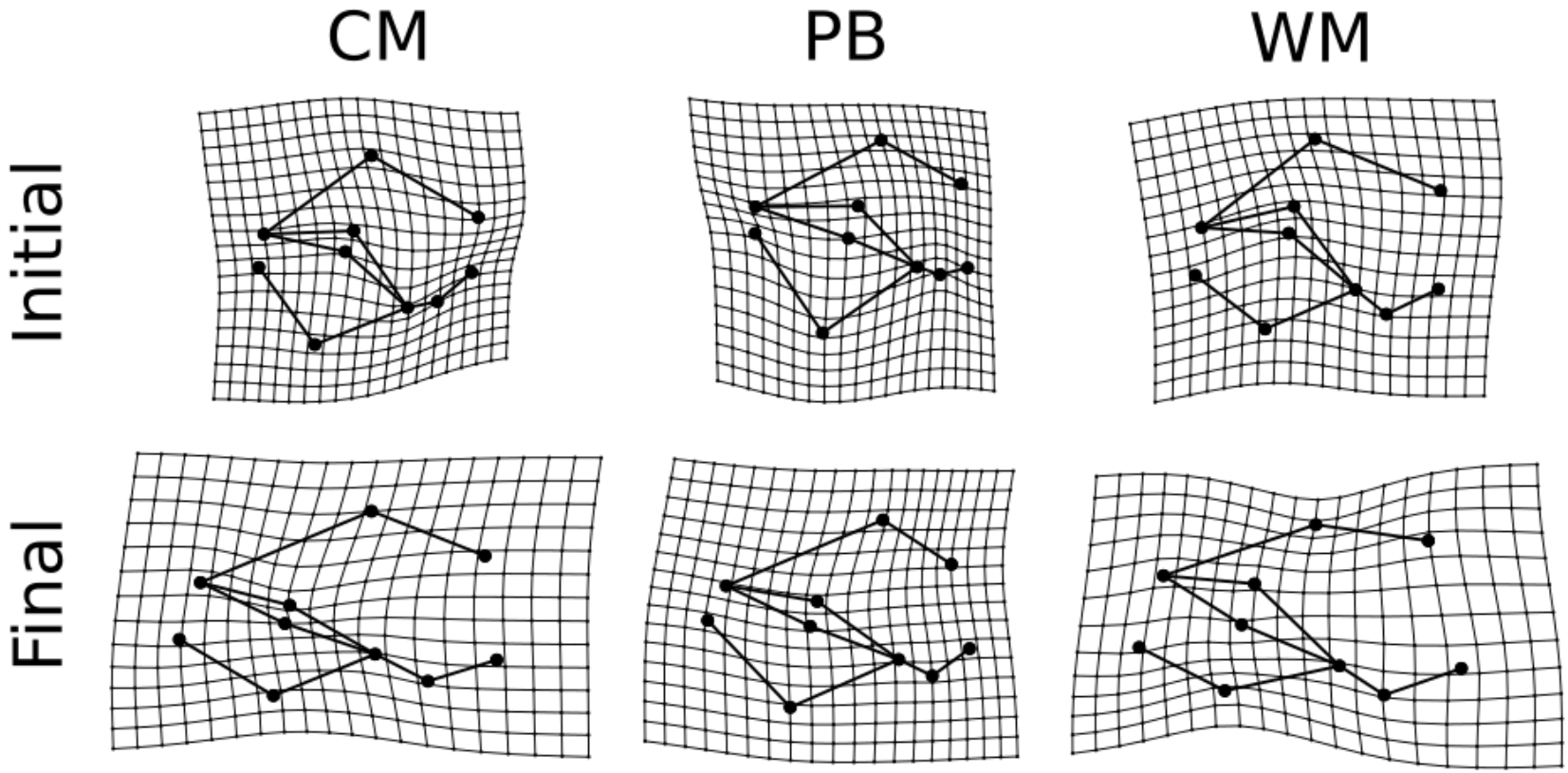

Figure 5-2. Thin-plate splines (TPS) illustrate average initial and final shapes of snails collected from different sites (CM= Crab Meadow Beach; PB= Old Ponquogue Bridge Marine Park; WM= West Meadow Beach). The reference for these TPS grids is the average of all snail shapes both before and after the experiment. Thin-plate splines are exaggerated by 5x to make shape differences easier to visualize.

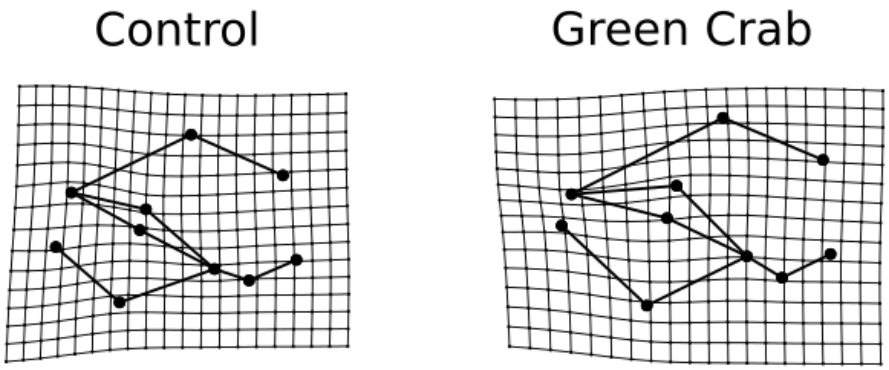

Figure 5-3. Thin-plate splines (TPS; exaggerated by 10x to make the shape changes easier to visualize.) show the mean shape of snails that were in the control and green crab chemical cue treatments. These TPS grids are used to compare the average morphology seen in each treatment to the average snail shape of all snails after the experiment was completed.

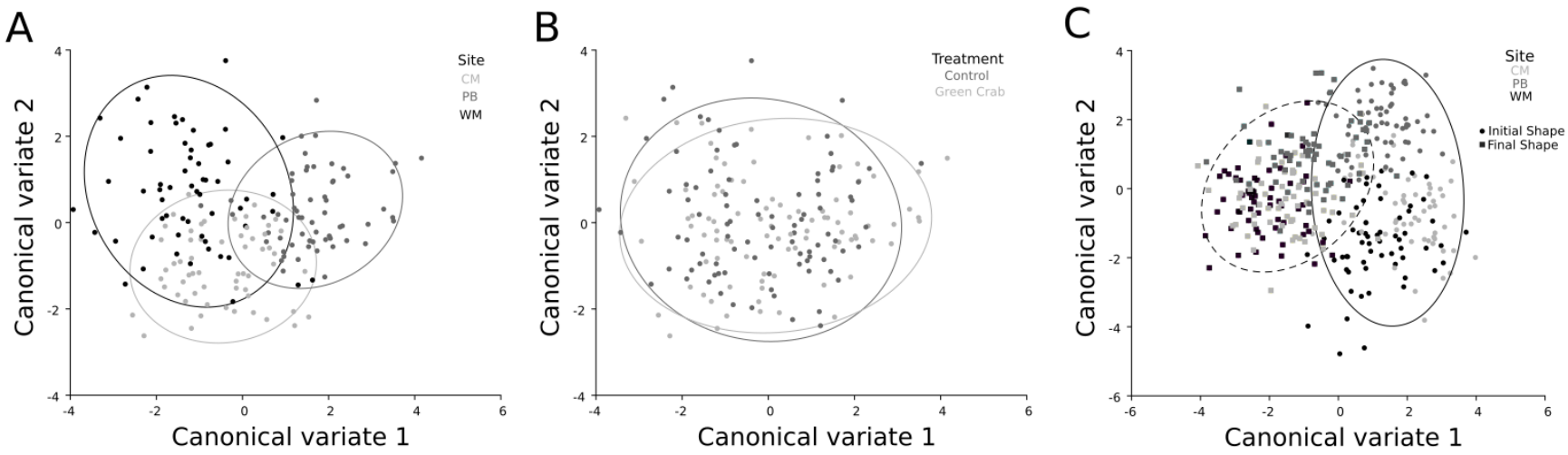

Figure 5-4. Canonical Variate Analysis plot of snail morphology from different sites (A) and of snails in the different experimental treatments (B). CVA plot of snail shell shape from different sites at the beginning and end of the experiment (C). The CVAs demonstrate how the morphologies of different groups (collection site or chemical cue treatment) compare while showing the largest differences between the groups.

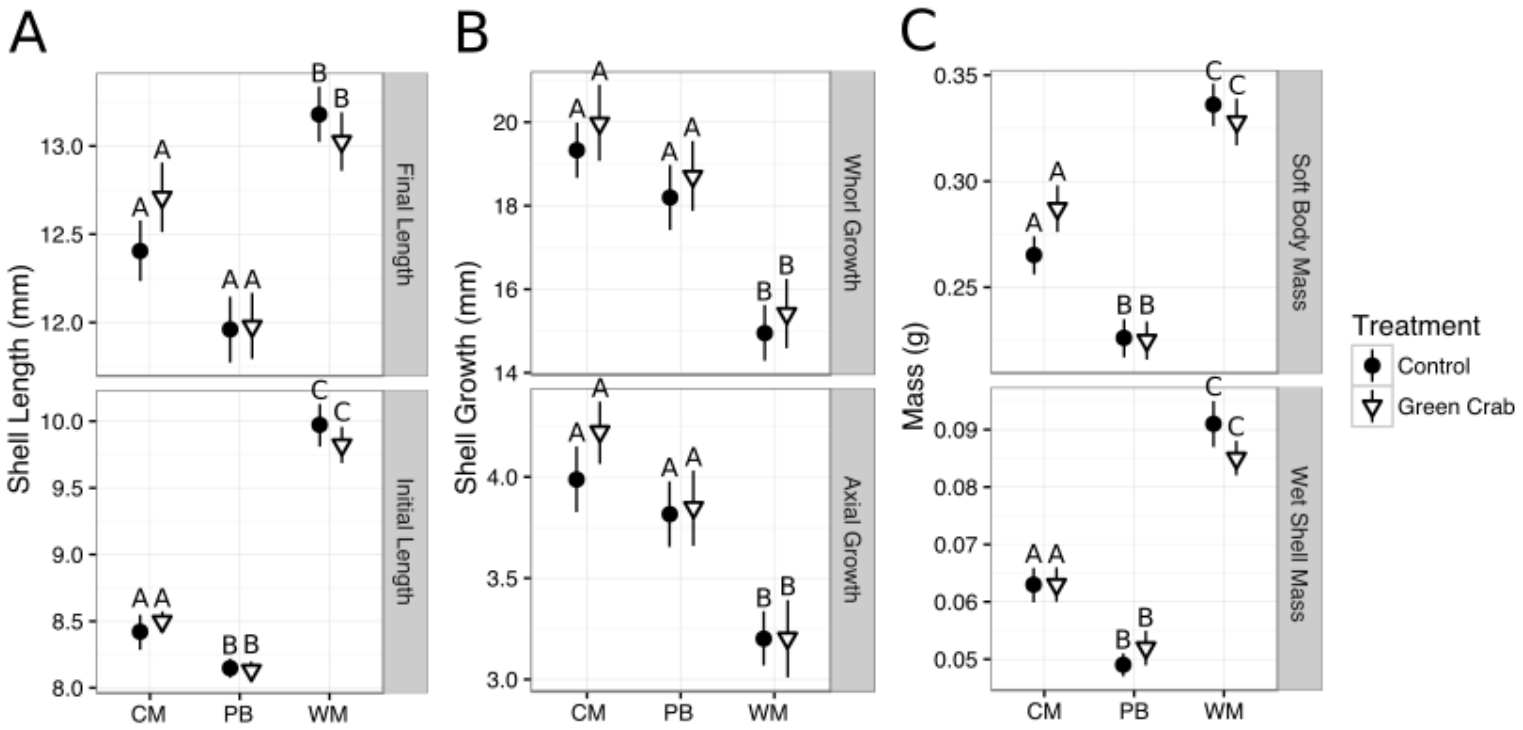

Figure 5-5. Snail shell length, growth, and mass by treatment and collection site. Snail measurements (A: initial and final shell length, B: axial and whorl growth, C: final shell and body mass) are separated by collection site and by chemical cue treatment (control = black circle, green crab treatment = open triangle). Points represent the mean value, and error bars represent the standard error. Points labeled with the same letter are not statistically different from each other. Collection Site Codes: CM- Crab Meadow Beach, PB- Old Ponquogue Bridge Marine Park, WM- West Meadow Beach.

Chapter 6

The impact of parasites on the size and morphology of a marine gastropod

Abstract

Animals with parasites often display altered phenotypes, which can include distinctive behavior, physiology and/or morphology. Trematode parasites, which commonly infect gastropods, are can be associated with changes in host phenotype, including host castration, and alteration of host behavior and shell morphology. Furthermore, gastropod shell shape is important in that it protects the individual from predators, desiccation, and heat stress. There is a close link between shell growth and morphology; therefore, if parasitized gastropods have different shell morphologies, it could be due to changes in growth rate. I used geometric morphometrics to determine if shell morphology of a marine snail, *Tritia obsoleta*, was different in parasitized and unparasitized individuals. Both parasite species and collection site were found to affect shell shape, but there was no interaction between these two factors. Snails infected with the trematode *Himasthla quissetensis* had a significantly different shell shape that was more elongate and narrow compared to unparasitized snails. This altered morphology, along with the fact that parasitized snails are larger than unparasitized snails, is consistent with an increased growth rate. However, the snails infected by two other parasite species, *Lepocreadium setiferoides* and *Stephanostomum dentatum*, did not have different morphologies than uninfected individuals, suggesting that these parasites may not alter snail growth rates. Rather, snails parasitized by these species could be older, and therefore larger individuals.

Introduction

Parasitism is common among most species, and there is thought to be at least one species of parasite that is specialized to infect every species of plant and animal (Begon et al. 2006). It is well known that parasites can have large impacts on the phenotype of their hosts, including host behavior, physiology, and morphology (reviewed in Poulin & Thomas 1999, Moore 2002, Lefèvre et al. 2009). Many species of trematode parasites have been shown to affect host morphology; for example trematodes can alter head morphology in their secondary fish host (Sandland & Goater 2001). Also, these parasites can affect the penis length (Merlo et al. 2017) and shell morphology (Levri et al. 2005, Żbikowska & Żbikowski 2005, Thieltges et al. 2009) of their gastropod hosts.

The gastropod shell has many roles including protecting the animal from heat stress, desiccation and predation. Shell morphology impacts the degree of protection from these different abiotic (Chapman 1994, Urabe 1998, Morley et al. 2010) and biotic stressors (Johannesson 1986, Bourdeau 2009). Parasite infections have been found to alter the shell shape of their gastropod host in many ways; infected gastropods can have narrower (*Zeacumantus subcarinatus*: Hay et al. 2005, *Lymnaea stagnalis*: Żbikowska & Żbikowski 2005, *Cominella glandiformis*: Thieltges et al. 2009) or stouter (*Potamopyrgus antipodarum*: Levri et al. 2005) shells than unparasitized individuals. However, for other gastropods, parasites do not affect morphology (*Elimia livescens*: Krist 2000, *Potamopyrgus antipodarum*: Haase 2003, *Lymnaea stagnalis*: Żbikowska & Żbikowski 2005, *Physa acuta*: Gustafson & Bolek 2016). These studies also demonstrate that different parasite species can impact behavior differently (Żbikowska & Żbikowski 2005).

This study examined the impact of parasitism on the shell morphology of *Tritia obsoleta*, a marine gastropod that can be infected by nine different species of trematode parasites (reviewed in Blakeslee et al. 2012, Phelan et al. 2016). Many studies have been conducted on parasitism in *T. obsoleta*, especially on the parasite communities (e.g., Esch & Fernandez 1994, Hendrickson & Curtis 2002), but little is known about how these parasites alter the shell morphology of their host. To examine the effects of parasitism on snail morphology, I used geometric morphometric analyses to determine if snails infected by different species of parasite had different shell shapes (independent of size), and whether there were any shell shape differences among collections sites. Additionally, gastropod growth rate is closely linked to shell morphology (Kemp & Bertness 1984, Urdy et al. 2010), so altering growth rate is one way in which parasites could impact host shell morphology. Therefore, I also examined shell length of parasitized (by one of three parasite species) and unparasitized *T. obsoleta* to determine if there was a difference in shell size between parasitized and unparasitized snails.

Materials and Methods

Study System

The eastern mudsnail, *Tritia obsoleta* (Say, 1822), formerly known as *Ilyanassa obsoleta*, is a marine intertidal zone gastropod that is abundant along the Atlantic coast of North America. These snails are found in a variety of habitats including mudflats, marshes, and beaches, and are usually found at high densities, occasionally with more than 500 snails per square meter (Curtis & Hurd 1983, Kelaher et al. 2003). *Tritia obsoleta* is a scavenger, herbivore and detritivore (Curtis & Hurd 1981, Feller 1984). Because of the wide variety of food sources that it can use and high population densities, *T. obsoleta* can have large impacts on communities they inhabit,

especially the abundance and distribution of benthic invertebrates and algae in the sediment (Connor et. al 1982, Kelaher et. al 2003).

Tritia obsoleta is the first intermediate host to nine species of trematode parasites: *Austrobilharzia variglandis* (Miller & Northup 1926), *Diplostomum nassa* (Martin 1945), *Gynaecotyla adunca* (Linton 1905), *Himasthla quissetensis* (Miller & Northup 1926), *Lepocreadium setiferoides* (Miller & Northup 1926), *Pleurogonius malaclemys* (Hunter 1961), *Stephanostomum dentatum* (Linton 1900), *Stephanostomum tenue* (Linton 1898), and *Zoogonus lasius* (Leidy 1891). Eight of these species (all except *P. malaclemys*) are found along the shores of Long Island, NY, and between 7-40% of adult *T. obsoleta* within a population are generally infected by one of these species (infection by multiple species of parasites is rare, Appendix 2). It is unknown whether these parasites alter *T. obsoleta* shell morphology, but snails parasitized with *Z. lasius* have been found to have heavier shells than unparasitized snails (Cheng et al. 1983), which has been associated with slower growth (Trussel & Nicklin 2002).

Trematodes are parasitic flatworms with complex life cycles. They can have two to six different larval stages (miracidium, sporocyst, redia, cercaria, metacercaria, mesocercaria) that can inhabit two to four different host species. The first intermediate host is usually a species of gastropod, and the final host, where they sexually reproduce, is usually a vertebrate (fish, bird, terrapin, reviewed in Blakeslee et al. 2012, Phelan et al. 2016). Snails are infected after consuming trematode eggs or after being infected by a newly hatched miracidium larvae. The miracidia feed on and reproduce within the gonads, and eventually the digestive gland of the host snail. Miracidia produce sporocysts, which can then produce more sporocysts or rediae larvae. Cercariae larvae are then produced asexually by either sporocysts or rediae, and leave the snail to infect the next host (Stunkard 1938, 1983, Moore 2002). Because the parasites inhabit and

consume the gonads, the snail host is fully or partially castrated (Cheng et al. 1973, Hoskin 1975, Sullivan et al. 1985).

Data Collection

Adult *T. obsoleta* were collected from three beach sites on Long Island, NY during the summer of 2014 (near Shinnecock Canal [SC] 40.88, -72.48, Old Ponquogue Bridge Marine Park [PB] 40.84, -72.50, and West Meadow Beach [WM] 40.94, -73.15; Appendix 3). All snails collected were greater than or equal to 13 mm in length. Snails reach sexual maturity when they are approximately 12-14 mm in length and can then become infected with trematodes (Scheltema 1964). I collected 1400 adults from each site for a total of 4200 snails. The snails were placed in containers marked with the date and site of collection, returned to the lab, and were kept in a recirculating seawater tank until processed. During a field experiment (Chapter 4) some snails were not recovered, which left 3853 snails that could be used to examine shell morphology.

Shell length was measured for each snail with digital calipers (± 0.01 mm), and a digital photograph was taken of each shell with the aperture facing up and with the axis of coiling parallel to the camera lens (Fig. 6-1A). Snails were then dissected (Chapter 2) and viewed under a dissecting microscope to determine if they were parasitized. If the snails were parasitized, parasites were identified to species by examining the morphologically distinct cercaria (McDermott 1951, Stunkard 1983). In some cases, cercariae were not present and the parasites could not be identified to species (58 snails had unidentifiable parasites, ~5 % of all parasitized snails, Table 6-1).

Data Analysis

Since *T. obsoleta* shell morphology can vary among sites (Chapter 5, Appendix 5), analyses were conducted to test for differences in shell length and shape among sites and among snails with and without parasites. Few snails (< 14) were parasitized by *A. variglandis*, *D. nassa*, and *S. tenue* at any site, and *G. adunca* and *Z. lasius* were only abundant at a few of the collection sites. Therefore, they were not included in the morphological analysis (Table 6-1). Only snails parasitized by *H. quissetensis*, *L. setiferoides*, and *S. dentatum* were abundant at each site; 14 snails infected with each parasite were randomly chosen from each population for morphological analysis. An additional 14 unparasitized snails were randomly chosen from each site to be included in the morphological analysis. The shell lengths of these snails fell within the distribution of shell lengths of all snails collected during the summer of 2014.

An ANOVA was used to test whether the shell lengths of snails infected by different parasite species differed from those of unparasitized snails, and to determine if there was an effect of site or an interaction between parasite species and collection site on shell length. Post-hoc pairwise comparisons were made using Tukey's HSD test to determine parasite and collection site differences.

Ten landmarks were digitized on the image of each gastropod using TpsDig2 (Rohlf 2006, Fig. 6-1A). Landmarks 1 and 2 were placed on either side of the siphonal canal, and landmark 3 was placed on the opposite end of the aperture from 1 and 2, to represent the length of the aperture (opening into the shell). Landmarks 4 and 5 were placed on either side of the widest part of the aperture, perpendicular to the axis determined by landmark 3 and the point between landmarks 1 and 2. Landmark 6 was placed on the outermost edge of the apertural lip on the axis determined by landmarks 4 and 5. Landmark 7 was placed at the widest point of the body whorl (shell whorl where the body of the snail resides) on the opposite side of the shell as

the aperture, and landmark 8 was placed at the widest point on the body whorl on the same side of the shell as the aperture. Landmarks 9 and 10 were placed on the widest points on the apical whorl (older smaller whorls) that is next to the body whorl. Landmarks 7-10 were all placed on the widest points of the body or apical whorl perpendicular to the axis of coiling. The apex (tip of the shell) of *T. obsoleta* is usually very eroded, so although it is a common landmark location for geometric morphometrics analyses in gastropods (e.g., Bourdeau 2009, Gustafson & Bolek 2016), it was not used as a landmark in this study (Fig. 6-1A).

The following analyses were performed using the geomorph package in R (Adams & Otarola-Castillo 2013; R version 3.1.1). A generalized Procrustes analysis was used to remove size and orientation from the landmark data, and then a MANOVA was used to determine whether there were shell shape differences among sites and among unparasitized snails and those infected with various parasite species. Pairwise comparisons were used to test for shape differences between uninfected snails and snails infected with different parasite species, and to test for shape differences among snails collected from different sites. Thin-plate splines were produced to show the average shape of snails from each collection site as well as unparasitized snails and those infected by each species of parasite (Fig. 6-2, 6-3). The thin-plate spline grid is based on the average unparasitized adult snail from all sites to standardize the grid (Figure 6-1B), and the alteration of the grid demonstrates how the shape of snails in each category (site, uninfected snails and those infected with each parasite species) differed from that of the average unparasitized snail from all sites. Canonical variate analyses (CVA) were also performed to help visualize shell shape differences by distilling the multivariate morphological variance into two-dimensions (Klingenberg 2011, Fig 6-4.). CVAs are similar to Principal Component Analyses,

except that CVAs maximize differences among predetermined groups (in this case, grouped by collection site or by parasite species).

Results

When examining shell length, I found a significant difference among snails infected with different parasite species (ANOVA, $F_{3, 166} = 7.302$, $p = 0.0001$, Table 6-2), and among snails collected from different sites (ANOVA, $F_{2, 166} = 50.293$, $p < 0.0001$, Table 6-2). There was no interaction between collection site and parasite species (ANOVA, $F_{6, 166} = 1.814$, $p = 0.0997$, Table 6-2). Post-hoc tests (Tukey's HSD) were used to examine pairwise differences in shell lengths, and found a difference between unparasitized snails and snails infected with each of the three parasite species (*H. quissetensis*: $p = 0.007$; *L. setiferoides:* $p = 0.032$; and *S. dentatum*: $p < 0.0001$), and unparasitized snails were shorter than all parasitized snails (Table 6-3). There was no difference between shell lengths when examining the pairwise comparisons between the snails parasitized by different species (Table 6-4). Snails from PB were significantly longer than those from SC ($p < 0.0001$) and WM ($p < 0.0001$, Table 6-3), but there was no size difference between snails collected from SC and WM ($p = 0.543$, Table 6-3).

There was a significant difference in shell morphology, independent of size, among snails infected by different species of parasites (MANOVA, $F_{3, 167} = 1.808$, $p = 0.021$, Table 6-5), and among snails from different sites (MANOVA, $F_{2, 167} = 6.104$, $p = 0.001$, Table 6-5). Again, there was no site by parasite interaction on morphology (MANOVA, $F_{6, 167} = 1.2992$, $p = 0.161$, Table 6-5). Pairwise comparisons were conducted to examine the effect of parasite species on shell morphology while taking the collection site into account. There were significant differences in morphology between unparasitized snails and snails infected with *H. quissetensis*

($p = 0.012$). There were also significant differences in the shape of snails parasitized with *H. quissetensis* and snails parasitized with *L. setiferoides* ($p = 0.009$). There were no significant shape differences found in the remaining comparisons between snails infected with different species of parasites (Table 6-4). When compared to unparasitized individuals, snails infected with *H. quissetensis* had a narrower, more elongate aperture, and a more elongate shell overall. *Himasthla quissetensis* infected snails also had relatively wider apical whorls than those of the uninfected conspecifics (Fig. 6-2). Pairwise comparisons were also used to examine the effect of collection site on shell shape while taking the parasite species the snail was infected with into consideration. Morphology significantly differed between snails from each site ($p = 0.001$ for each pairwise comparison, Table 6-4). When comparing the average snail shapes from each site, I found that snails from PB had relatively slender apical whorls and more elongate apertures than snails from SC and WM. Snails from SC had the most globose shells, with a relatively squatter aperture. WM snails had elongate shells like snails at PB, but with a broader body whorl and apertural lip (Fig. 6-3).

For both shell length and shell shape, there was no interaction between collection site and the parasite species with which a snail was infected (Length: ANOVA, $F_{6, 166} = 1.814$, $p = 0.010$; Shape: MANOVA, $F_{6, 167} = 1.299$, $p = 0.161$, Table 6-2, 6-5). The CVAs indicate that there was overlap in the shell morphologies of snails from different sites and of snails infected by different species of parasites (Fig. 6-4). By examining site and parasite effect separately, snails from each site appeared to have fairly distinct shapes (Fig. 6-4A), while the snails infected by different parasite species had shapes similar to those of unparasitized snails (Fig. 6-4B).

Discussion

I found that the shell lengths of parasitized *T. obsoleta* were larger than those of unparasitized snails across all collection sites. Since gastropod shell shape is closely linked to growth rate, I examined whether these morphologies could be the result of changes in growth rates. Snails infected by three individual species of parasite were examined, but only snails parasitized by *H. quissetensis* had shell morphologies different than those of unparasitized snails. Snails infected with *H. quissetensis* were more elongate and slender than their unparasitized conspecifics, which could be the result of altered growth rates (Kemp & Bertness 1984, Boulding & Hay 1993, Urdy et al. 2010). The shape differences of *H. quissetensis* parasitized snails coupled with the fact that they were larger than unparasitized individuals suggests that these infected snails may be growing faster than unparasitized *T. obsoleta*. However, Curtis (1995) previously found that parasitized *T. obsoleta* have slower growth rates than unparasitized snails, and that growth rate may depend on body size with small, parasitized snails having growth rates similar to unparasitized snails. *Himasthla quissetensis* could be infecting small snails, which then grow quickly into the large snails while altering their shell morphology. However, it is possible is that *H. quissetensis* is not altering snail growth rate but is affecting other shell growth parameters (e.g., rotation angle around the coiling axis), which can also affect shell shape (Urdy 2010). Another possibility is that the shape differences seen in *H. quissetensis* infected snails are the result of snail age (Urdy 2010).

Tritia obsoleta infected with *L. setiferoides* and *S. dentatum* did not have different shell morphologies than unparasitized snails, however snails parasitized by either of these two species were larger than unparasitized snails. These results suggest that parasites *L. setiferoides* and *S. dentatum* do not alter the growth rate (or other growth parameters) of their host, and that larger shell size is the result of another process. One possible explanation is that older, and therefore

larger snails have had more time to come in contact with and become infected by parasites (Sousa 1983, Krist 2000). Another possibility is that parasitized snails live longer than unparasitized snails, which would allow them to achieve these large shell sizes. Both of these mechanisms could also apply to the shell length and shell morphology differences seen in snails infected by *H. quissetensis* as well.

The size and morphological differences seen in *T. obsoleta* infected with different species of parasites could have large impacts on both the parasites and the host. Large shell size could be beneficial for the host if larger body mass increases host survival and chance of outliving the infection (Minchella 1985, Esch & Fernandez 1994, Genner et al. 2007). But it could also benefit the parasite as a larger snail body may allow parasites to produce more offspring and potentially increase transmission to the next host (McCarthy et al. 2004, Levri et al. 2005, Genner et al. 2007). The morphological differences seen in snails infected by *H. quissetensis* could influence the ecology of *T. obsoleta* in both negative and positive ways. The narrow, more elongate aperture seen in *H. quissetensis* infected snails is a shape thought to protect gastropods from seastar predators (Bourdeau 2009), but this same apertural shape could also make it harder for these snails to live in environments with a high water flow rate or large waves (Haase 2003, Gustafson & Bolek 2016).

Parasite infections are often associated with changes in host phenotype, but it is often unclear whether these phenotypic changes are caused by the parasites (parasite manipulation of host phenotype), if they are due to the host's response to being parasitized, or if they are only a by-product of parasitism (Minchella 1985, Sorensen & Minchella 2001, Thomas et al. 2005). If changes in phenotype were the by-product of parasitism, infection by any parasite species would likely result in the same phenotypic change in all hosts. This is not the case with *T. obsoleta*

where different parasite species have different effects on shell morphology, which is also seen for other species of gastropods: *Cominella glandiformis* (Thieltges et al. 2009), *Lymnaea stagnalis* (Zbikowska & Zbikowski 2005), and *Zeacumantus subcarinatus* (Hay et al. 2005). Hence, the difference in shell morphology that is seen in snails parasitized by *H. quissetensis* is either the result of parasite manipulation or the snail responding to being parasitized by that specific species of parasite.

I found that shell morphology of *T. obsoleta* differed among collection sites. These different shell shapes were likely the result of habitat differences among the sites, since environmental conditions, such as water flow (Haase 2003, Marquez et al. 2015, Gustafson & Bolek 2016), temperature (Trussell 2000, Dunithan et al. 2012), substrate (Rueda et al. 2011, Dunithan et al. 2012), and food availability (Bourdeau 2010, Hooks & Padilla 2014) which are known to impact snail shell morphology. All snails used in these analyses were collected along Long Island, NY with two sites in Shinnecock Bay along the south shore (PB and SC) and one in Long Island Sound on the north shore (WM). Sites PB and SC would be expected to have similar water temperatures since they are in the same body of water (Appendix 3), but water flow, substrate, predator presence, and food availability could all differ among sites. More work is needed to determine environmental factors that may be influencing the shell morphology differences among sites.

The snails used in this experiment were naturally infected with parasites when collected; therefore, the shape differences that were found are not necessarily just the result of parasitism. Parasitized and unparasitized snails do not always reside in the same place within a habitat (Miura et al. 2006, O'Dwyer et al. 2014, Byers et al. 2015). Snails from the same site might not experience the same microhabitat, intensity of predation, food type and food availability.

Although there is a possibility that spatial distributions of parasitized and unparasitized *T. obsoleta* differed within a site, all snails were collected at a single location at each site, so microhabitat differences between parasitized and unparasitized snails are unlikely to be a cause of morphological variation. Also, the shape differences in *H. quissetensis* infected snails were seen across sites, so it is unlikely that their shell morphology was shaped completely by environmental factors.

It appears that parasites may have an impact on gastropod shell shape, with snails infected with *H. quissetensis* having different shell morphology than unparasitized snails. The shell shape of *H. quissetensis* infected snails is consistent with the morphology of snails with altered growth rates, however this relationship could not be tested in this study. Gastropods infected by the other two parasite species examined were larger than uninfected snails, but did not have different shell shapes; this may be due to older, and larger, snails being more likely to be parasitized, or that snails parasitized by those two species of trematodes live longer. These results indicate that shell shape changes seen in *T. obsoleta* are not necessarily a by-product of parasitism, since not all parasite species were associated with the same shell morphology. More work is needed to determine whether the parasites or the host are responsible for the morphological differences, and which environmental factors are responsible for the shape differences among sites.

Table 6-1. Infection status of *Tritia obsoleta* collected during the summer of 2014. The species of parasites in bold indicate that a subset of these snails infected by these species were used in the morphological analysis.

Parasite Species	**Total**	**Collection Site**		
		PB	**SC**	**WM**
Unparasitized Snails	**2648**	**1040**	**821**	**787**
Himasthla quissetensis	**213**	**161**	**14**	**38**
Lepocreadium setiferoides	**398**	**24**	**58**	**316**
Stephanostomum dentatum	**176**	**57**	**89**	**30**
Austrobilharzia variglandis	22	8	7	7
Diplostomum nassa	6	2	4	0
Gynaecotyla adunca	249	2	235	12
Stephanostomum tenue	11	1	3	7
Zoogonus lasius	55	14	34	7
Double Infection	17	2	15	0
Unidentified Parasites	58	7	4	47

Table 6-2. ANOVA table of results indicating how the shell lengths of snails differed by parasite species and collection site. P-values less than 0.05 in bold. Post-hoc test results are in Table 6-4.

	df	SS	MS	F	P-value
Site	**2**	**0.0018977**	**0.0009489**	**50.293**	**< 0.0001**
Parasite Species	**3**	**0.0004133**	**0.0001378**	**7.302**	**0.0001**
Site*Parasite	6	0.0002053	0.0000342	1.814	0.0997
Residuals	155	0.0029243	0.0000189		

Table 6-3. Average shell length of *T. obsoleta* by parasite species and by collection site. The average shell length (± standard error) of all parasitized snails, and the average shell length (± SE) of unparasitized snails from each collection site used in the morphological analysis of shell shape.

Parasite Species	**Number of Snails**	**Average Shell Length**
Himasthla quissetensis	42	21.60 ± 0.451
Lepocreadium setiferoides	42	21.25 ± 0.364
Stephanostomum dentatum	42	22.06 ± 0.416
Unparasitized Snails	42	20.12 ± 0.319
Collection Site	**Number of Unparasitized Snails**	**Average Shell Length**
Ponquogue Bridge	14	21.78 ± 0.519
Shinnecock Canal	14	18.80 ± 0.379
West Meadow Beach	14	19.78 ± 0.446
Total	*42*	*20.12 ± 0.319*

Table 6-4. Pairwise comparisons of shell lengths (Tukey's HSD) and shape differences between sites (model comparison test), and between snails infected by various parasite species. For the model comparison test, collection site was taken into account when examining shape differences by parasite species, and parasite species was taken into account when examining shape differences between sites. Collection Site Key: PB = Ponquogue Bridge Marine Park, SC = Shinnecock Canal, WM = West Meadow Beach. Parasite Species Key: UN = unparasitized snails, HQ = snails infected with *H. quissetensis*, LS = snails infected with *L. setiferoides*, SD = snails infected with *S. dentatum*. Comparisons with p-values less than 0.05 are in bold.

Pairwise Comparison		P-value	
		Morphology	Shell Length
Between Collection Sites	**PB * SC**	**0.001**	**< 0.0001**
	PB * WM	**0.001**	**< 0.0001**
	SC * WM	**0.001**	0.543
Between Uninfected and Parasitized Snails	**UN * HQ**	**0.012**	**0.007**
	UN * LS	0.309	**0.032**
	UN * SD	0.214	**< 0.0001**
Between Parasite Species	HQ * LS	**0.009**	0.954
	HQ * SD	0.267	0.612
	LS * SD	0.089	0.299

Table 6-5. MANOVA table of results from the geometric morphometric analysis on how shell morphology differs by parasite species and by collection site. P-values less than 0.05 are bolded. Post-hoc test results are in Table 4.

	df	SS	MS	Rsq	F	Z	P-value
Site	**2**	**0.04303**	**0.0215134**	**0.067290**	**6.1041**	**5.1951**	**0.001**
Parasite Species	**3**	**0.01912**	**0.0063724**	**0.029897**	**1.8081**	**1.5810**	**0.021**
Site*Parasite	6	0.02747	0.0045789	0.042966	1.2992	1.1709	0.161
Residuals	156	0.54981	0.0035244				
Total	167	0.63942					

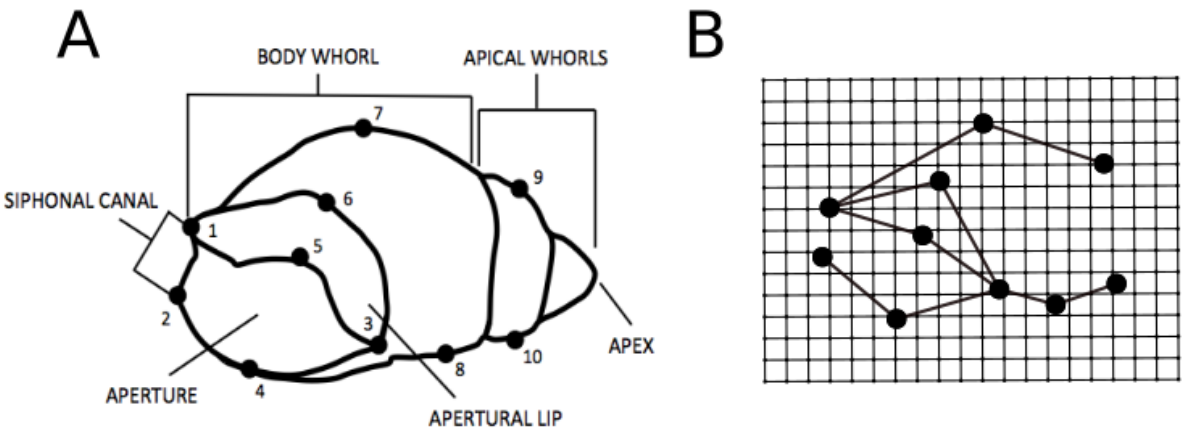

Figure 6-1. Diagram of *T. obsoleta* with labeled shell terminology and the placement of all 10 landmarks (A), and a picture of the reference thin-plate spline made using the average of unparasitized snails from all three sites (B).

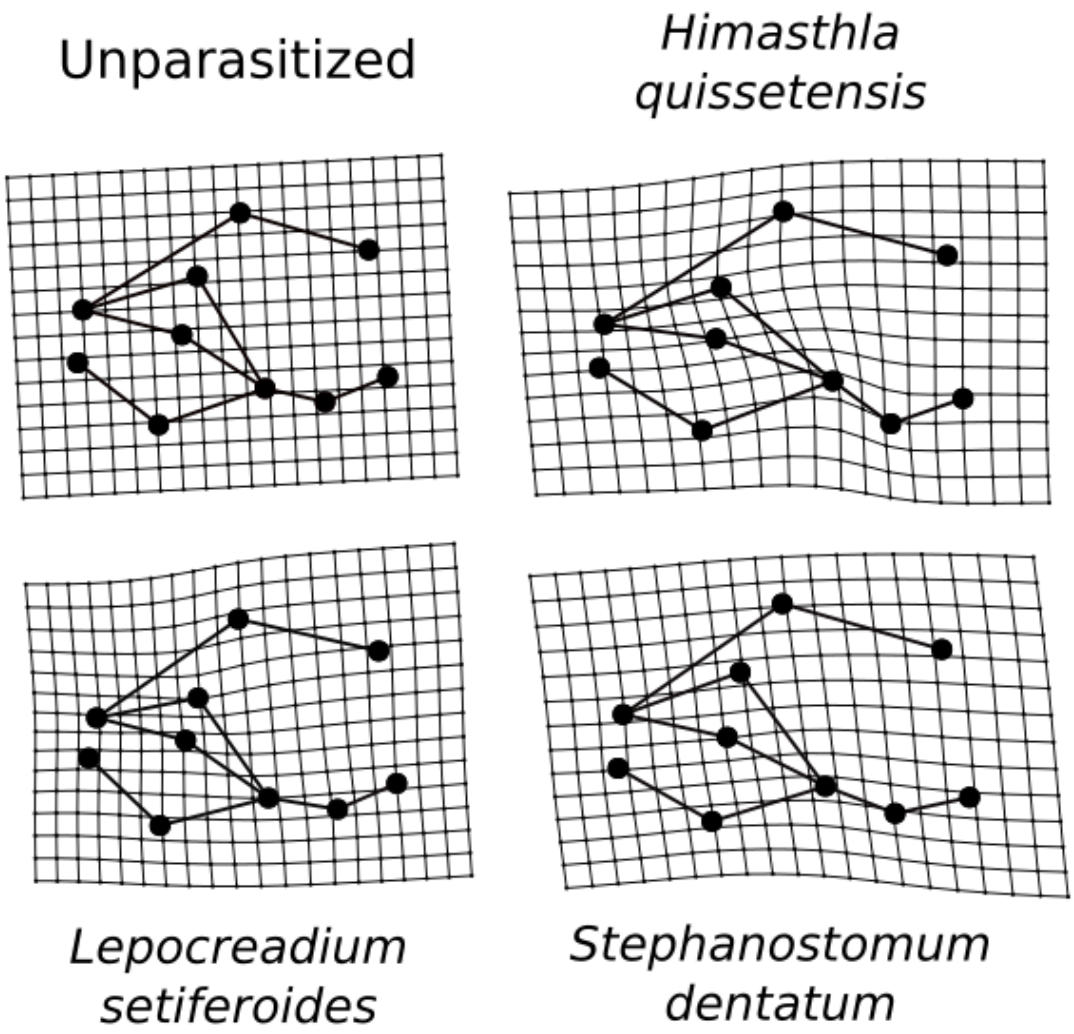

Figure 6-2. Thin-plate splines comparing the shapes of snails infected with each parasite species (HQ, LS, SD and unparasitized snails). Reference grids were determined by using the average shape of unparasitized snails from all sites (Fig. 6-1B). Shape changes indicated by thin-plate splines are exaggerated by 5x in order to make changes in morphology more apparent.

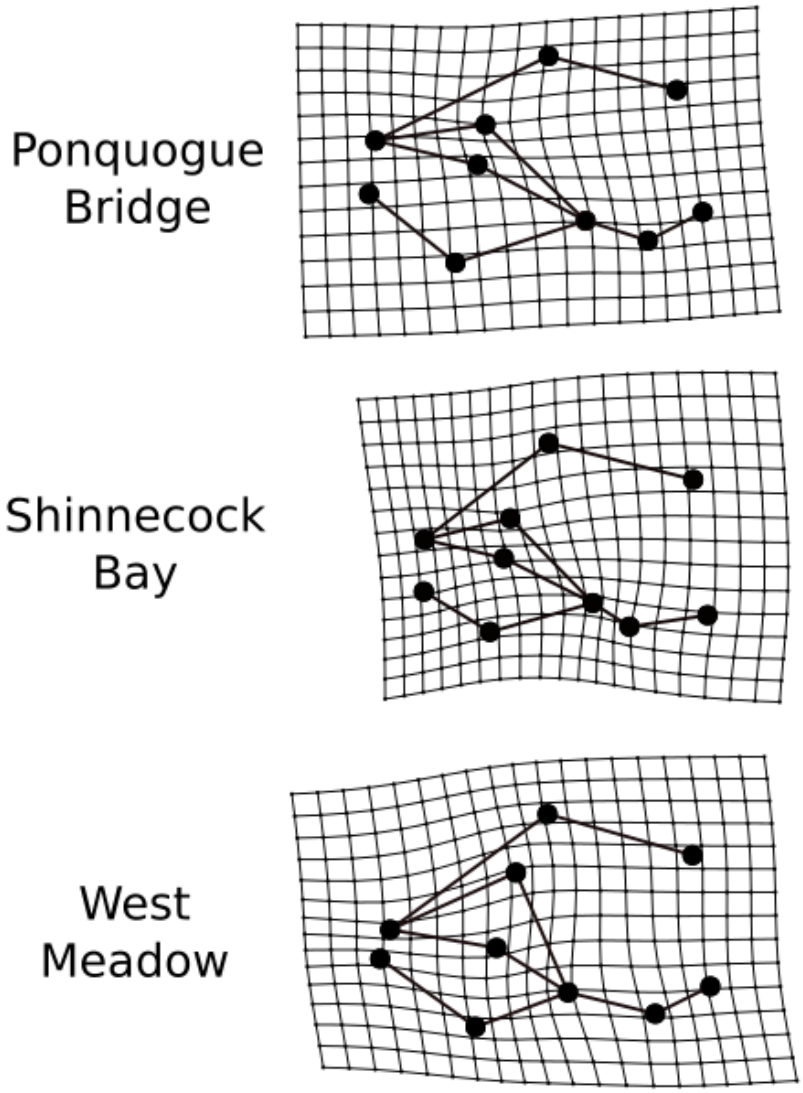

Figure 6-3. Thin-plate splines comparing the shapes of snails from each site (PB, SC, and WM). Reference grids were determined by using the average shape of unparasitized snails from all sites (Fig. 6-1B). Shape changes indicated by thin-plate splines are exaggerated by 5x in order to make changes in morphology more apparent.

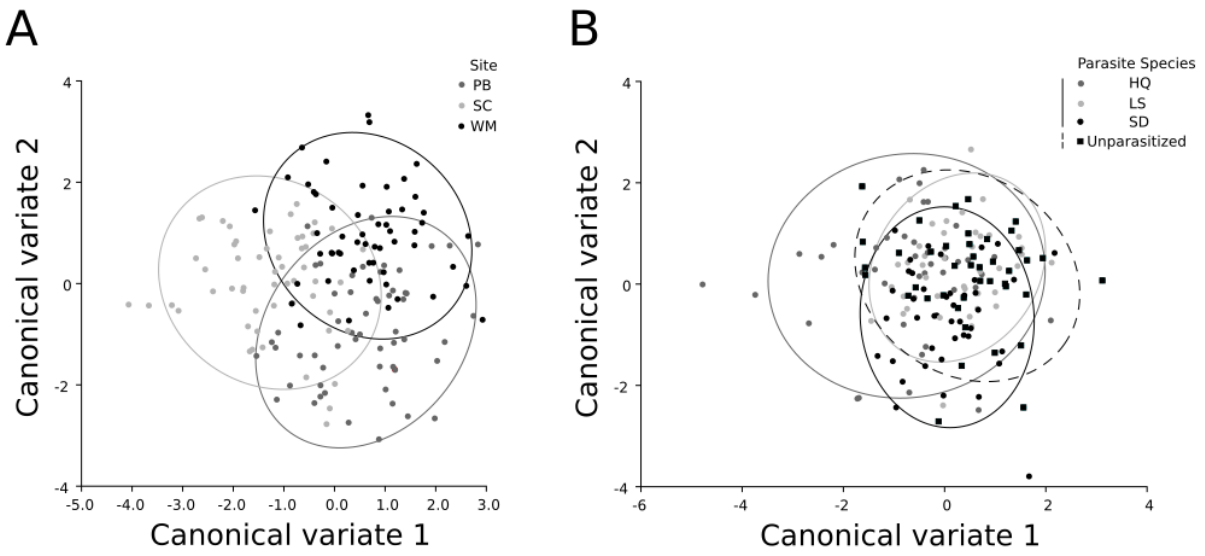

Figure 6-4. Canonical Variate Analysis plot of snails from different sites (A) and snails infected with different parasite species (B). The CVAs are used to demonstrate how the groups (collection site or parasite species) overlap while showing the largest differences between the groups.

Chapter 7

Conclusions

Species interactions can have huge impacts on communities, and can affect community biodiversity and structure (e.g., Stachowicz 2001, Wardle 2006, Gross et al. 2009). Within communities, species are involved in multiple types of interactions, and increasing the number of interactions increases the number of potential outcomes for an ecological process of interest (Hatcher et al. 2006). Also, it is possible that multiple species interactions can have synergistic or antagonistic effects, rather than just additive effects, which has been seen for predation and parasitism (e.g., Johnson et al. 2006, Hesse et al. 2012). This dissertation aimed to examine the effects of parasitism and predation on the marine snail *T. obsoleta*. I determined how these two types of species interactions altered the behavior and shell morphologies of these snails, as well as how responses to threat of predation changed over ontogeny.

Currently little is known about the mechanisms by which trematode parasites can alter the phenotypes of their gastropod hosts, but trematodes have been shown to affect the antipredator behavior, feeding behavior and morphology of their host (Fig. 7-1). Contrary to other trematode snails systems, parasite infection did not alter feeding or antipredator behaviors (Chapter 2, Appendix 4), but parasitism did seem to affect behavior by decreasing the proportion of snails that crawled out of the water in laboratory experiment (Chapter 4). Overall, in the laboratory parasitized snails moved down-slope more than unparasitized individuals. However, this pattern was not seen in the field, where the behavior expressed depended on the species of parasite that infected a snail. Parasitism did not have a consistent overall effect on adult shell morphology, however snails infected with *H. quissetensis* had different shell shapes compared to

unparasitized snails. It is unclear if these different behaviors and different shell morphologies are caused by the parasites, or by the host in response to being parasitized.

When examining common gastropod antipredator behaviors, crawling out of water or burrowing into sediment, juvenile and adult *T. obsoleta* did not behave as expected (Chapter 3). Adults increased the rate at which they crawled out of the water and burrowed into the sand in response to increased predation risk, as seen in most gastropods, but juveniles did not alter their crawl out behavior and were less likely to burrow in predator risk treatments. Juveniles are more vulnerable to predation as it is easier for predators, like crabs (Appendix 1), to consume them. So, it was surprising that juveniles did not display any of the antipredator behaviors typically seen in the other gastropod species that have been studied (Table 7-1). Although adults did display common antipredator behaviors when exposed to predator chemical cues, only a small proportions of adults tested responded as predicted (~50% of adults crawled out of water and ~20% of adults burrowed). *Tritia obsoleta* are gregarious and often found in very dense populations (Kelaher et al. 2003), which is thought to be the result of the conspecific trail-following behaviors that they exhibit (Trott & Dimock 1978). Because of large population densities, antipredator behavior may be unnecessary since safety-in-numbers will help protect from predation.

Fast growing juveniles were used to determine whether exposure to predator chemical cues could alter shell growth or morphology. No differences were found between snails in the predator risk and the control treatments (Chapter 5). The threat of predation did not alter *T. obsoleta* feeding rates (Chapter 2), and feeding rates are closely related to shell growth and morphology in most gastropods (Kemp & Bertness 1984, Boulding & Hay 1993). However, in most species of gastropods, feeding and growth rates decrease when the risk of predation

increases, such as in the presence of predator chemical cues, which leads to an altered shell morphology (Fig. 7-1, Table 7-1). For *T. obsoleta*, dense populations may provide some protection from predation, so instead of engaging in antipredator behaviors, which would give them short-term predation protection, juveniles may benefit more by continuing to feed and grow. This increased feeding and growth would result in increased sizes, which would give them a long-term defense against predation.

Since *T. obsoleta* seems to respond so differently to predation risk compared to other gastropod species, I compiled a list of snail species in which at least two different response types (behavior, growth, or morphology) have been examined when exposed to increased threat of predation (Table 7-1). The effect of risk of predation has been examined in an additional 46 species; however, those studies only examined one of the three types of responses (behavior, growth, or morphology). Most species were found to increase antipredator behaviors, decrease feeding and growth rates, and have altered morphology in response to predation risk. There were a few exceptions to this pattern, but the negative results were always included in studies that also presented species that displayed some expected results. Most of the snail species that have been tested were marine Caenogastropods, almost evenly split between the Neogastropoda (including *T. obsoleta*) and Littorinomorpha clades, with a few freshwater Heterobranchs. Within each clade, only a few families have been examined (Neogastropoda: Muricidae, Nassariidae (=*T. obsoleta*); Littorinomorpha: Littorinidae, Strombidae; Heterobranchia: Physidae, Planorbidae). This table indicates that although a significant amount of work has been done to examine the effects of predation on behavior, growth, and morphology of multiple gastropod species, only a small range of gastropod groups have been examined.

To determine why *T. obsoleta* responds differently to risk of predation than these other gastropod species, I examined the natural history traits of each species (Table 7-1). Most species of gastropod are temperate marine gastropods like *T. obsoleta*. Also, most species are either herbivores or carnivores, but there are a few species that consume both algae and detritus like *T. obsoleta* (although no other opportunistic scavengers have been tested). Tested gastropod species also varied greatly in adult shell length (6 mm – 90 mm), so shell size does not seem to determine if there will be behavioral and morphological responses to predation risk. The largest natural history difference between these other gastropod species and *T. obsoleta* is that *T. obsoleta* lives in high density populations (Table 7-1). For most species there was no mention of congregating behaviors or high density populations, therefore it was assumed that this did not occur in that species. A few species of gastropods only congregate in groups for short periods of time, e.g., as juveniles (*Littorina saxatilis, Lobatus gigas*), during the winter (*Littorina siktana*), during reproduction (*Nucella lamellosa*), and not throughout their lives as seen in *T. obsoleta*. This suggests that the gregarious behavior of *T. obsoleta* might be protecting individuals from predation, and that it may be why the species does not respond to predation like other gastropod species.

I consistently found differences in behavior and morphology among the snails from different collection sites (Chapter 3, 4, 5, 6). It is unlikely that these behavioral and morphological differences were the result of genetic differences between sites since *T. obsoleta* has a long larval period, and because no genetic differences have been found at this spatial scale among populations of other gastropod species with similar larval durations (Berger 1973, Riquet et al. 2013). Therefore, behavioral and morphological differences among sites are probably the result of environmental differences among sites. Some environmental factors that may be

influencing these differences, and are known to alter behavior and/or morphology in other species of snail, include: water flow/ wave strength (Levinton et al. 1995, Marquez et al. 2015, Gustafson & Bolek 2016), temperature (Trussell 2000, Dunithan et al. 2012), substrate type (Rueda et al. 2011, Dunithan et al. 2012), food type (Rueda et al. 2011, Saura et al. 2012), and food availability (Kelaher et al. 2003, Bourdeau 2009, Hooks & Padilla 2014).

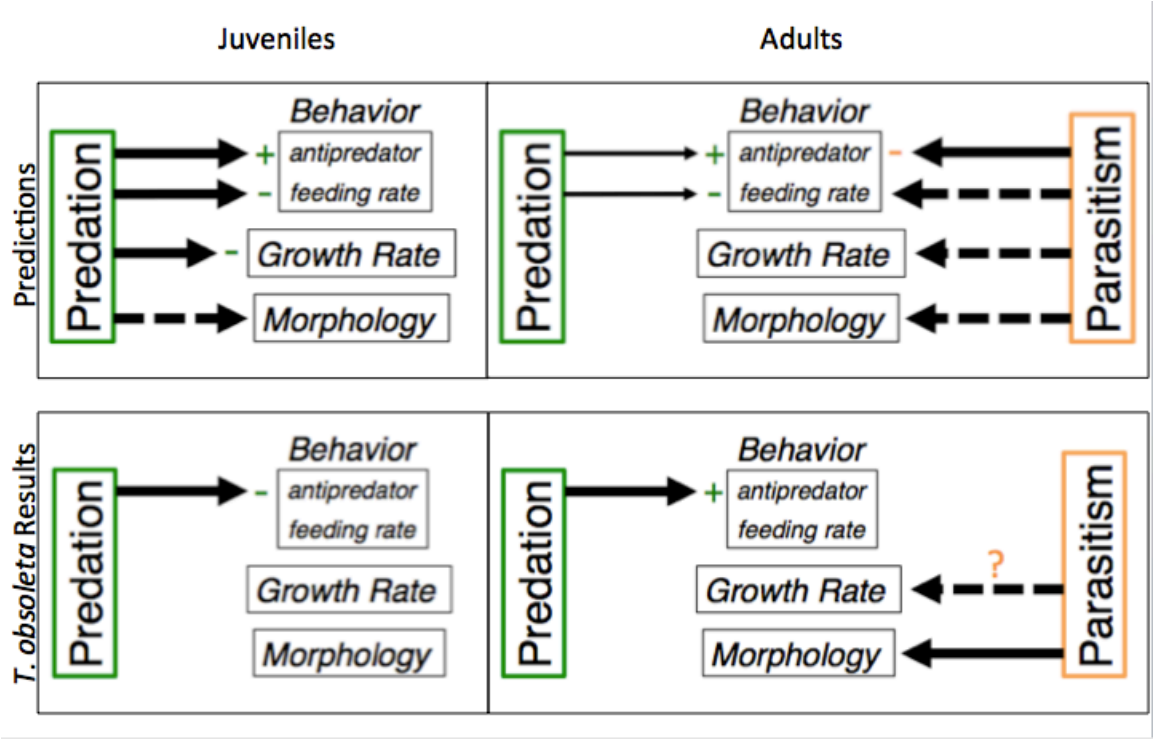

Figure 7-1. How predation and parasitism affect juvenile and adult *Tritia obsoleta* compared to a priori predictions. Predictions were based on how most gastropods respond to predation and parasitism. Arrows indicate where predation and parasitism are expected to alter behaviors, growth rate, or morphology. The direction of the effect is indicated by the plus (increase) and minus (decrease) signs, while dashed lines signify uncertainty. Line thickness indicates predicted impact on traits, with thicker lines representing stronger impacts.

This dissertation provides a better understanding of how *T. obsoleta* responds to both parasitism and predation. Although there were no synergistic or antagonistic interactions between these two species interactions as expected, the species did not follow predicted responses based on studies of other gastropod species (Fig. 7-1). Most gastropods that have been

studied have similar responses to risk of predation with increased avoidance behavior, reduced feeding rates, reduce growth rates, and altered shell morphology (Table 7-1). *Tritia obsoleta* does not exhibit most of these typical responses, but adults do display antipredator behaviors, although less frequently than expected. Juveniles, on the other hand, did respond to predation risk by burrowing less, which is the opposite of what is seen in most species of gastropods. More work is needed over a broader range of gastropod taxa, including species that have the population and habitat characteristics of *T. obsoleta* to determine if *T. obsoleta* is indeed an outlier, or if aspects of its natural history and lifestyle impact expected responses to increase risk of predation. Since little is known about how trematode parasites impact their snail hosts, the fact that they only alter the morphology of *T. obsoleta* is not as surprising. These results indicate that more work needs to be done over a broader range of taxa in order to examine how both predation risk and parasitism impact gastropods. Moreover, changes in snail responses over ontogeny need to be considered to gain a better understanding of how species interactions are affecting individuals throughout their lifetime, and how this might influence communities.

Table 7-1. Natural History of Gastropods that exhibit behavioral, growth, and/or morphological responses to threat of predation. Species included in this table have been examined for at least two of the four responses to predation risk that I examined in my research on T. obsoleta (antipredator behavior, feeding behavior, growth rate, morphology).

Gastropod Species	Predator Species	Response to Predation				Natural History				
		Anti-predator Behavior	Feeding Behavior	Growth Rate	Mor-phology	Range	Habitat	Diet	Adult Shell Size (length)	Gregarious behavior
Caenogastropoda (Neogastropoda), Family Nassariidae										
Tritia obsoleta	*Carcinus maenas*	crawl out [1, 2] burrowing [2]	(no change)[2]	(no change)[2]	(no change)[2]	Northwest Atlantic Ocean; Gulf of St. Lawerence – Florida [3]	marine intertidal, sandy-muddy sediment [4]	omnivore; algae, detritus, scavenged food [5,6]	12-20 mm [7]	Yes [8]
Caenogastropoda (Neogastropoda), Family Muricidae										
Nucella lamellosa	*Cancer productus*	refuge seeking[9, 10], fleeing behavior[11]	reduced feeding[9, 10]		rotund shell[12, 13]	Northwest Pacific Ocean; Alaska – Monterey Bay [14]	marine intertidal, rocky shores and jetties [14]	carnivore; barnacle[15]	~50 mm [15]	Yes, when reproducing [16]
	Pisaster ochraceus	refuge seeking[12]			elongate shell[12]					

Concholepas concholepas	*Homalapis plana*	predator avoidance [17]	reduced feeding[1] [7]	decreased growth[17]		Southeast Pacific Ocean; Chilean coast [18]	marine intertidal and subtidal, rocky intertidal [18]	carnivore [19]	90 mm diameter [20]	
	Acanthocyclus hassleri		reduced feeding[1] [7]	decreased growth[17]						
	Heliaster helianthus	predator avoidance [17]	reduced feeding[1] [7]	decreased growth[17]						
Bedeva vinosa	*Carcinus maenas*		reduced foraging [21]	decreased shell growth[21]		Southwestern Australian coast – New South Wales [22]	marine intertidal [21]	predator [21]	10-15 mm[23]	
Caenogastropoda (Littorinomorpha), Family Littorinidae										
Littoraria irrorata	*Callinectes sapidus*	predator avoidance [24]			narrower aperture[25]	Northwest Atlantic Ocean; New York – Florida[26]	marine/ brackish water, on salt marsh grasses [16, 26]	herbivore; periphyton and fungus on marsh grass [16, 26]	19-32 mm[26]	

Littorina obtusata	*Carcinus maenas*		reduced feeding [27, 28]	decreased growth [27, 28]	smaller aperture area [28]	North Atlantic; New England, Canada, Greenland, Iceland, White Sea - Portugal[29]	marine rocky intertidal, low wave shores, close association with macrophyte [29]	herbivore; soft tissue of fucoids where they live[29]	4.6-19.8 mm [29]	
Littorina saxatilis	*Dyspanopeus sayi*		reduced feeding[30]	decreased growth[30]		North Atlantic Ocean, Virginia - subarctic – Northern Africa [29]	marine upper intertidal, sheltered microhabitat [29]	herbivore & detritivore; diatoms, algae, detritus [29]	1.2-25.8 mm [29]	Yes, as juveniles, in rock crevices [29]
	Hemigrapsus sanguineus		reduced feeding, (no change) * [30]	decreased growth, (no change) * [30]						
Littorina sitkana	*Cancer productus*	up-shore movement [31]	reduced feeding [31]	decreased growth [32]		Northern Pacific; Bering Sea – Oregon and Okhotsk Sea – Northern Japan Sea [29]	marine intertidal, rocky boulder shores [29]	opportunistic omnivore; plant litter, micro- & macroalgae, lichens, epiphytes [29]	4.7-25 mm [29]	Yes, in winter, under boulders and on macroalgae [29]
Caenogastropoda (Littorinomorpha), Family Strombidae										

Lobatus gigas	*Panulirus argus*	reduced movement, burrowing [33]		decreased growth [33]		Caribbean; Florida – Venezuela [20]	marine, shallow water [3]	herbivore; algae [20]	200-300 mm [3]	Yes, as juveniles [34]
Heterobranchia, Family Physidae										
Physa pomila	*Procambarus clarkia*	predator avoidance [35]			more elongate, narrower aperture [35]	patchy distribution across the United States [36, 37]	freshwater; large rivers and ponds [36]	herbivore & detritivore; aquatic vegetation, detritus[37]	4-6 mm [37]	
Heterobranchia, Family Planorbidae										
Planorbella trivolvis	*Belostoma flumineum*	(no change)[38]			wider shell[38, 39, 40, 41]	North & South America [42]	freshwater; shallow areas of small lakes or bays of large lakes [42]	herbivore; algae & other vegetation [42]	~20 mm diameter [42]	
	Oronectes rusticus	crawl out [38]			narrower shell, (no change) ** [38, 39, 40, 41]					
Helisoma anceps	*Oronectes rusticus*	(no change)[43]			smaller aperture [41]	North & South America [42, 44]	freshwater; rivers, lakes or creeks [42, 44]	herbivore & detritivore; algae, detritus [44]	8-16 mm diameter [44]	

* results depend on snail population

** different findings among studies

1 Atema & Stenzler 1977

2 This dissertation

3 Abbot & Morris 1995

4 Curtis & Hurd 1983

5 Curtis & Hurd 1981

6 Feller 1984

7 Scheltema 1964

8 Kelaher et al. 2003

9 Bourdeau 2010

10 Bourdeau 2012

11 Marko & Palmer 1991

12 Bourdeau 2009

13 Bourdeau 2013

14 Ricketts & Calvin 1968

15 Kozloff 1973

16 Personal communication with Dianna Padilla

17 Manriquez et al. 2013

18 Vargas et al. 2013

19 Manriquez et al. 2008

20 Gabbi 1999

21 Freeman et al. 2013

22 Coulson et al. 2011

23 Beechey 2000

24 Duval et al. 1994

25 Moody & Aronson 2012

26 Mulcrone 2013b

27 Brookes & Rochette 2007

28 Trussel 2000b

29 Reid 1996

30 Hooks & Padilla 2014

31 Rochette & Dill 2000

32 Yamada & Boulding 1996

33 Delgado et al. 2002

34 Ray & Stoner 1994

35 Salice & Plautz 2011

36 Wethington 2013

37 Kimberley & Salice 2013

38 Hoverman et al. 2005

39 Hoverman & Relyea 2007

40 Hoverman & Relyea 2009

41 Hoverman et al. 2014

42 Baker & Van Cleave 1945

43 Covich et al. 1994

44 Mulcrone 2013a

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Appendix 1: Prey Preferences of *Carcinus maenas*

Tritia obsoleta were collected from West Meadow Beach on Long Island, NY (Appendix 3). They were transported to the laboratory and kept in recirculating aquaria and fed *Ulva lactuca* and Tetramin fish flakes *ad libitum* until used in the experiment. Parasitism of living adults was determined using the same methods as those used in Chapter 2. Parasitized snails were then labeled with one dot of nail polish on the top of the shell, and placed in containers labeled with the species by which they were parasitized.

Green crabs were collected at Stony Brook Harbor (40.9023, -73.1748), using raw chicken as bait. Crabs were transferred to the laboratory and kept in recirculating aquaria and fed tilapia until the experiments were complete. Sex was determined for each individual, and only male crabs were used. To keep track of individual crabs, a bee tag was glued onto the top of the carapace of each individual with a cyanoacrylate adhesive. 24 hours prior to each experimental trial, crabs were placed in separate aquaria without food.

Each crab was used in six preference trials, 3 trials were to examine potential preferences between adult and juvenile *T. obsoleta*, and 3 trials were used to determine potential preferences between parasitized and unparasitized adult *T. obsoleta*. For the three adult snail trials, one parasitized snail and a size-matched unparasitized snail (within 0.5 mm) was placed into the tank so that the crab was equidistant from each snail (Fig. A1-1). For the 3 trials with juvenile snails, the same design was used except that one snail was a juvenile (less than 12 mm in length) and one was an unparasitized adult (larger than 15 mm).

A digital camcorder (Sony HDR-CX580V) was placed above each tank with the lens parallel to the water to record the crab and snail movements. Each trial was run for 1 hour, after which the crab was returned to the recirculating aquarium and fed tilapia. If not consumed, the snails were then dissected to determine if they were parasitized and that species of parasite was identified. Some trials were no included in the analysis if the “unparasitized” snail (based on light detection methods) was found via dissection to be parasitized.

The following data were then collected from the videos: which snail the crab attacked first, whether the snails were consumed, and which snail had the longest handling time (Table A1-1). Contingency table analyses were used to determine if preferences were significantly different among individual crabs (G test, Table A1-2). If there was no difference among crabs (preferences were the same among all crabs), a t-test was then used to determine if one type of snail was preferred more than the other for each response variable (Table A1-2).

When examining preference between parasitized and unparasitized adults, there was no difference among individual crabs for any of the response variables examined (G tests, Table A1-2). Furthermore, there was no preference for parasitized or unparasitized snails when examining the snail that was attacked first and the snail that the crab spent the most time

handling (Table A1-2). No snails were consumed in these trials, so snail consumption was not examined (Table A1-1).

When examining potential preferences between juvenile and adult *T. obsoleta*, there was no difference among individual crabs for most response variables. However, individual crabs varied on which snail they spent the most time handling (Table A1-2). This was probably due to the amount of time it took to consume the snail. If the crab consumed the first snail quickly, it then spent more time on the second snail, but if it took a lot of time to consume the first snail, that was the snail that the crab spent the most time on. There was no preference difference among individual crabs for the snail first chosen, and all crabs were more likely to attack adult snails first than juvenile snails (Table A1-2). There was also no effect of individual crab preference on which snail was consumed, however this was because only juvenile snails were consumed (Table A1-2). This is likely due to the one-hour trial period. Juvenile snails could be consumed in one hour, but it seems that adult *T. obsoleta* require longer handling times.

Table A1-1. Prey preference of *C. maenas* when given the choice between juvenile or adult *T. obsoleta*, or choice between parasitized or unparasitized adult *T. obsoleta.* Only one snail is in each category, either a juvenile, uninfected adult, or infected (parasitized) snail. Grey shading indicates that a crab did not attack or consume either snail. Data from this table were used in the statisctial analyses.

Prey Preference Trial	Response Variable	Trial	Individual Crabs						
			77	78	79	80	82	85	86
Juvenile vs. Adult *T. obsoleta*	First Snail Chosen	1	Adult	Adult	Adult	Adult	Adult	Juvenile	Adult
		2	Adult	Juvenile	Adult	Juvenile	Juvenile	Adult	Adult
		3	Adult	Juvenile	Adult	Adult	Juvenile	Juvenile	Adult
	Most time spent on snail	1	Adult	Juvenile	Juvenile		Adult	Juvenile	Adult
		2	Adult	Juvenile	Juvenile	Juvenile	Adult	Adult	Adult
		3	Adult	Adult	Adult	Juvenile	Adult	Adult	Adult
	Snail consumed	1		Juvenile	Juvenile		Juvenile		
		2			Juvenile	Juvenile	Juvenile		
		3		Juvenile	Juvenile	Juvenile	Juvenile	Juvenile	Juvenile
Infected vs. Uninfected adult *T. obsoleta*	First Snail Chosen	1	Infected	Infected	Uninfected	Infected	Infected	Infected	Infected
		2	Infected	Infected	Uninfected	Uninfected	Uninfected	Uninfected	Uninfected
		3		Uninfected	Uninfected	Uninfected	Infected	Uninfected	Infected

	Most time spent on snail	1	Infected	Infected	Uninfected	Infected	Infected	Infected	Infected
		2	Infected	Infected	Uninfected	Uninfected	Uninfected	Uninfected	Uninfected
		3		Infected	Uninfected	Uninfected	Infected	Uninfected	Infected
	Snail consumed	1							
		2							
		3							

Table A1-2. Results of statistical analyses conducted to determine prey preference of *Carcinus maenas* on *Tritia obsoleta*. G tests were used to determine if each response variable was affected by which crab used for the trial. If there was no crab effect, T tests were used to determine which snail was preferred for each response variable. P-values less than 0.05 were considered significant, and are bolded.

Prey Preference Trial	Response Variable	G test p-value	Effect of Individual Crabs?	T test p-value	Snail preference?
Juvenile vs. Adult *T. obsoleta*	First Snail Chosen	> 0.10	No	**0.0287**	**Yes, adults attacked first**
	Most time spent on snail	**< 0.05**	**Yes**		
	Snail consumed	>0.995*	No	**0.0065**	**Yes, juveniles more likely to be consumed**
Parasitized vs. Unparasitized *T. obsoleta*	First Snail Chosen	> 0.05	No	0.6189	No
	Most time spent on snail	> 0.05	No	0.6189	No
	Snail consumed	**	Unknown		

*No adult snails were consumed

**No snails were consumed in the parasitized/ unparasitized snail trials

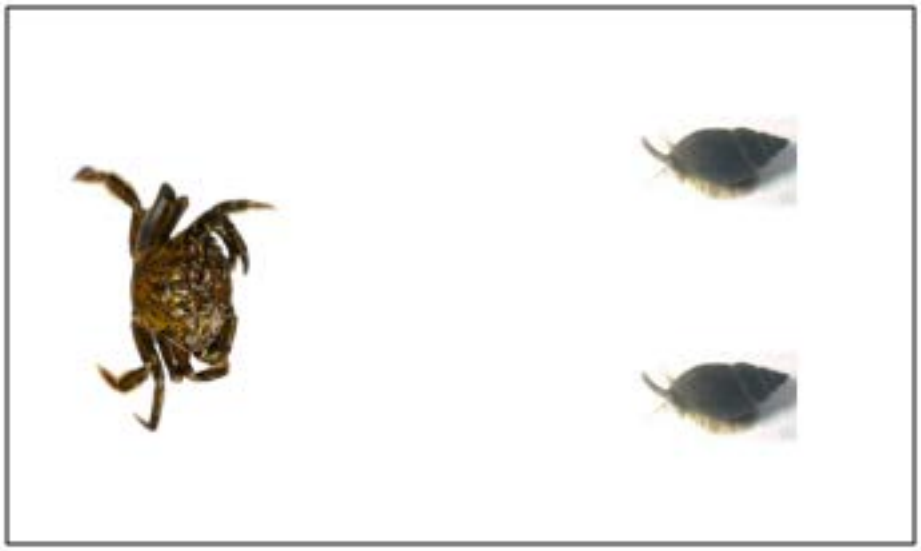

Figure A1-1. An aerial view of the tank setup to determine *Carcinus maenas* prey preferences. Two snails (one unparasitized adult and either one parasitized adult or one juvenile *T. obsoleta*) were placed into the tank. The crab was then placed in the tank so that it was equidistance from both snails.

Appendix 2: Prevalence of *T. obsoleta* trematode parasites across Long Island, NY

Tritia obsoleta were collected from 4 study sites (CM, PB, SC, and WM) during 3 summers (2013, 2014, 2015) for use in experiments, and the species of parasite that infected each snail was recorded (Table A2-1). General linear models (glm) were used to determine which factors best explained the variation in parasite prevalence of each species of parasite (including double infections and infections by unknown parasite species). The response variable was the proportion of snails infected with parasite each parasite species compared to the proportion of all other snails. Collection site, collection transect, month, and year were the independent factors. For each species, I began with a full model examining all of the four factors and all possible interactions. Interactions, and factors not in significant interactions, that were not statistically significant (chi-test, $p < 0.05$) were removed in order to find the best-fit model for each species of parasite. The best-fit model was considered to be the combination of factors with the smallest AIC value, and that was significantly different from other models. If the model with the lowest AIC value was not significantly different than another model, the model with the fewest number of factors was considered to be the best-fit model (only occurred when examining *Stephanostomum dentatum* infected snails and snails with double infections). All glm were fitted in R, using a binomial distribution.

Model Factors

Snails collected from 3 different sites (Old Ponquogue Bridge Marine Park (PB) 40.8433, -72.4985, near Shinnecock Canal (SC) 40.8835, -72.4848, and West Meadow Beach (WM) 40.9443, -73.1466) were used in these glms.

To examine how parasite prevalence varies throughout the summer, snails were separated into groups that were collected in early summer (5/10- 6/10), in the middle of the summer (6/11-7/10), or collected in late summer (7/11-8/25), hereafter referred to as month. All snails collected during 2013 were collected in mid- to late summer, so only snails collected during 2014 and 2015 were used in the models. The number of snails collected differed between years 2014 and 2015 (Table A2-2).

To look at variation of snail distribution on the shoreline, snails were collected along two 50 m transects parallel to the shore at low tide. One transect was high on the shoreline (shore that had been exposed at least 2 hours prior to low tide; PB, 0.04 m above mean low water (MLW); SC, 0.06 m above MLW; WM, 0.14 meters above MLW) and the other transect was low on the shore (shore exposed at low tide; at or 0.14 m below MLW at each site). Tidal elevation varied by site because the slope of the shore varied across sites which affected the horizontal distance that was exposed when comparing high tide and low tide marks.

Tables A2-3 - 6 display the number of snails parasitized by each species across sites (Table A2-3), transects (Table A2-4), months (Table A2-5) and years (Table A2-6). Tables A2-7 - 16 display the glm results of the best-fit model for each species of parasite (parasites in alphabetical order, followed by snails with double infections, and snails infected by an unknown parasite species.

Table A2-1. The total number of *T. obsoleta* collected and dissected from summer 2013 through the summer of 2015. The total number of snails infected by each parasite species by collection site and year. These numbers include snails collected from Crab Meadow Beach and snails collected during 2013, which were not incorporated into the general linear models. Grey shading indicates that no snails were collected from that site during that year.

Parasite Species	Crab Meadow			Ponquogue Bridge			Shinnecock Canal			West Meadow		
	2013	2014	2015	2013	2014	2015	2013	2014	2015	2013	2014	2015
Austrobilharzia variglandis	0			3	8	3	1	7	2	8	7	1
Diplostomum nassa	0			1	2	1	1	4	0	1	0	0
Gynaecotyla adunca	0			1	2	0	25	235	104	7	12	31
Himasthla quissetensis	3			33	161	74	2	14	17	22	38	6
Lepocreadium setiferoides	3			1	24	7	3	58	53	4	316	32
Stephanostomum dentatum	1			10	57	31	18	89	130	8	30	2
Stephanostomum tenue	1			1	1	2	1	3	4	1	7	1
Zoogonus lasius	0			1	14	9	4	34	25	0	7	0
double infection	0			0	2	1	0	15	10	1	0	0
unknown species	14			14	7	10	14	4	5	27	47	10
Unparasitized snails	278			235	1040	462	231	821	250	221	787	517
Total Snails	300			300	1318	600	300	1284	600	300	1251	600
	300			2218			2184			2151		

Table A2-2. The number of *T. obsoleta* collected and dissected from each site and transect during each year. All snails that were dissected in 2014 and 2015 were used in the general linear models. *Snails collected during 2013 were mostly collected during the late summer month.

Collection site	Year	Number of snails collected per transect	Number of snails collected per month	Total number of snails collected	**Total Number of snails dissected**
Ponquogue Bridge Marine Park	2013	150	*	300	300
	2014	702	468	1404	**1318**
	2015	300	200	600	**600**
Shinnecock Canal	2013	150	*	300	300
	2014	702	468	1404	**1284**
	2015	300	200	600	**600**
West Meadow Beach	2013	150	*	300	300
	2014	702	468	1404	**1251**
	2015	300	200	600	**600**

Table A2-3. Number of parasitized *T. obsoleta* by collection site. Parasite species names are bolded if site was a significant predictor of where snails with this species would be collected, with significance determined by glm. This table contains snails collected in all three summers, but only snails collected in 2014 and 2015 were used in the glms.

Parasite Species	**Ponquogue Bridge**	**Shinnecock Canal**	**West Meadow**
Austrobilharzia variglandis	14	10	16
Diplostomum nassa	4	5	1
Gynaecotyla adunca	**3**	**364**	**50**
Himasthla quissetensis	**268**	**33**	**66**
Lepocreadium setiferoides	**32**	**114**	**352**
Stephanostomum dentatum	**98**	**237**	**40**
Stephanostomum tenue	4	8	9
Zoogonus lasius	**24**	**63**	**7**
double infection	**3**	**25**	**1**
unknown species	**31**	**23**	**84**
Total Snails	2218	2184	2151

Table A2-4. Number of parasitized *T. obsoleta* by collection transect. Parasite species names are bolded if transect was a significant predictor of where snails with this species would be collected, with significance determined by glm. This table contains snails collected in all three summers, but only snails collected in 2014 and 2015 were used in the glms.

Parasite Species	**High Shore**	**Low Shore**
Austrobilharzia variglandis	17	23
Diplostomum nassa	7	3
Gynaecotyla adunca	**368**	**49**
Himasthla quissetensis	**244**	**123**
Lepocreadium setiferoides	**158**	**340**
Stephanostomum dentatum	**216**	**159**
Stephanostomum tenue	10	11
Zoogonus lasius	47	47
double infection	**26**	**3**
unknown species	59	79
Total Snails	3298	3255

Table A2-5. Number of parasitized *T. obsoleta* based on whether they were collected in early summer (5/10- 6/10), mid summer (6/11- 7/10), or late summer (7/11-8/25). Parasite species names are bolded if time during the summer (month) was a significant predictor of when snails with this species would be collected, with significance determined by glm. This table contains snails collected in all three summers, but only snails collected in 2014 and 2015 were used in the glms.

Parasite Species	Early Summer	Mid Summer	Late Summer
Austrobilharzia variglandis	**9**	**4**	**27**
Diplostomum nassa	**0**	**2**	**8**
Gynaecotyla adunca	**134**	**76**	**207**
Himasthla quissetensis	**90**	**83**	**194**
Lepocreadium setiferoides	**248**	**181**	**69**
Stephanostomum dentatum	**83**	**123**	**169**
Stephanostomum tenue	5	7	9
Zoogonus lasius	30	29	35
double infection	**20**	**5**	**4**
unknown species	**6**	**20**	**112**
Total Snails	1888	1944	2721

Table A2-6. Number of collected *T. obsoleta* infected by each parasite species in each collection year. Parasite species names are bolded if year was a significant predictor of when snails with this species would be collected, with significance determined by glm. This table contains snails collected in all three summers, but only snails collected in 2014 and 2015 were used in the glms.

Parasite Species	**2013**	**2014**	**2015**
Austrobilharzia variglandis	12	22	6
Diplostomum nassa	3	6	1
Gynaecotyla adunca	33	249	135
Himasthla quissetensis	57	213	97
Lepocreadium setiferoides	8	**398**	**92**
Stephanostomum dentatum	36	**176**	**163**
Stephanostomum tenue	3	11	7
Zoogonus lasius	5	55	34
double infection	1	17	11
unknown species	55	58	25
Total Snails	900	3853	1800

Table A2-7. General Linear Model Analysis of Deviance output showing the factors and interactions included in the best fit model for collection of snails infected by *Austrobilharzia variglandis*. The factors and interactions that are significant predictors of the collection of AV parasitized snails are bolded.

	df	deviance	residual df	residual deviance	p (chi-test)
NULL			35	48.307	
Site	2	0.3948	33	47.912	0.8209
Transect	1	0.6422	32	47.270	0.4229
Month	**2**	**7.4850**	**30**	**39.785**	**0.02370**
Site*Month	**4**	**17.3012**	**26**	**22.483**	**0.00169**
Transect*Month	**2**	**6.3208**	**24**	**16.163**	**0.04241**

Table A2-8. General Linear Model Analysis of Deviance output showing the factors and interactions included in the best fit model for collection of snails infected by *Diplostomum nassa*. The factors and interactions that are significant predictors of the collection of DN parasitized snails are bolded.

	df	deviance	residual df	residual deviance	p (chi-test)
NULL			35	25.192	
Site	2	5.7195	33	19.472	0.0573
Month	**2**	**7.0736**	**31**	**12.399**	**0.0291**

Table A2-9. General Linear Model Analysis of Deviance output showing the factors and interactions included in the best fit model for collection of snails infected by *Gynaecotyla adunca*. The factors and interactions that are significant predictors of the collection of GA parasitized snails are bolded.

	df	deviance	residual df	residual deviance	p (chi-test)
NULL			35	1377.55	
Site	**2**	**590.83**	**33**	**786.72**	**< 0.0001**
Transect	**1**	**315.81**	**32**	**470.92**	**< 0.0001**
Month	**2**	**63.14**	**30**	**407.77**	**< 0.0001**
Year	1	2.43	29	405.34	0.11884
Site*Transect	**2**	**35.56**	**27**	**369.78**	**< 0.0001**
Site*Month	**4**	**18.88**	**23**	**350.91**	**0.00083**
Transect*Month	**2**	**8.67**	**21**	**342.24**	**0.01312**
Site*Year	**2**	**28.16**	**19**	**314.07**	**< 0.0001**
Transect*Year	1	2.51	18	311.56	0.11283
Month*Year	**2**	**218.46**	**16**	**93.10**	**< 0.0001**
Site*Month*Year	**4**	**68.06**	**12**	**25.04**	**< 0.0001**
Transect*Month*Year	**2**	**20.77**	**10**	**4.27**	**< 0.0001**

Table A2-10. General Linear Model Analysis of Deviance output showing the factors and interactions included in the best fit model for collection of snails infected by *Himasthla quissetensis*. The factors and interactions that are significant predictors of the collection of HQ parasitized snails are bolded.

	df	deviance	residual df	residual deviance	p (chi-test)
NULL			35	423.51	
Site	**2**	**243.939**	**33**	**179.57**	**< 0.0001**
Transect	**1**	**37.279**	**32**	**142.29**	**< 0.0001**
Month	**2**	**20.134**	**30**	**122.16**	**< 0.0001**
Year	1	0.005	29	122.15	0.9447
Site*Transect	**2**	**8.561**	**27**	**113.59**	**0.01384**
Site*Month	4	6.986	23	106.61	0.13664
Transect*Month	**2**	**39.491**	**21**	**67.12**	**< 0.0001**
Site*Year	**2**	**16.006**	**19**	**51.11**	**0.00033**
Transect*Year	1	3.121	18	47.99	0.07730
Month*Year	**2**	**8.248**	**16**	**39.74**	**0.01618**
Site*Transect*Month	**4**	**16.779**	**12**	**22.96**	**0.00213**
Site*Transect*Year	2	3.419	10	19.54	0.18096
Site*Month*Year	4	5.032	6	14.51	0.28398
Transect*Month*Year	2	1.840	4	12.67	0.39845

Table A2-11. General Linear Model Analysis of Deviance output showing the factors and interactions included in the best fit model for collection of snails infected by *Lepocreadium setiferoides*. The factors and interactions that are significant predictors of the collection of LS parasitized snails are bolded.

	df	deviance	residual df	residual deviance	p (chi-test)
NULL			35	894.33	
Site	**2**	**383.26**	**33**	**511.06**	**< 0.0001**
Transect	**1**	**82.10**	**32**	**428.96**	**< 0.0001**
Month	**2**	**126.40**	**30**	**302.56**	**< 0.0001**
Year	**1**	**52.68**	**29**	**249.89**	**< 0.0001**
Site*Transect	**2**	**49.81**	**27**	**200.07**	**< 0.0001**
Site*Month	**4**	**9.81**	**23**	**190.27**	**0.04382**
Transect*Month	**2**	**11.94**	**21**	**178.33**	**0.00256**
Site*Year	**2**	**94.34**	**19**	**83.99**	**< 0.0001**
Transect*Year	1	0.01	18	83.98	0.92354
Month*Year	2	2.38	16	81.60	0.30353
Site*Transect*Month	**4**	**24.60**	**12**	**56.99**	**< 0.0001**
Site*Transect*Year	**2**	**27.10**	**10**	**29.90**	**< 0.0001**
Site*Month*Year	**4**	**19.51**	**6**	**10.39**	**0.00063**

Table A2-12. General Linear Model Analysis of Deviance output showing the factors and interactions included in the best fit model for collection of snails infected by *Stephanostomum dentatum*. The factors and interactions that are significant predictors of the collection of SD parasitized snails are bolded.

	df	deviance	residual df	residual deviance	p (chi-test)
NULL			35	499.64	
Site	**2**	**173.580**	**33**	**326.06**	**< 0.0001**
Transect	**1**	**5.648**	**32**	**320.41**	**0.01748**
Month	**2**	**14.335**	**30**	**306.08**	**0.00077**
Year	**1**	**43.490**	**29**	**262.59**	**< 0.0001**
Site*Transect	**2**	**9.709**	**27**	**252.88**	**0.00779**
Site*Month	4	6.177	23	246.70	0.18632
Transect*Month	**2**	**125.462**	**21**	**121.24**	**< 0.0001**
Site*Year	**2**	**55.663**	**19**	**65.58**	**< 0.0001**
Transect*Year	**1**	**12.689**	**18**	**52.89**	**0.00037**
Month*Year	2	1.108	16	51.78	0.57468
Site*Transect*Month	**4**	**13.142**	**12**	**38.64**	**0.01060**
Transect*Month*Year	**2**	**16.172**	**10**	**22.47**	**0.00031**

Table A2-13. General Linear Model Analysis of Deviance output showing the factors and interactions included in the best fit model for collection of snails infected by *Stephanostomum tenue*. The factors and interactions that are significant predictors of the collection of ST parasitized snails are bolded.

	df	deviance	residual df	residual deviance	p (chi-test)
NULL			35	36.870	
Site	2	2.7919	33	34.078	0.2476
Transect	1	0.2507	32	33.828	0.6166
Year	1	0.3778	31	33.450	0.5388
Site*Transect	2	0.7503	29	32.700	0.6872
Site*Year	2	4.8191	27	27.880	0.0899
Transect*Year	**1**	**3.9227**	**26**	**23.958**	**0.0476**
Site*Transect*Year	**2**	**7.8661**	**24**	**16.092**	**0.0196**

Table A2-14. General Linear Model Analysis of Deviance output showing the factors and interactions included in the best fit model for collection of snails infected by *Zoogonus lasius*. The factors and interactions that are significant predictors of the collection of ZL parasitized snails are bolded.

	df	deviance	residual df	residual deviance	p (chi-test)
NULL			35	97.700	
Site	**2**	**49.419**	**33**	**48.281**	**< 0.0001**
Transect	1	0.173	32	48.108	0.67734
Month	2	0.146	30	47.962	0.92972
Year	1	1.677	29	46.285	0.19528
Site*Transect	2	4.795	27	41.490	0.09094
Transect*Month	**2**	**11.242**	**25**	**30.248**	**0.00362**
Site*Year	**2**	**7.482**	**23**	**22.766**	**0.02373**
Transect*Year	1	3.092	22	19.674	0.07868

Table A2-15. General Linear Model Analysis of Deviance output showing the factors and interactions included in the best fit model for collection of snails infected by two species of parasite. The factors and interactions that are significant predictors of the collection of multiple infection snails are bolded.

	df	deviance	residual df	residual deviance	p (chi-test)
NULL			35	102.483	
Site	**2**	**42.556**	**33**	**59.927**	**< 0.0001**
Transect	**1**	**19.490**	**32**	**40.437**	**< 0.0001**
Month	**2**	**18.836**	**30**	**21.602**	**< 0.0001**

Table A2-16. General Linear Model Analysis of Deviance output showing the factors and interactions included in the best fit model for collection of snails infected by an unknown species of parasite. The factors and interactions that are significant predictors of the collection of snails with unknown parasites are bolded.

	df	deviance	residual df	residual deviance	p (chi-test)
NULL			35	184.601	
Site	**2**	**47.808**	**33**	**136.793**	**< 0.0001**
Transect	1	1.084	32	135.709	0.29779
Month	**2**	**59.096**	**30**	**76.614**	**< 0.0001**
Year	1	0.337	29	76.277	0.56179
Site*Transect	**2**	**14.895**	**27**	**61.382**	**0.00058**
Site*Month	**4**	**13.613**	**23**	**47.769**	**0.00864**
Transect*Month	2	2.159	21	45.610	0.33973
Site*Year	**2**	**15.729**	**19**	**29.881**	**0.00038**
Transect*Year	1	1.395	18	28.485	0.23749
Month*Year	2	4.042	16	24.443	0.13253
Site*Transect*Year	**2**	**10.893**	**14**	**13.550**	**0.00431**
Transect*Month*Year	**2**	**8.696**	**12**	**4.854**	**0.01293**

Appendix 3: Map of Collection Site

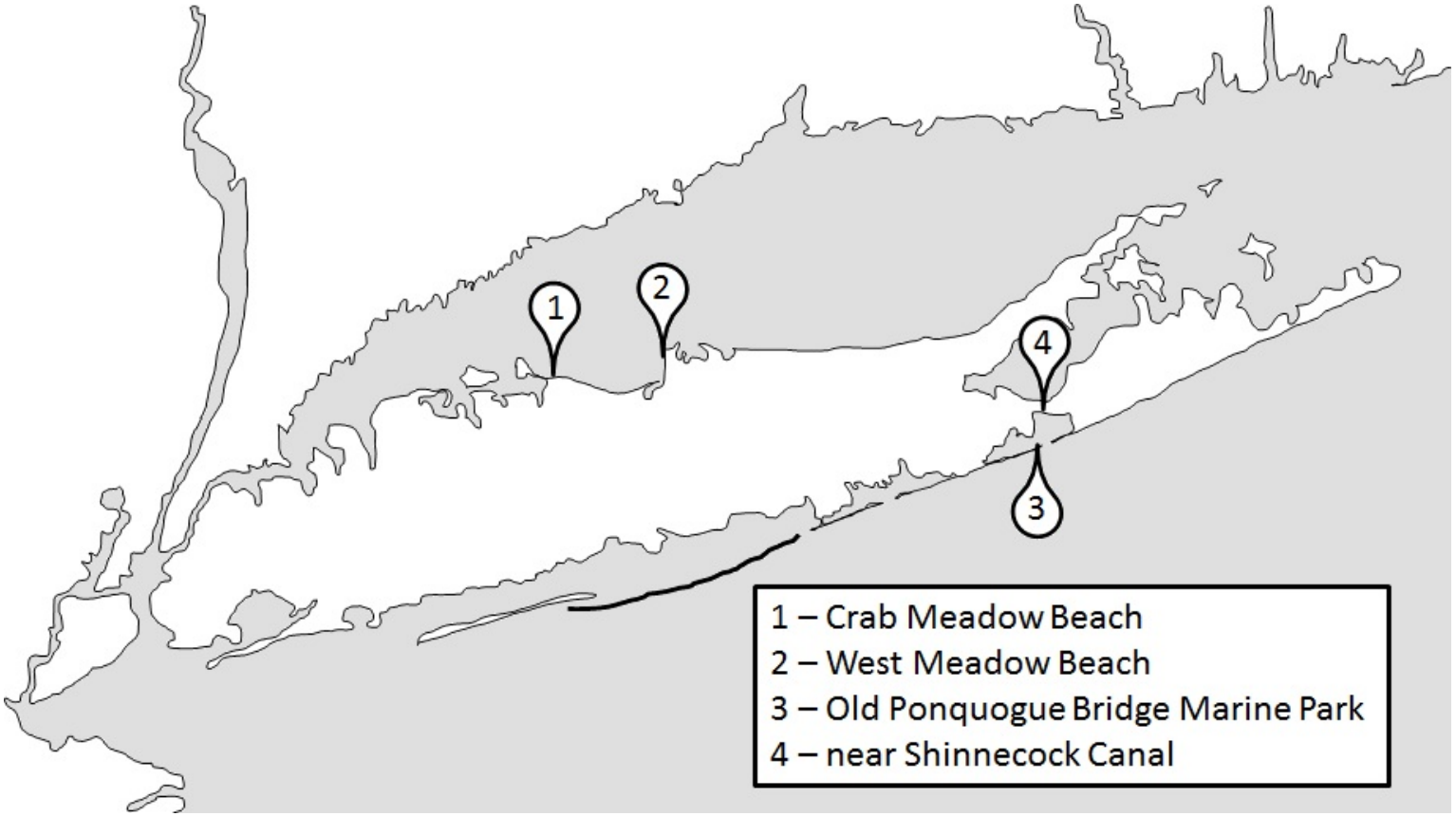

Figure A3-1. Map of collection sites across Long Island, NY.

The four sites where *Tritia obsoleta* was collected (site 1- 40.9293, -73.3281; site 2- 40.9443, -73.1466; site 3- 40.8433, -72.4985; site 4- 40.8835, -72.4848). White indicates land while grey indicates bodies of water.

Appendix 4: Antipredator behavior in parasitized and unparasitized snails

In the two adult snail experiments (Chapter 3) approximately 20 % of snails were parasitized (368 out of 1920 snails). Contingency table analyses (G-tests) were used to determine if there was a difference in *T. obsoleta* behavior based on the infection status (parasitized, unparasitized) or on the predator chemical cue treatment (Control, green crab chemical cues, seastar chemical cues).

There was no effect of infection status on crawl out behavior of *T. obsoleta* (3-way G test, df = 2, G = 0.05, p >0.75), but there was an effect of predator chemical cue treatment (3-way G test, df = 2, G = 10.78, p <0.005, Fig. A4-1 A) with snails more likely to crawl out of water. There was also no effect of infection status on the burrowing behavior of *T. obsoleta* (3-way G test, df = 2, G = 2.21, p >0.10), however there was an effect of chemical cue treatment (3-way G test, df = 2, G = 14.59, p <0.005, Fig. A4-1 B) where snails were more likely to burrow when exposed to predator chemical cues. Pairwise comparisons were used to determine that for both experiments, snails in the control treatment behaved differently than those in the green crab chemical cue (G test; Crawl out: df = 1, G = 7.25, p < 0.01; Burrow: df = 1, G = 11.57, p < 0.005) and seastar chemical cue treatments (G test; Crawl out: df = 1, G = 8.84, p < 0.005; Burrow: df = 1, G = 10.78, p < 0.005), and that there were no differences in behavior between the two predator chemical cue treatments (G test; Crawl out: df = 1, G = 0.08, p < 0.90; Burrow: df = 1, G = 0.01, p < 0.90).

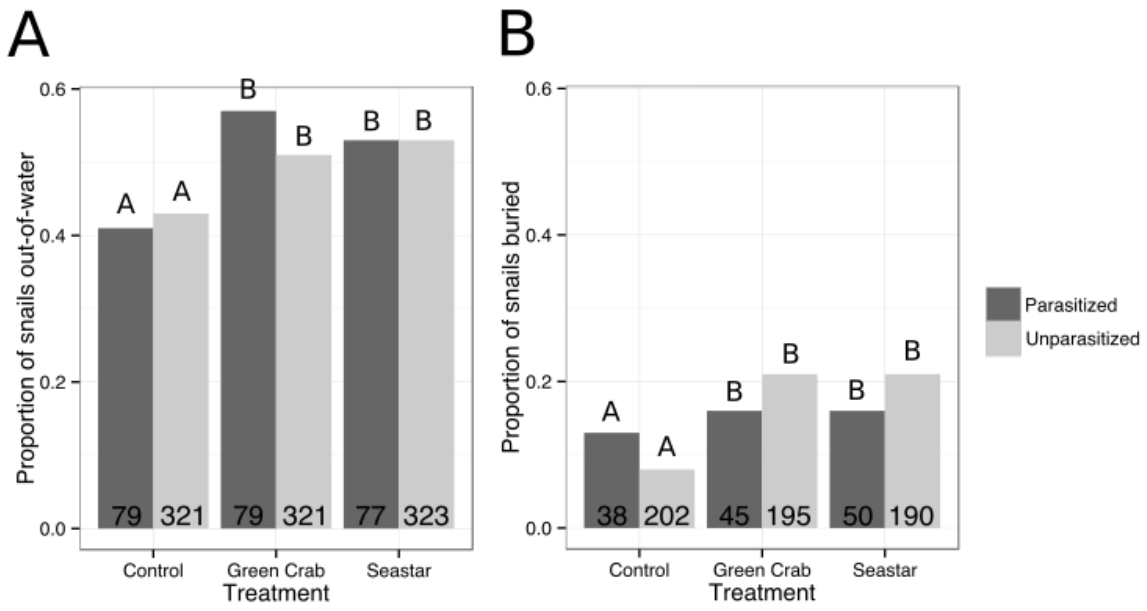

Figure A4-1. Antipredator behavior in parasitized and unparasitized snails. The proportion of parasitized and unparasitized snails that moved crawled out of the water (A) in each predator chemical cue treatment, and the proportion of parasitized and unparasitized snails that burrowed (B) in each chemical cue treatment. Sample sizes for each group are listed at the bottom of each bar, and letters indicate statistical significance.

Appendix 5: Shell morphology of juvenile and adult *T. obsoleta*

Adult and juvenile snails were collected from sites Old Ponquogue Bridge Marine Park (PB: 40.8433, -72.4985) and West Meadow Beach (WM: 40.9443, -73.1466, Appendix 3) during the summer of 2014. 60 juveniles and 60 unparasitized adults were randomly chosen from each site for use in the analysis, for a total of 240 snails. Pictures were taken and analyzed using the same methods described in Chapters 5 and 6. Adults were dissected to determine if they were parasitized, and only unparasitized snails were used in this analysis.

When examining the effects of life stage and site, I found a significant difference in shell shape between sites (MANOVA, $F_{1, 239} = 251.38$, $p = 0.001$, Fig. A5-1), and between life stages (MANOVA, $F_{1, 239} = 23.91$, $p = 0.001$, Fig. A5-1). There is also an interaction between life stage and site (MANOVA, $F_{1, 239} = 8.429$, $p = 0.017$, Fig. A5-1, Table A5-1). A canonical variate analysis (CVA) was also performed in order to visualize shape differences by refining the multivariate data into two-dimensions. The CVA indicates that adults and juveniles had different shell shapes, and that snails from different sites had significantly different shell shapes, but that adults from different sites have more similar morphologies than juveniles from different sites. It appears that *T. obsoleta* from different sites tend to converge on a similar shape over ontogeny (Fig. A5-2).

Juvenile snails had more stout shells compared to the more elongate shells of adults, and they also had a much narrower apertural lip. Juveniles also had a wider aperture compared to the adults. Snails collected from PB were stouter, and had a narrower aperture and narrower apical whorls than snails from WM. Adult snails collected from PB were more elongate and narrower than the adults from WM. Adults from PB also had a more elongate and narrower aperture. Juvenile snails from PB were stouter and wider than conspecifics from WM. Juveniles from WM had wider apical whorls and a wider siphonal canal than PB juveniles (Fig. A5-1).

Table A5-1. MANOVA table from geometric morphometric analysis of the affect of life history stage and collection site on *T. obsoleta* shell shape.

	df	SS	MS	Rsq	F	Z	P-value
Life History Stage	**1**	**0.57018**	**0.57018**	**0.48368**	**251.376**	**29.685**	**0.001**
Collection Site	**1**	**0.05424**	**0.05424**	**0.04601**	**23.9117**	**8.0887**	**0.001**
Life History Stage * Collection Site	**1**	**0.01912**	**0.01912**	**0.01622**	**8.4287**	**2.8517**	**0.017**
Residuals	236	0.53531	0.00227				
Total	239	1.17884					

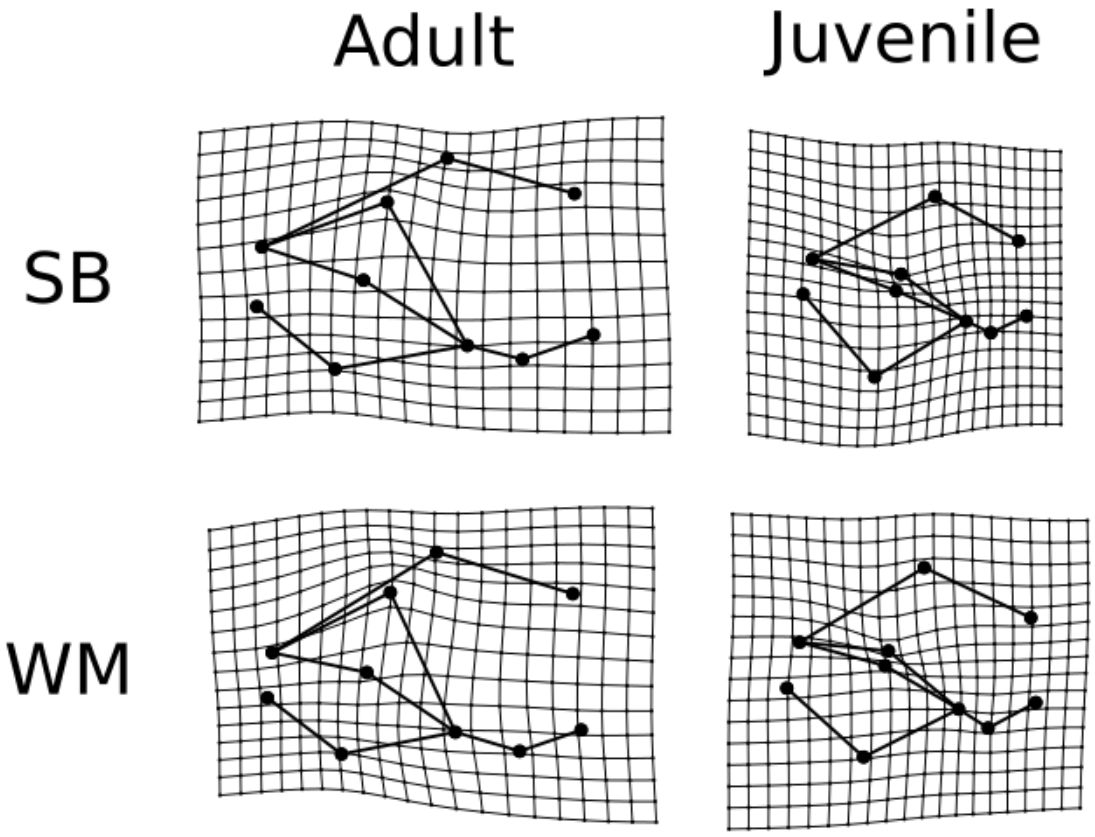

Figure A5-1. Interaction between life stage and collection site on the morphology of *T. obsoleta.* Thin-plate splines illustrating how the morphologies of adult (A) and juvenile snails (B) collected from Ponquogue Bridge and adult (C) and juvenile snails (D) collected from West Meadow Beach differed from the overall mean snail shape. Thin-plate splines are exaggerated by 2x to better indicate how the morphologies differ.

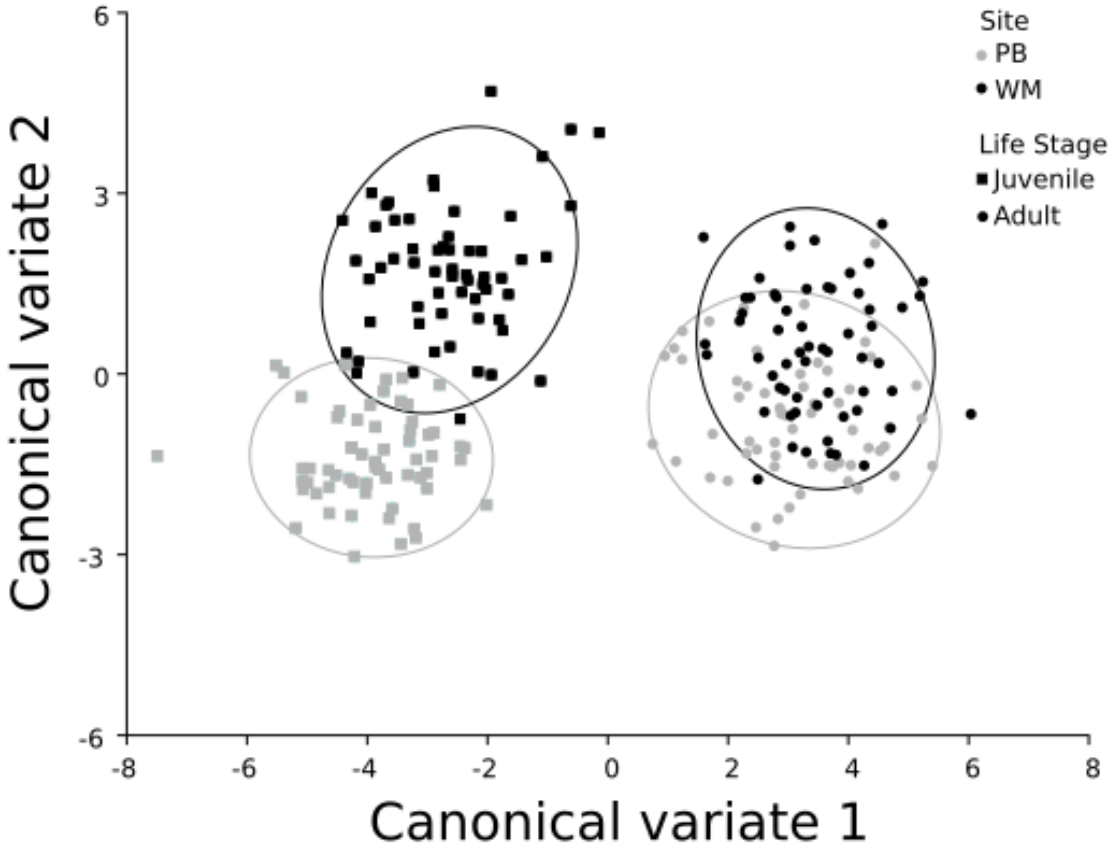

Figure A5-2. Canonical Variate Analysis plot of juvenile and adult snail morphology from different sites. The CVA indicates that there are shape differences between juveniles and between adults from different sites, but that adults seemed to be converge on a similar shape (greater overlap in the CVA plot).