Stony Brook University

Prey Plasticity Responses to a Native and Nonnative Predator

A Thesis Presented

by

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to

The Graduate School

in Partial Fulfillment of the

Requirements

for the Degree of

Master of Science

in

School of Marine and Atmospheric Sciences

Stony Brook University

August 2013

Stony Brook University

The Graduate School

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Abstract of the Thesis

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Phenotypic plasticity in response to environmental stimuli is exceedingly common across systems and taxa. For instance, predation risk in many gastropods can induce a variety of defenses including growing thicker shells, growing shells of different shapes, and developing apertural teeth. However, the role of coevolution between species that produce these defense responses and their consumers is not well known. This thesis examines the responses of an ovoviviparous gastropod (*Littorina saxatilis*) with low dispersal from three different habitats (marsh habitat, rocky habitat, and cobble stone habitat) to the presence of chemical cues from a native (*Dyspanopeus sayi*) and nonnative (*Hemigrapsus sanguineus*) crab predator. This work tested the potential role of coevolution in shaping phenotypically plastic responses, and whether responses to both a native and a nonnative predator differed for snails from different source sites. The morphological responses I tested for included axial growth, width growth, whorl growth, changes in total mass, and shell shape changes. I found that many measures of growth were needed in the investigation of plastic responses. Overall snails exposed to native predator cues had a similar response as those in the reduced diet treatment in both growth measurements and shell shape change, indicating a behavior response of reduced feeding in the presence of the native predator. Snails from the marsh and rocky habitats displayed a reduced response to cues from the nonnative predator, suggesting that they recognized this predator as a risk, but did not show as strong of a response as they did to the native predator. Snails in the rocky habitat, which live in barnacle tests, also had a slower growth rate than snails from the other two source sites. These results suggest the possibility of local adaptation and genetic differences between snails in these different source sites.

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Introduction

Phenotypically plastic responses, where different phenotypes are produced by a single genotype under different environmental conditions, are expected if different phenotypes produce a greater fitness advantage in some habitats versus others, and producing different phenotypes under different environmental conditions has a greater fitness advantage than solely producing the phenotype matching the dominant environmental type (e.g., Padilla & Adolph 1996; DeWitt *et al.* 1998; Agrawal 2001; Miner *et al.* 2005; Bourdeau 2011). For plasticity to be favored, there must be reliable, detectable cues correlated with each of the environmental types, the lag time required to produce the different phenotypes must be much less than the time course of environmental change (Padilla & Adolph 1996), and the cost of plasticity (e.g., regulatory and sensory machinery, or an opportunity cost) must be less than the advantage of having a phenotype matched to a given environment (Dewitt *et al.* 1998; Trussell & Nicklin 2002). If these conditions are met, plasticity will have an advantage over other fixed phenotypes when there is no one optimal phenotype (Dewitt *et al.* 1998).

Phenotypically plastic responses have been observed in a wide range of vertebrate and invertebrate animals, algae, fungi and plants (reviewed in Sultan 2000; Miner *et al.* 2005; Padilla & Savedo 2013) and can include changes in any trait including morphology, life history, physiology, or behavior (e.g., Clark & Harvell 1992; Hoverman *et al.* 2005; Fisk *et al.* 2007). Phenotypically plastic responses triggered by predators, or inducible defenses, have received particular attention because of the importance of predation risk and especially when predator risk varies in both time and space (reviewed in Adler & Harvell 1990; Harvell 1990; Tollrian & Harvell 1999). As for other plastic phenotypes, inducible defenses can be more advantageous

than constitutive defenses (defenses that are always present) because the cost associated with maintaining a defense is experienced only under certain conditions. However, for inducible defenses to be favored over constitutive defenses the presence of the predator has to be variable, there must be reliable cues correlated with predation risk, and the cost of plasticity must be less than the cost of maintaining a constitutive defense (reviewed in Dewitt *et al.* 1998; Tollrian & Harvell 1999). Thus, accurate and reliable predictors of predator risk are essential for increasing the adaptive value of a plastic trait to reduce mismatches between traits and environmental conditions (Padilla & Adolph 1996; Dewitt *et al.* 1998; Trussell & Nicklin 2002).

Novel, introduced predators pose a particular challenge for prey. The lack of a co-evolutionary history between predator and prey may result in prey not recognizing the introduced predator as a risk. Whether prey can recognize a novel predator may depend on the duration of time (number of generations) they have co-occurred. For example, the Australian ringtail possum (*Pseudocheirus peregrinus*) shows a similar flee response time to their native predator, the lace monitor (*Varanus varius*), and the introduced predator, the European red fox (*Vulpes vulpes*), with which it has coexisted for approximately 130 years (more than 60 ringtail generations) (Anson & Dickman 2013). Similarly, populations of *Daphnia melanica* have a smaller body size and delayed time to first reproduction when exposed to either native or introduced salmonids, which have been stocked in lakes at various times since 1913 (Fisk *et al.* 2007). Trussell and Nicklin (2002) found that the snail *Littorina obtusata* (generation time ~2 years) from two different populations with different exposure times to the predatory green crab *Carcinus maenas* (50 versus 100 years) respond similarly by growing a thicker shell, regardless of evolutionary time with the predator. However, Edgell and Neufeld (2008) found that the snail *Nucella lamellosa* (generation time ~4 years) produces morphological and behavioral responses

to its native predator, *Cancer productus,* but does not respond to the green crab, *Carcinus maenas,* which was introduced to their study site approximately 10 years prior.

Gastropods have proven to be a good system for studies of inducible defenses and predator recognition. They show a high degree of behavioral and morphological plasticity, are abundant and easy to collect, have small body sizes, and are very amenable to laboratory experiments. Gastropods have been shown to have predator induced morphological responses, including changes in shell thickness (Appleton & Palmer 1988; Trussell & Nicklin 2002), reduction in both somatic and shell growth (Edgell & Neufeld 2008; Bourdeau 2010), and changes in shell shape corresponding to the mode of feeding of their predator (Bourdeau 2009). In some cases, these morphological responses appear to be the result of changes in growth rate due to behavioral changes, such as reduced foraging, in response to predation risk (reviewed in Bourdeau & Johansson 2012).

This study focused on the response of the gastropod *Littorina saxatilis* (Olivi, 1792) to the presence of chemical cues from a native predator, the mud crab *Dyspanopeus sayi* (Smith, 1869), and from the introduced predatory crab, *Hemigrapsus sanguineus* (De Haan, 1835), which it has coexisted with for 20 years. *L. saxatilis* has a short generation time and low dispersal, increasing the likelihood of developing local differences among potential populations in response to changes in the environment. Due to this possibility of local differences among snails from different locations, *L. saxatilis* were collected from three different habitat types to test whether there were differences in response to the different predators among snails from the different sites.

Material and Methods

(a) Study System

Littorina saxatilis is a small bodied (1-9 mm) herbivorous snail found high on the shore along Atlantic coastlines in North America and Europe (Reid 1996). This species is sexually dimorphic; males are generally smaller than females. *L. saxatilis* is ovoviviparous with newly born juveniles ~1 mm, is thought to live approximately one year, and is reproductive year round (Reid 1996). *L. saxatilis* can be found in a wide range of habitats including rocky and cobble shores (Reid 1996), as well as in salt marshes (personal observation). *Dyspanopeus sayi* is an abundant native predatory crab on the east coast of the United States and is common on Long Island, NY (Strieb *et al.*1995). It is found on shores with *L. saxatilis*, and is a known predator of molluscs (Strieb *et al.* 1995). *Hemigrapsus sanguineus* was introduced to North America from Asia in 1988, and was first found on Long Island in the early 1990's (McDermott 1998). It is now a common predator of molluscs, especially littorinid snails (Bourdeau & O'Connor 2003; Kraemer *et al.* 2007).

(b) Experimental Design

Juvenile *Littorina saxatilis* (< 2 mm in shell dimension along the axis of coiling) were collected from three different habitat types on Long Island, NY: a salt marsh (Flax Pond, 40° 57' 41.7"N and 73° 8' 17.1"W), a rocky shore with barnacles, which are used for refugia (Crane Neck Point, 40° 58' 4.1"N and 73° 9' 29.5"W), and a cobble beach (Crab Meadow, 40° 55' 47.7"N and 73° 19' 44.3"W). *H. sanguineus* is the most abundant crab predator at the rocky shore site, but is also a common crab predator at the other two sites. *D. sayi* and other mud crabs species are abundant in both the marsh and cobble site, but not at the rocky (barnacle) site where *H. sanguineus* dominates. Overall, crab predator abundance is low at the cobble site compared to the other source sites where snails were collected. Due to their relatively low abundance at the cobble site, *H. sanguineus* and *D. sayi* were collected from the marsh and barnacle site.

To test whether *Littorina saxatilis* responded to cues associated with predator risk from a native and a nonnative predator, I performed an experiment with four treatments and 5 replicate aquaria (30.5 cm long x 19.1 cm wide x 20.3 cm high) of each treatment. The treatments were: 1) chemical cues from *H. sanguineus,* the nonnative predator*,* 2) chemical cues from *D. sayi,* the native predator, and 3) a control with no predator chemical cues. To test whether responses to predators were likely due to the behavioral response of reduced foraging (reviewed in Bourdeau & Johansson 2012), I also included a 4th treatment with no predator chemical cues, but where snails were fed a reduced diet.

Prior to experimentation, snails were weighed and photographed with their aperture up (Figure 1a), and the initial total damp mass of each snail was measured. Due to their small size, it was not possible to use techniques common for gastropods that allow separation of somatic and shell mass (Palmer 1982), thus it was not possible to estimate changes in shell mass in response to predators. The edge of the apertural lip of each snail was marked with one of four colors of nail polish to facilitate measuring whorl growth, and to provide a means of identifying individuals that were housed in the same cage.

Each experimental aquarium had a perforated cover, and was provided with an individual seawater supply (1.5 L/hr) and an air stone for aeration. Aquaria were arranged such that no two replicates of the same treatment were adjoining, in a block-like design (Figure 2b). Snails and crabs were kept in separate cages to prevent direct contact. Snails were housed in cylindrical mesh cages (interior length 6.14 cm x interior diameter 4.32 cm, 1 mm mesh). For each source population there were two cages, each with four snails, in each replicate aquarium for a total of six snail cages and 24 snails per aquarium (Figure 2a). The initial sizes of snails did not differ among replicates and treatments for each source site. Each cage of snails was provided with a ~

2 x 3 cm piece of the green alga, *Ulva lactuca*, replaced once each week. Snails never consumed more than half of the *Ulva* provided. The snails in the reduced diet treatment had access to this food three days per week. The snails were moved into clean cages each week to remove the accumulation of diatoms and feces, and dead snails were removed at that time.

H. sanguineus individuals ranged in carapace width from 1.9-2.5 cm. Because adult *D. sayi* only reach 3 cm maximum carapace width (Strieb *et al.* 1995), large individuals were scarce and in some cases two smaller crabs were used in a single cage and, in those cases, the sum of their carapace widths fell within 1.9-2.5 cm. Due to the aggressive nature of these crabs, only male-female pairs were housed together. Crabs were kept in 9.3 x 10 x 6 cm cages with 1.5 mm mesh and fed pieces of clam (*Mercenaria mercenaria*) three times a week. To control for the addition of food, the no predator cue treatments had a piece of clam tissue placed into the tank for 24 hours the same days that the crabs were fed. All crabs were replaced if they died during the experiment. All ovigerous females were replaced with non-reproductive individuals.

After 15 weeks all snails were reweighed (to determine the increase in damp mass) and photographed. Start and end photographs were used with a computer assisted image analysis system (ImagePro Premier v. 9.0, Media Cybernetics) to determine shell growth. Growth measurements included axial length (greatest distance from apex to the base of the shell along the axis of coiling), width (the widest portion of the shell perpendicular to the axis of coiling), and whorl expansion (growth along the shell margin; Figure 1b).

c) Statistical Analysis

Due to unequal mortality among replicates, replicate means were used for analyses. However, there was no differential mortality among treatments. Three-way ANOVAs (fixed factors: treatment and source, and random factor: tank replicate) were used to test whether there

were significant differences among replicate aquaria. There was no significant aquarium effect for any measures of growth except whorl growth, driven by snails from the barnacle habitat, for which all snails in the reduced diet treatment in three replicate aquaria died. Due to the overall lack of a significant aquarium effect, this factor was dropped in subsequent analyses. Two-way ANOVAs (fixed factors treatment and source) were used to analyze all growth data. In all cases growth per day was used to account for small differences in the time between when initial measurements were made and when the experiment was initiated. These differences were spread across all replicates of all treatments for snails in each source site.

Prior to analysis, the data were tested for normality (Shapiro test) and homogeneity of variance (Levene test). When necessary, data were transformed to meet these assumptions of an ANOVA. Data for change in mass were log transformed; both axial growth and whorl growth were square root transformed. Transforming whorl growth corrected the heteroskedasticity, but not normality. The robustness of the ANOVA allows for such violations of assumptions as long as other assumptions are met. When significance was found with an ANOVA, Fisher's Least Significant Difference (LSD) *post hoc* test was used for planned comparisons. Statistica (v. 6.1, StatSoft) was used for analyses.

Results

Significant differences among snails from the different source sites were seen in axial growth (ANOVA, $p = 0.001$, Table 1, Figure 3), width growth (ANOVA, $p = 0.005$, Table 1, Figure 4), whorl growth (ANOVA, $p = 0.001$, Table 1, Figure 5), and increase in mass (ANOVA, $p < 0.001$, Table 1, Figure 6).

Post hoc tests showed that snails from the barnacle source site had significantly lower

growth than those from the marsh source site in terms of axial growth (Fisher's LSD, $p = 0.021$, Table 2, Figure 3), change in width (Fisher's LSD, $p = 0.012$, Table 2, Figure 4), whorl growth (Fisher's LSD, $p = 0.018$, Table 2, Figure 5), and in terms of increase in mass (Fisher's LSD, $p < 0.001$, Table 2, Figure 6). Snails from the barnacle source site also had significantly lower growth than those from the cobble source site in terms of axial growth (Fisher's LSD, $p < 0.001$, Table 2, Figure 3), change in width (Fisher's LSD, $p = 0.003$, Table 2, Figure 4), whorl growth (Fisher's LSD, $p < 0.001$ Table 2, Figure 5), and in terms of increase in mass (Fisher's LSD, $p < 0.001$, Table 2, Figure 6). There were no significant differences between snails from the cobble and marsh source sites for all growth measures (Fisher's LSD, Table 2; axial growth $p = 0.109$, Figure 3; change in width $p = 0.446$, Figure 4; whorl growth $p = 0.143$, Figure 5; and increase in mass $p = 0.104$, Figure 6).

For snails from the barnacle source site, there were no significant treatment effects for either axial growth (ANOVA, $F_{2,36} = 2.55$, $p = 0.070$) or width growth (ANOVA, $F_{2,36} = 2.04$, $p = 0.125$). There were significant differences for whorl growth (ANOVA, $F_{2,36} = 5.19$, $p = 0.004$); the *post hoc* test confirmed that snails in the reduced diet treatment grew less than those in the control treatment (Fisher's LSD, $p = 0.047$, Table 3, Figure 5), but were not significantly different than the native (Fisher's LSD, $p = 0.412$, Table 3) and nonnative (Fisher's LSD, $p = 0.619$, Table 3) predator treatments. Also, for whorl growth, snails in the control treatment did not significantly differ from the native (Fisher's LSD, $p = 0.114$, Table 3) and nonnative (Fisher's LSD, $p = 0.091$, Table 3) predator treatments. However, Figure 5 qualitatively shows a difference between the control and both predator treatments, where the predator treatments had less growth than the control but more growth than the reduced diet treatment. There was also a significant treatment effect for increase in mass (ANOVA, $F_{2,36} = 4.22$, $p = 0.011$, Table 1); the

post hoc test confirmed that snails from this source site had a significantly lower growth in the reduced diet treatment compared to the control (Fisher's LSD, $p = 0.019$, Table 4, Figure 6) and nonnative predator treatment (Fisher's LSD, $p = 0.048$, Table 4, Figure 6). The native predator treatment, however, was not significantly different from the control (Fisher's LSD, $p = 0.339$, Table 4, Figure 6), nonnative predator ($p = 0.638$), or reduced diet treatment ($p = 0.077$). Snails in the nonnative predator treatment were not significantly different from the control (Fisher's LSD, $p = 0.660$, Table 4, Figure 6). Overall, snails in the control treatment were larger than those in the native and nonnative predatory treatments, and all three were larger than those in the reduced diet treatment (Figures 3, 4, 5, and 6).

For snails from the marsh source site, there were no significant treatment effects for either axial growth (ANOVA, $F_{2,36} = 2.55$, $p = 0.070$) or width growth (ANOVA, $F_{2,36} = 2.40$, $p = 0.125$). There were significant differences among treatments for whorl growth (ANOVA, $F_{2,36} = 5.19$, $p = 0.004$), and *post hoc* test confirmed that snails from the marsh site exposed to cues from the native predator grew less than those from the control (Fisher's LSD, $p = 0.005$, Table 3, Figure 5) and reduced diet ($p = 0.023$) treatments. Snails exposed to cues from the nonnative predator had less whorl growth than those in the control treatment (Fisher's LSD, $p = 0.006$, Table 3, Figure 5) and those given a reduced diet ($p = 0.026$). Snails from the marsh site exposed to cues from the native and nonnative predator did not differ in whorl growth (Fisher's LSD, $p = 0.950$, Table 3, Figure 5). Likewise, snails in the control and reduced diet treatments for the marsh site did not differ in terms of whorl growth (Fisher's LSD, $p = 0.465$, Table 3, Figure 5). There was also a significant treatment effect for increase in mass (ANOVA, $F_{2,36} = 4.22$, $p = 0.011$, Table 1), and the *post hoc* test showed that snails in the native predator treatment grew less in mass than those in the control treatment (Fisher's LSD, $p = 0.041$, Table 4, Figure

6). All other treatment combinations did not differ for the marsh source site (Fisher's LSD, in all cases $p > 0.14$, Table 4, Figure 6). Overall, the snails in the control treatment were larger than those in the reduced diet treatment and both were larger than snails in the native and nonnative predatory treatments (Figures 3, 4, 5, and 6).

For snails from the cobble source site, there were no significant treatment effects for any measure of growth: axial growth (ANOVA, $F_{2,36} = 2.55$, $p = 0.070$), width growth (ANOVA, $F_{2,36} = 2.40$, $p = 0.125$), whorl growth (Fisher's LSD, in all cases $p > 0.084$, Table 3, Figure 5), or increase in mass (Fisher's LSD, in all cases $p > 0.091$, Table 4, Figure 6). However, all figures qualitatively show a trend of reduced growth for the reduced diet and native predator treatments compared to the control (Figures 3, 4, 5, and 6).

Discussion

Unlike most studies of inducible defenses, I was able to test not only for responses to a common native predator, but also to an introduced predator, and examine differences in responses among different source sites. I found that snails from different sites showed different growth responses to cues from a native and nonnative predator. In all cases, these responses appear to be due to reduced feeding, a behavioral plasticity that is common for snails and other taxa exposed to the risk of predation (reviewed in Bourdeau & Johannson 2012).

I used several different measures of growth to detect responses of snails. In previous work, Bourdeau (2009) found that for another species of snail, *Nucella lamellosa*, detecting phenotypically plastic responses to predator risk depended on which measures of growth were used, indicating that multiple measures of growth should be used to test for inducible defense responses. Using different measures of growth are also important if animals respond by

changing shape or other aspects of their morphology. Like Bourdeau, I found that understanding whether *L. saxatilus* responded to cues from predators required multiple measures of growth as snails from different populations responded differently. Many differences in growth were found among treatments, however, due to large variance, some trends in response where not statistically significantly different. Lack of statistical significance in these cases is likely due to low statistical power, but interesting patterns in response did emerge among habitat and predator treatments.

Snails from the marsh habitat had significantly slower growth in response to chemical cues from both the native predator and the nonnative predator. The response to both predators was more extreme than the reduced food treatment (Figures 3, 4, and 5), indicating that the growth response was likely due to the behavioral plasticity of extremely reduced feeding. In the reduced food treatment, they had access to food three of seven days, or a 57% reduction in access to food. Thus, the marsh snails were likely eating much less than 57% of the time when exposed to cues from either predator. It is interesting that these snails responded similarly to the native and the nonnative predator. Although *H. sanguineus* has been on Long Island shores for 20 years, it has been common at this marsh site for only about 12 years (DK Padilla, personal observation).

Snails from the barnacle site did not have a significant reduction of growth in response to either predatory treatment, but there was a trend of reduced growth in the presence of cues from both the native and nonnative crab. However, in this case the snails exposed to cues from predators were larger than those given a reduced diet (Figures 3, 4, 5, and 6), indicating that their feeding activity, while depressed, was still greater than the reduced diet treatment and was less of a reduction in feeding than that seen for snails from the marsh habitat. Living in barnacle tests

can be advantageous to intertidal snails because they can provide protection from dislodgement (Catesby & McKillup 1998), prevent desiccation (Jones & Boulding 1999), and provide refuge from predation (Gosselin & Chia 1995). Snails from the marsh habitat had a stronger response to predators than snails from the barnacle source site; this may indicate that snails at the barnacle site experience lower predation risk by inhabiting empty barnacle tests.

Snails from the barnacle habitat grew less overall than snails from the other sites, even when given an unlimited amount of food (Table 2, Figures 3, 4, 5, and 6). Several ecotypes (*sensu* Bradshaw 1965), genetically and morphologically distinct populations that occur in particular habitat types or tidal heights, have been identified for *L. saxatilis* in Europe (e.g., Johannesson *et al.* 1997; Johannesson 2003; Conde- Padín *et al.* 2007). The results of my experiments were consistent with previous studies in Europe where the ecotype of *L. saxatilis* that lives among barnacles and inhabits dead barnacle tests matures at a smaller size (< 3 mm) and remains smaller than other ecotypes (Reid 1993). This may be the first evidence of similar ecotypes of *L. saxatilis* in the western Atlantic, and further research should be done to confirm these possible ecotypes.

Snails from the cobble site differed from snails from both the marsh and barnacle sites by showing a trend of slower growth in the presence of cues from the native crab but not the nonnative crab (Table 5). In this case, the reduction in growth of snails in response to cues from the native predator was similar to that seen in the reduced diet. Thus, only snails from this site distinguished between these two predators. While snails from the cobble site have fewer refugia from predation than the other source sites, their lack of a strong response to the predators may be due to lower predator risk experienced by snails at this source site.

Snails from the barnacle and marsh sources had a reduction of growth in the presence of

both the native and nonnative predatory crabs. Although these snails have been exposed to this nonnative predator for only 20 years, they responded to chemical cues from *H. sanguineus* in a similar fashion as to a predator with which they have had a long evolutionary history. In contrast, snails from the cobble source site tended to have reduced growth when exposed to cues from the native crab, but did not recognize the nonnative crab similarly (Table 5). These results suggest that there may be local adaptation and genetic differences in responses to predators for this species of snail. Further testing is needed to determine if there are genetic differences among source sites of *L. saxatilis* in different habitat types, and whether there are indeed habitat specific ecotypes of *L. saxatilis* as has been found in Europe. It will be important to examine animals from multiple populations of each habitat type to determine the generality of the observed inducible responses.

The differences that were seen among different measures of growth for different treatments and source sites may suggest that these snails experienced not only differences in absolute growth, but also differences in morphology. For example, snails that did not grow in axial length but did increase in width would be rounder than snails that experienced more growth in axial length. Further work is needed to determine if the growth responses seen among treatments and among source sites of snails translates into differences in morphology. Finally, to determine if these responses of snails are inducible defenses, it is important to determine if changes in behavior and morphology alter risk to predation. Due to the small size of specimens used in this study, shell thickness could not be measured. This trait would be important to explore because other studies have shown that many species of gastropods produce thicker shells in the presence of predators, particularly predators that crush their prey (e.g., Appleton & Palmer 1988; Trussell & Nicklin 2002; Bourdeau 2009). For many animals, reduced activity in the

presence of predators will reduce predation rates, especially for species that have access to spatial refugia from predation (e.g., Rahel & Stein 1988; Sparrevik & Leonardsson 1995; Krupa & Sih 1998). Also, changes in morphology in the presence of predators may be dependent on the type of predator. For instance, Bourdeau (2009) found that the marine snail *Nucella lamellosa* produced a rounder shell in response to a crab predator (*Cancer productus*) and an elongated shell shape in the presence of a sea star predator (*Pisaster ochraceous*). Further work is needed to explore if these snails show shell shape changes that are different for these two different predators, and whether such changes protect snails from predation.

Table 1. Analyses of variance of shell growth (mm per day) and increase in mass (mg per day) for *Littorina saxatilis* after 105 days of exposure to experimental conditions. Replicate means were used for each analysis. Fixed factors included treatment and source.

Dependent Variable	**Source**	**Df**	**MS**	**F**	**P**
Axial Growth	Treatment	3	0.000675	2.554	0.070
	Source	2	0.002052	7.760	0.001
	Treatment*Source	6	0.000404	1.529	0.196
	Error	36	0.000264		
Width Growth	Treatment	3	0.000012	2.0407	0.125
	Source	2	0.000034	6.1107	0.005
	Treatment*Source	6	0.000008	1.4467	0.224
	Error	36	0.000006		
Whorl Growth	Treatment	3	0.008605	5.1986	0.004
	Source	2	0.012723	7.6861	0.001
	Treatment*Source	6	0.001682	1.0162	0.430
	Error	36	0.001655		
Increase in Mass	Treatment	3	0.14395	4.224	0.011
	Source	2	0.53247	15.625	< 0.001
	Treatment*Source	6	0.02862	0.840	0.547
	Error	36	0.03408		

Table 2. *Post hoc* Fisher's LSD for shell growth (mm per day) and increase in mass (mg per day), comparing among *Littorina saxatilis* from three source sites, a cobble site, a marsh site, and a rocky site where snails live in barnacle tests, after 105 days of exposure to experimental conditions. Replicate means were used for each analysis. Significant differences are in bold.

Axial Growth

	Cobble	Barnacle
Barnacle	**< 0.001**	
Marsh	0.109	**0.021**

Width Growth

	Cobble	Barnacle
Barnacle	**0.003**	
Marsh	0.446	**0.012**

Whorl Growth

	Cobble	Barnacle
Barnacle	**< 0.001**	
Marsh	0.143	**0.018**

Increase in Mass

	Cobble	Barnacle
Barnacle	**< 0.001**	
Marsh	0.104	**< 0.001**

Table 3. Fisher's LSD for whorl growth (mm per day) in *Littorina saxatilis* from three different source sites, a cobble site, a marsh site, and a rocky site where snails live in barnacle tests, comparing among treatments after 105 days of exposure to experimental conditions. Replicate means were used for each analysis. Significant p values are in bold.

Cobble	Control	Nonnative	Native
Nonnative	0.221		
Native	0.084	0.570	
Reduced	0.192	0.976	0.569

Barnacle	Control	Nonnative	Native
Nonnative	0.091		
Native	0.114	0.749	
Reduced	**0.047**	0.619	0.412

Marsh	Control	Nonnative	Native
Nonnative	**0.006**		
Native	**0.005**	0.950	
Reduced	0.465	**0.026**	**0.023**

Table 4. Fisher's LSD for increase in mass (mg per day) comparing treatments for *Littorina saxatilis* from three different source sites, a cobble site, a marsh site, and a rocky site where snails live in barnacle tests, after 105 days of exposure to experimental conditions. Replicate means were used for each analysis. Significant p values are in bold.

Cobble	Control	Nonnative	Native
Nonnative	0.616		
Native	0.091	0.193	
Reduced	0.104	0.223	0.875

Barnacle	Control	Nonnative	Native
Nonnative	0.660		
Native	0.339	0.638	
Reduced	**0.019**	**0.048**	0.077

Marsh	Control	Nonnative	Native
Nonnative	0.146		
Native	**0.041**	0.504	
Reduced	0.198	0.852	0.394

Table 5. Summary of native and nonnative predator treatment effects and trends for snails from each source site, a cobble site, a marsh site, and a rocky site where snails live in barnacle tests. Direction of arrow indicates magnitude of growth relative to the control treatment. Thick arrows indicate statistical significance based on *post hoc* tests, thin arrows indicate trends.

Site	Barnacle		Cobble		Marsh	
	Native	Nonnative	Native	Nonnative	Native	Nonnative
Increase in mass	**0**	**0**	↓	**0**	⇓	↓
Axial Growth	↓	**0**	↓	**0**	↓	↓
Width Growth	↓	**0**	↓	**0**	↓	↓
Whorl Growth	↓	↓	↓	**0**	⇓	⇓

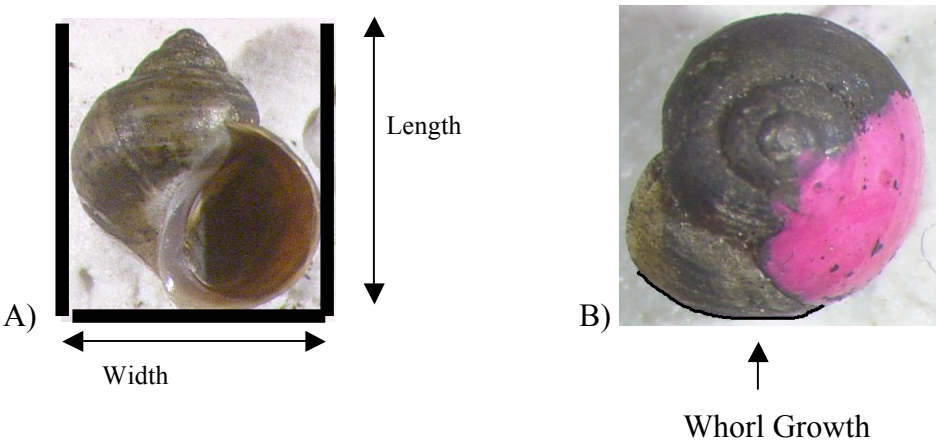
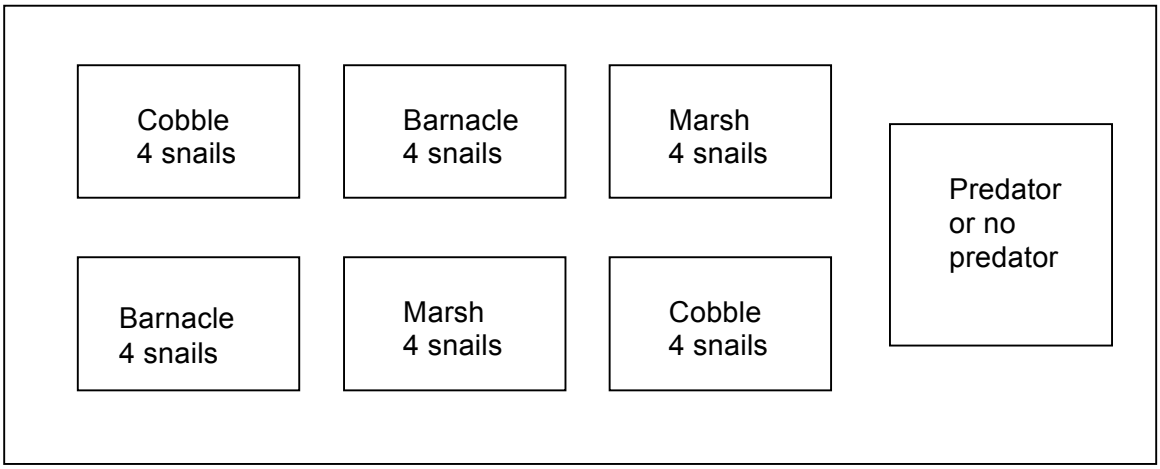


Figure 1: A) Shell dimensions used to quantify growth. For this snail, axial length was 1.552 mm and width was 1.522 mm. B) Whorl growth was measured as the length of shell added at the apertural lip of the snail (thick black line). For this snail whorl growth was 2 mm.

A)



B)

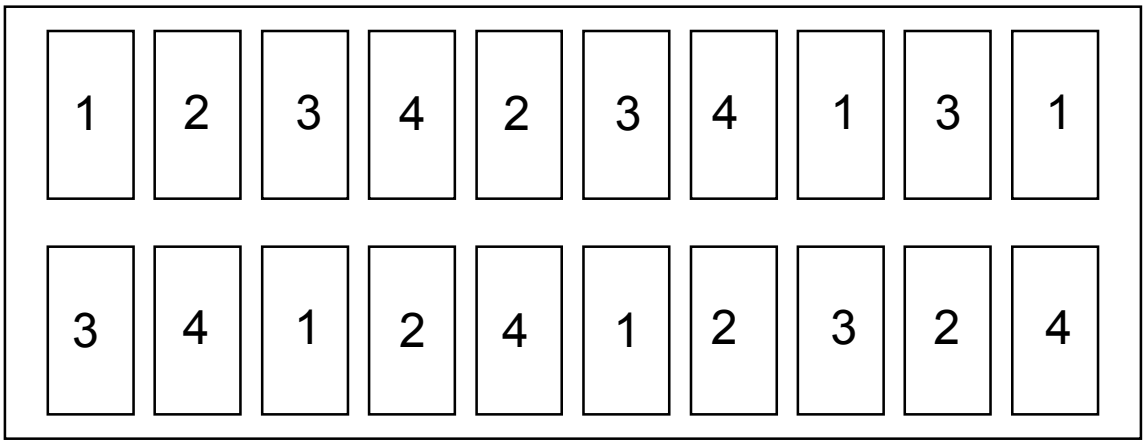


Figure 2: Depiction of experimental design. A) Within each aquarium, there were two cages for snails from each source site, with four individually marked snails in each, for a total of 24 snails per aquarium. There was also one cage with predatory crabs (either the native or introduced predator) or without a predator (control and reduced food treatments). Cages were free floating and readily moved throughout the aquarium. B) Layout of experimental treatments: 1) Nonnative predator, 2) Native predator, 3) Control, 4) Reduced Food.

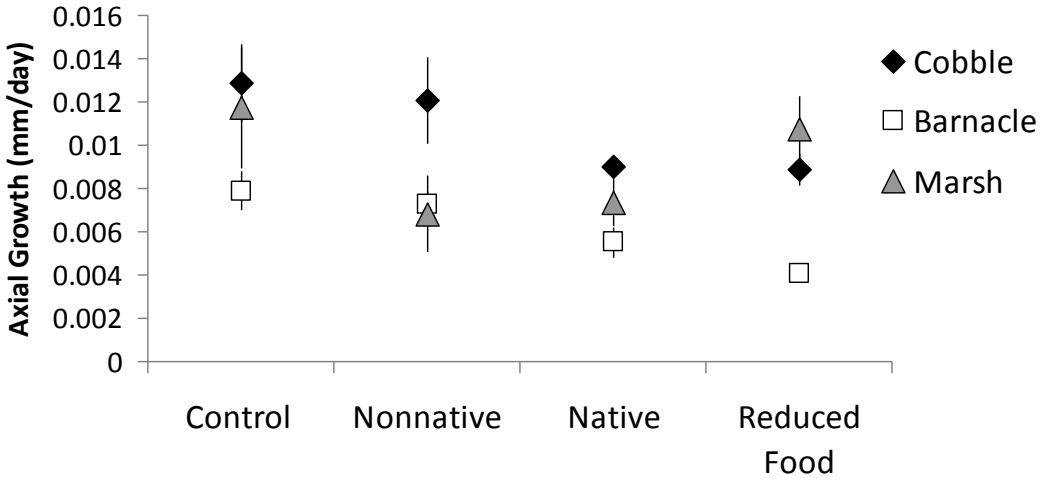


Figure 3: Average increase in axial length (mm per day) for snails from each source site (cobble, barnacle and marsh) for each of the four treatments, (Control – no exposure to crab cues, Nonnative – exposure to chemical cues from the nonnative crab, Native – exposure to chemical cues from the native crab, and Reduced Food – no exposure to chemical cues from crabs, and fed only three days per week) for the 105 day experimental period. Replicate means (n = 5) were used to calculate treatment means. Whiskers are standard error bars calculated using replicate means.

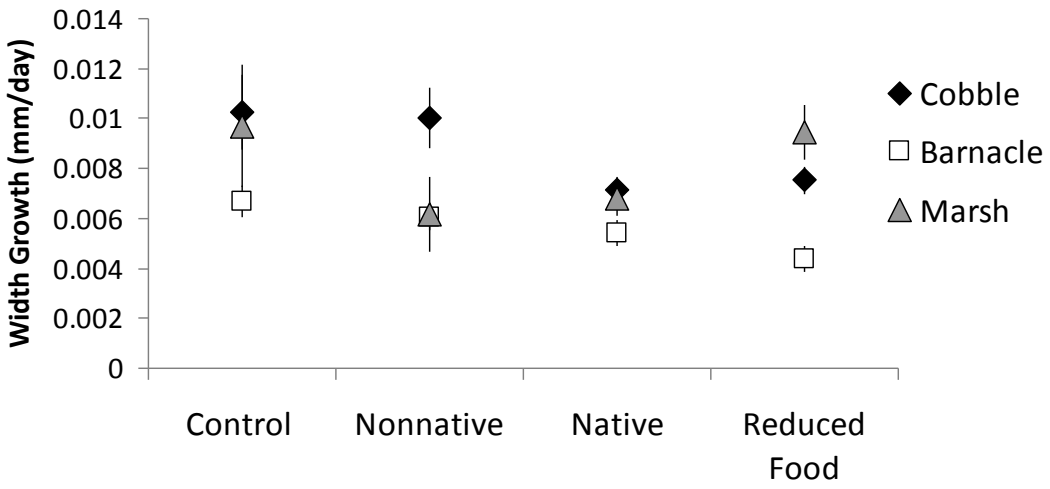


Figure 4: Average increase in width (mm per day) for snails from each source site (cobble, barnacle and marsh) for each of the four treatments, (Control – no exposure to crab cues, Nonnative – exposure to chemical cues from the nonnative crab, Native – exposure to chemical cues from the native crab, and Reduced Food – no exposure to chemical cues from crabs, and fed only three days per week) for the 105 day experimental period. Replicate means (n = 5) were used to calculate treatment means. Whiskers are standard error bars calculated using replicate means.

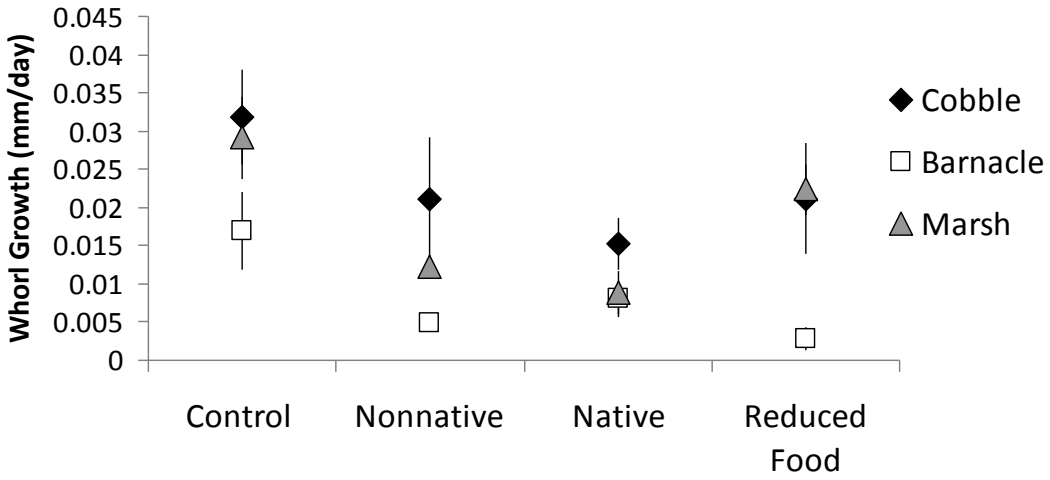


Figure 5: Average whorl growth (mm per day) for snails from each source site (cobble, barnacle and marsh) for each of the four treatments, (Control – no exposure to crab cues, Nonnative – exposure to chemical cues from the nonnative crab, Native – exposure to chemical cues from the native crab, and Reduced Food – no exposure to chemical cues from crabs, and fed only three days per week) for the 105 day experimental period. Replicate means (n = 5) were used to calculate treatment means. Whiskers are standard error bars calculated using replicate means.

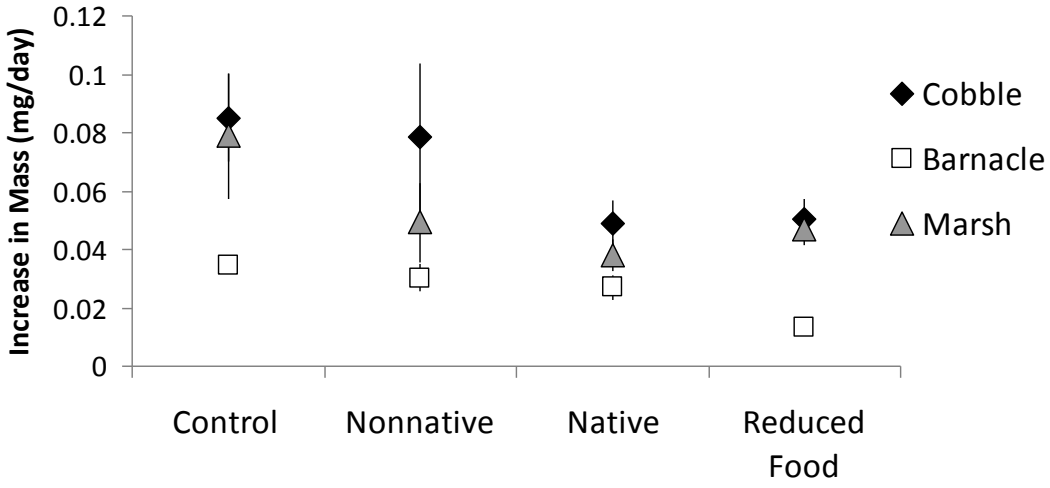


Figure 6: Average increase in mass (mg per day) for snails from each source site (cobble, barnacle and marsh) for each of the four treatments, (Control – no exposure to crab cues, Nonnative – exposure to chemical cues from the nonnative crab, Native – exposure to chemical cues from the native crab, and Reduced Food – no exposure to chemical cues from crabs, and fed only three days per week) for the 105 day experimental period. Replicate means (n = 5) were used to calculate treatment means. Whiskers are standard error bars calculated using replicate means.

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Chapter 2: Prey Shape Changes in the Presence of a Native and Nonnative Predator

Introduction

Phenotypic plasticity, when a single genotype produces different phenotypes in different environmental conditions, is expected when the fitness advantage of each phenotype depends on the habitat or when having differing phenotypes has a greater fitness advantage than producing a single, dominant phenotype (e.g., Padilla & Adolph 1996; DeWitt *et al.* 1998; Agrawal 2001; Miner *et al.* 2005; Bourdeau 2011). Phenotypic plasticity is exceedingly common across systems and taxa including plants and algae, as well as vertebrate and invertebrate animals (reviewed in Sultan 2000; Miner *et al.* 2005; Padilla & Savedo 2013). Plastic responses triggered by the presence of predators, or inducible defenses, have been studied extensively. Inducible defenses can include chemical, morphological, and behavioral changes in the presence of a predator (reviewed in Harvell 1990a,b; Adler & Harvell 1990; Tollrian & Harvell 1999).

Many studies of phenotypic plasticity have found inducible morphological defenses, changes in prey morphology in the presence of predators, reduce predation risk. For instance, Havel and Dodson (1984) compared the ability of the predator *Chaoborus americanus* to consume *Daphnia pulex* that had an induced toothed dorsal crest and *D. pulex* that did not have this induced defense. They found that *Chaoborus americanus* consume fewer *Daphnia* with the induced defense. Similarly, Laforsch and Tollrian (2004) found that *Daphnia cucullata* produce longer helmets and tail spines in the presence of the predators *Chaoborus flavicans*, *Leptodora kindtii*, and *Chaoborus americanus*. Bourdeau (2009) found that the snail *Nucella lamellosa* produces different shell morphologies in the presence of different predators that feed in different ways. *N. lamellosa* produces a rounder shell in the presence of a crushing, crab predator (*Cancer productus*) and an elongated shell shape in response to the shell-entry sea star predator (*Pisaster*

ochraceous). In this case, the different shapes produced were effective at deterring predation from the predator inducing that shape. However, quantifying morphological change in shape is difficult without the use of geometric morphometrics. Geometric morphometrics is the statistical multivariate study of shape variation independent of size (e.g., Bookstein 1991; Rohlf & Marcus 1993).

Littorina saxatilis (Olivi, 1792) is a common intertidal zone gastropod on shores in both the eastern and western Atlantic. *Hemigrapsus sanguineus* (De Haan, 1835), the Asian shore crab, was first introduced to North America in New Jersey from Asia in 1988 and was first found on Long Island in Rye, NY in the early 1990's (McDermott 1998). It is now a common predator on mid-Atlantic shores, consuming molluscs, including littorinid snails (Bourdeau & O'Connor 2003; Kraemer *et al.* 2007). *Dyspanopeus sayi* (Smith, 1869) is common native predatory crab on Long Island, NY and also preys on molluscs (Strieb *et al.* 1995). In this study, I investigated two questions. First, does *Littorina saxatilis* change shell shape in response to chemical cues from a native crab predator (*D. sayi*) and a nonnative crab predator (*H. sanguineus*). All three species co-occur on the shores of Long Island, NY, and thus have the potential to interact. Because *L. saxatilis* is ovoviviparous with low dispersal potential, I also asked whether morphological responses differed among snails from different source sites.

Methods and Materials

(a) Experimental Design

Juvenile (< 2 mm) *L. saxatilis* were collected from three sites on Long Island, NY, each with a different habitat type: Crab Meadow (40° 55' 47.7"N and 73° 19' 44.3"W) is a cobble beach, Crane Neck (40° 58' 4.1"N and 73° 9' 29.5"W) is rocky and the snails live in barnacle

tests, which provide refugia, and Flax Pond (40° 57' 41.7"N and 73° 8' 17.1"W) is a salt marsh. The two crab species, *D. sayi* (native predator) and *H. sanguineus* (nonnative predator) were collected from the marsh and barnacle habitat, where they are abundant. While *D. sayi* and *H. sanguineus* were present in the cobble stone site, overall predator abundance was lower at this site than the other two sites. In the experiment, a target carapace width of 1.9-2.5 cm was used for both crab species, and in most cases only one crab was used. However, in some cases two smaller *D. sayi* were used, and the sum of their carapace widths fell within this range. In cases where two individuals were used only male-female pairs were used to reduce aggressive interactions.

The experiment included four treatments with five replicate aquaria (30.5 cm long x 19.1 cm wide x 20.3 cm high), for a total of 20 aquaria. Each aquarium had a perforated lid and a separate seawater intake with a flow rate of 1.5 L/hr. Aquaria were aerated with individual air stones and situated such that no two replicates of the same treatment were adjacent. The four treatments were: 1) chemical cues from *H. sanguineus*, the nonnative predator, 2) chemical cues from *D. sayi,* the native predator, 3) no predator chemical cues (control), and 4) no predator chemical cues, but with snails fed a reduced ration to determine if changes seen in the predator treatments were due to reduce feeding in the presence of these predators (reviewed in Bourdeau & Johansson 2012).

A total of 24 juvenile snails (< 2 mm in shell dimension along the axis of coiling) were housed in each replicate aquarium (eight snails from each of the three source sites). Snails from the same source site were placed into two mesh cylindrical cages (6.14 cm interior length x 4.32 cm interior diameter, 1 mm mesh), each with four snails marked with nail polish such that each snail could be individually identified. Differences in starting sizes of snails among treatments

were minimized; initial total damp mass of each snail was determined, and the average starting mass of snails from each source site was not different across treatments and replicates. Snails in each cage were given a ~ 2 x 3 cm piece of the green alga, *Ulva lactuca*, once per week and had continual access to food. Snails did not consume more than one half of the food provided before it was changed. In the reduced diet treatments, snails had access to food three days per week. Cages were cleaned once per week to prevent the build up of diatoms and fecal matter.

Crabs were held in a separate cage (9.3 cm x 10 cm x 6 cm, 1.5 mm mesh) in each test aquarium, and fed tissue from the clam *Mercenaria mercenaria.* Similar sized empty cages were placed in the control and reduced diet treatments. Clam tissue was placed in the control and reduced treatment for 24 hours each time the crabs were fed to control for any effect the *M. mercenaria* may have had.

The experiment lasted 15 weeks after which snails were photographed in the same orientation with aperture up, and the apex pointed in the same direction for every picture (Figure 1). All photos were processed with ImagePro Premier (v. 9.0, Media Cybernetics).

(b) Geometric Morphometric Analysis

For each source site, ten snails were selected from across replicate aquaria for each treatment. However, only eight snails were used from the barancle site in the reduced diet treatment due to the high mortality of snails in this treatment from this source site. Nine landmarks were digitized as x, y coordinates with the software TpsDig2 v.2.17 (http://life.bio.sunysb.edu/morph/) on the same location on each shell in the same order (Figure 1). LM1 was the top of the apex, LM2 was placed on the widest point of the body whorl opposite the aperture, LM3 was placed at the base of the coiling axis, LM4 was placed such that it corresponded to the width of the apertural flare from the base of the shell (LM3) to the inside

edge of the aperture (where the operculum is seated), LM5 and LM 6 were placed such that they represented the longest axis of the aperture, LM7 and LM8 represented the widest portion of the aperture perpendicular to LM5 and LM6, and LM9 was placed at the widest point of the body whorl above the aperture.

The landmark coordinates obtained from TpsDig2 were used for a generalized procrustes analysis (GPA) to produce generalized warp scores with Morpholigika v. 2.5. The generalized warp scores were then used for a principal component analysis (PCA) in full tangent space. Principal component scores in this case are relative warp scores (Bookstein, 1991; Rohlf, 1993) and the first four principal components (which explained approximately 80% of variation) were analyzed with a MANOVA (SPSS v. 14.0, IBM Corporations) to test for differences among treatments and source sites. A *post hoc* Tukey Honest Significant Difference (HSD) was then used to determine differences between source sites and treatments. For all analyses, a critical alpha of 0.05 was used to indicate statistical significance.

PCA plots of the first two principal components were generated in Morpholigika v. 2.5. Line drawings of shell shape were also added to plots using TpsRelw v.1.36 (http://life.bio.sunysb.edu/morph) to visualize shell shape variation.

Results

The first two principal components of the PCA explained almost 50 percent of the variation in shell shape (31.7% and 17.6% respectively). Principal components 3 and 4 explained 15.2% and 7.8% of the variation respectively. Thus, the first four components explained near 80% of the variation in shell shape, and were used in a MANOVA to test for significant differences among treatments and source sites.

Plots of the first two principal components (Figure 2, all points; Figure 3, treatment means and standard errors), showed that the control treatment snails from all three source sites were clumped, and separate from the reduced feeding treatment snails, which were also clumped, along PC1. Snails from the native crab treatment clumped with the reduced feeding treatment, while the snails from the nonnative predator treatment were closer to the control treatment snails.

The MANOVA showed a significant difference for all four PC scores (Table 1, PC1, 2, and 3 $p < 0.001$, PC4 $p = 0.001$). The Tukey Honest Significant Difference (HSD) *post hoc* test on the results of the MANOVA found that snails exposed to chemical cues from the native predator and those that had a reduced diet were significantly different from the control treatment snails from all source sites for PC1 (Table 2, all p-values < 0.001). Snails exposed to chemical cues from the nonnative predator were not significantly different than those in the control treatment for all source sites for PC1 (cobble, $p = 1$; barnacle, $p = 0.885$; marsh, $p = 0.381$). There were no significant differences among the three source sites for PC1 (all p values > 0.22). There were also no significant differences among treatments or among source sites for any other principal component.

Snails from all three source sites responded to the native crab and reduced diet treatment similarly by producing a shell shape change that was rounder with an aperture that protruded less from the body whorl. Snails from the control treatment produced a taller shell with a larger aperture that extended further from the body whorl. Snails from the cobble stone site exposed to chemical cues from the nonnative predator were similar to snails from that source site in the control treatment. Snails from the marsh and barnacle sites exposed to the nonnative crab treatment tended toward a squatter shape than those in the control treatment for each site, but this difference was not statistically significant ($p > 0.381$).

Discussion

Snails from all three source sites exposed to cues from a native predator produced shells that were significantly different in shape than control snails, but were similar in shape to snails exposed to no predator cues but fed a reduced diet. These results suggest that the impact of the native predator was to induce the behavioral response of reduced feeding in the presence of cues associated with predation risk.

Many species of prey reduce their activity to reduce predation risk (Lima & Dill 1990; Sih 1992), especially when the movement of a slow moving animal will likely attract a predator (Ajie *et al.* 2007). However, this avoidance behavior is at the cost of time spent foraging (Lima & Dill 1990; Sih 1992; Luttbeg & Sih 2004), and this reduction in foraging can lead to slower growth (Kemp & Bertness 1984). Behavioral plasticity in terms of reduced feeding when exposed to greater risk of predation is believed to be responsible for a variety predator induced changes in morphology seen across taxa (reviewed in Bourdeau & Johansson 2012). If the shell shape change found in this study is a consequence of reduced feeding, shell shapes of snails responding to a cue associated with risk of predation are expected to be the same as those fed a reduced diet, as was found here for snails exposed to chemical cues from the native predator.

In this study, for the multivariate analysis of warp scores, PC1 was the only component with significant differences among treatments; therefore, warps along PC1 explained most of the shell shape differences among snails in different treatments. Snails in the native predator and reduced diet treatments produced rounder shells with an aperture that protruded less from the body whorl of the shell compared to snails in the control treatment (as seen on PC1 in Figure 3). Similarly, crab predators in other studies have been shown to induce a more rotund, thicker shell

in other species of gastropods (e.g., Palmer 1990; Trussell & Nicklin 2002; Bourdeau 2009), and that shape has been shown to deter crab predation (Quensen III & Woodruff 1997; Bourdeau 2009). *D. sayi*, the native mud crab in this study, feeds by handling the shell and positioning it so that it can peel the shell at the aperture. A more rotund shell may increase handling time for the mud crab, and snails with an aperture that protrudes less from the body whorl may be less vulnerable to peeling by the predator.

Contrary to the snails exposed to the native predator, snails from each of the three source sites exposed to cues from the nonnative crab predator did not significantly differ from the control snails in shape (Table 2). Snails from the marsh and barnacle source sites exposed to the nonnative predator did not differ from those in the reduced diet treatment or the control, but had a shell shape that was intermediate between these two treatments. However, shell shape of snails from the cobble source site exposed to cues from the nonnative predator did not significantly differ from those of control animals, but did significantly differ from those in the reduced diet treatment (Table 2, Figure 3). These results suggest that snails from the barnacle and marsh source sites showed an intermediate level of response to the nonnative predator, but snails in the cobble source site did not.

The results of the geometric morphometric shape analysis are consistent with growth results found for snails in these same treatments in Chapter 1. For both growth measures (Chapter 1) and shape changes (Chapter 2), snails from the barnacle and marsh source site responded strongly to the native predator, while showing a reduced response to the nonnative predator. Snails from the cobble site only responded to the native predator, and not the nonnative predator. These results suggest that snails from different source sites can have different abilities to recognize potential risk from different predators, which may be correlated

with relative predation risk among sites. Further work is needed to determine if predator risk from each of these predators differs among sites for *L. saxatilis*. Additional work is also needed to test whether the shell shape changes observed here affect predation risk and whether they are indeed a secondary effect of behavioral plasticity and reduced feeding or activity.

Because *L. saxatilis* has a short generation time and very limited dispersal, studies in Europe have shown morphologically and genetically distinct populations that occur at different tidal heights and habitat types (e.g., Johannesson *et al.* 1997; Johannesson 2003; Conde- Padín *et al.* 2007). There is the possibility that the snails in this study have locally adapted populations and may form ecotypes as have been seen in Europe. The difference in size, shape, and response to predators found in this study suggests that such genetic differences among source sites are possible. Genetic studies are needed to confirm whether differences among source sites are due to local adaptation. Continued studies of future generations of snails from these habitats will be important to determine if snails in these source sites are adapting to the introduced predator, and could provide interesting insight into how plasticity evolves in the presence of novel threats, which is of concern with the increasing amount of anthropogenic impacts on many ecosystems.

Table 1. Multivariate analyses of variance of principal components for the digitized landmarks for *Littorina saxatilis* after 105 days of exposure to experimental conditions.

Dependent Variable	Df	MS	F	P
PC1	11	0.009	15.22	< 0.001
PC2	11	0.002	3.62	< 0.001
PC3	11	0.002	4.09	< 0.001
PC4	11	0.001	3.295	0.001

Table 2. Matrix of p-values for the Tukey *post hoc* test for principal component 1. Results are grouped by source site (Cobble, Barnacle, and Marsh). Treatments included: Control – no exposure to crab cues, Nonnative – exposure to chemical cues from the nonnative crab, Native – exposure to chemical cues from the native crab, and Reduced Food – no exposure to chemical cues from crabs, and fed only three days per week. P-values are reported for pairwise comparisons. None of the other principal components were significantly different among source sites or treatments. Significant differences are in bold.

Cobble

	Control	Nonnative	Native
Nonnative	1.000		
Native	**< 0.001**	**0.003**	
Reduced	**< 0.001**	**< 0.001**	0.832

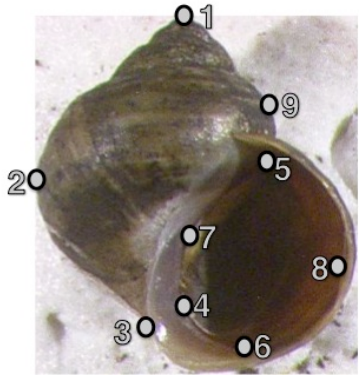
Barnacle

	Control	Nonnative	Native
Nonnative	0.885		
Native	**< 0.001**	0.063	
Reduced	**< 0.001**	0.066	1.000

Marsh

	Control	Nonnative	Native
Nonnative	0.381		
Native	**< 0.001**	**0.001**	
Reduced	**< 0.001**	0.060	0.966

Figure 1: Orientation of snails and placement of landmarks for geometric morphometric analyses.



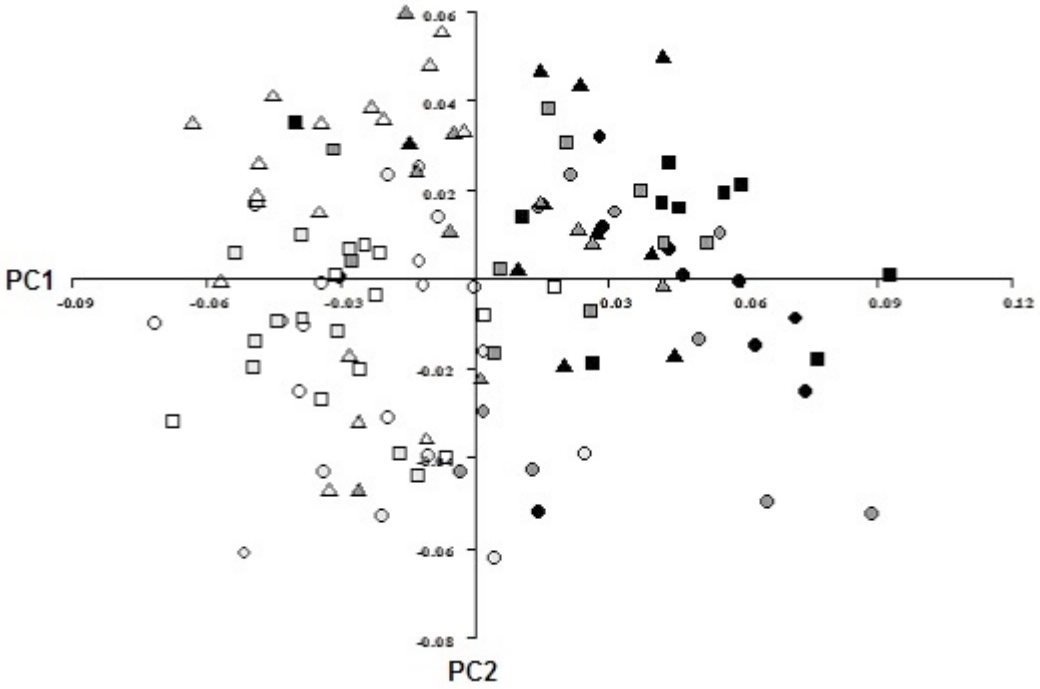


Figure 2: Plot of the two most informative components, PC1 and PC2, from a PCA performed on shell shape landmarks. Every point represents a different individual. Shapes represent source sites (Cobble – circle, Barnacle– triangle, and Marsh – square) and shade represents treatment (control – black, nonnative predator – dark gray, native predator – light gray, and reduced food – white).

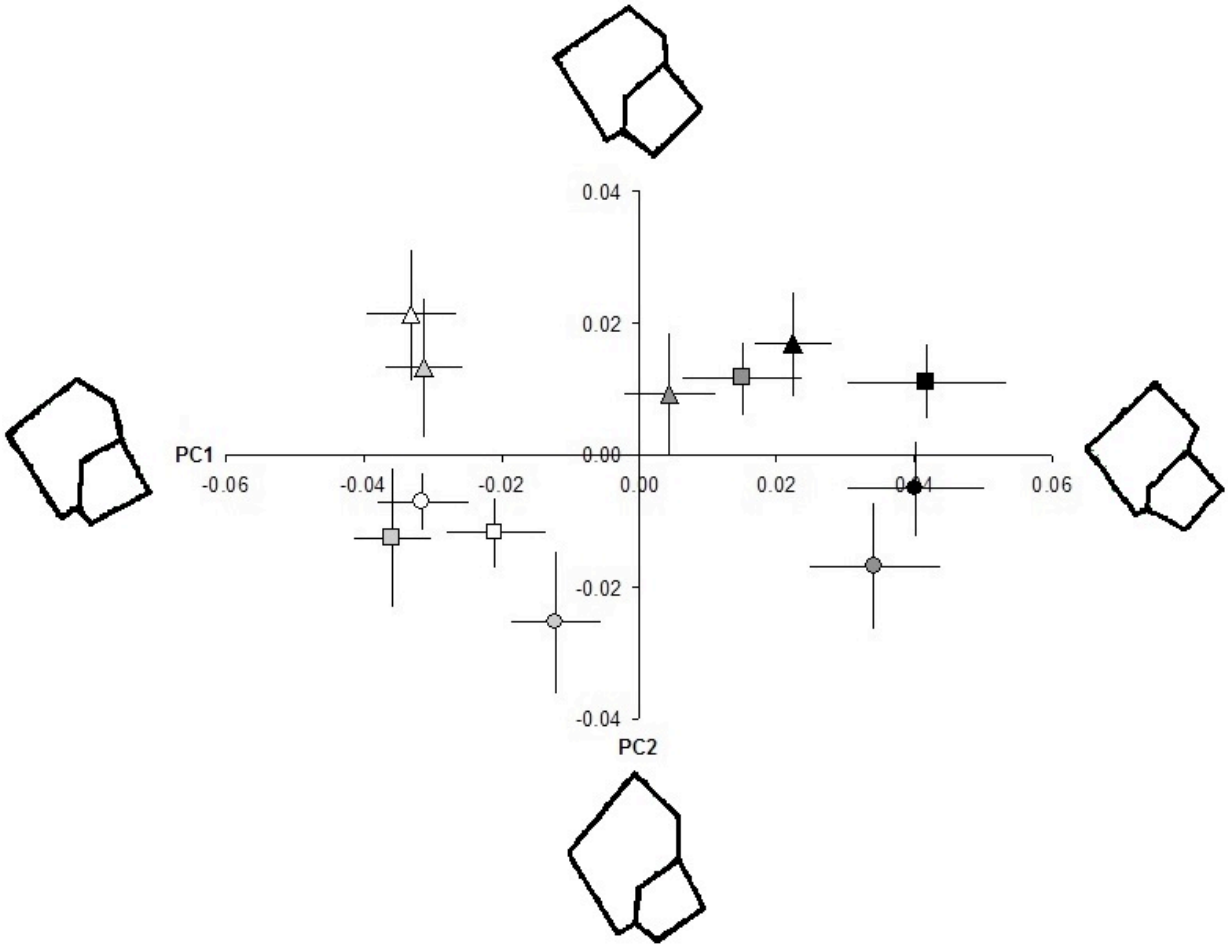


Figure 3: Plot of the two most informative components (PC1 and PC2) from a PCA performed on shell shape landmarks, including stick drawings of shell shape at extremes of each axis. Points represent treatment means with standard error bars for each treatment by source site combination. Shapes represent source sites (Cobble – circle, Barnacle – triangle, and Marsh – square) and shade represents treatment (control – black, nonnative predator – dark gray, native predator – light gray, and reduced food – white).

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