BIOGEOGRAPHIC PATTERNS, PREDATOR IDENTITY, AND CHEMICAL SIGNALS INFLUENCE THE OCCURRENCE AND MAGNITUDE OF NON-LETHAL PREDATOR EFFECTS

A Dissertation

by

SCOTT ISAAC LARGE

Submitted to the Office of Graduate Studies of Texas A&M University
and the Graduate Faculty of The Texas A&M University – Corpus Christi
in partial fulfillment of the requirements for the joint degree of

DOCTOR OF PHILOSOPHY

August 2011

Major Subject: Marine Biology

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Major Subject: Marine Biology

ABSTRACT

Biogeographic Patterns, Predator Identity, and Chemical Signals Influence the Occurrence and Magnitude of Indirect Predator Effects

(August 2011)

Scott I. Large, B.A., Hendrix College

M.S., Texas A&M University—Corpus Christi

Chair of Advisory Committee: Dr. Delbert L. Smee

Predators can have large effects on prey populations and on the structure and function of communities. In addition to direct consumption of prey, predators often cause prey to alter their foraging behavior, habitat selection, and morphology. These non-lethal effects of predators can propagate to multiple trophic levels and often exert equal or larger effects upon communities than those of direct consumption. For non-lethal predatory effects to occur, prey must detect and respond to predation risk. While the importance of information transfer in this process has been realized, few studies explore how prey responses are influenced by predator characteristics and environmental conditions that influence the transmission of cues indicative of predation risk. In this dissertation I investigate factors that influence how a single prey species evaluates and responds to predation risk. Here, I examined: 1) the type and nature of cues prey use to evaluate predator risk; 2) how predator identity, predator diet, and the relative risk of predators influence prey response to predation risk; 3) how hydrodynamic conditions influence the delivery of predator cues; 4) how biogeographic trends in predator

distribution influence prey response to predation risk; and 5) how genetic structure might vary according to prey geographic location and habitat. To address these questions, I used a common intertidal model system consisting of the rocky intertidal whelk *Nucella lapillus* (Linnaeus, 1758) and a suite of its predators, the native rock crab *Cancer irroratus* (Say, 1817), Jonah crab *Cancer borealis* (Stimpson, 1859), and the invasive green crab *Carcinus maenas* (Linnaeus, 1758). *Nucella* use chemical cues emanating from their most common predator (*Carcinus maenas*) and crushed conspecifics to evaluate predation risk. *Nucella* from different habitats experience different levels of predation risk, and *Nucella* from habitats with high levels of predation had larger anti-predatory responses to predator risk cues than *Nucella* that experienced less predation. These chemical cues indicative of predation risk are influenced by hydrodynamic conditions, and *Nucella* have the strongest anti-predatory response in flow velocities of u= ~4- 8 cm s^{-1}. Furthermore, *Nucella* from geographic regions where green crabs are historically absent did not elicit anti-predatory responses, while *Nucella* from regions where green crabs are common frequently responded. Findings from my dissertation research demonstrate that prey detection and response to predation risk is highly dependent upon predator identity, predator diet, environmental forces, and biogeographic patterns in predator and prey distributions.

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DEDICATION

In dedication to my loving Grandparents: Mary and Lynn Bender, Hobart and Adeline Large, and Emma Marie Bender

INTRODUCTION

Predators often shape community structure and function through lethal and non-lethal interactions with prey (Paine 1969, Carpenter et al. 1985, Turner and Mittelbach 1990, Abrams et al. 1996, Schmitz et al. 1997, Menge 2000, Trussell et al. 2003), which may propagate to lower trophic levels via trophic cascades (Carpenter et al. 1985). By consuming lower trophic levels (lethal effects) or altering prey traits including morphology, behavior, or habitat selection (non-lethal effects) (Paine 1969, Carpenter et al. 1985, Abrams et al. 1996, Schmitz et al. 1997, Menge 2000, Trussell et al. 2003), predators can influence community structure and initiate trophic cascades (Schmitz 1998, Trussell et al. 2003, Trussell et al. 2006a). While lethal predator effects are often identified as strong factors in determining community structure, non-lethal predator effects may result in a larger influence upon community structure. Non-lethal predator effects occur when prey reliably detect and respond (i.e., alter morphology, behavior, or life history into a more predator resistant form) to predation risk; whereas, lethal predator effects occur regardless of prey detection and response to predation risk (Lima 1998). Therefore, to understand how non-lethal predator effects occur in nature, ecologists should examine how cues indicative of predation risk are transferred through a system.

In marine systems, both predators and prey often detect each other using waterborne chemical cues (Zimmer and Butman 2000, Weissburg et al. 2002). For prey to reliably detect and respond to the threat of predation, these sensory cues must accurately reflect predator risk (Kats and Dill 1998). Altering morphology, behavior, or life history in response to predation risk is costly, and to minimize costs associated with

anti-predator responses, prey require accurate information about the presence and motive of consumers. Prey use many types of chemical cues to evaluate predator risk. These cues may emanate directly from predators (Dix and Hamilton 1993), originate from something in the predator's diet (Rahman et al. 2000), or come from injured conspecifics (Burks and Lodge 2002) or heterospecifics (Schoeppner and Relyea 2005). To understand what enables prey to detect predators, it is important to understand characteristics of chemical cues that elicit anti-predator responses in prey.

As chemical signals are transferred through advection over a scale of centimeters to meters, hydrodynamic forces such as flow velocity and turbulence influence the structure of chemical odor plumes (Weissburg 2000, Webster and Weissburg 2001) as well as the perception of the odor plumes by predators (Finelli 2000, Powers and Kittinger 2002, Weissburg et al. 2003, Ferner and Weissburg 2005) and prey (Smee and Weissburg 2006a, Smee et al. 2008). Under hydrodynamic conditions that enable prey to detect and respond to predation risk, non-lethal predator effects might be more prominent; however, under conditions that prevent prey detection, lethal predator effects might occur (Smee and Weissburg 2006a, Smee et al. 2008). Therefore, when prey use chemical cues indicative of predation risk, hydrodynamic forces may influence prey detection of predators, thereby influencing the outcome of lethal versus non-lethal predator effects.

Prey develop responses to predators based upon previous experience with predation risk (Lima 1998). As predator and prey distributions often do not fully overlap, predation risk is likely not homologous over geographic regions (Bertness et al. 1981,

Smee and Weissburg 2008), and these biogeographic patterns suggest that predation is stronger in lower latitudes than higher latitudes (Schemske et al. 2009). Prey in lower latitudes may be more sensitive to chemical cues indicative of predation risk than conspecifics in higher latitudes. Many studies examining the strength of non-lethal predator effects do not address geographic or between habitat variation in predator and prey distributions (but see Bertness et al. 1981, Fawcett 1984, Smee and Weissburg 2008). Furthermore, if predation intensity varies according to geographic ranges, prey may be under different selective pressure, which may influence genetic diversity. Therefore, to understand how prey detect and respond to predation risk, it is important to explore multiple spatial scales and explore how genetic variation might influence or be influenced by predator-prey interactions.

The goal of this dissertation research was to investigate factors that influence how a single prey species evaluates and responds to predation risk. To achieve this goal, I examined: 1) the type and nature of cues prey use to evaluate predator risk; 2) how predator identity, predator diet, and the relative risk of predators influence prey response to predation risk; 3) how hydrodynamic conditions influence the delivery of predator cues; 4) how biogeographic trends in predator distribution influence prey response to predation risk; and 5) how genetic structure might vary according to prey geographic location and habitat. To address these questions, I used a common intertidal model system consisting of the rocky intertidal whelk *Nucella lapillus* and a suite of its predators, the native Jonah (*Cancer irroratus*) and rock crabs (*Cancer irroratus*), and the invasive green crab (*Carcinus maenas*). I used this model system for several reasons.

First, these organisms are common throughout the northwestern Atlantic and are readily collected within the rocky inter- and subtidal zones. Second, a large body of research emerged using these species to model lethal predator effects (Menge and Sutherland 1987), non-lethal predator effects (Trussell et al. 2003, Freeman and Hamer 2009), community influences of invasive species (Trussell and Smith 2000, Fisher et al. 2009), and genetic variation across marine habitats (Colson and Hughes 2004, Colson and Hughes 2007, Bell 2008). Therefore, by using a commonly studied model system, the findings from my dissertation will be useful to explain the mechanisms that contribute to these interspecific processes. Furthermore, findings from my dissertation will be useful to both empirical and theoretical ecologists to compare and contrast how prey evaluate and respond to predation risk and how these processes influence community structure. My dissertation research demonstrates that prey detection and response to predation risk is highly dependent upon predator identity, predator diet, environmental forces, and biogeographic patterns in predator and prey distributions.

Chapter 1 Type and nature of cues used by *Nucella lapillus* to evaluate predation risk

ABSTRACT

The ability of prey to detect and adequately respond to predation risk influences immediate survival and overall fitness. Chemical cues are commonly used by prey to evaluate risk, and the purpose of this study was to elicit the nature of cues used by prey hunted by generalist predators. *Nucella lapillus* are common, predatory, intertidal snails that evaluate predatory risk using chemical cues. Using *Nucella* and a suite of its potential predators as a model system, I explored how: 1) predator type, 2) predator diet, and 3) injured conspecifics and heterospecifics influence *Nucella* behavior. Using laboratory flumes, I determined that *Nucella* responded only to the invasive green crab (*Carcinus maenas*), the predator it most frequently encounters. *Nucella* did not respond to rock crabs (*Cancer irroratus*) or Jonah crabs (*Cancer borealis*), which are sympatric predators but do not frequently encounter *Nucella* because these crabs are primarily subtidal. Predator diet did not affect *Nucella* responses to risk, although starved predator response was not significantly different from controls. Since green crabs are generalist predators, diet cues do not reflect predation risk, and thus altering behavior as a function of predator diet would not likely benefit *Nucella*. *Nucella* did however react to injured conspecifics, a strategy that may allow them to recognize threats when predators are difficult to detect. *Nucella* did not react to injured heterospecifics including mussels (*Mytilus edulis*) and herbivorous snails *Littorina littorea*, suggesting that they are

responding to chemical cues unique to their species. The nature of cues used by *Nucella* allows them to minimize costs associated with predator avoidance.

INTRODUCTION

The ability of prey to detect, and respond to the presence of potential predators has important implications for the structure and function of communities (Paine 1966, Carpenter et al. 1985, Turner and Mittelbach 1990, Abrams et al. 1996, Schmitz et al. 1997, Menge 2000, Trussell et al. 2003). Predators can affect prey populations and community structure by consuming lower trophic levels (lethal effect) and by altering prey traits including morphology, behavior, or habitat selection (non-lethal effect) (Paine 1966, Carpenter et al. 1985, Abrams et al. 1996, Schmitz et al. 1997, Menge 2000, Trussell et al. 2003, Werner and Peacor 2003). Both types of predator effects may propagate to lower trophic level through trophic cascades (Schmitz 1998, Trussell et al. 2003, Trussell et al. 2006a). The responses of prey to potential consumers may determine whether cascades are driven by lethal or non-lethal predator effects. If prey detect and respond to predation risk, their likelihood of survival may increase, but anti-predatory responses may limit time spent foraging or reproducing, and have detrimental impacts upon overall prey fitness. In these conditions, non-lethal predator effects are likely to be prevalent. In contrast, if prey fail to detect and respond to predation risk, they will likely succumb to predation, and lethal predator effects will be more widespread.

To prevent consumption, prey must reliably detect predation risk and respond to it appropriately (Chivers and Smith 1998). Since responses are costly, prey require reliable

information regarding the presence and intention of consumers to minimize costs associated with predator avoidance. That is, prey need to limit predator avoidance to truly risky situations, and they require sensory cues that accurately reflect risk levels. Clearly, detecting predators is advantageous, and prey use many types of chemical cues to evaluate risk. In aquatic systems, predators and prey often detect one another via reciprocal detection of waterborne chemical cues (Weissburg et al. 2002, Zimmer and Zimmer 2008). These cues may emanate directly from predators (Dix and Hamilton 1993), originate from something in the predator's diet (Rahman et al. 2000, Turner 2008), or come from injured conspecifics (Burks and Lodge 2002) or heterospecifics (Schoeppner and Relyea 2005).

The purpose of this study was to determine how prey responses to predators vary between different predator species, predators fed different diets, and other indicators of predation risk such as injured conspecific and heterospecifics. The dogwhelk (*Nucella lapillus,* hereafter *Nucella*) is an intermediate consumer in rocky intertidal food webs of New England (Trussell et al. 2003). Green crabs (*Carcinus maenas*) consume *Nucella*, which leads to an increase in barnacles (*Semibalanus balanoides*), *Nucella* primary prey. In addition, *Nucella* also alter their behavior and morphology in the presence of green crabs (Appleton and Palmer 1988, Palmer 1990, Vadas et al. 1994, Trussell et al. 2006b). Therefore, this trophic cascade is driven by both lethal and non-lethal interactions as *Nucella* are both consumed by green crabs and react to green crabs by seeking refuge and decreasing their foraging time (Trussell et al. 2003). *Nucella* provide an excellent model organism for investigating how prey evaluate and respond to predation risk because

decisions made by *Nucella* in response to predators may affect the strength and type of indirect effects (i.e., lethal or non-lethal) seen in this system. Previous research using caged predators provided strong evidence that chemical signals mediated non-lethal effects in this system (e.g. Trussell et al. 2003). Here, I verified that chemical cues alone were mediating *Nucella* reactions to consumers. *Nucella* react primarily to their most common predator, regardless of that predator's diet, but they did not to other sympatric predators that they are unlikely to encounter. *Nucella* reacted to the scent of injured conspecifics, a strategy that may allow them to recognize risk when a predator is otherwise undetectable. *Nucella* did not respond to damaged heterospecifics, suggesting that the risk cues released by injured conspecifics are unique to the species.

MATERIALS AND METHODS

General protocol

In the presence of green crab predators, *Nucella* decrease their activity. Therefore, *Nucella* movement frequency was used as a proxy for response to perceived predation risk. Behavioral assays were conducted in flumes at the Darling Marine Center (DMC) in Walpole, Maine and at Texas A&M University—Corpus Christi (TAMU-CC) in Corpus Christi, Texas. *Nucella* reactions to predation risk were measured by comparing the frequency of *Nucella* movements in the presence vs. absence of predators. The flume at the DMC was useful for behavioral investigations as the animals could be collected from nearby study sites and placed in water from their natural habitat. Replicate behavioral assays were also conducted in the TAMU-CC flume to insure that behaviors were not unique to the DMC flume and for logistical purposes.

DMC flume

Behavioral assays were conducted in a flow-through laboratory flume at the DMC. The flume was 2.2 m long, 0.53 m wide, and was able to reliably reproduce free-stream flow velocities between 3.0 cm s^{-1} and 8.0 cm s^{-1} with an approximate water depth of 10.0 cm (see Smee and Weissburg 2008 for detailed flume description). Ceramic tiles lined the entire bottom of the flume to emulate the natural rocky substrate where *Nucella* are commonly found. Water was pumped from the Damariscotta River into the flume, and then released back into the river. The Damariscotta River is a well-mixed estuary, and during the summer months little variation was measured in both salinity (32-34) and temperature (10-15°C).

TAMU-CC flume

Behavioral assays were also conducted in a recirculating laboratory flume at TAMU-CC. The flume was 4.25 m long, 0.75 m wide, and was able to reliably reproduce free-stream flow velocities between 0.5 cm s^{-1} and 25 cm s^{-1} at a water depth of 20 cm. Ceramic tiles identical to those used in the DMC flume were used to form the substratum. The flume was filled with seawater drawn from a local estuary and passed through sand, UV, and carbon filtration systems as well as a 50.0 μm biological filter. Water was chilled to ~13°C and salinity was maintained at ~32. These values were within the range experienced by organisms in the DMC flume.

Animal collection and care

Organisms used in behavioral assays were collected from the Damariscotta River, ME and held in flowing seawater tables at the DMC. Jonah (*Cancer borealis*), rock

(*Cancer irroratus*), and green crabs (*Carcinus maenas*) were captured using lobster traps, by hand using SCUBA, and with recreational crab nets. These predators were maintained on an *ad libitum* diet of *Nucella,* mussels (*Mytilus edulis*), and clams (*Mercenaria mercenaria*), except when used in predator diet experiments. *Nucella* were collected by hand and held in flowing seawater tables and fed an *ad libitum* diet of mussels and barnacles (*Semibalanus balanoides*). Water temperature ranged between 12-16°C and salinity remained at approximately 32 in the sea tables. *Nucella* were acclimated for at least 24 hours before being used in behavioral assays and were assayed within one week of collection. Each snail was used in a single behavioral assay before being returned to the river, except for those organisms used as food. Green, Jonah, and rock crabs were fed and acclimated for two weeks before being used in behavioral assays, and were used within two weeks after the acclimation period. Green crabs used in diet assays were maintained on each diet for two weeks before use in behavioral assays. All crabs were used in a single assay before being released back into the estuary.

For experiments conducted in Texas, green crabs and *Nucella* were collected from the Damariscotta River and shipped overnight in refrigerated containers to TAMU-CC. They were then housed in insulated tanks with filtered and circulating seawater chilled to approximately 13°C. In all assays conducted at TAMU-CC, organisms were used in a single assay and were then humanely euthanized and discarded in a land-based facility.

Behavioral assay

In each assay, flow velocity was maintained at 4 cm s^{-1}, and this flow velocity is within the range experienced by *Nucella* in the field (Large, *unpublished data*). The

experimental area of each flume was lined with 15 x 15 cm ceramic tiles to mimic the rocky habitat encountered by *Nucella*. The tiles were spaced 1.5 cm apart to provide crevices similar to those in which *Nucella* are typically found in the field (Large, *personal observation*). In the presence of predators, *Nucella* reduce movement and increase use of crevices or other refuge habitats (Gosselin and Bourget 1989, Vadas et al. 1994, Trussell et al. 2003). Therefore, movement was used as a proxy for risk response. Small *Nucella* (<20 mm, with a "sharp" shell margin) have a greater propensity to predation than do larger snails (Etter 1989, Vadas et al. 1994) and moved more frequently than larger individuals in preliminary assays. Therefore, smaller, more motile *Nucella* were used in behavioral assays.

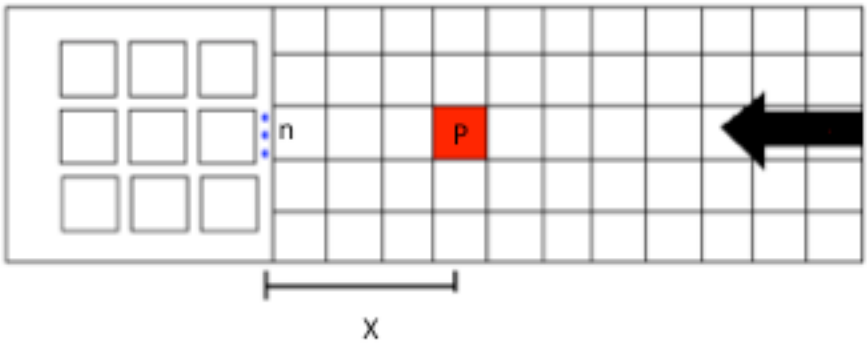

Figure 1.1 Diagram of flume setup for behavioral assays, where x) indicates the distance from p) predator to n) *Nucella* started in crevices between ceramic tiles. The arrow indicates the direction of flow.

To begin the assay, *Nucella* were placed in a crevice between ceramic tiles (Fig. 1.1). Refuge habitat was selected as the starting location for three reasons. First, *Nucella*

were commonly collected from crevices and other refuge habitats in the field (e.g., heterogeneous structure of mussel beds). Second, I wanted to determine if *Nucella* would leave a refuge habitat in the presence of predators. Third, starting *Nucella* in a refuge removes potential observational ambiguity. That is, if snails were not started in a refuge and found to be actively moving, it would not be possible to determine if the *Nucella* were unresponsive to the predator and foraging or detecting the predator and seeking refuge. Thus, starting snails in a refuge allowed me to assess *Nucella* response to predators as well as mimic the location these animals were most often collected from in the field.

Three *Nucella* were placed into a crevice within the experimental area and allowed to acclimate for 5 min. After the 5 min acclimation period, the group of *Nucella* was observed for 20 sec and movement activity of each snail was recorded. All observable activity including climbing from refuge, lifting or rotating their shells, or crawling within the crevice was scored equally. After the initial observation, a tethered predator, crushed conspecific or heterospecific, or the tethering apparatus without a predator (control) was introduced at a fixed distance upstream from the *Nucella* being observed. All snail groups in each assay were observed for 20 sec and any movement was noted during this time. Observations were performed immediately before the addition of the predator and at 5 min intervals thereafter for 30 min. Thus, each *Nucella* could have been observed moving a maximum of seven times during each assay.

Cue characteristics: examining the nature of the cue

Nucella react to green crab predators by reducing their movement (Appleton and Palmer 1988, Palmer 1990, Vadas et al. 1994, Trussell et al. 2006b), and these reactions are thought to be chemically mediated (Appleton and Palmer 1988, Vadas et al. 1994, Trussell et al. 2003). A chemical cue delivery device (adapted from Smee and Weissburg 2006b) was constructed to deliver predator exudates to *Nucella* without predators present in the experimental arena to verify that chemical cues mediate *Nucella* response to predators. In each assay, one live, male green crab (Carapace Width =72.7 mm, SE= 1.2 mm) was held in a 5 liter, flow-through plastic box with a 5.0 cm diameter tygon delivery tube carrying water from the plastic box into the flume. The delivery tube was placed 0.5 m upstream from the *Nucella* and 1.0 cm above the substrate. Water velocity in the flume was maintained at 4.0 cm s^{-1}, and the chemical cue was delivered into the flume at the same velocity to ensure a similar amount of predator cue would reach the *Nucella* as when a predator was caged upstream. Control treatments without a predator were also conducted to ensure the device itself did not significantly influence snail behavior. Each treatment and control was replicated ten times and treatments were interspersed with controls. Tethered green crabs were also placed in the flume 0.5 m upstream from the *Nucella* or an empty tethering device as a control. *Nucella* responses to green crabs and green crab exudates were compared to determine if chemical signals alone caused a similar effect on *Nucella* behavior as to a tethered predator. This created four treatments: tethered predator, tethering device without predator (control), predator exudates from delivery system, delivery system without predator releasing predator-free water (control).

Response of Nucella *to three common predators and crushed conspecifics*

Assays were performed to evaluate *Nucella* responses to risk posed by common predators. Treatments consisted of placing one of three sympatric predators (rock, Jonah, or green crabs) 0.5 m upstream from *Nucella.* As in earlier assays, flow velocity was maintained at 4 cm s^{-1}. After the 5 min acclimation period, *Nucella* were exposed to a tethered Jonah (CW= 106.8 mm, SE= 2.23 mm), rock (CW= 89.0 mm, SE= 1.6 mm), or green crab (CW= 77.6 mm, SE=0 .51 mm) that had been fed an *ad libitum* diet of mussels and *Nucella* daily for two weeks, or the *Nucella* were exposed to injured conspecifics that were manually crushed. Predator and crushed conspecific treatments were each replicated 10 times, and the order of treatments was randomly selected. No-predator control treatments were also interspersed between experimental treatments.

Behavioral response of Nucella *to a predator fed different diets*

Nucella significantly decreased their movement in response to green crabs and injured conspecifics but not Jonah and rock crabs (see results). With this information, I performed a separate series of experiments to determine how changes in the green crab diet affected *Nucella* responses. Behavioral assays were performed to determine what aspect of the green crab chemical cue elicits *Nucella* behavioral responses. Three predator diet treatments were used: mussel fed (CW= 81.7 mm, SE= 0.74 mm), *Nucella* fed (CW= 79.4 mm, SE= 0.66 mm), and starved (CW= 78.9 mm, SE= 0.66 mm) male green crabs. Green crabs were maintained on these diets for 14 days prior to behavioral assays. Each diet treatment and no-predator control was replicated ten times and the order

of treatments was randomly selected each day. No-predator control treatments were performed several times daily and interspersed within predator treatments.

Behavioral response of Nucella *to crushed conspecifics and heterospecifics*

Nucella significantly decreased their movement in response to injured conspecifics. Therefore, an additional series of experiments were performed to determine how chemical cues indicative of crab predation influenced *Nucella* behavior. Three cues indicative of predation risk were used: crushed mussels (Shell Length= 30.3 mm, SE= 0.3 mm), crushed *Littorina* (Shell Length= 21.3 mm, SE= 0.4 mm), and crushed *Nucella* (Shell Length= 27.5 mm SE= 0.5 mm). These prey treatments were selected because they are common within the rocky intertidal zone, and form a large portion of the green crab diet. Each predatory indicator treatment and appropriate control was replicated five times, and the order of treatments was randomly selected.

Data analysis

Initial trials in both Maine and Texas were performed to verify that behaviors of *Nucella* were similar between these locations to investigate possible lingering effects of shipping animals. In these assays, *Nucella* responses to controls and green crab predators were compared using a two-factor ANOVA with experimental location and risk as fixed factors. No significant differences in *Nucella* behaviors were found when tested in Maine or Texas, and data were combined from behavioral experiments conducted in both flumes for analysis.

In the Damariscotta River, *Nucella* are usually found in groups throughout the intertidal zone (Large, *personal observation*). Therefore, groups of *Nucella* were used in

behavioral assays. To insure that interactions between individual *Nucella* did not bias results, a series of assays were performed with a single vs. group of *Nucella.* The responses of individual *Nucella* to the presence of green crabs was compared to those exhibited by groups of *Nucella* (three *Nucella* per group) using a two-factor ANOVA where risk level (predator or control) and prey density (one or three *Nucella*) were fixed factors (Sokal and Rohlf 1995). There was a significant effect of risk but significant density or interaction effects were not found.

Four separate experiments were performed in this study to assess the nature of cues used by *Nucella* to evaluate predation risk and included comparing *Nucella* responses to: 1) tethered predators vs. predator exudates, 2) sympatric predators and crushed conspecifics, 3) green crabs fed different diets, and 4) crushed con- and heterospecifics. Described below are the four nested ANOVAs used to analyze these data, one ANOVA for each of these experiments.

The lack of a significant prey density effect on *Nucella* response to risk suggested that interactions between *Nucella* did not affect their responses. Therefore, a nested ANOVA (see Smee and Weissburg 2006a) was used to compare the effects of predator treatment and trial nested within treatment on the number of *Nucella* movements (Sokal and Rohlf 1995). A nested ANOVA was used to show if variations in *Nucella* responses were affected by variability in cue quality or quantity across replicate treatments, which is a source of uncontrolled variation in the experiments. The P-value for the nested effect was greater than 0.25 in all experiments, indicating that *Nucella* in different groups were reacting similarly to the same treatments. The lack of a significant nested effect enabled

individual snail responses to be grouped within treatments to test the significance of the main effect using the pooled error variance (Sokal and Rohlf 1995). The absence of a nested effect suggests that cues from predators and *Nucella* responses were not significantly different between replicate trials. Since the nested effect was not significant nor was *Nucella* behavioral response when assayed individually or in groups, individual snail responses were treated as independent replicates. Pair-wise differences in treatments were compared using Tukey-Kramer post hoc tests (Sokal and Rohlf 1995). All statistical analyses were performed using SPSS software for Windows (SPSS 2005), and all data met assumptions of ANOVA.

RESULTS

Behavior in Maine and Texas

Nucella responses were not significantly different when performed in Maine or Texas (Fig. 1.2). The two-factor ANOVA revealed that *Nucella* movements were significantly less in response to green crab predators ($F_{1, 89} = 9.48$, $P < 0.01$), but flume location was not significantly different ($F_{1, 89} = 0.45$, $P = 0.50$), nor was there an interaction between these factors ($F_{1, 89} = 0.30$, $P = 0.58$). Thus, I did not consider the location the assay was performed in subsequent analysis.

Density

I compared grouped and individual *Nucella* movements in the presence of a tethered green crab predator and a no-predator control to verify that *Nucella* reactions to consumers were independent. The number of observed movements for each snail was treated as an individual measurement. The presence of a green crab caused a significant

reduction in *Nucella* movement (Fig. 2, $F_{1, 67} = 14.83$, $P < 0.001$), but effects of *Nucella* density ($F_{1, 67} = 0.003$ $P = 0.96$) and interactive effects between density and risk ($F_{1, 67} = 0.12$ $P = 0.73$) were not detected (Fig. 1.3). Thus, interactions between *Nucella* were not influencing their reactions to green crab predators.

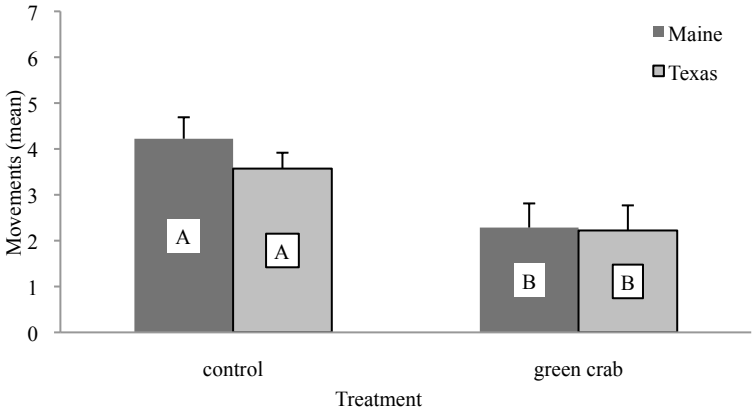

Figure 1.2 Mean number (+ SE) of *Nucella* movements in response to control and green crabs assayed in flumes located in Maine and Texas. *Nucella* movements were significantly less in response to green crab predators (two-factor ANOVA; $P < 0.01$, $n = 15$), but flume location was not significantly different ($P = 0.50$, $n = 15$), nor was there an interaction between these factors ($P = 0.58$, $n = 15$). Letters denote pair-wise differences as determined with a Tukey-Kramer post hoc test.

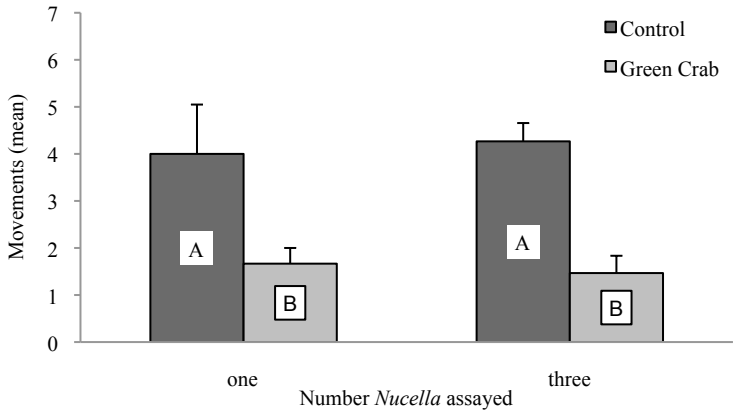

Figure 1.3 Mean number (+ SE) of *Nucella* movements in response to controls and green crabs when assayed individually or in groups of three. The presence of a green crab caused a significant increase in *Nucella* responses (two-factor ANOVA; $P < 0.001$), but effects of *Nucella* density ($P = 0.96$ $n = 15$) and interactive effects between density and risk ($P = 0.73$ $n = 15$) were not detected. Letters denote pair-wise differences as determined with a Tukey-Kramer post hoc test.

Cue characteristics

Previous studies produced strong evidence that *Nucella* detect predators using chemical signals. I empirically tested *Nucella* behavioral responses to tethered predators, to predator exudates, and to controls. When presented with a tethered green crab or with green crab exudates, *Nucella* significantly reduced the number of movements, as compared to no-predator controls ($F_{3, 116}= 29.04$ $P < 0.001$, Fig. 1.4). *Nucella* responses to predators and predator exudates were not significantly different but both predator treatments resulted in a significant reduction of movement compared to controls, suggesting that chemical cues alone can modulate *Nucella* reactions to predators.

Behavioral response to predators

I compared *Nucella* responses to three common crab predators: green crabs, Jonah crabs, and rock crabs, as well as to manually crushed conspecifics and no-predator controls. When presented with a tethered green crab or crushed conspecifics, the mean number of *Nucella* movements decreased to 2.9 as compared to 4.8 in controls, a change of approximately 40%, which was significantly less than the number of movements in the presence of tethered Jonah crabs, rock crabs, and no-predator controls ($F_{4, 145}$= 28.16, P < 0.001, Fig. 1.5). *Nucella* were unresponsive to rock and Jonah crabs as significant differences were not observed in *Nucella* movement when compared to rock crab, Jonah crab, and control treatments.

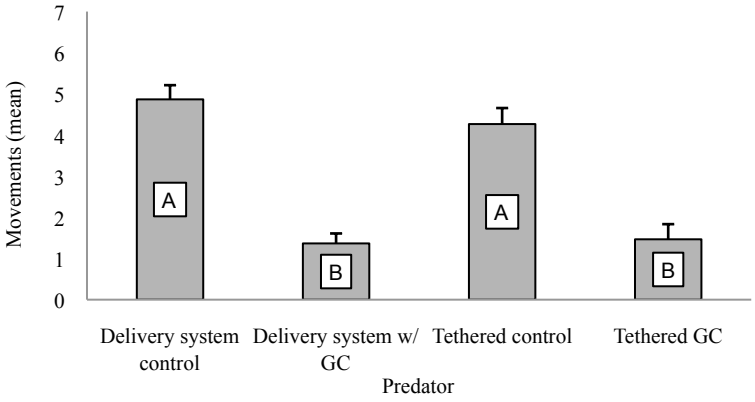

Figure 1.4 Mean number (+ SE) of *Nucella* movements in response to predator chemical cues. *Nucella* movements were significantly less in response to green crab predators and green crab predator exudates compared to no-predator controls (one-factor ANOVA; P < 0.001, n = 30), but there was no significant difference between responses to predators and predator exudates. Letters denote pair-wise differences as determined with a Tukey-Kramer post hoc test.

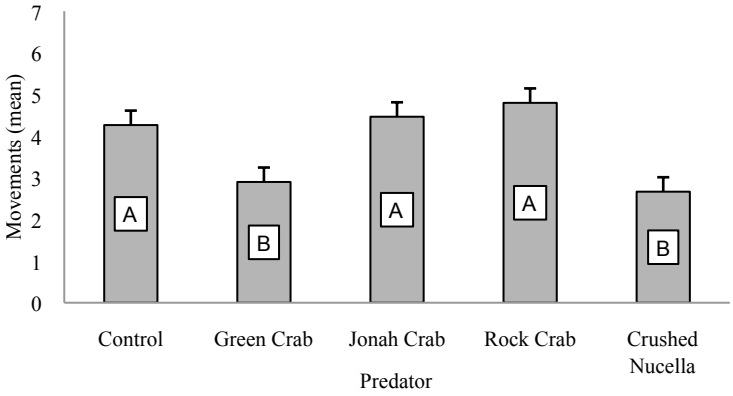

Figure 1.5 Mean number (+ SE) of *Nucella* movements in response to sympatric predator cue, crushed conspecifics, and a no-predator control. *Nucella* movements were significantly less in response to green crab predators and crushed conspecifics (one-factor nested ANOVA; $P < 0.001$, $n = 30$), but there was not a significant difference between the no-predator controls, rock, and Jonah crabs. Letters denote pair-wise differences as determined with a Tukey-Kramer post hoc test.

Behavioral response to predator diet

Nucella had a significant response to the presence of green crabs that were fed either mussels or conspecifics, but their response to starved green crabs was not significantly different from no-predator controls ($F_{3, 152}= 25.91$, $p< 0.001$, Fig. 1.6). The strongest *Nucella* response was to green crabs fed *Nucella,* and the mean number of *Nucella* movements in this treatment was 2.3. When green crabs were fed mussels, the mean number of *Nucella* movements was 2.9, and in both mussel-fed and *Nucella*-fed treatments movements were less than controls. Mean number of *Nucella* movements was 3.5 in response to starved green crabs, a value that was not significantly different from either controls or fed crab treatments.

Behavioral response of Nucella *to predatory indicators*

Green crabs are generalist predators and consume mussels, *Littorina* snails and *Nucella*. Since *Nucella* reacted to crushed conspecifics, I completed an additional study to determine if they would respond to crushed heterospecifics (mussels and *Littorina* snails). When presented with crushed conspecifics and heterospecifics, *Nucella* movements significantly decreased in the presence of crushed conspecifics, but their behavior did not change in the presence of crushed heterospecifics ($F_{3, 56}$= 30.28, P < 0.001, Fig. 1.7).

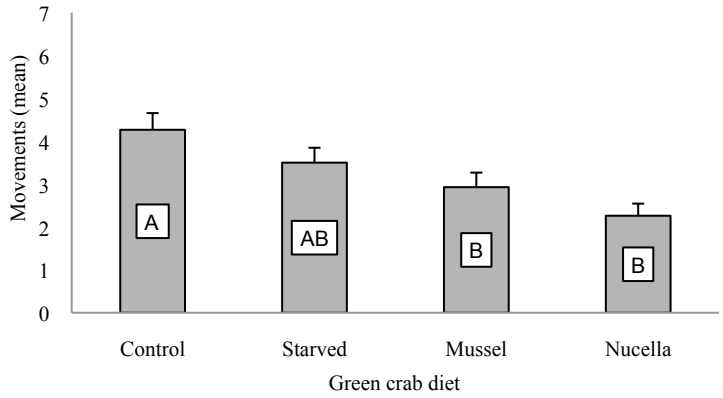

Figure 1.6 Mean number (+ SE) of *Nucella* movements in response to green crab diet. *Nucella* movements were significantly less in response to green crab predators fed *Nucella*, and mussels, but starved green crabs did not differ from no-predator controls (one-factor nested ANOVA; P < 0.001, n = 30). Letters denote pair-wise differences as determined with a Tukey-Kramer post hoc test.

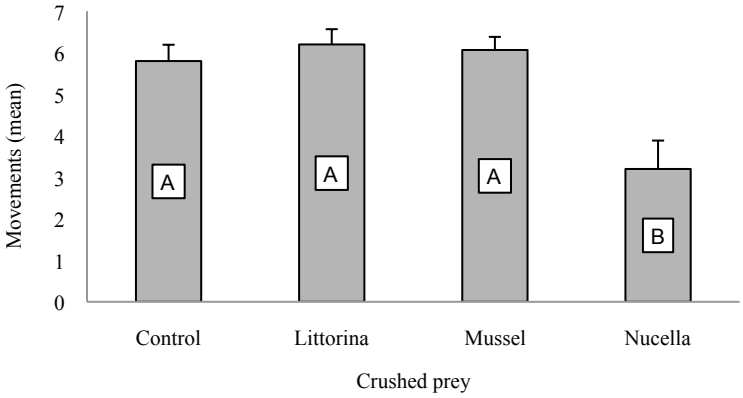

Figure 1.7 Mean number (+ SE) of *Nucella* movements in response to injured *Nucella*, injured *Littorina*, and injured mussels. *Nucella* movements were significantly less in response to crushed conspecifics, but crushed heterospecifics did not differ from no-predator controls (one-factor nested ANOVA; $P < 0.001$, $n = 15$). Letters denote pair-wise differences as determined with a Tukey-Kramer post hoc test.

DISCUSSION

Understanding factors that elicit prey behavioral response to predators continues to be an important aspect of behavioral and community ecology. How intermediate consumers evaluate and respond to predation risk is important because these foraging decisions contribute to the strength and prevalence of indirect predator effects. Although predatory avoidance tactics are costly for prey, the benefits of surviving a predatory encounter surpass any reduction in fitness (Dawkins and Krebs 1979, Kats and Dill 1998, Smee and Weissburg 2006b). Many studies have shown that prey minimize costs associated with predator avoidance by limiting the frequency of predator avoidance to dire situations (reviewed by Kats and Dill 1998). To make appropriate decisions regarding initiation of predator avoidance tactics, prey require reliable signals of risk to

avoid costs of unnecessary reactions to consumers (Kats and Dill 1998, Zimmer and Zimmer 2008).

Cue characteristics

Nucella use chemical cues to detect the presence and potential risk of predators. The presence of predators can alter the morphology (Palmer 1990, Trussell 1996) and foraging behavior (Geller 1982, Vadas et al. 1994) of intertidal snails. A simple experiment was performed to test if *Nucella* use chemical signals to detect predators. *Nucella* behavioral response did not differ between tethered green crabs and green crab chemical cues, suggesting that chemical exudates alone are modulating *Nucella* reactions to predators (Fig 1.4). It is not surprising that *Nucella* use chemical cues, as these cues are widely used by many aquatic predators and prey (Chivers and Smith 1998, Kats and Dill 1998, Smee and Weissburg 2006a), and in marine systems, visual and mechanical cues are often unreliable and unavailable (Zimmer and Butman 2000, Weissburg et al. 2002).

Behavioral response to predators

Nucella discerned between potential predators. In the presence of Jonah and rock crabs, *Nucella* did not display a significant behavioral response (Fig 1.5). These predators readily consumed *Nucella* in feeding assays (Large, *unpublished data*), and *Nucella* were expected to react similarly to rock, Jonah, and green crabs since these crabs occur sympatrically with *Nucella*. These results may be explained by the tidal distribution of these predators. Scuba surveys of intertidal and subtidal areas within the Damariscotta River, ME were conducted during high tide at sites where *Nucella* were collected. Green

crabs were exclusively found within the intertidal zone, and Jonah and rock crabs were only found only in the subtidal zone (Large, *unpublished data*). League-Pike and Shulman (2009) found similar crab distributions off Appledore Island, ME. *Nucella* were not collected or observed subtidally.

The lack of an observed response by *Nucella* to rock and Jonah crabs may have occurred because they either could not detect these crabs or because they were able to detect them but did not consider them to be a threat. All predators were maintained on the same diet, however, only the green crab produced behavior-inducing chemical cues (Chivers and Mirza 2001b). Until the chemical cues *Nucella* use to detect predators are identified, it will not be possible to determine if rock and Jonah crabs release signals that *Nucella* are unable to detect, or if *Nucella* are simply unresponsive to predators they do not perceive as posing a threat.

In the presence of both rock, and green crabs, Aschaffenburg (2008) reported a significant decrease in *Nucella* barnacle consumption rate, a result that differs from this study. I present three possible reasons why my results are inconsistent with his findings. First, my assays were performed in flowing water while Aschaffenburg (2008) performed trials in a static tank, which may have caused the predator cues to build-up beyond an ecologically realistic level. This may be especially important if rock crabs produce fewer chemical cues than green crabs. Aschaffenburg exchanged water in tanks every two weeks, which may have further concentrated the levels of predator cues. Secondly, my experiment was designed to elicit short-term changes in behavior. Aschaffenburg's experiment lasted 40 days and examined changes in barnacle consumption not refuge use.

Nucella may not react to rock crabs initially, but may develop a response over time, and neither my experiment nor that of Aschaffenburg compared initial responses to rock crabs vs. responses after a long term exposure to rock crabs. Finally, these studies compare different parameters (movement vs. barnacle consumption), and separately each may fail to fully capture the consequences of *Nucella* reactions to consumers. Clearly, the results from both studies suggest that additional research on *Nucella*-predator interactions is needed to answer these lingering questions.

Prior to the introduction of the green crab in the early 19th century, rock crabs were believed to be the common intertidal predator in this system (Grosholz and Ruiz 1996). Therefore, over the past 200 years *Nucella* may have lost the behavioral response to rock crabs or may have not reacted to these predators historically. Presently, green crabs are commonly found within the same intertidal range as *Nucella*, and a strong behavioral response occurs (Fig. 1.5), as green crabs are the largest threat to *Nucella*. The lack of any behavioral response to *Nucella* most likely historic predators indicates that anti-predatory behavior might be highly plastic, perhaps even based on ecological time scales, assuming that rock crabs did indeed inhabit the intertidal zone prior to the arrival of green crabs. Determining whether this response is based upon ecological versus evolutionary time would be beneficial to further understand these predator-prey interactions and provide insight as to how *Nucella* survived the green crab invasion.

When presented with crushed conspecifics, *Nucella* responses rivaled their response to green crabs (Fig. 1.6). The response to crushed conspecifics may be an important mechanism that allows *Nucella* to avoid reacting to predators that pose little

threat, while still having the ability to detect rare or cryptic predators when they indeed pose a threat by consuming neighboring conspecifics. Additionally, the ability to detect crushed conspecifics appears to be robust, and species selective (Fig. 1.7), as crushed heterospecifics did not elicit a behavioral response. The lack of response to crushed heterospecifics was a bit surprising given that green crabs readily consumed these species and all occur sympatrically.

Behavioral response to predator diet

Many prey species react differently to the same predator when the predator is maintained on different diets. Diet-dependent responses are considered adaptive by allowing prey to save costs by not reacting to predators that are not consuming them. Anurans, (Wilson and Lefcort 1993, Chivers and Smith 1998), fish (Chivers and Mirza 2001a), and larval invertebrates (Chivers et al. 1996), vary their responses to predators depending upon predator diet, yet this response is not ubiquitous among all species (Relyea and Werner 2000, Bryer et al. 2001). The pattern of diet-dependent responses is thought to be adaptive when predator diet is a true reflection of risk. For instance, if a predator preys on prey species A in the spring and prey species B in the fall, the prey species could reliably use diet cues as an estimation of risk (Chivers and Mirza 2001b). However, in systems where generalist predators can consume prey at any time, limiting reactions to predators that are eating conspecifics does not appear to be an adaptive response (Bryer et al. 2001, Smee and Weissburg 2006a). Green crabs are generalist predators that opportunistically forage for *Nucella* and remain a threat to *Nucella* regardless of their diet. Diet did not significantly influence *Nucella* responses to green

crabs, a result that seems logical given the generalist feeding behavior of green crabs. *Nucella* minimize costs associated with anti-predator behavior by responding only to common predators, which elicit the highest predator risk. In the presence of their most common generalist predator, a behavioral response to one diet over another does not diminish the risk of predation, and a nearly equal response between diets is seen. Reacting to a common predator, regardless of predator diet, demonstrates an adaptive predator response, where over time prey experience a constant level of predation and develop a response to a predator (Chivers et al. 2001).

Behavior-inducing chemicals emanating from predators may be broken down metabolically (Chivers and Mirza 2001b). *Nucella* demonstrated a response to starved green crabs that is not significantly different from the no-predator control. Starved predators may be a higher risk, and the lack of a response by *Nucella* to starved green crabs does not seem adaptive. Therefore, either the quantity or quality of behavior-inducing chemicals changes when green crabs are unable to forage. Smee and Weissburg (2006a) found a similar result with hard clam- blue crab interactions. Clams reacted to crabs regardless of crab diet, but were unresponsive to starved crabs. Like the present study, a hungry crab likely posses a significant risk to its prey and the lack of a response does not appear adaptive. Rather, I propose, as did Smee and Weissburg (2006a) that starved crabs simply exude fewer metabolites and are thus harder to detect. Although this is not beneficial for *Nucella*, it may benefit green crabs as their foraging success may increase with time since their previous meal. Clearly additional studies are needed to

quantitatively study how cue quantity and quality affect the strength of response in intermediate consumers and the consequences of diet in a broader, community context.

Another possibility for *Nucella* not responding to starved crabs may be explained by green crab behavior before molting. During the molting process, green crab feeding is significantly reduced, and it is unlikely that soft chelae present after molting could crack a *Nucella* shell (Crothers 1968). If *Nucella* frequently encounter molting green crabs, the risk of consumption from a molting, and not readily feeding crab, may indeed be slight. Future studies comparing the response to molting, and starved green crabs would help clarify this discrepancy. I suspect, however, that fed crabs release larger quantities of metabolites than starved crabs, and thus, are easier for *Nucella* to detect.

Conclusions

Intermediate consumers must gain reliable information about predator risk to maximize time spent foraging and to avoid consumption. By altering behavior in response to predator risk, the strength of non-lethal interactions within a system may vary based upon factors such as predator identity, predator feeding behavior, substrate complexity (Grabowski and Kimbro 2005, Jackson et al. 2007), and hydrodynamic conditions (Smee and Weissburg 2006a, b). To understand top-down forces in natural communities, it is important to examine the mechanisms that may dictate the type of predator-prey interactions within a system. The simple model system used in this series of experiments demonstrates that intermediate consumer response is variable dependent upon the types of chemical signals received. Similarly, intermediate consumer response is also variable based upon abiotic conditions such as flow velocity (Large et al. 2011), and

wave stress (Freeman and Hamer 2009). Therefore, to understand how prey interpret and react to predation risk, we must examine biotic and abiotic factors that can significantly alter anti-predatory responses.

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Chapter 2 Predator identity and diet influences the expression of *Nucella lapillus* behavioral and morphological inducible defenses

ABSTRACT

In addition to consumption, predators often affect community structure through non-consumptive interactions with prey that influence prey morphology, life history, and behavior. For these non-consumptive effects to occur, prey must reliably detect cues indicative of predation risk. In aquatic environments, prey frequently use chemical signals to detect predation risk, and often, these chemical cues are developed through: 1) previous experience with a predator, 2) predator diet, or 3) the relative risk of a predator. Each of these factors was tested to see how they influences prey activity, foraging, and morphology using a model system consisting of the intertidal whelk *Nucella lapillus* and two sympatric, decapod, generalist predators *Carcinus maenas* and *Cancer irroratus*. *Nucella* are common along wave-exposed and wave-protected intertidal shores throughout the northwestern Atlantic. Both crab species are unable to forage along wave-exposed shorelines and populations of wave-exposed *Nucella* experience little predation risk, so I compared wave-exposed and wave-protected *Nucella* response to predation pressure. *Carcinus maenas* dominates the intertidal shoreline, while *C. irroratus* generally exists subtidally; therefore, *Nucella* experiences more contact with *C. maenas*. *Nucella* from both types of populations significantly decrease their activity when in the presence of either predator; however, activity of *Nucella* from wave-protected shorelines

decreased further in the presence of *C. maenas*. After continuous exposure cues emanating from predators maintained on *Nucella* or *Littorina littorea* (a sympatric, heterospecific snail) for 45 d induction period, predators consuming *Nucella* caused a significant decrease in *Nucella* mussel consumption, resulting in significantly less shell and body growth. Compared to the no-predator controls, wave-exposed populations grew considerably less in response to either predator. Aspects such as previous exposure to predation risk, predator diet, and the relative risk of a predator can influence multiple factors of prey behavior and morphology. Further, each of these factors does not act independently, but interactively, and examining a single factor or response may misrepresent how prey detect and respond to predation risk.

INTRODUCTION

Predators moderate community structure through interactions with prey that influence prey density, termed lethal predator effects, or behavior, morphology, and life history, termed non-lethal predator effects (Paine 1966, Carpenter et al. 1985, Turner and Mittelbach 1990, Abrams et al. 1996, Schmitz et al. 1997, Menge 2000, Trussell et al. 2003). Traditionally ecologists focused on the consumptive effects of predators on prey density (Sih et al. 1985), but many recent studies suggest that non-lethal predator effects exert equal or perhaps larger influences on communities than those of direct consumption (Werner and Peacor 2003, Preisser et al. 2005, Preisser et al. 2007). For non-lethal effects to propagate through a system, prey must reliably detect and respond to predation risk (Chivers and Smith 1998). Despite the growing appreciation for the importance of non-lethal effects in structuring communities, some research has characterized how signals

transmitted between predators and prey influences the prevalence and magnitude of non-lethal predator effects (but see Turner et al. 2000, Relyea 2001a, Smee and Weissburg 2006b, Edgell 2010, Large and Smee 2010, Large et al. 2011); however, more research should quantify the influence of information transfer upon non-lethal predator effects.

In aquatic systems, predatory interactions are often chemically mediated (Weissburg et al. 2002, Preisser et al. 2005, Preisser et al. 2007, Zimmer and Zimmer 2008) and result resultant non-lethal predator effects (Chivers and Smith 1998, Kats and Dill 1998, Werner and Peacor 2003, Preisser et al. 2005, Preisser et al. 2007). Chemical signals are frequently used because they are the most reliable indicators of predator presence and intention (Brown et al. 2000) and across taxa, prey use chemical cues to from a variety of sources to detect and evaluate predation risk (reviewed by Chivers and Smith 1998, Ferrari et al. 2010). Thus, determining the origin and effects of chemical cues that reflect predation risk is necessary to understand the ubiquity and importance of non-lethal predator effects. The chemical cues prey respond to are often species specific and are generally selected for based upon: 1) previous experience with a predator (Appleton and Palmer 1988), 2) predator diet (Chivers et al. 1996, Brodin et al. 2006, Smee and Weissburg 2006b, Large and Smee 2010), or 3) the relative risk of a predator (Schoeppner and Relyea 2005, Turner 2008). Previous research has explored how these factors separately contribute to the induction of prey behavioral (Chivers and Mirza 2001b, Smee and Weissburg 2006b, Large and Smee 2010) and morphological defenses (Appleton and Palmer 1988). However, few studies have investigated the combined

effect of how these factors might interact and further influence the behavior and morphological defenses of prey (but see Chivers et al. 2007).

Research exploring how chemical cues transfer non-lethal predator effects through systems and the resultant community effects often measure factors such as changes in activity (Large and Smee 2010), foraging behavior (Aschaffenburg 2008, Freeman and Hamer 2009), morphology (Appleton and Palmer 1988, Palmer 1990), or other aspects of prey life history (Hoverman et al. 2005). Often, single responses to predation risk are investigated, (but see Palmer 1990, Hoverman et al. 2005), and rarely the quantity, quality, or delivery of chemical cues are varied to measure effects on prey responses (Smee and Weissburg 2006a, b, Smee et al. 2008, Large and Smee 2010, Large et al. 2011). Prey may use different anti-predatory strategies for different predators; suggesting that type and nature of risk cues are important and can mediate different types or degrees of non-lethal predator effects (Sih et al. 1985, Relyea 2001b, Chivers et al. 2007, Turner 2008, Freeman and Hamer 2009).

When estimating non-lethal predator effects, single cues or single prey responses may not adequately quantify how prey determine and respond to predation risk. For example, in short-term behavioral assays, Large and Smee (2010) exposed the intertidal dogwhelk *Nucella lapillus* (hereafter, *Nucella*) collected from shorelines that experience high predation risk to three different predators. *Nucella* responded to green crabs (*Carcinus maenas*), but not sympatric rock (*Cancer irroratus*) or Jonah (*Cancer borealis*) crabs, and the authors posited that rock and Jonah crabs are subtidal and pose little risk to intertidal snails like *Nucella* (Large and Smee 2010). Further, *Nucella* reduce their

activity level in the presence of green crabs regardless of the green crab's diet, and also reduce activity level in response to injured conspecifics but not injured heterospecifics (Large and Smee 2010). In contrast, Freeman and Hamer (2009) found *Nucella* responded to Jonah crabs more than green crabs, a result differing from that noted by Large and Smee (2010). However, Freeman and Hamer (2009) measured *Nucella* consumption of mussels while Large and Smee (2010) measured *Nucella* activity during short term assays. Further, Palmer (1990) noted that *Nucella* developed more predator resistant shell morphology in response to *Cancer pagurus,* a result dependent upon *Nucella* habitat and the diet of *C. pagurus*. Therefore, based upon the anti-predatory response measured and the chemical cues presented to prey, these three studies came to different conclusions.

Nucella are direct-developing whelks and are common along wave-exposed and wave-protected rocky intertidal shorelines along the northwestern Atlantic from Long Island to Greenland. *Nucella* spp. respond to predation risk by: decreasing their activity and remaining in refuges (Vadas et al. 1994, Large and Smee 2010), reducing their foraging rate (Burrows and Hughes 1991, Vadas et al. 1994, Aschaffenburg 2008), and changing their morphology (Appleton and Palmer 1988, Palmer 1990, Bourdeau 2009). Each of these responses can be influenced by wave exposure (Boulding et al. 1999). On wave-protected shorelines, *Nucella* are preyed upon by both rock and green crabs, however, on wave-exposed shorelines, foraging of both predators is inhibited by wave energy, releasing wave-exposed populations from decapod predation risk (Kitching et al. 1966, Menge 1983, Menge and Sutherland 1987, Leonard et al. 1998, Boulding et al.

1999). I hypothesized that in areas of differential predation risk, the response to risk, and the importance of predator diet, and further, the behavioral, morphological, and life history response would differ. *Nucella* respond to risk from both rock and green crabs, and that regardless of previous exposure to predation risk, predator diet is an important factor in determining this response.

The purpose of this study was to determine how different chemical cues emanating from predators affect multiple types of prey responses. Since previous exposure to chemicals from predators and injured conspecifics can influence prey responses to risk (Smee and Weissburg 2008, Edgell 2010), I used prey from two distinct habitats that experience high vs. low levels of predation. For these experiments, a rocky intertidal model system consisting of a carnivorous snail, *Nucella,* and two common predators of *Nucella,* green and rock crabs was used. I explored how previous exposure to each of these predators fed diets of hetero- or conspecific prey affected *Nucella* behavior (i.e., activity and consumption of blue mussels *Mytilus edulis*) and resulting plastic inducible defenses.

MATERIALS AND METHODS

General protocol

To examine how prey from habitats that experience different levels of predation risk respond to predators based upon their diet, I measured initial behavioral response (i.e., activity level) to sympatric predators shortly after collection, and then assessed changes in *Nucella* activity level, *Nucella* consumption of mussels, and the change of *Nucella* morphology after 45 d of continuous exposure to predators consuming hetero- or

conspecific prey. Heterospecific prey used was the grazing periwinkle snail, *Littorina littorea* (hereafter *Littorina*).

Animal collection and care

Approximately 90 *Nucella* of similar size (mean shell length= 13.56 mm SE= 0.08 mm) were collected from both wave-protected and wave-exposed shorelines and immediately transferred them to flowing seawater tanks at the Darling Marine Center (DMC) in Walpole, ME. Wave-protected and wave-exposed shorelines were <10 km apart. Within each of these habitats, *Nucella* were collected from two different sites < 2 km apart to minimize localized bias (Table 1). In the lab, *Nucella* were maintained in flow-through tanks and fed an *ad libitum* diet of mussels *Mytilus edulis*. Male green and rock crabs with carapace widths of 75 mm SE= 4.0 mm and 78 mm SE= 3.6 mm, respectively, were captured from the Damariscotta River using recreational crab traps. Crabs were immediately transferred to flowing seawater tanks at the DMC and maintained on an *ad libitum* diet of *Nucella*, *Littorina*, and mussels until used in behavioral assays or placement into an induction chamber where their diets were changed to consist of con- or heterospecific prey (see below). During the experiment, water temperatures ranged from 12 to 16°C and salinity remained at ~32 in all the seawater tanks. After collection, each snail was allowed a 24 h acclimation period before behavior was observed.

Table 2.1 Description of sites where *Nucella* were collected during the summer of 2010.

Site description	Habitat	Latitude	Longitude
Pemaquid Point, South Bristol, ME	Exposed	43.836960	-69.508040
Long Cove Point, Chamberlain, ME	Exposed	43.885190	-69.473940
Lower Narrows (East), Walpole, ME	Protected	43.891380	-69.583300
Lower Narrows (West), Boothbay, ME	Protected	43.894440	-69.576990

Behavioral assay to measure activity

Behavioral assays were conducted in a flow-through laboratory flume (2.2 m long x 0.53 m wide x 0.1 m deep) at the DMC (for description of flume see Smee and Weissburg 2008). This flume is able to reliably maintain free-stream flow velocities between 3.0 and 8.0 cm s^{-1} and these flow velocities are well within the range experienced by *Nucella* in the field (Leonard et al. 1998). This design has been used successfully for many studies (for detailed description see Smee and Weissburg 2008). Ceramic tiles were used as the bottom substrate to imitate natural substrate and flow velocity remained at ~ 4 cm s^{-1} for all behavioral assays (see Large et al. 2011).

To examine how prey from each habitat initially responded to predation risk, *Nucella* were exposed to chemical cues from green and rock crab predators maintained on mixed diets. As both crabs are generalist predators, mixed diets were used to assay a

baseline response to each of these predators. Further, in short-term behavioral assays, there were no differences in *Nucella* responses to green crabs maintained on mussels vs. *Nucella* (Large and Smee 2010). In the presence of predator cues, *Nucella* decrease their activity (Vadas et al. 1994, Large and Smee 2010); therefore, *Nucella* movement was used as a proxy for response to predation risk. To begin each assay, three *Nucella* were placed within a crevice between the ceramic tiles that served as a predation refuge (for description of behavioral assay see Large and Smee 2010). Groups of three *Nucella* were used because *Nucella* are typically in groups in the field and previous empirical data had shown that *Nucella* responses to predators are not statistically different when assayed individually or in groups (Large and Smee 2010). *Nucella* were started within a refuge to provide them the option to exit the refuge in a risky situation, thereby limiting behavioral ambiguity. If *Nucella* were placed away from a refuge on the bare substrate, any subsequent movements could either be: 1) failed response to predator risk, or 2) an active search for refuge in response to predation risk. *Nucella* were allowed to acclimate for five minutes before one of two predator treatments or a control were introduced 0.5 m upstream: 1) green crab, 2) rock crab, or 3) no-predator control. Predators were tethered to a ceramic tile preventing them from moving throughout the flume, but allowing them to still expose *Nucella* to chemical cues from predators (Large and Smee 2010). After the acclimation period, *Nucella* movement was monitored for 20 s every 5 min for 30 m creating a total of 7 observations (see Large and Smee 2010). All *Nucella* movements such as climbing from the refuge, lifting or rotating their shells, or crawling within the

refuge were scored equally, and the order of predator treatments was performed randomly.

Inducing anti-predatory defenses

Nucella were exposed to predator cues in chambers consisting of a large plastic aquarium (60.45 cm x 39.63 cm x 22.61 cm) with a perforated barrier bisecting the tank. Seawater was pumped from the Damariscotta River into a header tank where it was drained into each aquarium and allowed to drain from the opposite end creating a gentle current (~2 l min^{-1}). Within each large aquarium, two small, mesh-sided containers (25.4 cm x 17.78 cm x 10.16 cm, 1.50 mm vexar meshing) were placed downstream of the perforated barrier. The mesh permitted water to pass through the container containing *Nucella*. Within each mesh-sided container 15 *Nucella* from a single habitat were placed, along with mussels of three size classes: shell length = small (13–17.5 mm), medium (17.5– 20 mm), and large (20–23.5 mm). For each mesh-sided container, 15 small, 6 medium, and 4 large mussels were included as food for the *Nucella* (Freeman and Hamer 2009).

Upstream of the perforated barrier bisecting the tank, one of 4 predator treatments plus a no-predator control were placed: 1) green crab fed *Nucella*, 2) green crab fed *Littorina*, 3) rock crab fed *Nucella*, or 4) rock crab fed *Littorina*. *Nucella* and *Littorina* used for predator food were ~25 mm shell length and were collected from wave-protected shorelines near the DMC. This experiment was run for a period of 45 d, and previous studies demonstrated that this time period was sufficient to observe differences in anti-predatory morphological responses (Trussell and Smith 2000). Predators were fed 3-5

snails every other day, and to facilitate crab feeding snail shells were carefully cracked and placed near each crab. Each experimental chamber contained one predator upstream of 15 *Nucella* from wave-exposed and wave-protected habitats. Deceased crabs were immediately replaced with conspecifics maintained on the same diet. Each predator and diet combination was simultaneously replicated four times.

Foraging

Nucella food supply was replaced weekly with 25 living mussels (15 small, six medium, and four large), and drilled mussel valves were counted to measure consumption rate. Some *Nucella* perished during the experiment and these snails were subsequently removed. *Nucella* mortality was similar among populations and predator induction treatments. To account for different numbers of *Nucella* in each container, the number of mussels consumed each week was divided into the number of living *Nucella* for data analysis.

Post-induction Nucella *activity*

After the 45 d induction period, behavioral assays were repeated with *Nucella* to determine if exposure to different predator risk cues influenced short-term behavioral response to predators. Response to the presence of predation risk was compared, and each population was held in the presence of crab predators maintained on diets consisting of hetero- and conspecific prey. Since both green and rock crabs are generalist predators, to determine if different levels of exposure influenced *Nucella* anti-predatory behavior predators were maintained on mixed diets. Additionally, *Nucella* were only tested against the predator it was held with, that is, *Nucella* in induction treatments where the predator

was fed *Littorina* were only assayed with green crabs and no predator controls and they were not assayed with rock crabs.

Change in shell morphology

To determine how *Nucella* from different habitats alter shell morphology in response to predator and predator diets, shell mass and body mass were predicted using a nondestructive technique (see Palmer 1982 for detailed process). After the initial behavioral assay, I attached apiary tags to each *Nucella* with cyanoacrylate adhesive to uniquely identify them. Then, prior to induction, each *Nucella* was weighed submerged in seawater using an Ohaus SP602 balance readable to 0.01 g. Each snail was then allowed to dry for 30 m and coaxed back into its shell with an absorbent tissue to collect any residual water and then re-weighed dry. Actual shell mass was predicted from submerged mass using regressions from a destructive sampling of *Nucella* from all populations (Palmer 1982). As with other experiments utilizing this method (Burrows and Hughes 1990, Freeman and Hamer 2009), regression curves were highly significant ($R^2 < 0.99$). Upon completion of the 45 d induction period, each snail was re-weighed to compare the change in body and shell mass between predator treatments, predator diets, and habitats.

Statistical analysis: behavioral assay to measure activity

Initial behavioral response was analyzed using a two-factor ANOVA with predator (green crab, rock crab, and no-predator controls) and habitat (wave-exposed and wave-protected shorelines) as main, fixed effects. As a significant interaction was present, so predator and habitat combinations were condensed into a single variable and used a simple main effects one-factor ANOVA design. Pair-wise differences were

compared using Tukey-Kramer *post hoc* tests. Induced behavioral response was analyzed using a 3-factor ANOVA with predator (green crab, rock crab, and no-predator controls), predator diet (*Nucella* and *Littorina*), and habitat (wave-exposed and wave-protected shorelines) as main, fixed effects.

Statistical analysis: foraging

The design of this experiment was not balanced because diet treatments were not applied to the no-predator controls. Therefore, in the initial analysis I combined predator and predator diet into a single factor ("predator x diet"). To measure how consumption changed a two-factor repeated measure ANOVA was used with "predator x diet" and habitat as fixed, main effects and week as a repeated measure. All tests met the assumptions of ANOVA (Sokal and Rohlf 1995). Repeated-measures analysis were tested for violation of sphericity using the Mauchly's *W* test in the "ez" package (Lawrence 2010) of R (R Core Development Team 2010).

Statistical analysis: change in shell morphology

To compare how morphological measures were influenced by long-term exposure to predation risk, final body mass and shell mass was subtracted from the initial body mass and shell mass. This standardized differences present in initial snail size. For both measures a 3-factor ANOVA was used, with predator, predator diet, and habitat as main, fixed effects. All models met the assumptions of ANOVA (Sokal and Rohlf 1995). In comparing induced behavioral response and morphology the number of replicates were not equal among treatments and I used Type III sum of squares to properly calculate the F- ratios (Sokal and Rohlf 1995).

RESULTS

Behavioral response to predation risk: activity

Behavioral responses to predation risk from *Nucella* from wave-exposed and wave-protected shorelines were compared using movement frequency as a proxy for risk response. Using a two-factor ANOVA with habitat (wave-exposed and wave-protected shores), and predator type (green crab, rock crab, and no-predator control) as fixed factors in the model, a significant interaction was found between habitat and predator ($F_{2, 252} = 7.37$, $P < 0.001$). Therefore, each habitat and predator treatment combination was compared in a simple main effects design ANOVA ($F_{5, 252} = 48.05$, $P < 0.001$). In the presence of a rock crab, both wave-protected and wave-exposed populations of *Nucella* significantly decreased their activity relative to the controls (Fig. 2.1). The strongest anti-predatory behavioral response was from wave-protected snails in response to green crabs, which was significantly different from all other treatments. Wave-exposed snails also responded to green crabs; however, their response was intermediate between wave-protected response to green crabs and both habitats response to rock crabs (Fig. 2.1).

Nucella behavior was reevaluated after the 45 d induction and *Nucella* responses compared: 1) green crab fed *Nucella*, 2) green crabs fed *Littorina*, 3) rock crabs fed *Nucella*, 4) rock crabs fed *Littorina*, or 5) a no-predator control. The behavior of induced was qualitatively similar to pre-induction *Nucella*. A three-factor ANOVA was used with habitat (wave-exposed and wave-protected), predator type (green crab, rock crab, and no-predator control), and predator diet (*Nucella* and *Littorina*) as fixed factors in the ANOVA model (Fig. 2.2). There were no significant interactions and the only significant

main effect was predator type ($F_{2, 234} = 92.65$, $P < 0.001$). As the only significant main effect was predator type, a Tukey-Kramer *post hoc* test was used on this factor to explore pair-wise differences between treatments. Significant differences existed between green crab and no-predator controls, and rock crab and no-predator controls. No significant pair-wise difference occurred between green and rock crabs.

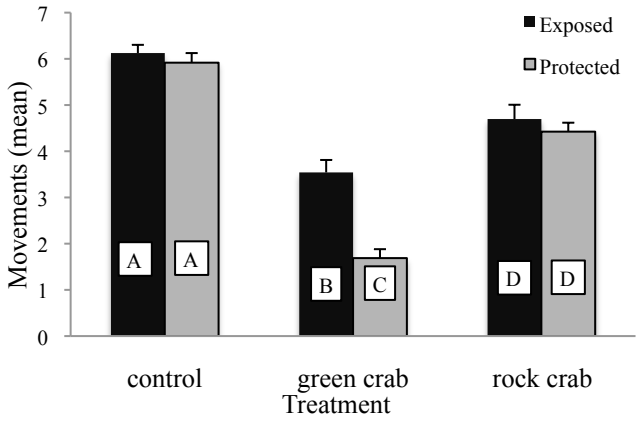

Figure 2.1 Mean (+ SE) response of *Nucella* from wave-exposed and wave-protected shorelines in response to green and rock crab chemical cues. There was a significant interaction between predator and habitat (one-factor nested ANOVA; $F_{2, 102} = 3.41$, $P = 0.03$, $n = 42$), so treatments were combined in a simple main effects design. Letters denote pair-wise differences as determined with a Tukey-Kramer post hoc test.

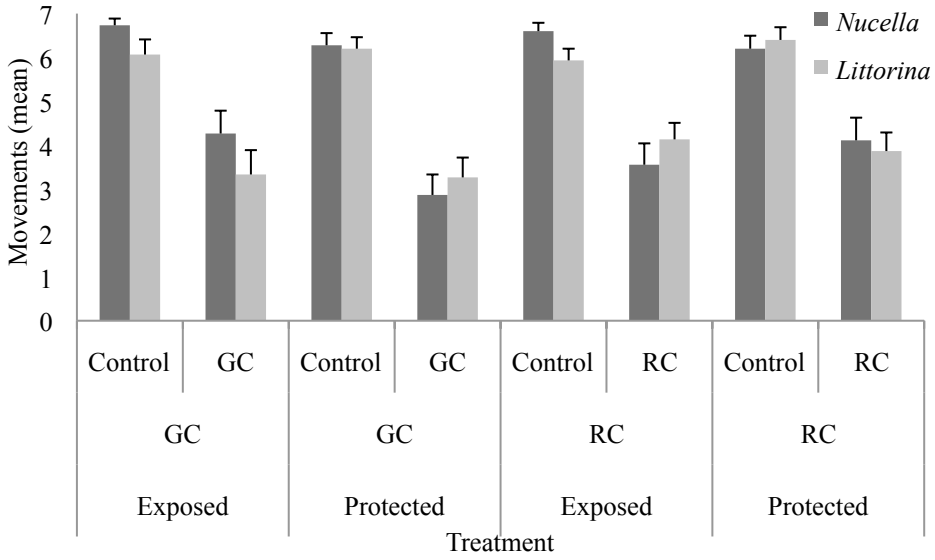

Figure 2.2 Mean (+ SE) response of *Nucella* from wave-exposed and wave-protected shorelines held in containers with green crabs (GC) or rock crabs (RC) maintained on diets of *Nucella* or *Littorina* for 45 d. There was a significant effect of predator type (three-factor ANOVA; $F_{2, 234} = 92.65$, $P < 0.001$, $n = 15$). Pair-wise differences were calculated using a Tukey-Kramer post hoc test, and all controls were significantly different from all predator treatments.

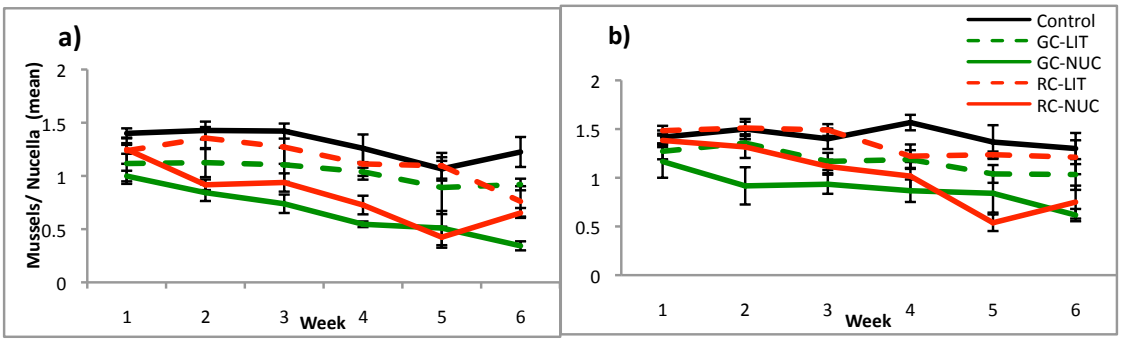

Figure 2.3 Mussels consumed per *Nucella* (Mean ± SE) from a) wave-exposed and b) wave-protected shorelines in response to chemical exudates from green (GC) and rock (RC) crabs maintained on a diet of *Nucella* (NUC) and *Littorina* (LIT) over a 45 d induction period ($n = 4$). There was a significant interaction between diet and week (three-factor ANOVA; $F_{20, 150} = 1.89$, $P < 0.001$), but not a significant effect of habitat. For clarity I separated wave-exposed and wave-protected habitats in this figure.

Foraging

Weekly, I measured consumption of mussels by populations of *Nucella* from different habitats held in chambers containing a no-predator control, green crabs or rock crabs maintained on diets of *Nucella* or *Littorina*. To maintain a balanced design, predator type and predator diet treatments were combined into a single factor (i.e., green crabs fed *Nucella)*, called "predator x diet" and used a repeated-measures ANOVA to compare "predator x diet" and habitat using week as the repeated measure. To test for sphericity, Mauchly's *W* test was used in the "ez" package (Lawrence 2010) in R (R Development Core Team 2010), and there was not a violation of sphericity (Mauchly's $W = 0.51$, $P = .17$). There was a significant interaction between "predator x diet" and week ($F_{20, 150} = 1.89$, $P < 0.001$). To determine if the rates of consumption were similar between habitats, another repeated-measures ANOVA was run, using only the no-predator controls and habitat as the fixed factor (Fig. 2.3). There was not a significant difference between habitat ($F_{1, 30} = 1.34$ $P = 0.094$), week ($F_{5, 30} = 2.39$, $P = 0.17$), or an interaction between the two factors ($F_{5, 30} = 1.26$, $P = 0.10$). Therefore, I dropped the no-predator control from the ANOVA model and compared differences between predator type and predator diet. There was a significant interaction between predator diet and week ($F_{5, 120} = 2.76$, $P = 0.002$). When in the presence of predators consuming conspecifics, *Nucella* consumed fewer mussels than when predators consumed *Littorina*.

Morphological response to predation risk

In response to predator exudates, *Nucella* are known to induce thicker, heavier shells. After a 45 d exposure to exudates from green, and rock crabs maintained on diets of *Nucella*, or *Littorina*, and a no-predator control, body mass differed significantly based upon "predator x diet" ($F_{4, 487}$ == 23.06, $P < 0.001$), and habitat ($F_{1, 487} = 4.55$, $P < 0.03$), yet there was not a significant interaction between these factors ($F_{4, 487} = 1.31$, $P = 0.26$, Fig. 2.4). Similarly, shell mass differed based upon "predator x diet" ($F_{4, 487} = 16.18$, $P < 0.001$), and habitat ($F_{1, 487} = 17.00$, $P < 0.001$) but there was not a significant interaction between the factors ($F_{4, 487} = 1.16$, $P = 0.32$). As controls did not vary between the two factors, were dropped from the ANOVA model and compared the treatment effects using a three-factor ANOVA with predator type, predator diet, and habitat as fixed, main effects. In both shell ($F_{1, 384} = 50.23$, $P < 0.001$) and body mass ($F_{1, 384} = 66.33$, $P < 0.001$) there was a significant effect of diet and habitat (shell mass $F_{1, 384} = 19.74$, $P < 0.001$; body mass $F_{1, 384} = 8.60$, $P = 0.003$); however, there were no significant interactions, or a significant effect of predator type. Comparing each predator diet separately, *Nucella* responded to the presence of predators by growing less shell and body mass when in the presence of predators consuming *Nucella*, whereas in the presence of crab predators consuming *Littorina*, they grew more shell and body mass. In response to both predator diets, wave-exposed populations of *Nucella* grew less shell and body mass compared to *Nucella* from wave-protected populations.

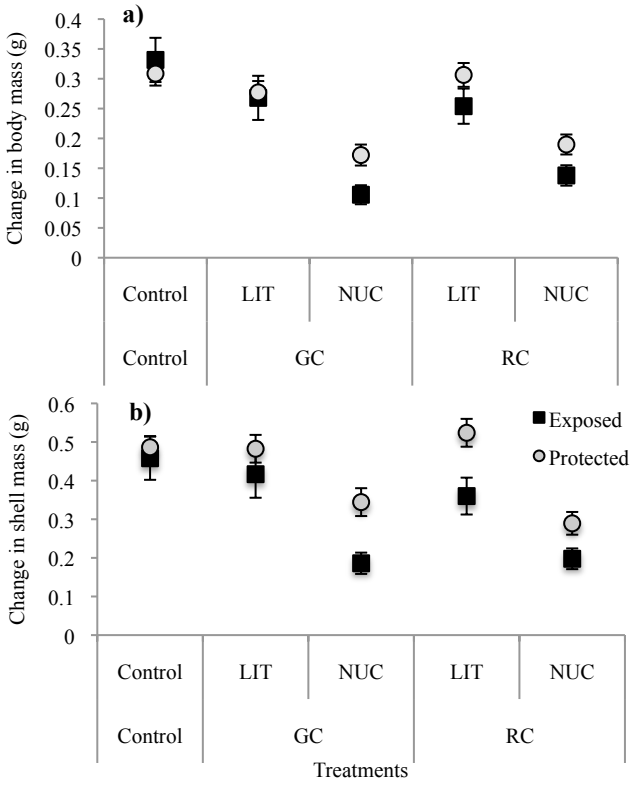

Figure 2.4 Change in *Nucella* a) body and b) shell mass from wave-exposed and wave-protected shorelines over a 45 d induction period in response to green (GC) and rock (RC) crabs maintained on diets of *Littorina* (LIT) *Nucella* (NUC). Significant differences were present between habitat (Two separate two-factor ANOVAs; shell mass: $F_{1, 487} = 17.00$, $P < 0.001$; body mass: $F_{1, 487} = 4.55$, $P = 0.03$) and "predator x diet" (shell mass: $F_{4, 487} = 16.18$, $P < 0.001$; body mass: $F_{4, 487} = 23.61$, $P < 0.001$) for both shell and body mass, however, there was not a significant interaction for either (shell mass: $F_{4, 487} = 1.16$, $P = 0.32$; body mass: $F_{4, 487} = 1.31$, $P = 0.26$). Exposed: Control, n = 45; GC-LIT, n = 39; GC-NUC, n = 45; RC-LIT, n = 40; RC-NUC, n = 43. Protected: Control, n = 60; GC-LIT, n = 55; GC-NUC, n = 57; RC-LIT, n = 59, RC-NUC, n = 54.

DISCUSSION

To minimize predation risk, prey use multiple strategies, such as decreasing activity (Vadas et al. 1994, Large and Smee 2010), reducing foraging behavior (Appleton

and Palmer 1988, Aschaffenburg 2008, Freeman et al. 2009), altering habitat selection (Turner and Mittelbach 1990), or developing (Relyea 2001a, Relyea 2001b) a more predator resistant morphology (Vermeij 1982, Appleton and Palmer 1988, Palmer 1990, Relyea and Werner 2000). Predator avoidance behaviors are costly, reducing prey growth, fitness, and competitive ability (Kats and Dill 1998, Nakaoka 2000, Relyea and Werner 2000, Relyea 2001a, Relyea 2001b). Prey use plastic responses to predators to limit costly avoidance strategies to situations where predators pose significant risk of injury or death.

If plastic responses are to be effective, prey must reliably detect and respond to cues indicative of predation risk. In aquatic environments, prey frequently use chemical cues to detect and evaluate risk (reviewed by Chivers and Smith 1998, Ferrari et al. 2010), perhaps because chemical cues provide the most reliable indication of predator presence. That is, predators may be able to reduce activity and hide or disguise visual or mechanical signals, and may restrict their own emission of dissolved compounds through morphological or behavioral adaptation, but it is unlikely they are able to completely avoid releasing metabolites through waste products or body secretions (Brown et al. 2000). Moreover, chemical signals include variation in both chemical components and ratios, making a virtually infinite number of distinct signals prey may use to detect predatory threats (Buck 1996).

In this study, predator diet was important in some aspects of prey anti-predator response but not others. To determine how prey respond to differing degrees of predation risk, many studies have exposed prey to a variety of chemical cues such as conspecific

alarm cue, injured con- or heterospecifics, predators, and different predators fed different diets (Chivers and Smith 1998, Kats and Dill 1998, Ferrari et al. 2010). While all of these cues can be indicative of risk, prey responses to each tend to be highly context-dependent, and few studies have examined how changes in cue quality or quantity affect multiple prey responses. For example, in some instances prey show sensitivity to variation in predator diet (Palmer 1990, Chivers et al. 1996, Relyea and Werner 2000, Turner 2008), while in some cases they do not (Bryer et al. 2001, Smee and Weissburg 2006b, Large and Smee 2010), or may express different types of responses for different predators (Freeman and Hamer 2009). Large and Smee (2010) found *Nucella* did not alter their activity level in response to risk cues emanating from Jonah crab (*C. borealis*) or rock crabs (*C. irroratus*), while Freeman and Hamer (2009) and Aschaffenburg (2008) both found *Nucella* consumption rates to drop significantly in response to both of these predators. The type and nature of cues, as well the type of prey response measured, can affect the interpretation of results, which may partially account for different conclusions drawn from studies using *Nucella*.

Relative risk of predator

Predator-induced defenses can mirror the dangerousness of predators (Bourdeau 2009, Hettyey et al. 2011). In this study I used two generalist predators, and the risk associated with each predator was dependent upon the likelihood *Nucella* would encounter each predator. Green crabs likely pose the largest threat to *Nucella* since they both inhabit the intertidal zone. In the initial assay, the strongest behavioral response observed was to this predator was (Fig. 2.1). Rock crabs generally occur subtidally, and

there was a slight, albeit significant, decrease in activity compared to the no-predator controls. In short-term behavioral assays using movement frequency as a metric for response to predators fed mixed diets, *Nucella* significantly reduced their movement frequency in the presence of both rock and green crabs as compared to controls (Fig. 2.1). Wave-protected populations decreased their activity in response to green crabs more than wave-exposed populations, presumably because wave-protected populations experience significant risk from crab predators while wave-exposed populations do not. Both wave-protected and wave-exposed populations demonstrated a slight response to rock crab predators, but there were no differences between populations. As rock crabs are generally found in the subtidal zone, *Nucella* from both shorelines likely do not frequently encounter this predator. Therefore, prey populations exposed to higher levels of predation risk may show more sensitivity and stronger responses to risk cues compared to populations were predation pressure is lower (Chivers and Smith 1998, Smee and Weissburg 2008, Edgell 2010). However, after the 45 d induction experiment, responses to predators fed mixed diets of *Nucella* and mussels were not significantly different between populations with both wave-protected and exposed *Nucella* populations reducing movement to both predators. Therefore, *Nucella* response to predator risk cue was variable based upon the response measured.

Foraging behavior, defined as the number of mussels consumed, was not significantly different between wave-protected and wave-exposed populations, nor was mussel consumption different in the presence of rock vs. green crabs. Instead, predator diet produced the only significant effect (Fig. 2.3). *Nucella* from both populations

consumed significantly fewer mussels and produced less body and shell mass when exposed to predators consuming conspecific *Nucella* than the heterospecific snail *Littorina*. After the 45 d induction period, there was a significant effect of habitat and "predator x diet" in change of morphology. Wave-protected populations grew less body and shell mass only in response to conspecific-fed predators while *Nucella* from wave-exposed populations grew less shell mass in response to both predators, albeit the strongest effect was still to conspecific fed predators (Fig. 2.4). Larger morphological changes noted in wave-exposed populations were somewhat surprising given that wave-protected populations had a larger decrease in activity in behavioral assays. Wave-exposed populations may show a much stronger morphological change in response to predator exudates because they have not previously been exposed to risk cues, while *Nucella* from wave-protected shores might have already initiated morphological changes due to higher ambient predator cues in these areas.

Predator diet

Some prey species limit reactions to predators only when predators have recently eaten conspecifics (Chivers et al. 1996). These situations occur when predator diet can reliably indicate predator risk, such as when predators switch between prey seasonally (Chivers and Mirza 2001b). *Nucella* exposed to predators consuming conspecifics foraged and grew less than predators consuming heterospecifics (Figs. 2.3 and 2.4), but different diets did not influence *Nucella* behavioral responses to either predator. Large and Smee (2010) found that in short-term behavioral assays *Nucella* did not respond differently to green crabs fed diets of *Nucella* vs. mussels. However, crushed conspecifics

did elicit a strong anti-predatory behavioral response, but crushed heterospecifics and mussels did not (Large and Smee 2010). Since both rock and green crabs reduced *Nucella* foraging behavior (Fig. 2.3) and injured conspecifics can limit activity (Large and Smee 2010), I hypothesized that in induction treatments with green crabs consuming *Nucella*, the experimental *Nucella* were receiving cues both form predators and injured conspecifics simultaneously. This combined cue may have introduced a synergistic cue larger than green crab or crushed conspecifics separately (Bourdeau 2009, Ferrari et al. 2010). More research should explore how combined cues influence prey anti-predatory behavior and morphology (but see Bourdeau 2009).

Non-consumptive predator effects

Numerous studies during the past two decades have shown predators exert significant effects on prey populations and entire communities through non-consumptive mechanisms including reducing the foraging rates (Palmer 1990, Freeman and Hamer 2009) and habitat selection of prey (Turner and Mittelbach 1990). Recent reviews suggest that non-lethal predator effects exert equal or larger effects than those of direct consumption (Preisser et al. 2005, Preisser et al. 2007). For non-lethal predator effects to occur, prey must detect and respond to cues emanating from predators or other indicators of predation risk (e.g., injured conspecifics). Surprisingly, few studies have attempted to empirically test how changes in the type or nature of cues or cue delivery would affect the occurrence and magnitude of non-lethal predator effects (Relyea 2001a, Turner and Montgomery 2003, Smee and Weissburg 2006a, b, Smee and Weissburg 2008, Turner 2008, Large and Smee 2010, Large et al. 2011). Non-lethal predator effects can vary

depending upon the type and quantity of risk cues used as well as the metric used to quantify non-lethal predator effects (i.e., changes in activity, foraging, and morphology).

Conclusions

Aspects such as previous exposure to predation risk, predator diet, and the relative risk of a predator can influence multiple aspects of prey behavior and morphology. Additionally, each of these factors does not act independently, and measuring a single factor or a single response might misrepresent if and to what degree prey react to predators after detection of chemical cues indicative of risk. Measuring non-lethal predator effects in nature is important, but may be affected by many factors including predation pressure in study animals, quality and quantity of cue source, cue delivery, and the type of prey response(s) measured. Future studies that seek to understand and predict the occurrence and magnitude of non-lethal predator effects in nature should empirically test these factors.

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Chapter 3 Environmental conditions influence the frequency of prey responses to predation risk

ABSTRACT

Predators can strongly influence prey populations and the structure and function of communities by altering the foraging behavior and/or habitat selection of prey. For these non-lethal predator effects to occur, prey must be able to detect and respond to cues indicating predation risk. The ability of prey to detect and respond to predator signals likely varies with environmental conditions. To better understand how the environment can modify non-lethal predator effects by influencing the frequency of prey responses to predators, I examined how hydrodynamic conditions influence predator avoidance behavior in the dogwhelk (*Nucella lapillus*), a carnivorous snail found on rocky intertidal shores. When confronted with predation risk, such as that signaled by water-borne chemical cues released by predatory green crabs, *Nucella* often reduce their movement and foraging activity. Using laboratory flumes, I explored how flow velocity and turbulence influenced *Nucella* responses to predator risk cues. The influence of hydrodynamic conditions on predator avoidance behavior was nonlinear. *Nucella* responded to predators most frequently in intermediate flow velocities but less in high and low velocities, suggesting that the effects of flow on predator avoidance behaviors are complex. Abiotic factors like flow can strongly influence the

behavioral responses of intermediate consumers, which may propagate to other trophic levels via trait-mediated trophic cascades.

INTRODUCTION

In the presence of cues signaling predation risk, prey often decrease foraging behavior, increase refuge use, and/or alter their habitat selections to reduce their vulnerability to consumers (Trussell et al. 2003, Turner and Montgomery 2003, Werner and Peacor 2003, Grabowski 2004, Valeix et al. 2009). Responding to risk may reduce prey growth or fecundity (Harvell 1990, Palmer 1990, Kats and Dill 1998, Nakaoka 2000, Bernot and Turner 2001) and have community level effects by generating trait-mediated trophic cascades that have a positive effect on the prey's resources (e.g., Trussell et al. 2003, Grabowski 2004). Thus, understanding how prey evaluate and respond to predation risk have been a key focus of behavioral and community ecology (reviewed by Werner and Peacor 2003, Preisser et al. 2005). While many studies have examined the types of signals prey use to detect and evaluate risk (reviewed by Chivers and Smith 1998, Kats and Dill 1998, Werner and Peacor 2003), few studies have explored how environmental conditions influence prey behavioral responses to predation risk by altering prey detection capability (but see Malmqvist and Sackmann 1996, Abrahams and Kattenfeld 1997, Smee and Weissburg 2006a, Smee and Weissburg 2008).

Predator avoidance behaviors are often costly (Harvell 1990, Palmer 1990, Kats and Dill 1998, Nakaoka 2000, Bernot and Turner 2001), and thus there is a premium on prey being able to use reliable sensory cues that accurately reflect risk levels before initiating predator avoidance strategies (Kats and Dill 1998). In aquatic systems,

predators and prey often detect one another via reciprocal detection of waterborne chemical cues (Zimmer and Butman 2000, Weissburg et al. 2002, Zimmer and Zimmer 2008). As these chemical signals are propelled by currents, hydrodynamic forces such as flow velocity and turbulence can influence the structure of chemical odor plumes (Weissburg 2000, Webster and Weissburg 2001), as well as the perception of the odor plumes by predators (Weissburg and Zimmer-Faust 1993, Powers and Kittinger 2002, Weissburg et al. 2003, Ferner et al. 2005, Ferner and Weissburg 2005) and prey (Smee and Weissburg 2008, Smee et al. 2008). Therefore, in chemically mediated predatory interactions, hydrodynamic conditions likely dictate the strength and frequency of prey responses to predators by altering their perceptive ability (Smee and Weissburg 2008, Smee et al. 2008, Smee et al. 2010).

Here I examine how hydrodynamic conditions affect the responses of an intermediate consumer to predation risk. In rocky intertidal systems, green crabs (*Carcinus maenas*) initiate trophic cascades by either consuming *Nucella lapillus* (dogwhelks, hereafter *Nucella*) or causing changes in foraging activity and refuge behavior of *Nucella* (e.g. increased use of refuge habitats) (Trussell et al. 2003, Trussell et al. 2006b). In both scenarios, green crabs can have positive indirect effects on resources of *Nucella* (mussels and barnacles), but recent evidence suggests that the non-lethal effects of green crabs are more important (Trussell et al. 2006b). Because *Nucella* clearly respond to chemical signals emanating from predatory green crabs, they are an excellent model system to explore how hydrodynamics affect assessment of predation risk and subsequent foraging decisions. Flow conditions strongly influence *Nucella*

responses to green crab predators and that the environment can may have complex effects on the role that non-lethal predator effects play in these communities.

MATERIALS AND METHODS

General protocol

In the presence of green crab predators, *Nucella* decrease their activity (Vadas et al. 1994). Therefore, *Nucella* movement frequency was used as a proxy for response to perceived predation risk. Behavioral assays were conducted in flumes at the Darling Marine Center (DMC) in Walpole, Maine and at Texas A&M University—Corpus Christi (TAMU-CC) in Texas. The effects of hydrodynamic conditions on *Nucella* behavior was measured by comparing the frequency of *Nucella* movements in the presence vs. absence of green crab risk cues. The flume at the DMC is useful for behavioral investigations because animals can be collected from nearby study sites and readily assayed with minimal disturbance. However, the DMC flume is incapable of producing flows above 8.0 cm s^{-1}, so I used the TAMU-CC flume, which is capable of producing the higher flow velocities necessary for a portion of my experiments.

Hydrodynamic environments: DMC flume

Behavioral assays were conducted in a flow-through laboratory flume (2.2. m L, 0.53 m W, 0.1 m D) at the DMC that reliably reproduced free-stream flow velocities between 3.0 cm s^{-1} and 8.0 cm s^{-1} (see Smee and Weissburg 2008 for detailed flume description). Ceramic tiles lined the entire bottom of the flume to simulate the natural rocky substratum typically occupied by *Nucella* in the field. Flow-through seawater pumped from the Damariscotta River was delivered to the flume and then released back

into the river. The Damariscotta River is a well-mixed estuary and during the summer months there is little variation in both salinity (32-34) and temperature (10-15°C).

Hydrodynamic environments: TAMU-CC flume

Behavioral assays were also conducted in a re-circulating laboratory flume at TAMU-CC. The flume was 4.25 m long, 0.75 m wide and was able to reliably reproduce free-stream flow velocities between 0.5 cm s^{-1} and 25 cm s^{-1} at a water depth of 20 cm. Ceramic tiles identical to those used in the DMC flume were used to form the substratum. The flume was filled with seawater drawn from a local estuary that had passed through sand, UV, and carbon filtration systems as well as a 50.0 μm biological filter before it entered the flume. Water was chilled to ~13°C and salinity was maintained at ~32, values that are similar to that experienced by organisms in the DMC flume.

Hydrodynamic methods

Flow conditions were measured in both flumes to ensure behavioral assays were conducted in similar and reproducible flow regimes. Flow was measured in each flume using an acoustic Doppler velocimeter (ADV; Vectrino model, NortekUSA™, Annapolis, MD) and vendor-supplied software. Free-stream flow velocity was measured 7 cm and 15 cm above the substratum in Maine and Texas respectively for 5 min at a sampling rate of 10 Hz for each flow condition. Flow velocity and turbulence were also measured 3.0 cm above the substratum in each flume to quantify the near-substratum hydrodynamic conditions experienced by *Nucella*. Previous authors have used this height as it is typically within the boundary layer, which is likely used for chemosensory

sampling (e.g., Smee and Weissburg 2006a). As above, flow velocity was measured for 5.0 minutes at 10 Hz at this height.

ADVs measure three-dimensional flow velocity, and the net flow velocity (U) was calculated using the formula $U = \sqrt{(u^2+v^2+w^2)}$ where u, v, and w are the velocity components in the x, y, and z dimensions, respectively. Turbulence was calculated using the root mean square (RMS) of the velocity time series. As with flow velocity, RMS was combined in the x, y, and z dimensions for each 5 min measurement period using the formula $RMS = \sqrt{(RMS_u^2+RMS_v^2+RMS_w^2)}$ where these values represent the RMS levels in the x, y, and z dimensions, respectively.

Hydrodynamic environment in the field

Flow velocities were measured *in situ* using Vector model ADVs (NortekUSA™, Annapolis, MD) and vendor-supplied software at six different sites in the Damariscotta River to ensure that the velocity ranges and RMS measured in behavioral assays were similar to those experienced by *Nucella* in the field. Flow velocities in the field ranged from ~0 at slack tide to 1.2 m s^{-1} and RMS ranged from ~0 to 0.17 m s^{-1}, which are similar to values reported by Leonard et al (1998).

Animal collection and care

Organisms used in behavioral assays were collected from the Damariscotta River, ME and held in flowing seawater tables at the DMC. Green crabs (*Carcinus maenas*) were captured using lobster traps, scuba, and recreational crab nets and maintained on an *ad libitum* diet of *Nucella,* mussels (*Mytilus edulis*), and clams (*Mercenaria mercenaria*). *Nucella* were collected by hand and maintained on an *ad libitum* diet of mussels and

barnacles (*Semibalanus balanoides*). Water temperature ranged between 12-16°C and salinity remained at approximately 32 in the sea tables. *Nucella* were acclimated for 24 hours in sea tables and used in behavioral assays within one week of collection. Each snail was used in a single behavioral assay before being returned to the river, except for those organisms used as food for green crabs. Green crabs were fed and acclimated for at least 48 hours before being used in behavioral assays and were used within two weeks of collection. Green crabs were only used in a single assay before being released.

For experiments conducted in Texas, green crabs and *Nucella* were collected from the Damariscotta River and shipped overnight in refrigerated containers to TAMU-CC. They were then housed in insulated tanks with filtered and circulating seawater chilled to approximately 13°C. Green crabs and *Nucella* were similarly housed and fed in Texas. In all assays conducted at TAMU-CC, organisms were used in a single assay and were then humanely euthanized and discarded in a land-based facility. TAMU-CC Institutional Animal Care and Use Committee (IACUC) approved this protocol.

Behavioral assay

The experimental area of each flume was lined with 15 x 15 cm ceramic tiles to mimic the rocky habitat encountered by *Nucella*. Tiles were spaced 1.5 cm apart to provide crevices similar to those in which *Nucella* are typically found in the field (Large *personal observation*). Because *Nucella* reduce movement and increase use of crevices or other refuge habitats in the presence of predation risk (Gosselin and Bourget 1989, Vadas et al. 1994, Trussell et al. 2003), movement was used as a proxy for risk response. Small *Nucella* (<20 mm, with a thin shell lip) are more vulnerable to crab predation than larger

snails (Hughes and Elner 1979, Vadas et al. 1994) and moved more frequently than larger individuals in preliminary assays. Hence, small *Nucella* were used in behavioral assays.

To begin the assay, *Nucella* were placed in the crevice between the tiles. A refuge habitat was selected as the starting location for three reasons. First, *Nucella* were commonly collected from crevices and other refuge habitats (e.g., mussel beds) in the field. Second, I wanted to determine if *Nucella* would leave a refuge habitat in the presence of predators. Third, starting *Nucella* in a refuge removes potential observational ambiguity. That is, if snails were not started in a refuge and found to be actively moving, it would not be possible to determine if the *Nucella* were unresponsive to the predator and foraging or detecting the predator and seeking refuge. Thus, starting snails in a refuge allowed us to assess *Nucella* response to predators as well as mimic the location these animals were most often collected from in the field.

In each assay, three *Nucella* were placed into a crevice within the experimental area and allowed to acclimate for 5 min. After the 5 min acclimation period, *Nucella* were observed for 20 sec and snails were recorded as moving, or not. All observable activity including climbing from refuge, lifting or rotating their shells, or crawling within the crevice was scored equally. After the initial observation, a tethered predator, or the tethering apparatus without a predator (control) was introduced at a fixed distance upstream from the *Nucella* being observed. Observations were made for 20 sec at 5 min intervals for 30 min. Thus, each *Nucella* could have been observed moving a maximum of seven times during each assay.

Additional trials were performed in Texas to verify that behaviors of *Nucella* were similar between Maine and Texas after shipping. In these assays (u= 4 cm s^{-1}), I compared *Nucella* responses to controls and predators with assays performed in Maine and Texas using a two-factor ANOVA with experimental location (Maine or Texas) and risk level (crab present or absent) as the main effects. These data met ANOVA assumptions. No significant differences in *Nucella* behaviors in Maine and Texas were found so the behavioral data from both flumes were combined for subsequent analyses (Fig. 1.2).

Data collection and analysis

In the Damariscotta River, *Nucella* were usually found in groups throughout the intertidal zone (Large, *personal observation*). Therefore, groups of *Nucella* were used in behavioral assays. To insure that interactions between individual *Nucella* did not bias results, a series of assays were performed with a single vs. group of *Nucella.* The responses of individual *Nucella* to the presence of green crabs was compared to those exhibited by groups of *Nucella* (three *Nucella* per group) using a two-factor ANOVA where risk level (predator or control) and prey density (one or three *Nucella*) were fixed factors (Sokal and Rohlf 1995). These data met ANOVA assumptions. There was a significant effect of risk but no significant density or interaction effects. The lack of a significant conspecific density effect suggests that interactions between *Nucella* did not affect their response to predation risk. Therefore, each *Nucella* was treated as an independent replicate in behavioral assays (Fig. 1.3).

Response of Nucella *to predators in differing flow conditions*

Flow velocity and turbulence can affect the advection of chemical signals and the distance over which prey respond to risk (Webster and Weissburg 2001, Smee and Weissburg 2006a, Smee et al. 2008). To determine the effect of flow velocity on *Nucella* behavioral responses to predators, the behavioral assays described above were performed in different flow regimes. Green crabs were presented to *Nucella* as a predator stimulus in five different flow velocities (u): u = 0, 4, 8, 12, and 20 cm s^{-1}, all of which are within the natural range experienced by *Nucella* (Leonard et al. 1998, Large, unpublished data). For each flow velocity, one male green crab (CW = 75.7 mm SE= 5.0 mm) was placed at one of two fixed distance, 0.5 or 1.0 m, upstream from the *Nucella*. *Nucella* distance from predator cue served as a different level of risk where 0.5 m was considered high risk, 1.0 m as low risk, and a no-predator control as no risk. For each flow velocity, *Nucella* responses to three risk treatments (i.e., no, low, high) were replicated at least 10 times (three *Nucella* per trial) with treatments randomly interspersed.

As flow velocity increases, turbulence also increases. In high velocity trials, turbulent mixing of odor plumes or a faster advection rate of chemical signals may have affected *Nucella* perceptive ability. To determine if *Nucella* perceptive ability is altered by higher flow velocity or increased turbulence, the substrate roughness was increased to generate turbulence in slower flows, thereby decoupling turbulence from flow velocity (see Weissburg and Zimmer-Faust 1993, Jackson et al. 2007 for discussion). Gravel (2.5 cm SE= 0.25 cm) was placed in the flume in lieu of ceramic tiles to create a longer hydraulic roughness length. Flow was maintained at an intermediate level of u = 8 cm s^{-1}.

By increasing the sediment roughness, turbulence was increased while maintaining equivalent flow velocity (Table 3.1).

Table 3.1 Flow conditions measured in Maine (DMC) and Texas (TAMU-CC) flumes.

Flume	Substrate	Free-stream (cm s^{-1})	RMS (cm s^{-1})
DMC	tile	0	0
DMC	tile	3.8	0.65
DMC	tile	7.2	0.92
DMC	gravel	6.9	1.15
TAMU-CC	tile	3.6	0.47
TAMU-CC	tile	8.67	0.62
TAMU-CC	tile	12.5	0.86
TAMU-CC	tile	19.4	2.66

A flow treatment was defined as a set of behavioral assays performed at one flow velocity and over one substrate type. Responses of *Nucella* in the six flow treatments were compared using a two-factor ANOVA with flow treatment and risk level (none, high, low) as fixed factors (Sokal and Rohlf 1995). A significant interaction was detected between flow and risk, so risk level was compared within each flow treatment using a simple main effects test to ascertain the variation in risk responses at a given flow. This was necessary because *Nucella* movement decreases in fast flows, and this analysis allowed us to compare differences in predator avoidance responses between risk levels at each flow condition and avoid ambiguity of a decrease in movement caused by predator detection vs. reduced movement due to hydrodynamic forces (e.g., drag). For each flow treatment, variance between risk levels was compared with nested one-way ANOVAs

with each risk level (no, high, low) as a fixed factor. Each set of flow treatments were conducted at different points in time and no predator controls were interspersed within all risk treatments. This approach allowed me to precisely measure how *Nucella* response to risk is affected by flow velocity and/or turbulence. Some ANOVAs did not yield significant differences, and *post hoc* analysis of power for each one-factor ANOVA was performed to minimize risk of Type II error using G*Power (Faul et al. 2007). All data met ANOVA assumptions of normality and equal variances. Pair-wise differences in treatments were compared using Tukey-Kramer post hoc tests (Sokal and Rohlf 1995).

The lack of a significant prey density effect on *Nucella* response to risk suggested that interactions between *Nucella* did not affect their responses. Therefore, a nested ANOVA (see Smee and Weissburg 2006a) was used to compare the effects of predator treatment and trial nested within treatment on the number of *Nucella* movements (Sokal and Rohlf 1995). A nested ANOVA was used to show if variations in *Nucella* responses were affected by variability in cue quality or quantity across replicate treatments, which is a source of uncontrolled variation in the experiments. The P-value for the nested effect was greater than 0.25 in all experiments, indicating that *Nucella* in different groups were reacting similarly to the same treatments. The lack of a significant nested effect enabled individual snail responses to be grouped within treatments to test the significance of the main effect using the pooled error variance (Sokal and Rohlf 1995). The absence of a nested effect suggests that cues from predators and *Nucella* responses were not significantly different between replicate trials. Since the nested effect was not significant nor was *Nucella* behavior when assayed individually or in groups, individual snail

responses were treated as independent replicates. All statistical analyses were performed using SPSS software for Windows (SPSS 2005), and all data met assumptions of ANOVA.

RESULTS

Hydrodynamic conditions

All flow velocities and RMS values were within the range *Nucella* experience in the field and roughly similar between Texas and Maine flumes (see Table 3.1). As expected, turbulence increased with flow velocity and substrate coarseness.

Behavior in Maine and Texas

Nucella responses to controls and predators were not significantly different regardless of whether assays were performed in Maine or Texas (Fig. 1.2). *Nucella* moved significantly less in the presence of green crabs ($F_{1, 89} = 9.48$, $P < 0.01$), but flume location had no effect ($F_{1, 89} = 0.45$, $P = 0.50$) and there was no interaction between these factors ($F_{1, 89} = 0.30$, $P = 0.58$). Thus, I did not consider assay location in subsequent analyses.

Density

I compared grouped and individual *Nucella* movements in the presence of a tethered green crab predator and a no-predator control to verify that *Nucella* reactions to consumers were independent. The number of observed movements for each snail was treated as an individual measurement. The presence of a green crab caused a significant reduction in *Nucella* movement (Fig. 1.3, $F_{1, 67} = 14.83$, $P < 0.001$), but effects of *Nucella* density ($F_{1, 67} = 0.003$, $P = 0.96$) and interactive effects between density and risk ($F_{1, 67} =$

0.12, P = 0.73) were not detected (Fig. 1.3). Thus, interactions between *Nucella* were not influencing their reactions to green crab predators.

Behavioral Response to Predators in Differing Hydrodynamic Conditions

A two-factor ANOVA revealed a significant interaction ($F_{10, 677} = 6.68$, $P < 0.001$; Fig. 3.1) between the effects of flow treatment ($F_{5, 677} = 15.42$, $P < 0.001$) and risk level ($F_{2, 677} = 29.98$, $P < 0.001$). To tease apart these effects, I compared risk levels within each flow condition using one-factor ANOVAs.

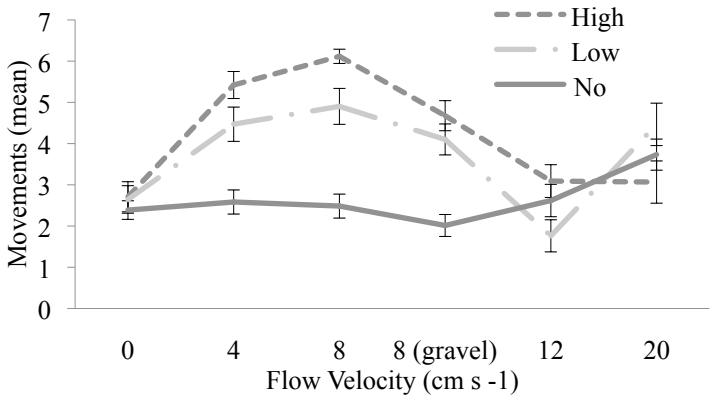

Figure 3.1 Mean number (+ SE) of *Nucella* movements in response to controls and green crabs in all flow treatments and risk levels. A two-factor ANOVA was used to compare *Nucella* behavioral responses to differing levels of predator risk (no predator, predator 0.5 m upstream, and predator 1.0 m upstream), in differing flow treatments using a two-factor ANOVA. Risk level ($F_{5, 677} = 15.42$, $P < 0.001$, $n = 15$) and flow treatment ($F_{2, 677} = 29.98$, $P < 0.001$, $n = 15$) were significant, as was the interaction between risk and flow ($F_{10, 677} = 6.68$, $P < 0.001$, $n = 15$).

In no flow (Fig. 3.2a, $u = 0$ cm s^{-1}), there was no significant difference in *Nucella* behavior between predator risk levels and the control ($F_{2, 139} = 0.38$, $P = 0.68$, $1-\beta = 0.99$).

Similarly, in 12 cm s^{-1} (Fig. 3.2e, $F_{2, 72} = 2.76$, $P = 0.07$, $1-\beta = 0.99$) and 20 cm s^{-1} (Fig. 3.2f, $F_{2, 57} = 1.78$, $P = 0.17$, $1-\beta = 0.99$) there were no significant differences in *Nucella* behavior among the no, low, and high risk treatments. In the slower flow treatments, compared to no-predator controls, risk significantly reduced the frequency of *Nucella* movements, regardless of the distance the green crabs were placed upstream. Differences between risk conditions, as determined by Tukey-Kramer *post hoc* tests are reported (Fig 3.2).

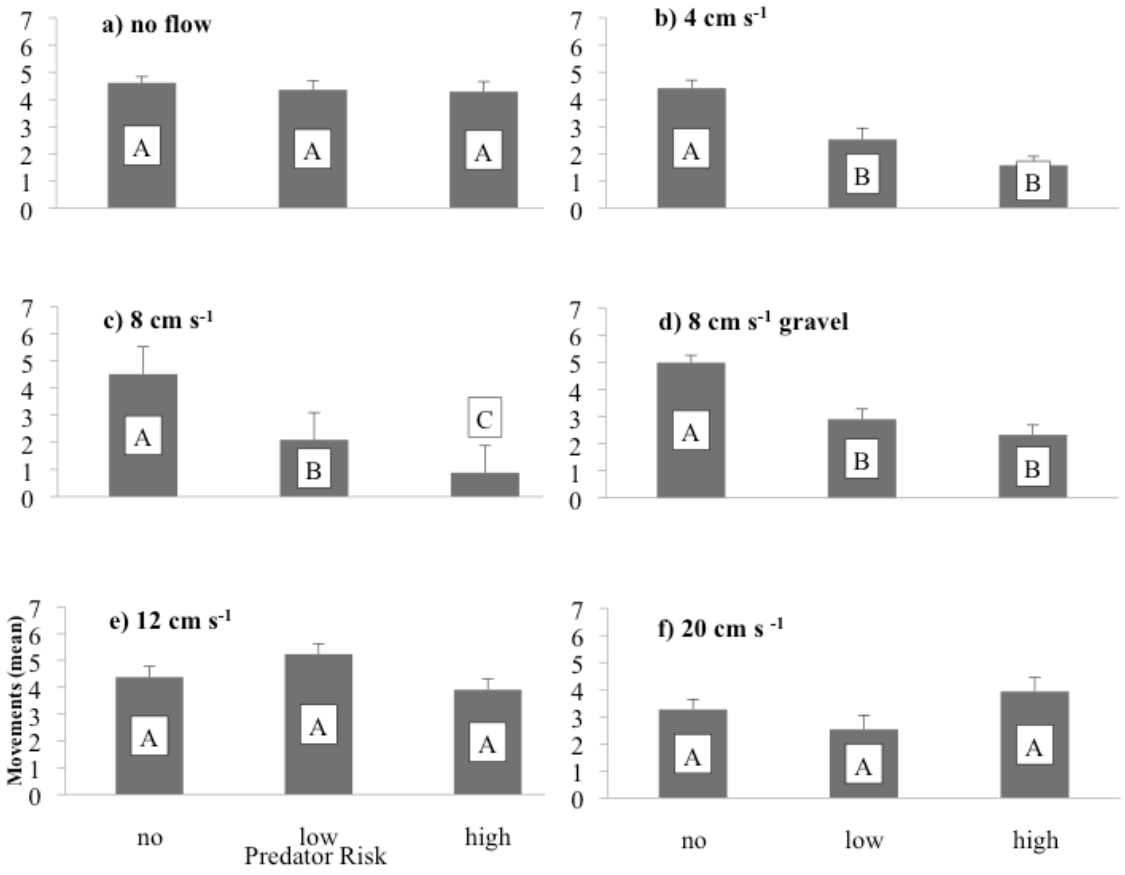

Figure 3.2 Mean number (+ SE) of *Nucella* movements in response to different risk levels in individual flow treatments. Each flow treatment is labeled and a one-way ANOVA was

used to compare *Nucella* responses to risk within that flow treatment. P-values are presented within text and letters denote significant differences ($P < 0.05$) in each flow treatment based upon Tukey-Kramer post hoc tests.

In the 8 cm s^{-1} flow treatment (Fig. 3.2c) over tile *Nucella* movement was reduced by approximately 50% in response to both high and low risk levels ($F_{2, 130} = 36.44$, $P < 0.001$). However, in slower (4 cm s^{-1}, Fig. 3.2b, $F_{2, 129} = 20.25$, $P < 0.001$) and more turbulent (8 cm $s^{-1}$with gravel, Fig. 3.2d, $F_{2, 133} = 20.85$, $P < 0.001$) flow conditions, *Nucella* did not respond differently between low and high predator risk levels. The fewest movements were observed in the highest risk level at 8 cm s^{-1} flow, suggesting that these flow conditions may be optimal for *Nucella* to detect and respond to predation risk.

DISCUSSION

Within a given system, environmental forces such as flow may play an important role in dictating how organisms detect and respond to risk and may ultimately affect the strength of emergent indirect predator effects on lower trophic levels (Post et al. 1999, Smee and Weissburg 2006a, Peckarsky et al. 2008). Factors such as predator identity (Turner et al. 2000, Bernot and Turner 2001, Relyea 2001b, Relyea 2004, Schmitz et al. 2004), habitat type (Trussell et al. 2006b) and complexity (Grabowski 2004, Grabowski and Kimbro 2005), and hydrodynamic conditions (Smee et al. 2008, Ferner et al. 2009) may influence the magnitude of prey responses. In this model system, flow significantly influenced the response of *Nucella* to predation risk across a relatively small range of flow conditions. Hence, the decision-making of intermediate consumers like *Nucella*

under predation risk can be significantly influenced by environmental conditions. The relationship between predator avoidance and flow velocity was nonlinear in this study, suggesting complex relationships between non-lethal predator effects and environmental conditions that enhance or attenuate the transmission of cues indicative of risk.

Turbulent flows strongly affect the advection of chemical odor plumes (Webster and Weissburg 2001) and the performance of organisms that use chemical signals to forage, find mates, and avoid predators (Weissburg and Zimmer-Faust 1993, Powers and Kittinger 2002, Ferner and Weissburg 2005, Smee and Weissburg 2006a, Vickers 2006, Jackson et al. 2007). Faster, more turbulent flows increase mixing of chemical signals, homogenize odor plumes, increase plume width, and decrease the range of concentration of odor filaments within the plume (Webster and Weissburg 2001, Rahman and Webster 2005, Jackson et al. 2007). By altering chemical signal structure, turbulent flows can affect the chemoreceptive abilities of organisms. For example, turbulence reduces the ability of blue crabs (*Callinectes sapidus*) to locate prey (Weissburg and Zimmer-Faust 1993, Powers and Kittinger 2002, Jackson et al. 2007). Similarly, green crab predation on *Nucella* declines sharply in fast flows, suggesting that green crabs may have a more difficult time foraging under these conditions (Leonard et al. 1998). Unlike crustaceans, some gastropods are more successful foragers in fast, turbulent flows (Powers and Kittinger 2002, Ferner and Weissburg 2005). For example, increased turbulence increases the foraging efficiency and success rates of knobbed whelks (*Busycon carica*) in the lab and field (Powers and Kittinger 2002, Ferner and Weissburg 2005, Ferner et al. 2009).

In this study, *Nucella* were most responsive to green crabs in intermediate flow velocities and turbulence levels. In the absence of flow, *Nucella* did not show significant behavioral responses to green crabs, presumably because advection of predator cues did not occur. Similarly, blue crabs are also unresponsive to chemical signals in the absence of flow (Weissburg and Zimmer-Faust 1993). Here, *Nucella* response to green crabs increased with flow until flow velocity and RMS exceeded 12 cm s^{-1} and 1.0 cm s^{-1}, respectively (Fig. 3.1). Previous research has shown that green crab predation on *Nucella* is highest in regions of slow flow (Leonard et al. 1998), and it was at such flow speeds that there was a significant reduction in *Nucella* movement in the presence of predators. Because faster flows tend to mix chemical signals, the increased behavioral response to predators at intermediate flows may, at first, seem counterintuitive. However, there are two possible mechanisms that may explain this observation. First, Weissburg (2000) proposed that slower moving animals, such as gastropods, might temporally average odor concentrations from turbulent odor plumes and forage more effectively in flow conditions that limit faster moving organisms like blue crabs and potentially green crabs. Alternatively, increased flow velocity and turbulence create a larger transfer of momentum in the form of eddies into the boundary layer. Such increased turbulence may deliver more predator cue to the substrate, which is closer to the primary chemosensory organs of *Nucella*. Regardless of the mechanism, like knobbed whelks, *Nucella* chemosensory performance is enhanced by moderate increases in turbulence.

When RMS exceeded 1.0 cm s^{-1}, *Nucella* ceased responding to green crabs and I propose two possible mechanisms to explain this finding. First, as turbulence increases,

the odor plume mixes such that it becomes undetectable to *Nucella*. Therefore, in the higher flow velocity and turbulence treatments, *Nucella* were not aware of the potential danger upstream. Conversely, green crab predation on *Nucella* declines sharply in fast flows (Leonard et al. 1998) and while *Nucella* may be aware of the danger, they continue to forage because the realized risk posed by these consumers is low in these conditions.

Prey responses to predators are often higher when predatory threats are first detected and may wane over time as prey are forced to accept riskier behavior to acquire sufficient energy for survival (Lima and Bednekoff 1999). The frequency of anti-predator behaviors observed may be larger in short-term experiments such as this study. Yet, flow still had significant effects on *Nucella* response to predators. Because predator avoidance tactics were most likely to be observed in this short-term behavioral experimental design, I attribute a lack of responses by *Nucella* in more turbulent flows to them being unable to detect chemical cues from potential predators. Additionally, while predator avoidance tactics are costly for prey, the benefits of surviving a predatory encounter clearly outweigh any short-term reduction in fitness (Dawkins and Krebs 1979, Chivers and Smith 1998, Kats and Dill 1998, Smee and Weissburg 2006a). Thus, the failure of *Nucella* to respond to green crabs in faster flows most likely results from their inability to detect predator signals in these flow conditions and not from *Nucella* detecting predators but electing to forage in faster flows. Future experiments will explore which of these mechanisms is responsible for changing the reaction of *Nucella* to predators. Regardless, these results clearly show that hydrodynamics can significantly influence how the intermediate consumer, *Nucella*, responds to predatory green crabs.

Prey responses to predators may vary between environments or habitats and be context dependent (Heithaus et al. 2009). For example, predators are much less common on wave-exposed shorelines of New England as compared to wave-protected habitats. *Nucella* from wave-exposed shorelines are less likely to respond to predatory threats than those from inland populations. Moreover, wave-exposed populations have thinner shells and larger feet that enable them prevent dislodgement by waves while wave-protect populations of *Nucella* possess thicker shells that help deter predators (Etter 1988, Freeman and Hamer 2009). Similarly, in the Damariscotta River, blue mussels found in low flow areas where predation pressure is highest have thicker shells and produce more byssal threads than do conspecifics in nearby high flow habitats where predation pressure is low (Leonard et al. 1998). Like this study, these examples suggest that predator effects on *Nucella* may vary with environmental conditions.

Along with earlier studies by Smee and Weissburg (2006a) and Smee et al. (2008), hydrodynamics can influence prey reactions to consumers. In other systems, environmental factors that differentially affect the transmission of visual, acoustic, or mechanical cues between predators and prey may similarly modify the frequency of prey responses to risk. I propose that measuring how environmental conditions affect the reciprocal responses of predators and prey will be important for understanding how predator effects propagate through natural communities.

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Chapter 4 Biogeographic variation in behavioral and morphological responses to predation risk

ABSTRACT

Prey often possess more defenses against predators based upon relevant predation pressure, but biogeographic patterns examining prey responses to predation risk are uncommon. I experimentally tested how a native rock crab (*Cancer irroratus*) and an invasive green crab (*Carcinus maenas*) predator influence behavioral and morphological defenses of a single prey (*Nucella lapillus*, hereafter *Nucella*) collected across a geographic range of ~230 km within the Gulf of Maine. Rock crabs are common on wave-protected shores throughout the Gulf of Maine, while green crabs are abundant only on southern, wave-protected shores. Both predators are absent from wave-exposed shorelines. *Nucella* responds to crab predators behaviorally by decreasing movement and increasing refuge use and morphologically by growing a thicker shell. I collected *Nucella* from wave-exposed and wave-protected shores in northern and southern latitudes and examined behavioral and morphological responses to both predators. All *Nucella* populations decreased activity and increased refuge use in the presence of both predators, but southern wave-protected populations reacted significantly more than other populations to green crabs. After continuous exposure to predator cues during a 45 d induction period, southern wave-protected populations produced thicker shells in response to green crabs only, northern wave-protected populations produced thicker

shells in response to rock and green crabs but to a lesser degree than southern individuals. Wave-exposed populations did not alter morphology in response to either predator. These findings demonstrate the importance of recognizing biogeographic patterns in predator and prey distributions, as non-lethal predator effects could vary between prey populations across their geographic range.

INTRODUCTION

Biotic processes such as predation are important community structuring forces but their intensity can vary across geographic ranges (Schemske et al. 2009). Biogeographic patterns have emerged showing that processes such as diversity (Fischer 1960, Mittelbach et al. 2007), herbivory (Coley and Aide 1991, Pennings et al. 2001, Long and Trussell 2007), and predation (Bertness et al. 1981, Smee and Weissburg 2008) are stronger in lower latitudes than higher latitudes. As predation intensity varies across geographic regions (Menge and Lubchenco 1981, Heck and Wilson 1987), differences in prey response to predation risk may also vary with risk levels and exhibit biogeographic patterns (Smee and Weissburg 2008). In lower latitudes where consumer pressure is higher, prey express increased morphological (Vermeij 1991), chemical (Bakus and Green 1974), or behavioral (Bertness et al. 1981, Fawcett 1984, Smee and Weissburg 2008) defenses compared to conspecifics or congeners in higher latitudes. Yet, prey defenses might also vary according to small-scale variation in predation intensity between habitats at the same latitude (Leonard et al. 1999, Moody and Aronson 2007, Freeman and Hamer 2009). Finally, distribution of predators may also vary according to

invasion history of non-native predators, thereby increasing variation in prey response to predation risk making biogeographic patterns difficult to distinguish.

Many recent studies have shown that by inducing changes in behavior or habitat selection of prey, predators can generate trophic cascades that affect entire communities (Preisser et al. 2005, Schmitz et al. 1997). These non-lethal predator effects are routinely considered to exert equal or larger effects in communities than those of direct consumption (Preisser et al. 2005). Yet, how variation in prey behavior among populations affects prey responses and resultant non-lethal predator effects have not been carefully considered (Bertness et al. 1981, Fawcett 1984, Smee and Weissburg 2008). To understand the occurrence and magnitude of non-lethal predator effects in nature, ecologists must understand how prey responses to predation risk vary in time and space.

In this study, I explored how prey from different geographic regions and habitat types within geographic regions respond to native and invasive predators. Using a model system with an invasive predator that differs in length of time present among different populations of prey (Trussell and Smith 2000), I hypothesized that prey from areas with higher predation rates would develop more acute responses to the risk of predation, and any resultant non-consumptive predator effects would also be more prevalent. Using a rocky intertidal model system consisting of a carnivorous snail, *Nucella lapillus* (hereafter, *Nucella*), and two common predators of *Nucella*, the invasive green crab (*Carcinus maenas*) and the native rock crab (*Cancer irroratus*), I explored how each of these predators affected the behavior (i.e., movement and consumption of blue mussels

(*Mytilus edulis*) and morphology for *Nucella* collected from populations in different habitats and different latitudes.

Nucella is a direct-developing, common intertidal whelk that is abundant along both wave-protected and wave-exposed shorelines along the northwestern Atlantic from Long Island to Greenland. *Nucella* spp. respond to predation risk (Vadas et al. 1994, Large and Smee 2010) and in turn alter foraging behavior (Burrows and Hughes 1991, Vadas et al. 1994, Aschaffenburg 2008) and morphology (Appleton and Palmer 1988, Palmer 1990, Bourdeau 2009), which can be strongly influenced by wave exposure (Boulding et al. 1999). In many instances, the influence of predation risk on *Nucella* results in reductions of *Nucella* feeding and an increase in basal food sources including barnacles (*Semibalanus balanoides*) and mussels (Trussell et al. 2003, Trussell et al. 2006a, b, Freeman and Hamer 2009).

The green crab preys upon *Nucella* and is also a successful invader. From the initial invasion in the mid- 1800s, the green crab has expanded its range from Cape Cod, MA northward to the Bay of Fundy by the 1950s (Scattergood 1952, Vermeij 1982, Carlton and Cohen 2003, Baldridge and Smith 2008). Currently, the green crab is abundant in southern Maine but populations near the Bay of Fundy remain small and ephemeral (Seeley 1986), so *Nucella* in the south experience more exposure to green crab predation risk than their northern counterparts. The rock crab is a predator native to the Gulf of Maine and is sympatric with all *Nucella* populations tested. Predation intensity from green crabs differs not only geographically, but also between habitats. On wave-protected shorelines, *Nucella* experience heavy predation from green crabs, whereas on

wave-exposed shorelines, both green and rock crabs are unable to successfully forage, releasing *Nucella* from predation risk (Kitching et al. 1966, Menge 1983, Menge and Sutherland 1987, Leonard et al. 1998, Boulding et al. 1999). Thus predation pressure varies between latitudes and within latitudes depending upon wave exposure.

Here I describe a series of experiments designed to assess how prey respond to predation risk of a native and an invasive predator across a geographic region and between habitats. Throughout these 45 d experiments, I monitored *Nucella* collected from a gradient of predation pressure and measured movement (Large and Smee 2010), prey consumption rate (Freeman and Hamer 2009), and changes in morphology (Trussell and Smith 2000) in response to the presence of predation risk from native and invasive predators. Geography and habitat may cause significant variation in prey behavioral and morphological response to predation risk. Also, inducible morphological and behavioral responses are strongest in regions that experience the highest predation risk and trophic cascades driven by non-consumptive predator effects are likely to be strongest in these areas.

MATERIALS AND METHODS

Animal collection and care

I assessed how prey occupying a geographic gradient of predation risk initially responds and ultimately alter inducible defensive mechanisms in response to common predators. Approximately 120 *Nucella* (mean shell length= 17.91 mm SE= 0.14 mm) were haphazardly collected from each location (Fig. 4.1 and Table 4.1) and were immediately transferred to flowing seawater tanks at the Darling Marine Center (DMC)

in Walpole, ME. Northern sites were ~230 km from southern sites, and wave-protected and wave-exposed sites were < 10 km apart within latitudes. Within each latitude and habitat, *Nucella* were collected from two different areas to minimize localized bias in results. In the lab, *Nucella* were maintained in flow-through tanks and fed an *ad libitum* diet of barnacles (*Semibalanus balanoides*) and mussels (*Mytilus edulis*). Green and rock crabs were captured from the Damariscotta River using recreational crab traps.

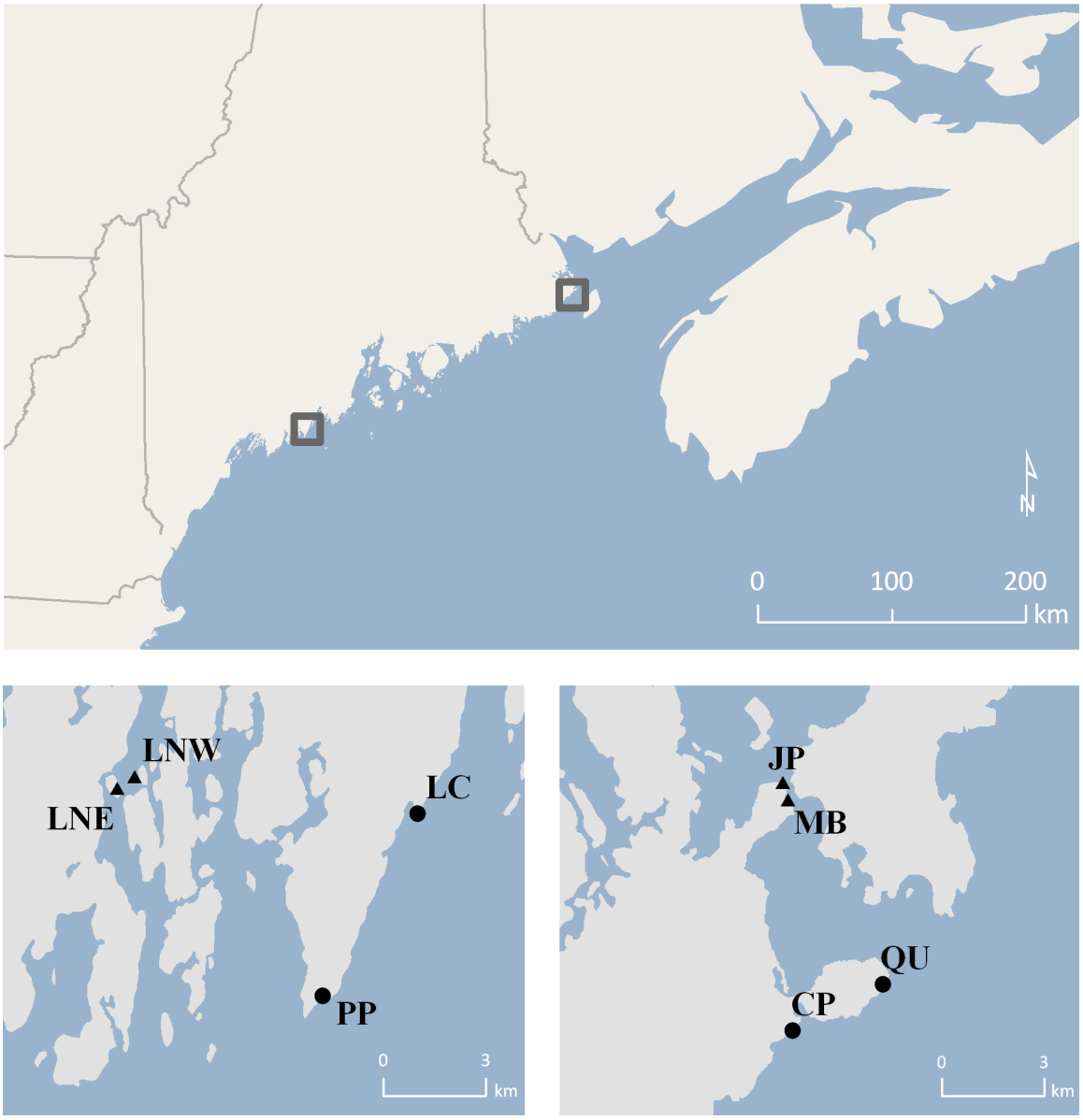

Figure 4.1 a) Map of sites *Nucella* were collected in June 2010. b) Southern protected sites were Lower Narrows-East (LNE) and Lower Narrows-West (LNW). Southern exposed sites were Long Point Cove (LC) and Pemaquid Point (PP). c) Northern protected sites were Johnson Point (JP) and FDR Memorial Bridge (MB). Northern exposed sites were Carrying Place Cove (CP) and West Quoddy Head (QU). Map by A. Reisinger.

Table 4.1 Details of the 8 sites *Nucella* were sampled from in June 2010

Site description	Location	Exposure	Latitude	Longitude
Quoddy Head, near Lubec, ME	North	Exposed	44.813892	-66.950825
Carrying Place Cove, near Lubec, ME	North	Exposed	44.803380	-66.981510
Johnson's Point, Lubec, ME	North	Protected	44.862852	-66.982749
FDR Bridge, Lubec, ME	North	Protected	44.858680	-66.981193
Pemaquid Point, South Bristol, ME	South	Exposed	43.836960	-69.508040
Long Cove Point, Chamberlain, ME	South	Exposed	43.885190	-69.473940
Lower Narrows (East), Walpole, ME	South	Protected	43.891380	-69.583300
Lower Narrows (West), Boothbay, ME	South	Protected	43.894440	-69.576990

Crabs were immediately transferred to flowing seawater tanks at the DMC and maintained on an *ad libitum* diet of *Nucella* and mussels. During the experiment, water temperatures ranged from 12 to 16°C and salinity remained at ~32 in all the seawater tanks.

Behavioral response to predation risk: movement

To examine how prey from different latitudes and habitats respond to short-term predation risk, *Nucella* were exposed to chemical cues indicative of risk (Large and Smee 2010). In the presence of predator cues, *Nucella* decrease their activity (Vadas et al. 1994, Large and Smee 2010); therefore, *Nucella* movement was used as a proxy for risk response. Behavioral assays were conducted in a flow-through laboratory flume (2.2 m long x 0.53 m wide x 0.1 m deep) at the DMC (for detailed behavioral assay description see Large and Smee 2010). This flume is able to reliably maintain free-stream flow velocities between 3.0 and 8.0 cm s^{-1} (for detailed flume description see Smee and Weissburg 2006a), and these flow velocities are well within the range experienced by *Nucella* in the field (Leonard et al. 1998). For these experiments, the substrate of the flume was lined with ceramic tiles to imitate natural substrate and flow velocity remained at~ 4 cm s^{-1}. After collection, each snail was allowed a 24 h acclimation period before behavior was observed.

To begin each assay, three *Nucella* were placed within a crevice between the ceramic tiles that served as a predation refuge. Starting *Nucella* in a refuge allows them the option to exit the refuge even in a risky situation and allows us to remove behavioral ambiguity. If *Nucella* were placed onto the substrate away from a refuge any subsequent movements could either be: 1) a failed response to predator risk or 2) an active search for refuge in response to predation risk. Once in the flume, *Nucella* were allowed to acclimate for 5 min before one of three predator treatments were introduced 0.5 m upstream: 1) green crab, 2) rock crab, or 3) no-predator control. Predators were tethered

to a ceramic tile preventing them from moving. *Nucella* movement was monitored for 20 s every 5 min for a total of seven observations. All *Nucella* movements such as climbing from refuge, lifting or rotating their shells, or crawling within the refuge were scored equally, and the order of predator treatments was performed randomly (see Large et al. 2011 for methods).

Inducing anti-predatory defenses

To examine how prey from different latitudes and habitats would use plastic anti-predator responses to predation risk, I assessed change in anti-predator behavioral response*, Nucella* consumption of mussels, and change in *Nucella* shell morphology after 45 d of continuous exposure to either an exotic or a native predator. This time is sufficient to observe changes in *Nucella* shell morphology in response to predator exudates (Trussell and Smith 2000). After initial behavior was scored, each *Nucella* was uniquely labeled with an apiary tag affixed with cyanoacrylate glue.

Induction chambers were used to expose *Nucella* to predator effluent. Chambers consisted of a large plastic aquarium (60.45 cm x 39.63 cm x 22.61 cm) with a perforated barrier bisecting the tank. Seawater was pumped from the Damariscotta River into a header tank where it was drained into each aquarium and allowed to drain from the opposite end creating a gentle current (~2 l min^{-1}). Within each large aquarium, 2 small mesh-sided containers (25.4 cm x 17.78 cm x 10.16 cm, 1.50 mm vexar meshing) were placed downstream of the perforated barrier. Within each mesh-sided container 15 *Nucella* from a single latitude and habitat (e.g. north wave-protected) were placed alongside 25 mussels of three size classes: shell length= small (13–17.5 mm), medium

(17.5– 20 mm), and large (20–23.5 mm). For each small container 15 small, six medium, and four large mussels were included. Mussels were collected weekly from southern wave-exposed sites, and held in flowing seawater. Upstream of the barrier one of three predator treatments: 1) green crab (Carapace width= 75.0 mm SE= 4.0 mm), 2) rock crab (Carapace width= 78.0 mm SE= 3.6 mm), or 3) a no-predator control were placed. Therefore, each experimental chamber contained one predator upstream of two separate populations of *Nucella*. Each predator and population combination was replicated four times for a total of 60 *Nucella* from each population. Crabs were fed mussels and *Nucella* every other day and deceased crabs were immediately replaced.

Behavioral response to predation risk: foraging

Nucella food supply was replaced weekly with 25 fresh mussels (15 small, six medium, and four large) and drilled mussels valves were counted to measure consumption rate. Some *Nucella* perished during the experiment and these snails were subsequently removed. *Nucella* mortality was similar among populations and predator induction treatments. To account for different numbers of *Nucella* in each container, the number of mussels consumed each week was divided into the number of living *Nucella* for data analysis.

Change in movement behavior

After the 45 d induction period, behavioral assays were repeated as previously described on *Nucella* to determine if exposure to predator risk cue influenced short-term behavioral response to predators. I compared how each population held in the presence of predators (i.e., green crab, rock crab, or no predator control) responded to the presence of

predation risk. I did not, however, compare crab species inductions to heterospecific behavioral assays. For example, *Nucella* held in green crab induction treatments were only assayed with green crabs and no-predator controls and they were not assayed with rock crabs.

Change in morphology

To determine how *Nucella* from different latitudes and habitats alter shell morphology shell mass and body mass were predicted using a nondestructive technique (see Palmer 1982 for a detailed description of process). Prior to induction, each *Nucella* was weighed submerged in seawater using an Ohaus SP602 balance readable to 0.01 g. Each snail was then allowed to dry for 30 m and "scared" back into its shell with an absorbent tissue to collect any residual water and then re-weighed dry. Actual shell mass (Y) was predicted from submerged mass (X) using a regression (Fig. 4.2a) from a destructive sampling of *Nucella* from all populations (Palmer 1982, Trussell and Smith 2000). Body mass was estimated by subtracting the shell mass from the whole air-dry mass (Fig. 4.2b). As with other experiments utilizing this method (Burrows and Hughes 1990, Freeman and Hamer 2009), regression curves were highly significant (Fig. 4.2).

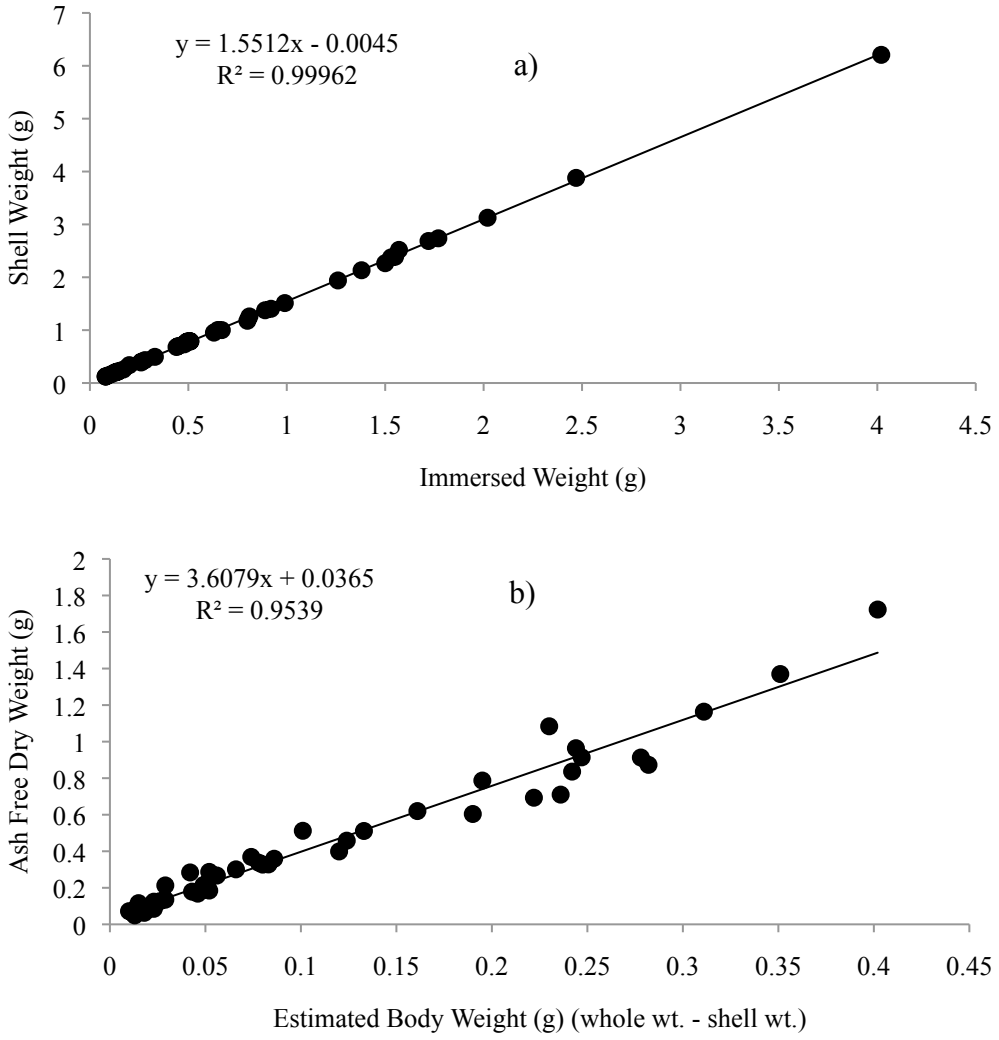

Figure 4.2 a) Non-destructive estimate of shell mass was calculated using a regression of actual shell weight on submerged weight. b) Non-destructive estimates of body mass were estimated by subtracting the estimated of shell mass (calculated from Fig. 4.1a) from the total mass of snails when weighed in the air. For each population I sampled 10 *Nucella*. No differences were evident between populations so the total n = 80).

Upon completion of the induction period, the change in body and shell mass was compared between predator induction treatments and populations. After the behavioral

assays, all *Nucella* were re-weighed both submerged and air-dried to determine the post-induction shell and body mass.

Analysis of behavioral response to predation risk: movement

These experiments were performed in the summers of 2009 and 2010. Behavioral data was analyzed using a 4-factor ANOVA with predator, geographic location, habitat, and year as main, fixed effects. There was no significant interaction or significant main effect of year ($P = 0.5$), so year was not included in the ANOVA model, making the analysis a three-factor ANOVA design (Sokal and Rohlf 1995). To compare behavior before and after the predator induction experiment, a three-factor repeated measure ANOVA was used, with predator (green crab, rock crab, or no-predator control), geographic location (northern or southern shorelines), and habitat (wave-exposed or wave-protected shorelines) as main, fixed effects and induction (i.e., before or after induction experiment) as a repeated measure. Significant interactions were present, so to determine how different populations of *Nucella* respond to predation risk, behavioral response for each predator was compared in separate two-factor repeated measure with location, and habitat as main effects, and induction as a repeated measure.

Analysis of behavioral response to predation risk: foraging

To analyze consumption, a three-factor repeated measure ANOVA was used with predator, location, and habitat as fixed, main effects and week as a repeated measure. Logistical reasons prevented recording of consumption during week two and was excluded from analysis. However, feeding was qualitatively similar in weeks one and three. Consumption has been noted to vary according to population and habitat.

Similarly, with the control groups there was a significant differences in consumption based on location, habitat, and week. To compare how *Nucella* consumption differs over time in response to predation risk, the consumption of mussels in predator treatments was divided into consumption of mussels in the control treatments. This provided a metric to compare consumption by *Nucella* in the presence of an induction predator standardized to any deviation in control treatments. This ratio was analyzed with two separate two-factor repeated measure ANOVAs using location and habitat as fixed, main effects, and week as a repeated measure for each ratio (i.e., *C. maenas*/ control or *C. irroratus*/ control). In all repeated measures ANOVAs, sphericity was tested using Mauchley's *W*.

Analysis of morphology

To compare how morphological measures were influenced by long-term exposure to predation risk, final body mass and shell mass was subtracted from the initial mass. This standardized between inherent differences in initial snail size. For both change in body mass and change in shell mass, a three-factor ANOVA was used with predator, location, and habitat as main, fixed effects. All models met the assumptions of ANOVA, and repeated-measures analyses did not violate the assumption of sphericity as tested with Mauchly's *W*. In comparing induced behavioral response and morphology the number of replicates were not equal among treatments, therefore I used Type III sum of squares to properly calculate the F- ratios (Sokal and Rohlf 1995). All analyses were conducted using R (R Development Core Team 2010 and www.R-project.org) and the 'car' package (Fox and Weisberg 2010).

RESULTS

Behavioral response to predation risk: movement

Nucella behavior from multiple populations within the northern and southern coast of Maine from wave-exposed and wave-protected shorelines were analyzed. Using movement frequency as a proxy for predation risk response, *Nucella* movement was compared using three predator treatments: 1) green crabs, 2) rock crabs, and 3) no-predator control. A three-factor ANOVA was used, with geographic location (north vs. south), habitat (wave-exposed vs. wave-protected shores), and predator type (green crabs, rock crabs, and no-predator control) as fixed factors in the ANOVA model. In the presence of both green and rock crabs, *Nucella* significantly decreased their activity relative to the controls ($F_{2, 168} = 84.40$, $P < 0.001$). However, there were also significant two-way interactions between geographic location and predator ($F_{2, 168} = 9.12$, $P < 0.001$), and habitat and predator ($F_{2, 168} = 4.75$, $P = 0.009$, Fig. 4.2). The strongest anti-predatory, behavioral response was from south, wave-protected populations in response to green crabs, where this invasive predator is most established. All other populations responded to both green and rock crabs by decreasing their movement relative to controls, but their responses did not significantly differ between predator treatments.

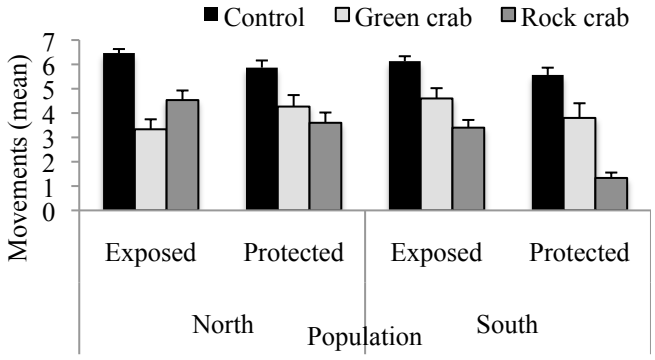

Figure 4.3 Behavioral response (mean + SE) of *Nucella* (n = 15) from different geographic locations (north and south) and habitats (exposed and protected) in response to a no-predator control, rock, or green crab. Significant two-way interactions occurred between predator and location ($F_{2, 168} = 9.12$, $P < 0.001$), and predator and habitat ($F_{2, 168} = 4.75$, $P = 0.009$; three-factor ANOVA).

To evaluate how exposure to predators influenced *Nucella* inducible morphological and behavioral anti-predator defenses, *Nucella* were placed in environmental chambers downstream of green crabs, rock crabs, and a no-predator control in laboratory flow-through tanks for 45 d. After this induction period, movement assays were repeated on *Nucella*. *Nucella* response did not significantly vary between initial and post-induction behavior ($F_{2, 685} = 0.34$, $P = 0.71$), suggesting that behavioral responses do not significantly change after 45 d exposure to predation risk. Because two-way interactions between predator and location ($F_{2, 685} = 17.55$, $P < 0.001$), and predator and habitat ($F_{2, 685} = 6.74$, $P = 0.001$) were significant I analyzed green crabs, rock crabs, and the no-predator controls separately to determine how each population responded to each predator. In response to rock crabs (Fig. 4.3b), *Nucella* activity was significantly less when exposed to no-predator controls ($F_{1, 315} = 125.22$, $P < 0.001$), and differences

between geographic location ($F_{1, 315} = 0.013$, $P = 0.91$), habitat ($F_{1, 315} = 1.27$, $P = 0.26$), or induction ($F_{1, 315} = 0.52$, $P = 0.47$) were not significant. In response to green crabs (Fig. 4.3c), two-way interactions between predator and location ($F_{1, 486} = 28.58$, $P < 0.001$), and predator and habitat ($F_{1, 486} = 4.90$, $P = 0.27$), were significant. Additionally, geographic location ($F_{1, 486} = 51.66$, $P < 0.001$), habitat ($F_{1, 486} = 31.27$, $P < 0.001$), and predator ($F_{1, 486} = 356.77$, $P < 0.001$) differed significantly, but induction ($F_{1, 486} = 0.06$, $P = 0.94$) was not significant. *Nucella* do not quickly induce behavioral responses to predators. In the no-predator control treatments (Fig. 3.3a), there was a significant effect of induction ($F_{1, 232} = 16.44$, $P < 0.001$) on *Nucella* behavior. As with initial control behaviors, there were no significant main effects of location, habitat and treatment. While statistically significant, the reduction of movement in controls was from approximately 6.3 to 5.6 movements per assay. Since I used a flow-through aquarium system, this slight behavioral change in *Nucella* response to controls may be caused by ambient predator cues in the crab-rich Damariscotta River.

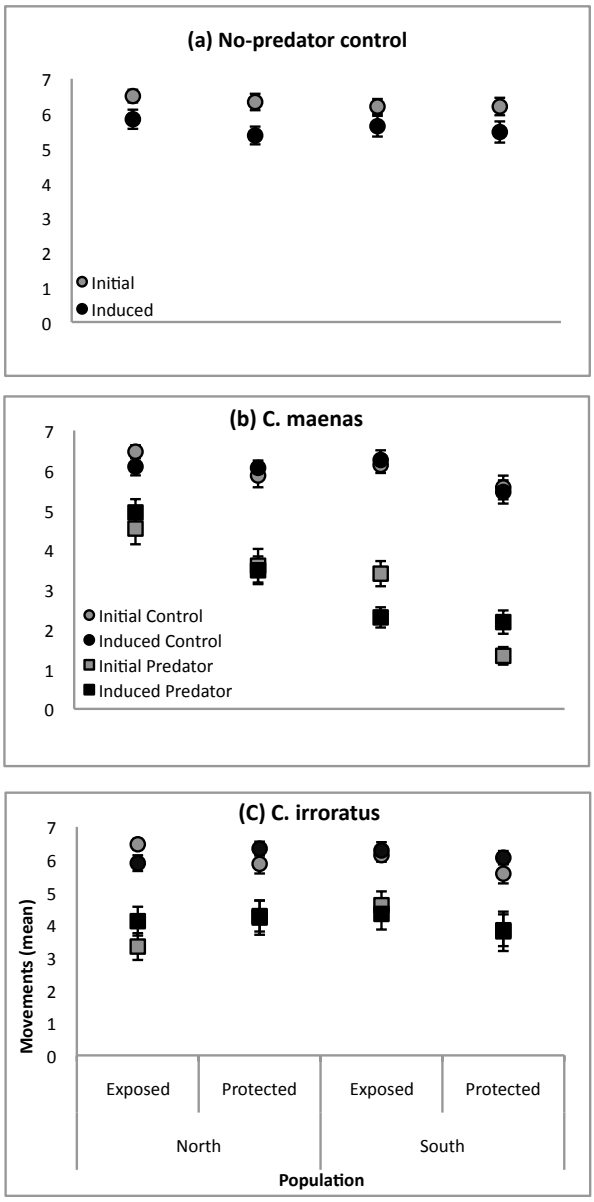

Figure 4.4 Number (mean + SE) of *Nucella* movements in response to (a) no-predator controls, (b) green, and (c) rock crabs from populations with varying predation risk. There was not a significant induction effect, yet there were significant differences based upon predator and location.

Resource acquisition

I measured the weekly consumption of mussels by each population of *Nucella* held in chambers containing green crabs, rock crabs, or no-predator control. Weekly consumption of mussels varied significantly over time ($F_{1, 227} = 60.57$, $P < 0.001$), between geographic locations ($F_{1, 227} = 34.12$, $P < 0.001$), predators ($F_{2, 227} = 17.45$, $P < 0.001$), and habitats ($F_{1, 227} = 38.35$, $P < 0.001$), as well as significant interactions between all main effects. *Nucella* initially consumed similar amounts of mussels in control treatments, but after 45 d displayed differences in consumption rates (Fig. 4.4a). Therefore, for each population and induction treatment, consumption in the predator treatment was divided by the consumption rate in the controls to create a ratio of consumption and standardized any natural variation between populations. Using this ratio, there were no significant differences in *Nucella* consumption rates between geographic location ($F_{1, 75} = 0.39$, $P = 0.53$) and habitats ($F_{1, 75} = 0.46$, $P = 0.49$) when exposed to chemical cues from rock crabs (Fig. 4.4c). There was however, a significant difference between habitats ($F_{1, 75} = 8.83$, $p = 0.004$), but not location ($F_{1, 75} = 0.69$, $P = 0.41$) in response to green crabs with wave-exposed populations consuming more mussels than those from protected shores (Fig. 4.4b). There is natural variation among populations of *Nucella* consumption of mussels, and over a 45 d period green crabs caused a significant increase in consumption for wave-exposed population snails (Fig. 4.4b).

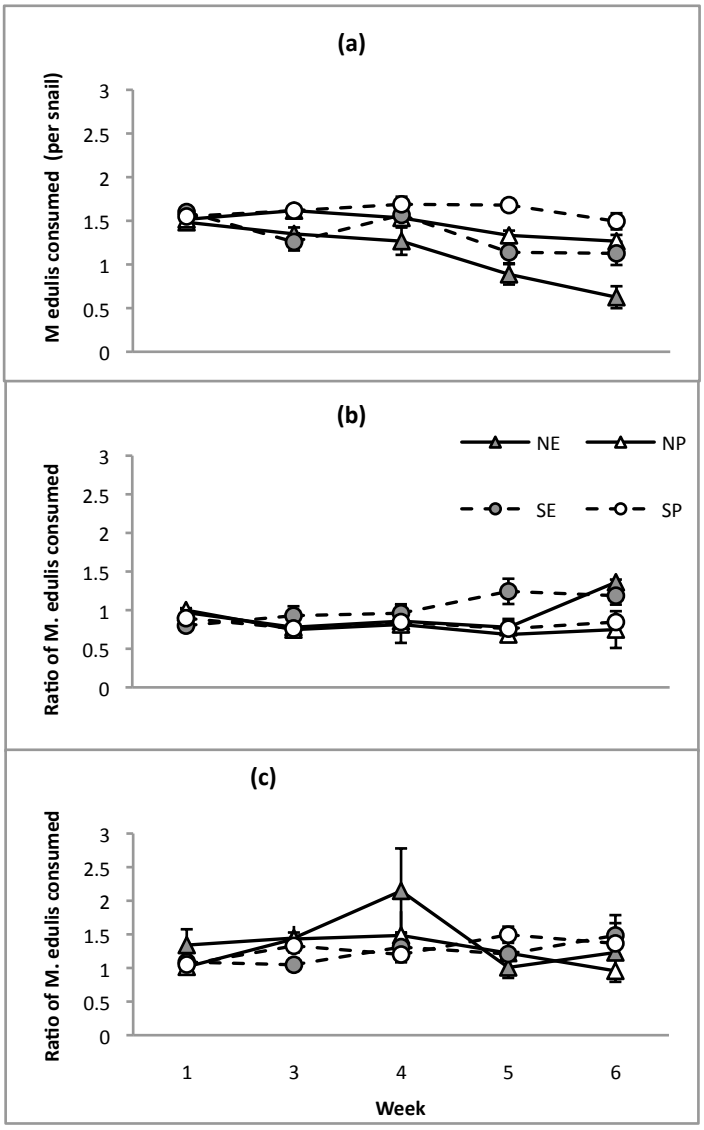

Figure 4.5 (a) Number of mussels consumed (mean + SE) per *Nucella* over a 45 d induction period. As differences existed between locations ($F_{1, 227} = 34.12$, $P < 0.001$) and habitats ($F_{1, 227} = 38.35$, $P < 0.001$), consumption of green and rock crab treatments were divided into no-predator controls to compare how predator treatments influence each population's consumption of mussels. In the presence of (b) green crabs, *Nucella* consumption was dependent upon habitat ($F_{1, 75} = 8.83$, $P = 0.004$), but not location ($F_{1, 75} = 0.69$, $P = 0.41$), with no significant interactions. In the presence of (c) rock crabs, *Nucella* consumption did not significantly vary according to location ($F_{1, 75} = 0.39$, $P = 0.53$) or habitat ($F_{1, 75} = 0.46$, $P = 0.49$), with no significant interactions present.

Morphological response to predation risk

In response to predator exudates, *Nucella* are known to produce thicker, heavier shells. I measured the difference between shell mass before and after a 45 d exposure to exudates from green crabs, rock crabs, or a no-predator control. The change in shell mass differed significantly based upon predator treatment ($F_{2, 615} = 6.32$, $P = 0.002$), location ($F_{1, 615} = 134.52$, $P < 0.001$), and habitat ($F_{1, 615} = 36.27$, $P < 0.001$), with a significant interaction between predator treatment and location ($F_{2, 615} = 6.16$, $P = 0.002$, Fig. 4.5a). Since there were interactions between predator treatments and locations, I combined factors and analyzed using a one-factor simple main effects design ANOVA, which was significant ($F_{11, 615} = 19.93$, $P < 0.001$). As all pair-wise comparisons are not necessary, I used *a priori* linear contrasts corrected with a Bonferroni procedure. *Nucella* from wave-protected shores increased shell mass significantly more in green crab inductions than did *Nucella* from wave-exposed shores ($F_{1, 203} = 15.36$, $P < 0.001$). The largest increase in shell mass was seen in southern wave-protected snails response to green crabs (Fig. 4.5a), the population that experiences most contact with this predator. In response to rock crabs, *Nucella* from northern wave-protected shorelines significantly increased shell mass compared to the controls, although at this location there were no significant difference between responses to rock and green crabs $F_{2, 292} = 6.74$, $P < 0.001$). In all wave-exposed habitats shell mass did not change significantly in response to either predator ($F_{2, 281} = 1.68$, $P = 0.19$, Fig 4.5a).

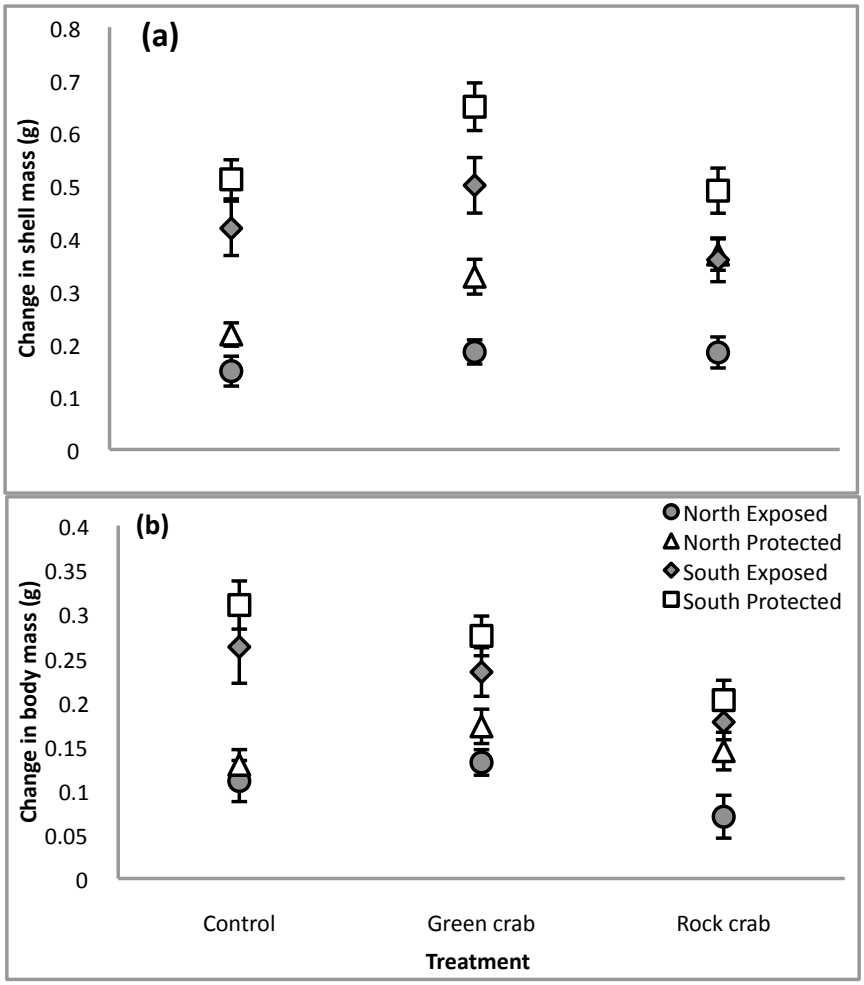

Figure 4.6 Change (mean ± SE) of *Nucella* (a) shell and (b) body mass from different geographic locations and habitats in response to a no-predator control, green, or rock crabs. Change was calculated as initial mass subtracted from the final mass after a 45 d exposure to predator cue. Using a three-factor ANOVA significant two-way interactions were present between predator and location ($F_{2, 615} = 6.17$, $P = 0.023$ and $F_{2, 615} = 17.06$, $P = 0.03$, for shell and body mass, respectively). *A priori* pair-wise comparisons are detailed in the text above.

Increasing shell mass may increase survival, but not without costs, such as decline in body mass and lower fitness. To assess these costs, I measured the change in body

mass before and after a 45 d exposure to exudates from green crabs, rock crabs, or a no-predator control. Southern *Nucella* produced significantly more body mass than northern populations regardless of habitat (Fig. 4.5b). Changes in body mass were significantly different based upon predator ($F_{2, 615} = 7.62$, $P < 0.001$), habitat ($F_{1, 615} = 9.340$, $P = 0.002$), and location ($F_{1, 615} = 75.97$, $P < 0.001$), as well as a significant interaction between predator treatment and location ($F_{2, 615} = 3.50$, $P = 0.03$). Since there were interactions between predator treatments and locations, I combined factors and analyzed using a one-factor simple main effects design ANOVA, which was significant ($F_{11, 615} = 10.10$, $P < 0.001$). As all pair-wise comparisons are not necessary, I used *a priori* linear contrasts corrected with a Bonferroni procedure. Body growth of northern populations did not significantly vary between treatments ($F_{2, 329} = 2.30$, $P = 0.10$), whereas *Nucella* from southern populations grew significantly less in rock crab treatments than green crab, or control treatments ($F_{2, 292} = 8.11$, $P < 0.001$). Although general differences occured between *Nucella* consumption of mussels based upon habitat and geographic location, no patterns between shell growth, body growth, and consumption rates were observed. Thus, differences in shell morphology are not likely caused by inherent differences in feeding, but rather to differences in resource allocation between populations and their recognition of predation risk.

DISCUSSION

Consumer pressure often varies across latitudinal scales (Fischer 1960, Coley and Aide 1991, Pennings et al. 2001, Long and Trussell 2007, Mittelbach et al. 2007) and prey respond to heightened consumer pressure by increasing inducible defenses such as

defensive morphology or anti-predatory behavior (Bertness et al. 1981, Fawcett 1984, Bolser and Hay 1996, Smee and Weissburg 2008, Freeman and Hamer 2009). Previous studies (Bertness et al. 1981, Fawcett 1984, Smee and Weissburg 2008) have explored biogeographic patterns between prey anti-predatory defenses and predation levels and note that prey populations in lower latitudes exhibited stronger anti-predator behaviors and morphologies (Trussell and Smith 2000) than those from northern populations. Yet within habitats predation and prey responses may also vary significantly, making true biogeographic patterns difficult to explore (Moody and Aronson 2007, Freeman and Hamer 2009). This study builds upon earlier research by examining phenotypic plasticity in both prey behavior and morphology between latitudes and between habitats within latitudes. Further, I measured several anti-predatory responses to multiple predators across geographic scales and compared responses of *Nucella* for each location and habitat to predators before and after a short, intense period of exposure to predation risk.

In the presence of green crabs, southern wave-protected populations of *Nucella* exhibited a strong predator avoidance behavioral response (Fig.4.2) and grew a significantly heavier shell (Fig. 4.5a) than northern populations. Southern populations produced less body mass in rock crab treatments than in controls, a pattern not observed in northern populations (Fig. 4.5b). Although the northern wave-protected populations had significant increases in shell mass in response to green crabs and rock crabs, there is no difference in body mass, which might indicate they are allocating more resources to morphological defenses, but this allocation did not influence their ability to add body mass. Therefore, these findings suggest that *Nucella* response to an invasive and native

predator may be based upon geographic location and habitat. In the north, green crab populations are small and ephemeral, and rock crabs are the more common predator. There were no significant increases in shell mass compared to the controls in wave-exposed habitats, the populations where *Nucella* experiences little risk predation due to wave exposure. Moreover, there were no clear patterns between shell weight, consumption of mussels, and increased body mass. Therefore, differences observed in shell allocation in predator treatments are most likely caused by differences in resource allocation and not simply caused by changes in feeding, as reported for *Nucella lamellosa* (Bourdeau 2010).

The biogeographic pattern seen in snail behavior in this study may result from a predator present in the southern part of the range that is rare to absent in the north. Similarly, Fawcett (1984) compared predation intensity and habitat choice by the herbivorous intertidal snail *Tegula funebralis* in sites in southern vs. northern California. *Tegula funebralis* migrated farther up the shore and into a less suitable habitat to reduce predation risk despite lower resource availability in the high intertidal zone in southern sites where predation intensity was most. *Tegula funebralis* transplanted between northern and southern sites exhibited similar behaviors and regardless of their original location, moved faster and farther up the shore in habitats where predation pressure was higher. Fawcett (1984) attributed the higher predation rates in the south to the presence of an octopus predator, which, like green crabs in the present study, were not present in northern study sites.

In wave-protected habitats where crab predators are able to effectively forage, *Nucella* significantly increased their shell mass in response to predation risk and this occurred in both northern and southern populations. Previously, Trussell and Smith (2000) found the grazing snail *Littorina obtusata* to increase shell thickness in response to green crabs when collected from northern and southern wave- protected habitats in the Gulf of Maine. Water temperature also had large effects on snail growth and shell thickness when *L. obtusata* were reared under different temperatures. In my study, southern populations to grew faster and produced thicker shells than those in the north even though all of the populations were maintained in the lab under identical temperatures. Thus, differences in shell thickening responses between northern and southern populations may be caused by selection for more phenotypic plasticity due to differences in predation pressure within each area. Further, habitat was also an important factor in determining morphological plasticity. *Nucella* collected from wave-exposed habitats in both the north and south did not produce thicker shells when exposed to predator-exudates.

Trophic cascades caused by changes in behavior of intermediate consumers

Predators are well known to affect entire food webs by generating trophic cascades. Many recent studies have shown that changes in prey behavior caused by predators may also cause trophic cascades. Trophic cascades resulting from changes in prey behavior have been shown to produce effects on food chains to an equal or higher degree than those caused by direct consumption (Preisser et al. 2005, Preisser et al. 2007, Preisser et al. 2009). Yet, most studies focus on predators and prey collected from a

single habitat type and geographic location. Based upon geographic location and habitat, the anti-predatory response of prey may vary, which potentially could influence the likelihood of trophic cascades occurring solely from changes in prey behavior. Future studies examining the occurrences of trophic cascades based upon non-lethal predator effects should consider how variation in prey responses caused by differential predation pressure between populations might influence results.

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Chapter 5 Genetic variation of *Nucella lapillus* between northwestern Atlantic habitats and geographic range

ABSTRACT

Nucella lapillus (hereafter *Nucella*) is a direct-developing rocky intertidal whelk that has recolonized the northwestern Atlantic shoreline within the past 13,000 years. Although the specific route remains in contention, northeastern Atlantic *Nucella* migrated to populate this region. Within the past ~200 years, the invasive green crab *Carcinus maenas* has expanded its range from Cape Cod northward, and many studies have observed significant behavioral and morphological response to this predator. As the green crab is unable to forage effectively in wave-exposed shorelines, I hypothesize that *Nucella* from areas of longer green crab exposure (i.e., southern shorelines), and more green crab predation pressure (i.e., protected shorelines) may experience higher selection than other habitats. *Nucella* were compared from two geographic locations (northern and southern Maine) and two habitats within each location (wave-exposed and wave-protected), and two sites within each habitat for a total of 8 populations. Population genetic structure was analyzed between these populations of *Nucella* using neutral microsatellite markers. Northwestern Atlantic *Nucella* have a lower allelic diversity, and a higher F_{IS} (inbreeding coefficient) compared to studies examining the same microsatellite markers in northeastern Atlantic *Nucella*. Furthermore, *Nucella* from southern wave-protected shorelines have significant genetic differentiation from all other

populations of *Nucella* in this study, and northwestern Atlantic *Nucella* may have experienced a genetic bottleneck during the recolonization of the American continent.

INTRODUCTION

Nucella lapillus (hereafter *Nucella*) is a common and abundant rocky intertidal whelk found on shorelines throughout the North Atlantic. Along the American continent, *Nucella* is found from Long Island (USA) northward to Greenland and Iceland. In Europe, *Nucella* is found from southern Portugal northward to Norway (Colson and Hughes 2007). During the last glacial maximum (LGM), approximately 18,000 years BP (Ingolfsson 1992), *Nucella* was extirpated from western Atlantic coasts, as ice sheets extended beyond the rocky intertidal habitat where *Nucella* persist (Colson and Hughes 2007). European populations, however, were not adversely affected and after the glacial thaw (~13,000 years BP), *Nucella* recolonized the northwestern Atlantic. While the precise route remains in contention, *Nucella* likely expanded along a trans-Atlantic route "hopping" across Iceland into the northwestern Atlantic (Colson and Hughes 2007) where it is currently found. Therefore, populations of *Nucella* from the northwestern Atlantic are relatively young compared to the northeastern Atlantic populations.

In marine systems the dispersal patterns of young generally connect adult populations. Free-swimming planktonic larvae (planktotrophic) often provide high connectivity and dispersal opportunity (Thorson 1950), whereas shorter-lived planktonic larvae (leciphotrophic) have lower levels of gene flow between populations. Larvae that lack a planktonic stage (direct-developing) generally are thought to have less gene flow

between populations (Levin 2006). *Nucella* lacks a pelagic stage, and direct-developing juvenile snails crawl away from encapsulated eggs upon maturity (Crothers 1985). Adult *Nucella* have low migration rates, and are not known to disperse distances more than 3-20 m yr^{-1} (Bell 2008), making it possible for *Nucella* to have strong population structure between habitats. Despite low adult migration rates and lack of pelagic larvae, Colson and Hughes (2004) found *Nucella* to have high dispersal ability after a localized extinction caused by the anti-fouling additive tributyltin (TBT) (Spence et al. 1990). *Nucella* likely use rafting, or other dispersal mechanisms that enable population connectivity more than allowed only through movement (Colson and Hughes 2004, Bell 2008).

Nucella from both Atlantic coasts have frequently been used as a model organism in many ecological studies (Menge and Sutherland 1987, Vadas et al. 1994, Trussell et al. 2003, Aschaffenburg 2008, Freeman and Hamer 2009), and *Nucella* from the northwestern Atlantic have been the focus of many studies exploring the influence of the invasive green crab (*Carcinus maenas*) upon *Nucella* morphology (Vermeij 1982, Seeley 1986, Fisher et al. 2009), behavior (Freeman and Hamer 2009, Large and Smee 2010, Large et al. 2011), and community effects (Trussell et al. 2003). Some northwestern populations of *Nucella* generally are under intense selection from the green crab, depending upon habitat and geographic location (Trussell and Smith 2000, Freeman and Hamer 2009). Along the northwestern Atlantic coast, *Nucella* in the south generally experience higher predation risk than those in the north, and as green crabs are unable to

forage in wave-exposed areas, *Nucella* experience more predation risk along wave-protected shorelines (Large, *in review)*.

Given the relatively young age of this meta-population (~13,000 years) structure may be less likely because drift-migration balance may not have reached. However, intense natural selection caused by an invading predator (Vermeij 1982, Seeley 1986, Fisher et al. 2009) may produce significant patters of genetic structure, but not detectable with neutral markers. With few exceptions (Day et al. 1993, Wares and Cunningham 2001, Colson and Hughes 2007), the majority of population genetic analysis has examined northeastern populations of *Nucella* from Great Britain (Colson and Hughes 2004, Bell 2008, McInerney et al. 2009), Spain (Rolan et al. 2004), and Norway (Ingolfsson 1992). In this study I examined the genetic structure of several northwestern Atlantic populations of *Nucella* in areas that experience different levels of predation risk. In *Nucella*, diversity in mitochondrial genes is relatively low (Wares and Cunningham 2001); therefore, neutral microsatellite markers were used to estimate genetic diversity and gene flow in *Nucella*.

MATERIALS AND METHODS

Sampling sites

Nucella were collected in June 2010 along the northwestern coast of the Atlantic from two geographic locations (northern and southern Maine) and two habitats (wave-exposed and wave-protected shorelines) within locations. Geographic locations were ~230 km apart, habitats within locations were ~10 km apart, and within each habitat, *Nucella* was collected from two sites < 2 km apart (Fig. 4.1). At each site I haphazardly

collected 40 *Nucella* (see Table 5.1), and shipped live organisms in refrigerated containers to Texas A&M University—Corpus Christi for genetic analysis.

Table 5.1 Description of sites *Nucella* were collected from for use in genetic analysis.

Site description	Location	Habitat	Latitude	Longitude
Quoddy Head, near Lubec, ME	North	Exposed	44.813892	-66.950825
Carrying Place Cove, near Lubec, ME	North	Exposed	44.803380	-66.981510
Johnson's Point, Lubec, ME	North	Protected	44.862852	-66.982749
FDR Bridge, Lubec, ME	North	Protected	44.858680	-66.981193
Pemaquid Point, South Bristol, ME	South	Exposed	43.836960	-69.508040
Long Cove Point, Chamberlain, ME	South	Exposed	43.885190	-69.473940
Lower Narrows- East, Walpole, ME	South	Protected	43.891380	-69.583300
Lower Narrows- West, Boothbay, ME	South	Protected	43.894440	-69.576990

Molecular approaches

Foot tissue (~1-2 mm^3) was dissected from *Nucella* and stored in ethanol (99%) at 5°C. DNA was extracted using DNEasy© blood and tissue extraction kits (Qiagen, Valencia, CA, USA) and stored in AE buffer (at –30°C). Each individual was genotyped at eleven microsatellite loci (Nlw2, Nlw3, Nlw5, Nlw8, Nlw11, Nlw13, Nlw14, Nlw18,

Nlw21, Nlw25 and Nlw27) (Kawai et al. 2001). PCR product was amplified in a final volume of 10 µl using 5 µl 2x GoTaq Hot Start polymerase Mix (PROMEGA), 0.04 µl of each primer, 2.42 µl nuclease-free H_2O and 2.5 ng of genomic DNA. An initial denaturation step of 2 min at 95°C was followed by 40 cycles (30 cycles for Nlw27 and 35 cycles for Nlw8) of 95°C for 40 s, 52°C for 40 s, 72°C for 60 s, followed by 72°C for 5 min. Forward primers were labeled with either 6-FAM, HEX, ROX or Tamara fluorescent dyes to allow loci to be simultaneously analyzed. PCR products were confirmed on a 2.5% agarose gel and genotypes were determined on an ABI Prism 3730XL genetic analyzer (MCLabs, San Francisco, CA). Alleles for each microsatellite were scored using Peak Scanner software version 1.0 (Applied Biosystems) and binned according to TANDEM (Matschiner and Salzburger 2009).

Genetic analysis

Gene frequency deviations from Hardy-Weinberg Equilibrium (HWE) and linkage disequilibrium were calculated using the exact tests within the computer program GDA (Lewis and Zaykin). Significance levels were calculated using the default Markov chain method and corrected using the Bonferroni procedure for each locus within each population. Genetic diversity was measured for each population as the mean number of alleles per locus (A), the expected heterozygotes (H_e), the observed heterozygotes (H_o), and the inbreeding coefficient (F_{IS}) using Arlequin 3.1 (Excoffier et al. 2006). I analyzed the genetic structure using the infinite alleles model (Excoffier et al. 2006) and the stepwise mutation model (Kimura and Ohta 1978). Arlequin was also used to calculate hierarchical analysis of molecular variance (AMOVA) and values of F_{ST} (Weir and

Cockerham 1984) and R_{ST} (Slatkin 1995). Significance levels were calculated for population pair-wise comparisons and overall values using 1000 permutations.

RESULTS

Genetic diversity`

The mean number of alleles varied from 3.00 to 5.38 per locus and mean gene diversity (H_e) from 0.30 to 0.44 per locus and locality (Table 5.2). The observed heterozygosity (H_o) ranged from 0.18- 0.30, and all populations were deficient in heterozygotes (Table 5.2). The difference in observed and expected heterozygosity corresponds to high inbreeding coefficients (F_{IS}) between 0.38- 0.61. Loci Nlw2, Nlw3, Nlw5, Nlw8, Nlw11, Nlw14, Nlw18, Nlw21 and Nlw25, were all deficient in heterozygotes in at least one population ($P < 0.001$, based on 1000 randomizations of alleles within samples). Between loci there was no linkage disequilibrium; therefore, I assumed that loci were independent (i.e., unlinked).

Table 5.2 The genetic diversity of populations of *Nucella*. The sample size (N), mean number of alleles per locus (± SD) (*A*), observed heterozygotes (H_o), expected heterozygotes (H_e), F_{IS} (inbreeding coefficient), and the F_{IS} significance value (where the null hypothesis is that F_{IS} is not significantly different from zero) across all microsatellite loci. For site descriptions see Table 5.1.

Population	N	*A*		H_0	H_e	F_{IS}	*P* value
Carrying Place	24	3.63	(±1.302)	0.18	0.30	0.61	< 0.001
Quoddy Head	37	3.89	(±2.088)	0.19	0.31	0.45	< 0.001
FDR Bridge	9	4.50	(±2.563)	0.27	0.39	0.38	< 0.001
Johnson Point	24	3.00	(±1.155)	0.30	0.44	0.45	< 0.001
Long Point	27	5.38	(±2.973)	0.27	0.43	0.49	< 0.001
Pemaquid Point	24	4.75	(±3.012)	0.26	0.40	0.46	< 0.001
Lower Narrows- East	24	4.63	(±3.159)	0.26	0.42	0.56	< 0.001
Lower Narrows- West	24	4.33	(±2.598)	0.23	0.38	0.40	< 0.001

Genetic differentiation between populations

There was significant heterogeneity in genotypic frequency distribution between several populations under both the infinite allele (F_{ST}) and stepwise mutation (R_{ST}) models (Bonferroni adjusted $P < 0.000625$) (Table 5.3), and for all populations the F_{ST} and R_{ST} values were generally similar. The southern wave-protected site Lower Narrows-West showed significant genetic differences between all other populations except Lower Narrows- East, with F_{ST} values ranging from 0.107- 0.195 and R_{ST} ranging from 0.073-

0.243. There was also a significant genetic difference between the northern wave-protected site FDR Bridge and the northern wave-exposed site Quoddy Head with a F_{ST} value of 0.05, and a R_{ST} value of 0.05. Since there were no significant pair-wise genetic differences between sites, I lumped sites within habitat and geographic locations (i.e., northern wave-protected). *Nucella* from southern wave-protected populations showed significant genetic differences between all other populations with F_{ST} ranging from 0.046- 0.125 and R_{ST} ranging from 0.044- 0.11. *Nucella* from southern wave-exposed populations and northern wave-protected populations also showed a significant genetic difference with F_{ST} value of 0.042, and R_{ST} value of 0.042. All other pair-wise combinations were not significant.

Table 5.3 Matrix of pair-wise comparisons of population genetic differentiation using F_{ST} (infinite allele model) on the upper diagonal and R_{ST} (stepwise mutation model) on the lower diagonal for all *Nucella* populations sampled. Bold numbers indicate a significant pair-wise comparison (Bonferroni corrected $P < 0.000625$).

	Carrying Place	Quoddy Head	Johnson Point	FDR Bridge	Pemaquid Point	Long Point	Lower Narrows-East	Lower Narrows- West
Carrying Place	-	0.009	0.001	0.014	0.049	0.024	0.043	**0.179**
Quoddy Head	0.009	-	0.054	**0.005**	0.075	0.027	0.018	**0.117**
Johnson Point	0.001	0.058	-	0.069	0.027	0.022	0.064	**0.195**
FDR Bridge	0.014	**0.005**	0.075	-	0.082	0.043	0.046	**0.168**
Pemaquid Point	0.051	0.081	0.028	0.090	-	0.004	0.029	**0.107**
Long Point	0.024	0.028	0.023	0.045	0.000	-	0.000	0.068
Lower Narrows-East	0.045	0.018	0.069	0.048	0.030	0.000	-	0.038
Lower Narrows-West	**0.217**	**0.133**	**0.243**	**0.202**	**0.120**	**0.073**	0.040	-

There were significant differences in allele frequencies between *Nucella* populations (Table 5.4). The hierarchical analysis of molecular variance (AMOVA) showed that approximately 4.8% of the total variation of microsatellite loci was explained by variation among populations. The largest proportion of variation was attributed to individuals within the total populations (49.79%), and the rest of the variation (45.41%) was among individuals within populations (Table 5.5). All components of genetic variance were significantly different from zero ($P < 0.001$).

Table 5.4 Matrix of pair-wise comparisons of population genetic differentiation using F_{ST} (infinite allele model) on the upper diagonal and R_{ST} on the lower diagonal for all habitat and geographic locations sampled. Bold numbers indicate significant pair-wise comparison (Bonferroni corrected $P < 0.0125$).

	North Exposed	North Protected	South Exposed	South Protected
North Exposed	-	0.012	**0.044**	**0.086**
North Protected	0.012	-	0.026	**0.125**
South Exposed	**0.042**	0.025	-	**0.046**
South Protected	**0.079**	**0.111**	**0.044**	-

Table 5.5 Hierarchical Analysis of Molecular Variance (AMOVA) of the allele frequencies between 4 populations sampled from different habitat and geographic locations

Source of variation	Degrees of freedom	Sum of squares	Variance components	Percentage of variation	Significance level
Among Populations	3	12.005	0.033	4.80	$P < 0.001$
Among individuals within populations	185	176.921	0.309	45.41	$P < 0.001$
Within individuals	189	64.000	0.339	49.79	$P < 0.001$
Total	377	252.926	0.680		

DISCUSSION

Within the past 13,000 years, *Nucella* has recolonized the northwestern Atlantic after being extirpated ~18,000 years BP (Ingolfsson 1992). Since its reintroduction, *Nucella* has become common along wave-exposed and wave-protected shorelines within the northwestern Atlantic rocky intertidal south to Long Island. Since the invasion of the green crab *Carcinus maenas* in the 1800s, *Nucella* has been exposed to selective pressure that has influenced *Nucella* morphology (Vermeij 1982, Seeley 1986, Fisher et al. 2009).

While microsatellite markers are neutral and are not able to detect selective forces, my research suggests that *Nucella* along the northwestern Atlantic shoreline may experience genetic drift or possibly differential selection.

Populations from southern wave-protected shorelines have the largest genetic differentiation from other populations. As *Nucella* lack pelagic larvae and have limited adult movement, rafting is commonly thought to be their main dispersal mechanism (Colson and Hughes 2004, Colson and Hughes 2007, Bell 2008), although there is not a published account of this phenomenon. I collected southern wave-protected *Nucella* from a submerged island (Lower Narrows- East) that is only accessible during low tide and from a shoreline directly to the west of this island (Lower Narrows- West), both within the Damariscotta River, ME, a tidal estuary. Given the hydrodynamic conditions of this estuary it seems reasonable that over evolutionary time, *Nucella* could raft towards wave-exposed shorelines (Table 5.3). However, as this estuary has a net outward flow (Leonard 1998), *Nucella* may not be able to raft into the more protected shorelines, which may explain the observed significant genetic variation between these two habitats.

In studies of northeastern Atlantic *Nucella* genetic differentiation between habitats ~10 km apart most pair-wise populations comparisons are significantly different using F_{ST} and R_{ST} (Bell 2008). However, in the northwestern Atlantic *Nucella*, with the exception of the southern wave-protected populations, most populations of *Nucella* are not significantly different (Table 5.3). As *Nucella* from the northwestern Atlantic are ~13,000 years old, there may not have been sufficient time for differentiation between sites. Further, northwestern Atlantic shorelines north of Long Island generally consist of

suitable *Nucella* habitat, potentially creating more connectivity between populations compared to northeastern Atlantic shorelines.

Green crabs are native decapod predators within the northeastern Atlantic (Scattergood 1952, Grosholz and Ruiz 1996) and coexist with northeastern Atlantic *Nucella*. As green crabs invaded the northwestern Atlantic ~200 years ago (Scattergood 1952), *Nucella* may be under selective pressure from a new threat. Since this invasion several studies document *Nucella* increasing shell thickness (Vermeij 1982, Fisher et al. 2009) in response the range expansion of the green crab. In this study, I analyzed genetic structure and diversity of several populations of *Nucella* that might experience differential levels of predation according to habitat (i.e., wave-protected experience higher predation risk), and geographic location (i.e., southern shorelines have longest exposure to green crabs). Microsatellite markers are neutral and we cannot say that there is selective pressure. However, there is a clear pattern of differentiation that coincides with predation risk and the selective pressure it may exert (Table 5.4).

All *Nucella* populations sampled had relatively low allelic diversity compared to similar microsatellite studies of northeastern Atlantic *Nucella* (Colson and Hughes 2004, Colson and Hughes 2007, Bell 2008). Here, the highest mean allelic diversity occurred in *Nucella* from the southern wave-protected site Long Point of 5.38 ($\pm$ 2.937 SD), whereas in *Nucella* sampled in Great Britain, Bell (2008) reported the lowest mean allelic diversity of 8.0 ($\pm$ 4.0 SD), a pattern mirrored by results from Colson and Hughes (2007). Similarly, in this study *Nucella* populations had high inbreeding coefficient (F_{IS}) of 0.40-0.61, where Bell (2008) reported inbreeding coefficients between 0.02- 0.07, which were

not significant from zero. My results and findings from Colson and Hughes (2007) suggest that compared to the more ancient northeastern populations, *Nucella* in the northwestern Atlantic have much lower genetic diversity.

Conclusions

Nucella in the northwestern Atlantic generally do not vary significantly between spatial scales up to hundreds of km. However, *Nucella* populations in habitats that experience higher predation risk (i.e., southern wave-protected) are significantly differentiated from other populations, regardless of distance. Furthermore, when compared to northeastern populations of *Nucella*, northwestern populations have much lower allelic diversity and much higher F_{IS} inbreeding coefficient, suggesting a strong likelihood of a bottleneck. Future research should compare more populations of *Nucella* along the northwestern Atlantic to populations along the northeastern Atlantic to compare large-scale genetic diversity (but see Ingolfsson 1992, Colson and Hughes 2007).

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SUMMARY AND CONCLUSIONS

Predators often heavily influence prey populations and the structure and function communities (Paine 1969, Carpenter et al. 1985, Trussell et al. 2003). In addition to direct consumption of prey, predators often cause prey to alter their foraging behavior (Freeman and Hamer 2009), habitat selection (Werner and Peacor 2003), morphology (Vermeij 1982, Boulding et al. 1999, Fisher et al. 2009) or life-history (Lima 1998, Preisser et al. 2005, Preisser et al. 2007, Preisser et al. 2009). These non-lethal effects can propagate to multiple trophic levels and have often been shown to exert equal or larger effects than those of direct consumption (Lima 1998). For non-lethal predator effects to occur, prey must detect and respond to predation risk, and despite the important role of information transfer in this process, few studies have explored how prey responses are influenced by predator characteristics and the environmental conditions that affect transmission of cues indicative of predation risk.

To prevent predation, prey must reliably detect predator risk (Kats and Dill 1998). In terrestrial systems, use chemical, visual, and mechanical signals to evaluate predation risk, however, in aquatic environments visual cues are often unreliable and/or unavailable and mechanical signals degrade quickly (Zimmer and Butman 2000, Weissburg et al. 2002). Therefore, in aquatic environments prey primarily use chemical cues to evaluate predation risk. Prey may use many different types signals to provide an accurate representation of predation risk.

Conclusions

In this dissertation, I examined factors that influence *Nucella* response to predator risk cues. Within this study system, predators that frequently target *Nucella* are generalists and do not specialize on *Nucella*. It is not surprising that the both injured conspecifics and green crabs elicit the strongest response, since injured conspecifics may be the most specific predator risk cue available, and green crabs were the most common predator. Furthermore, *Nucella* who do not frequently encounter predation risk, such as northern, or wave-exposed snails, either do not elect to respond to predation risk, or do not detect predation risk. However, my experimental design was not able to differentiate between these two responses. It would be beneficial for future studies to collect juvenile *Nucella* from multiple geographic locations and habitats and rear them for several generations in the presence of different predators. This design would enable an accurate designation between responses observed from different habitats and geographic locations.

Chemical cues used by both predators (Weissburg and Zimmer-Faust 1994, Robinson et al. 2011) and prey (Smee and Weissburg 2006, Large et al. 2011) can be positively and negatively influenced by environmental forces. In this dissertation, I examined how flow velocity and turbulence influence *Nucella* response to predation risk. *Nucella* response to predation risk is dependent upon these environmental conditions, and *Nucella* seem to be most adept at detecting predators in u = 4- 8 cm s^{-1}. *Nucella* are frequently found in both low wave-energy habitats such as tidal estuaries, and high wave-energy habitats such as wave-exposed shoreline. In both habitats, *Nucella* likely experience flow conditions that far surpass those that I tested. Future studies should

compare the ability of *Nucella* from habitats that experience different flow conditions to detect and respond to predators. That is, whether *Nucella* from high wave-energy habitats do not experience the same predation risk as low wave-energy habitats, or if *Nucella* from high-energy habitats have a different optimal range of cue detection. Empirically clarifying this issue would be helpful in determining the interactions between the identity of chemical signals and their delivery.

Predators and prey rarely share the same distribution over large spatial scales (Schemske et al. 2009). In this dissertation research I explored how prey from habitats and different geographic locations that experience differential predation risk respond to a native and invasive predator. *Nucella* that experience the highest predation risk show the strongest anti-predatory response. Wave-protected *Nucella* from southern shorelines increased morphological defenses and decreased activity in response to their most common predator, the green crab. Conversely, *Nucella* from wave-exposed shorelines did not significantly alter morphology or behavior. Therefore, non-lethal interactions do vary according to biogeographic scales and the distribution of predators. For example, *Nucella* for Chapters 1 and 3 were collected exclusively from southern, wave-protected sites (Lower Narrows-East and Lower Narrows-West) and I saw a strong behavioral response to green crab predators. Had I collected *Nucella* from a wave-exposed shoreline, or from northern locations, my conclusions may have been different. Exploring natural variation in predator-prey distributions will be important to understand the importance of non-lethal predator effects.

On evolutionary time-scales, *Nucella* have recently become established in the northwestern Atlantic. In this dissertation research I explored the genetic structure of 8 populations of *Nucella* that: 1) experience differential predation risk, and 2) may be isolated from each other. Comparing my results to findings from Great Britain (Bell 2008), Spain (Rolan et al. 2004), and Norway (Ingolfsson 1992), these populations have a much lower genetic diversity and are experiencing high levels of inbreeding, suggesting a recent genetic bottleneck. While Colson and Hughes (2007) compared northeastern and northwestern populations of *Nucella* to find putative expansion routes, further research to explain phylogenetic variation within this species is necessary. Furthermore, future studies should closely compare non-lethal predator effects with genetic diversity.

Overall my dissertation provides new insights into mechanisms that prey use to evaluate and respond to predation risk. These findings will be useful to both empirical and theoretical ecologists to compare and contrast how prey detection and response to predation risk is highly dependent upon predator identity, predator diet, environmental forces, and biogeographic patterns in predator and prey distributions.

REFERENCES

Abrahams, M. V. and M. G. Kattenfeld. 1997. The role of turbidity as a constraint on predator-prey interactions in aquatic environments. Behavioral Ecology and Sociobiology **40**:169-174.

Abrams, P. A., B. A. Menge, G. G. Mittelbach, D. A. Spiller, and P. Yodzis. 1996. The role of indirect effects in food webs. Pages 371–395 *in* G. Polis and K. Winemiller, editors. Food webs: dynamics and structure. Chapman and Hall, New York, New York, USA.

Appleton, R. D. and A. R. Palmer. 1988. Water-borne stimuli released by predatory crabs and damaged prey induce more predator-resistant shells in a marine gastropod. Proceedings of the National Academy of Sciences, USA **85**:4387-4391.

Aschaffenburg, M. 2008. Different crab species influence feeding of the snail *Nucella lapillus* through trait-mediated indirect interactions. Marine Ecology **29**:348-353.

Bakus, G. and G. Green. 1974. Toxicity in sponges and holothurians: a geographic pattern. Science **185**:951-953.

Baldridge, A. and L. Smith. 2008. Temperature constraints on phenotypic plasticity explain biogeographic patterns in predator trophic morphology. Marine Ecology Progress Series **365**:25-34.

Bell, J. 2008. Similarity in connectivity patterns for two gastropod species lacking pelagic larvae. Marine Ecology Progress Series **357**:185-194.

Bernot, R. J. and A. M. Turner. 2001. Predator identity and trait-mediated indirect effects in a littoral food web. Oecologia **129**:139-146.

Bertness, M. D., S. D. Garrity, and S. C. Levings. 1981. Predation pressure and gastropod foraging: A tropical-temperate comparison. Evolution **35**:995-1007.

Bolser, R. and M. Hay. 1996. Are tropical plants better defended? Palatability and defenses of temperate vs. tropical seaweeds. Ecology **77**:2269-2286.

Boulding, E., M. Holst, and V. Pilon. 1999. Changes in selection on gastropod shell size and thickness with wave-exposure on Northeastern Pacific shores. Journal of Experimental Marine Biology and Ecology **232**:217-239.

Bourdeau, P. E. 2009. Prioritized phenotypic responses to combined predators in a marine snail. Ecology **90**:1659-1669.

Bourdeau, P. E. 2010. An inducible morphological defence is a passive by-product of behaviour in a marine snail. Proceedings of the Royal Society B: Biological Sciences **277**:455-462.

Brodin, T., D. J. Mikolajewski, and F. Johansson. 2006. Behavioural and life history effects of predator diet cues during ontogeny in damselfly larvae. Oecologia **148**:162-169.

Brown, G. E., J. A. Paige, and J. G. J. Godin. 2000. Chemically mediated predator inspection behaviour in the absence of predator visual cues by a characin fish. Animal Behaviour **60**:315-321.

Bryer, P. J., R. S. Mirza, and D. P. Chivers. 2001. Chemosensory assessment of predation risk by slimy sculpins (*Cottus cognatus*): responses to alarm, disturbance, and predator cues. Journal of Chemical Ecology **27**:533-546.

Buck, L. B. 1996. Information coding in the vertebrate olfactory system. Annual Review of Neuroscience **19**:517-544.

Burks, R. L. and D. M. Lodge. 2002. Cued in: Advances and opportunities in freshwater chemical ecology. Journal of Chemical Ecology **28**:1901-1917.

Burrows, M. and R. Hughes. 1990. Variation in growth and consumption among individuals and populations of dogwhelks, *Nucella lapillus*: a link between foraging behaviour and fitness. Journal of Animal Ecology:723-742.

Burrows, M. and R. Hughes. 1991. Optimal foraging decisions by dogwhelks, *Nucella lapillus* (L.): influences of mortality risk and rate-constrained digestion. Functional Ecology:461-475.

Carlton, J. and A. Cohen. 2003. Episodic global dispersal in shallow water marine organisms: the case history of the European shore crabs *Carcinus maenas* and *C. aestuarii*. Journal of Biogeography **30**:1809-1820.

Carpenter, S. R., J. F. Kitchell, and J. R. Hodgson. 1985. Cascading trophic interactions and lake productivity. Bioscience **35**:634-639.

Chivers, D. and R. Mirza. 2001a. Predator diet cues and the assessment of predation risk by aquatic vertebrates: A review and prospectus. Page 277 *in* A. Marchlewska-Koj, J. Lepri, and D. Muller-Schwarze, editors. Chemical signals in vertebrates, Plenum, New York.

Chivers, D. P. and R. S. Mirza. 2001b. Importance of predator diet cues in responses of larval wood frogs to fish and invertebrate predators. Journal of Chemical Ecology **27**:45-51.

Chivers, D., B. Wisenden, and R. Smith. 1996. Damselfly larvae learn to recognize predators from chemical cues in the predator's diet. Animal Behaviour **52**:315-320.

Chivers, D. P., R. S. Mirza, P. J. Bryer, and J. M. Kiesecker. 2001. Threat-sensitive predator avoidance by slimy sculpins: understanding the importance of visual versus chemical information. Canadian Journal of Zoology **79**:867-873.

Chivers, D. P. and R. J. F. Smith. 1998. Chemical alarm signaling in aquatic predator-prey systems: A review and prospectus. Ecoscience **5**:338-352.

Chivers, D. P., X. Zhao, and M. C. O. Ferrari. 2007. Linking morphological and behavioural defences: prey fish detect the morphology of conspecifics in the odour signature of their predators. Ethology **113**:733-739.

Coley, P. D. and T. Aide. 1991. Comparison of herbivory and plant defenses in temperate and tropical broad-leaved forests. Plant-animal interactions: evolutionary ecology in tropical and temperate regions. Wiley, New York 25-49.

Colson, I. and R. Hughes. 2004. Rapid recovery of genetic diversity of dogwhelk (*Nucella lapillus* L.) populations after local extinction and recolonization contradicts predictions from life-history characteristics. Molecular Ecology **13**:2223-2233.

Colson, I. and R. N. Hughes. 2007. Contrasted patterns of genetic variation in the dogwhelk *Nucella lapillus* along two putative post-glacial expansion routes. Marine Ecology Progress Series **343**:183-191.

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

Crothers, J. 1985. Dog-whelks: an introduction to the biology of *Nucella lapillus* (L.). Field Studies **6**:291-360.

Dawkins, R. and J. R. Krebs. 1979. Arms races between and within species. Proceedings of the Royal Society B: Biological Sciences **205**:489-511.

Day, A., H. Leinaas, and M. Anstensrud. 1993. Allozyme differentiation of populations of the dogwhelk Nucella lapillus,(L.): the relative effects of geographic distance and variation in chromosome number. Biological Journal of the Linnean Society **51**:257-277.

Dix, T. L. and P. V. Hamilton. 1993. Chemically mediated escape behavior in the marsh periwinkle *Littoraria irrorata* Say. Journal of Experimental Marine Biology and Ecology **166**:135-149.

Edgell, T. C. 2010. Past predation risk induces an intertidal whelk (*Nucella lamellosa*) to respond to more dilute concentrations of its predator's scent. Marine Biology **157**:215-219.

Etter, R. 1989. Life history variation in the intertidal snail Nucella lapillus across a wave-exposure gradient. Ecology **70**:1857-1876.

Etter, R. J. 1988. Physiological stress and color polymorphism in the intertidal snail *Nucella lapillus*. Evolution **42**:660-680.

Excoffier, L., G. Laval, and S. Schneider. 2006. Arlequin ver 3.1--An Integrated Software Package for Population Genetics Data Analysis. Evolutionary Bioinformatics Online **1**:47-50

Faul, F., E. Erdfelder, A. Lang, and A. Buchner. 2007. GPower 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behavior Research Methods **39**:175-191.

Fawcett, M. 1984. Local and latitudinal variation in predation on an herbivorous marine snail. Ecology **65**:1214-1230.

Ferner, M. C., D. L. Smee, and Y. P. Chang. 2005. Cannibalistic crabs respond to the scent of injured conspecifics: danger or dinner? Marine Ecology Progress Series **300**:193-200.

Ferner, M. C., D. L. Smee, and M. J. Weissburg. 2009. Habitat complexity alters lethal and non-lethal olfactory interactions between predators and prey. Marine Ecology Progress Series **374**:13-22.

Ferner, M. C. and M. J. Weissburg. 2005. Slow-moving predatory gastropods track prey odors in fast and turbulent flow. Journal of Experimental Biology **208**:809-819.

Ferrari, M. C. O., B. D. Wisenden, and D. P. Chivers. 2010. Chemical ecology of predator-prey interactions in aquatic ecosystems: a review and prospectus. Canadian Journal of Zoology **88**:698-724.

Finelli, C. 2000. Velocity and concentration distributions in turbulent odor plumes in the presence of vegetation mimics: a flume study. Marine Ecology Progress Series **207**:297-309.

Fischer, A. G. 1960. Latitudinal variations in organic diversity. Evolution **14**:64-81.

Fisher, J., E. Rhile, H. Liu, and P. Petraitis. 2009. An intertidal snail shows a dramatic size increase over the past century. Proceedings of the National Academy of Sciences, USA **106**:5209-5212.

Fox, J. and S. Weisberg. 2010. An {R} Companion to Applied Regression. 2 edition. Sage, Thousand Oaks, California.

Freeman, A. and C. Hamer. 2009. The persistent effect of wave exposure on TMIIs and crab predation in *Nucella lapillus*. Journal of Experimental Marine Biology and Ecology **372**:58-63.

Freeman, A., J. Meszaros, and J. Byers. 2009. Poor phenotypic integration of blue mussel inducible defenses in environments with multiple predators. Oikos **118**:758-766.

Geller, J. B. 1982. Chemically mediated avoidance response of a gastropod, *Tegula funebralis* (A. Adams), to a predatory crab, *Cancer antennarius* (Stimpson). Journal of Experimental Marine Biology and Ecology **65**:19-28.

Gosselin, L. A. and E. Bourget. 1989. The performance of an intertidal predator *Thais lapillus*, in relation to structural heterogeneity. Journal of Animal Ecology **58**:287-303.

Grabowski, J. 2004. Habitat complexity disrupts predator-prey interactions but not the trophic cascade on oyster reefs. Ecology **85**:995-1004.

Grabowski, J. H. and D. L. Kimbro. 2005. Predator-avoidance behavior extends trophic cascades to refuge habitats. Ecology **86**:1312-1319.

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

Harvell, C. D. 1990. The ecology and evolution of inducible defenses. Quarterly Review of Biology **65**:323-340.

Heck, K. L. and K. Wilson. 1987. Predation rates on decapod crustaceans in latitudinally separated seagrass communities: a study of spatial and temporal variation using tethering techniques. Journal of Experimental Marine Biology and Ecology **107**:87-100.

Heithaus, M. R., A. J. Wirsing, D. Burkholder, J. Thomson, and L. M. Dill. 2009. Towards a predictive framework for predator risk effects: the interaction of landscape features and prey escape tactics. Journal of Animal Ecology **78**:556-562.

Hettyey, A., K. Vincze, S. Zsarnóczai, H. Hoi, and A. Laurila. 2011. Costs and benefits of defences induced by predators differing in dangerousness. Journal of Evolutionary Biology **32**:77-82.

Hoverman, J., J. Auld, and R. Relyea. 2005. Putting prey back together again: integrating predator-induced behavior, morphology, and life history. Oecologia **144**:481-491.

Hughes, R. N. and R. W. Elner. 1979. Tactics of a predator, *Carcinus maenas*, and morphological responses of the prey, *Nucella lapillus*. Journal of Animal Ecology **48**:65-78.

Ingolfsson, A. 1992. The origin of the rocky shore fauna of Iceland and the Canadian Maritimes. Journal of Biogeography **19**:705-712.

Jackson, J. L., D. R. Webster, S. Rahman, and M. J. Weissburg. 2007. Bed roughness effects on boundary-layer turbulence and consequences for odor-tracking behavior of blue crabs (*Callinectes sapidus*). Limnology and Oceanography **52**:1883-1897.

Kats, L. B. and L. M. Dill. 1998. The scent of death: chemosensory assessment of predation risk by animals. Ecoscience **5**:361-394.

Kimura, M. and T. Ohta. 1978. Stepwise mutation model and distribution of allelic frequencies in a finite population. Proceedings of the National Academy of Sciences of the United States of America **75**:2868-2872.

Kitching, J., L. Muntz, and F. Ebling. 1966. The ecology of Lough Ine. XV. The ecological significance of shell and body forms in *Nucella*. Journal of Animal Ecology **35**:113-126.

Large, S. and D. Smee. 2010. Type and nature of cues used by *Nucella lapillus* to evaluate predation risk. Journal of Experimental Marine Biology and Ecology **396**:10-17.

Large, S. I., D. L. Smee, and G. C. Trussell. 2011. Environmental conditions influence the frequency of prey responses to predation risk. Marine Ecology Progress Series **422**:41-49.

Lawrence, M. 2010. ez: Easy analysis and visualization of factorial experiments. R package version **2.1-0**.

League-Pike, P. and M. Shulman. 2009. Intraguild predators: behavioral changes and mortality of the green crab (*Carcinus maenas*) during interactions with the American lobster (*Homarus americanus*) and Jonah crab (*Cancer borealis*). Journal of Crustacean Biology **29**:350-355.

Leonard, G. H., M. D. Bertness, and P. O. Yund. 1999. Crab predation, waterborne cues, and inducible defenses in the blue mussel, *Mytilus edulis*. Ecology **80**:1-14.

Leonard, G. H., J. M. Levine, P. R. Schmidt, and M. D. Bertness. 1998. Flow-driven variation in intertidal community structure in a Maine estuary. Ecology **79**:1395-1411.

Levin, L. 2006. Recent progress in understanding larval dispersal: new directions and digressions. Integrative and Comparative Biology **46**:282-297.

Lima, S. 1998. Nonlethal effects in the ecology of predator-prey interactions. Bioscience **48**:25-34.

Lima, S. L. and P. A. Bednekoff. 1999. Back to the basics of antipredatory vigilance: can nonvigilant animals detect attack? Animal Behaviour **58**:537-543.

Long, J. and G. Trussell. 2007. Geographic variation in seaweed induced responses to herbivory. Marine Ecology Progress Series **333**:75.

Malmqvist, B. and G. Sackmann. 1996. Changing risk of predation for a filter-feeding insect along a current velocity gradient. Oecologia **108**:450-458.

Matschiner, M. and W. Salzburger. 2009. TANDEM: integrating automated allele binning into genetics and genomics workflows. Bioinformatics **25**:1982-1983.

McInerney, C., A. Allcock, M. Johnson, and P. Prodöhl. 2009. Understanding marine reserve function in a seascape genetics context: *Nucella lapillus* in Strangford Lough (Northern Ireland) as an example. Aquatic Biology **7**:45-48.

Menge, B. 2000. Top-down and bottom-up community regulation in marine rocky intertidal habitats. Journal of Experimental Marine Biology and Ecology **250**:257-289.

Menge, B. and J. Lubchenco. 1981. Community organization in temperate and tropical rocky intertidal habitats: prey refuges in relation to consumer pressure gradients. Ecological Monographs **51**:429-450.

Menge, B. and J. Sutherland. 1987. Community regulation: variation in disturbance, competition, and predation in relation to environmental stress and recruitment. American Naturalist **130**:730-757.

Menge, B. A. 1983. Components of predation intensity in the low zone of the New England rocky intertidal region. Oecologia **58**:141-155.

Mittelbach, G., D. Schemske, H. Cornell, A. Allen, J. Brown, M. Bush, S. Harrison, A. Hurlbert, N. Knowlton, and H. Lessios. 2007. Evolution and the latitudinal diversity gradient: speciation, extinction and biogeography. Ecology Letters **10**:315-331.

Moody, R. M. and R. B. Aronson. 2007. Trophic heterogeneity in salt marshes of the northern Gulf of Mexico. Marine Ecology Progress Series **331**:49-65.

Nakaoka, M. 2000. Nonlethal effects of predators on prey populations: predator-mediated change in bivalve growth. Ecology **81**:1031-1045.

Paine, R. 1969. A note on trophic complexity and community stability. American Naturalist **103**:91-93.

Paine, R. T. 1966. Food web complexity and species diversity. American Naturalist **103**:65-75.

Palmer, A. 1982. Growth in marine gastropods. A non-destructive technique for independently measuring shell and body weight. Malacologia **23**:63-74.

Palmer, A. R. 1990. Effect of crab effluent and scent of damaged conspecifics on feeding, growth, and shell morphology of the Atlantic dogwhelk *Nucella lapillus* (L.). Hydrobiologia **193**:155-182.

Peckarsky, B. L., P. A. Abrams, D. I. Bolnick, L. M. Dill, J. H. Grabowski, B. Luttbeg, J. L. Orrock, S. D. Peacor, E. L. Preisser, and O. J. Schmitz. 2008. Revisiting the classics: considering non-consumptive effects in textbook examples of predator-prey interactions. Ecology **89**:2416-2425.

Pennings, S. C., E. L. Siska, and M. D. Bertness. 2001. Latitudinal differences in plant palatability in Atlantic coast salt marshes. Ecology **82**:1344-1359.

Post, E., R. O. Peterson, N. C. Stenseth, and B. E. McLaren. 1999. Ecosystem consequences of wolf behavioural response to climate. Nature **401**:905-907.

Powers, S. P. and J. N. Kittinger. 2002. Hydrodynamic mediation of predator–prey interactions: differential patterns of prey susceptibility and predator success explained by variation in water flow. Journal of Experimental Marine Biology and Ecology **273**:171-187.

Preisser, E. L., D. I. Bolnick, and M. F. Benard. 2005. Scared to death? The effects of intimidation and consumption in predator-prey interactions. Ecology **86**:501-509.

Preisser, E. L., D. I. Bolnick, and J. H. Grabowski. 2009. Resource dynamics influence the strength of non consumptive predator effects on prey. Ecology Letters **12**:315-323.

Preisser, E. L., J. L. Orrock, and O. J. Schmitz. 2007. Predator hunting mode and habitat domain alter nonconsumptive effects in predator-prey interactions. Ecology **88**:2744-2751.

Pritchard, J. K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics **155**:945-959.

R Development Core Team. 2010. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna.

Rahman, S. and D. R. Webster. 2005. The effect of bed roughness on scalar fluctuations in turbulent boundary layers. Experiments in Fluids **38**:372-384.

Rahman, Y. J., R. B. Forward, and D. Rittschof. 2000. Responses of mud snails and periwinkles to environmental odors and disaccharide mimics of fish odor. Journal of Chemical Ecology **26**:679-696.

Relyea, R. 2001a. The relationship between predation risk and antipredator responses in larval anurans. Ecology **82**:541-554.

Relyea, R. 2004. Fine-tuned phenotypes: tadpole plasticity under 16 combinations of predators and competitors. Ecology **85**:172-179.

Relyea, R. A. 2001b. Morphological and behavioral plasticity of larval anurans in response to different predators. Ecology **82**:523-540.

Relyea, R. A. and E. E. Werner. 2000. Morphological plasticity in four larval anurans distributed along an environmental gradient. Copeia **2000**:178-190.

Robinson, E. M., D. L. Smee, and G. C. Trussell. 2011. Green Crab (*Carcinus maenas*) foraging efficiency reduced by fast flows. PLoS ONE **6**:e21025.

Rolan, E., J. Guerra-Varela, I. Colson, R. N. Hughes, and E. Rolan-Alvarez. 2004. Morphological and genetic analysis of two sympatric morphs of the dogwhelk *Nucella lapillus* (Gastropoda : Muricidae) from Galicia (northwestern Spain). Journal of Molluscan Studies **70**:179-185.

Scattergood, L. 1952. The distribution of the green crab, *Carcinus maenas* (L.) in the northwestern Atlantic. Department of Sea and Shore Fisheries Circular **8**:1-10.

Schemske, D. W., G. G. Mittelbach, H. V. Cornell, J. M. Sobel, and K. Roy. 2009. Is there a latitudinal gradient in the importance of biotic interactions? Annual Review of Ecology, Evolution and Systematics **40**:245-269.

Schmitz, O. J. 1998. Direct and indirect effects of predation and predation risk in old-field interaction webs. American Naturalist **151**:327-342.

Schmitz, O. J., A. P. Beckerman, and K. M. O'Brien. 1997. Behaviorally mediated trophic cascades: effects of predation risk on food web interactions. Ecology **78**:1388-1399.

Schmitz, O. J., V. Krivan, and O. Ovadia. 2004. Trophic cascades: the primacy of trait mediated indirect interactions. Ecology Letters **7**:153-163.

Schoeppner, N. M. and R. A. Relyea. 2005. Damage, digestion, and defense: the roles of alarm cues and kairomones for inducing prey defenses. Ecology Letters **8**:505-512.

Seeley, R. 1986. Intense natural selection caused a rapid morphological transition in a living marine snail. Proceedings of the National Academy of Sciences, USA **83**:6897-6901.

Sih, A., P. Crowley, M. McPeek, J. Petranka, and K. Strohmeier. 1985. Predation, competition, and prey communities: a review of field experiments. Annual Review of Ecology and Systematics **16**:269-311.

Slatkin, M. 1995. A measure of population subdivision based on microsatellite allele frequencies. Genetics **139**:457-462.

Smee, D., M. Ferner, and M. Weissburg. 2010. Hydrodynamic sensory stressors produce nonlinear predation patterns. Ecology **91**:1391- 1400.

Smee, D. and M. Weissburg. 2008. Heightened prey responses in risky habitats: predation pressure increases prey sensitivity to predation risk. Marine Ecology Progress Series **363**:39-50.

Smee, D. L., M. C. Ferner, and M. J. Weissburg. 2008. Alteration of sensory abilities regulates the spatial scale of nonlethal predator effects. Oecologia **156**:399-409.

Smee, D. L. and M. J. Weissburg. 2006a. Clamming up: environmental forces diminish the perceptive ability of bivalve prey. Ecology **87**:1587-1598.

Smee, D. L. and M. J. Weissburg. 2006b. Hard clams (*Mercenaria mercenaria*) evaluate predation risk using chemical signals from predators and injured conspecifics. Journal of Chemical Ecology **32**:605-619.

Sokal, R. R. and F. J. Rohlf. 1995. Biometry: the principles and practice of statistics in biological research 3rd edition. WH Freeman and Co., New York.

Spence, S., G. Bryan, P. Gibbs, D. Masters, L. Morris, and S. Hawkins. 1990. Effects of TBT contamination on *Nucella* populations. Functional Ecology **4**:425-432.

SPSS, I. 2005. SPSS 14.0 for Windows.

Thorson, G. 1950. Reproductive and larval ecology of marine bottom invertebrates. Biological Reviews **25**:1-45.

Trussell, G., P. Ewanchuk, and C. Matassa. 2006a. The fear of being eaten reduces energy transfer in a simple food chain. Ecology **87**:2979-2984.

Trussell, G., P. Ewanchuk, and C. Matassa. 2006b. Habitat effects on the relative importance of trait-and density-mediated indirect interactions. Ecology Letters **9**:1245-1252.

Trussell, G. and L. Smith. 2000. Induced defenses in response to an invading crab predator: an explanation of historical and geographic phenotypic change. Proceedings of the National Academy of Sciences, USA **97**:2123-2127.

Trussell, G. C. 1996. Phenotypic plasticity in an intertidal snail: The role of a common crab predator. Evolution **50**:448-454.

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

Turner, A. 2008. Predator diet and prey behaviour: freshwater snails discriminate among closely related prey in a predator's diet. Animal Behaviour **76**:1211-1217.

Turner, A. and G. Mittelbach. 1990. Predator avoidance and community structure: interactions among piscivores, planktivores, and plankton. Ecology:2241-2254.

Turner, A. and S. Montgomery. 2003. Spatial and temporal scales of predator avoidance: experiments with fish and snails. Ecology **84**:616-622.

Turner, A. M., R. J. Bernot, and C. M. Boes. 2000. Chemical cues modify species interactions: the ecological consequences of predator avoidance by freshwater snails. Oikos **88**:148.

Vadas, R., M. Burrows, and R. Hughes. 1994. Foraging strategies of dogwhelks, *Nucella lapillus* (L.): interacting effects of age, diet and chemical cues to the threat of predation. Oecologia **100**:439-450.

Valeix, M., A. Loveridge, S. Chamaille-Jammes, Z. Davidson, F. Murindagomo, H. Fritz, and D. Macdonald. 2009. Behavioral adjustments of African herbivores to predation risk by lions: spatiotemporal variations influence habitat use. Ecology **90**:23-30.

Vermeij, G. 1982. Phenotypic evolution in a poorly dispersing snail after arrival of a predator. Nature **299**:349-350.

Vermeij, G. 1991. Anatomy of an invasion: the trans-Arctic interchange. Paleobiology **17**:281-307.

Vickers, N. J. 2000. Mechanisms of animal navigation in odor plumes. The Biological Bulletin **192**: 203-212.

Wares, J. P. and C. W. Cunningham. 2001. Phylogeography and historical ecology of the North Atlantic intertidal. Evolution **55**:2455-2469.

Webster, D. R. and M. J. Weissburg. 2001. Chemosensory guidance cues in a turbulent chemical odor plume. Limnology and Oceanography **46**:1034-1047.

Weir, B. and C. C. Cockerham. 1984. Estimating F-statistics for the analysis of population structure. Evolution **38**:1358-1370.

Weissburg, M. J. 2000. The fluid dynamical context of chemosensory behavior. The Biological Bulletin **198**:188-202.

Weissburg, M. J., M. C. Ferner, D. P. Pisut, and D. L. Smee. 2002. Ecological consequences of chemically mediated prey perception. Journal of Chemical Ecology **28**:1953-1970.

Weissburg, M. J., C. P. James, D. L. Smee, and D. R. Webster. 2003. Fluid mechanics produces conflicting constraints during olfactory navigation of blue crabs, *Callinectes sapidus*. Journal of Experimental Biology **206**:171-180.

Weissburg, M. J. and R. K. Zimmer-Faust. 1993. Life and death in moving fluids: hydrodynamic effects on chemosensory-mediated predation. Ecology **74**:1428-1443.

Weissburg, M. J. and R. K. Zimmer-Faust. 1994. Odor plumes and how blue crabs use them in finding prey. Journal of Experimental Marine Biology and Ecology **197**:349-375.

Werner, E. E. and S. D. Peacor. 2003. A review of trait-mediated indirect interactions in ecological communities. Ecology **84**:1083-1100.

Wilson, D. J. and H. Lefcort. 1993. The effect of predator diet on the alarm response of red-legged frog, *Rana aurora*, tadpoles. Animal Behaviour **46**:1017-1019.

Zimmer, R. and C. Butman. 2000. Chemical signaling processes in the marine environment. The Biological Bulletin **198**:168-187.

Zimmer, R. and C. Zimmer. 2008. Dynamic scaling in chemical ecology. Journal of Chemical Ecology **34**:822-836.

BIOGRAPHICAL SKETCH

Scott Isaac Large received a Bachelor of Arts in Biology from Hendrix College (Conway, Arkansas) in 2006. Upon graduation, he entered the Master's of Science in Biology program at Texas A&M University—Corpus Christi in 2006 working under Dr. Delbert L. Smee. Scott conducted research at the Darling Marine Center under a National Science Foundation grant to Drs. Delbert L. Smee and Geoffrey C. Trussell (co-PIs). Scott is also a member of the Ecological Society of America, American Association for the Advancement of Science, and the Texas Academy of Science and has presented his work at conferences throughout the United States. To date, Scott has authored two peer-reviewed manuscripts on prey detection and response to predation risk. Scott now resides in Falmouth, MA and is employed as a Post-doctoral Research Fellow for the National Oceanic and Atmospheric Administration Fisheries Division at Woods Hole Oceanographic Institute.