EFFECT OF PREDATOR DIET ON FORAGING BEHAVIOR OF *PANOPEUS HERBSTII* IN RESPONSE TO PREDATOR URINE CUES

A Thesis
Presented to
The Academic Faculty

by

Lauren E. Connolly

In Partial Fulfillment
of the Requirements for the Degree
Master of Science in the
School of Biology

Georgia Institute of Technology
December 2013

EFFECT OF PREDATOR DIET ON FORAGING BEHAVIOR OF *PANOPEUS HERBSTII* IN RESPONSE TO PREDATOR URINE CUES

Approved by:

Dr. Marc Weissburg, Advisor
School of Biology
Georgia Institute of Technology

Dr. Don Webster
School of Civil and Environmental Engineering
Georgia Institute of Technology

Dr. Mark Hay
School of Biology
Georgia Institute of Technology

Dr. Lin Jiang
School of Biology
Georgia Institute of Technology

Date Approved:
November 11, 2013

ACKNOWLEDGEMENTS

I would like to thank my committee including my advisor for their help and ideas in developing this thesis. Particular thanks go to my advisor Marc Weissburg for his support and advice while I conducted my research. This thesis would not be possible without them.

I would further like to thank the Byers lab from the University of Georgia without whom the many months down at the Skidaway Institute of Oceanography would have been unbearable. Their friendship and support helped me through many a lonely day in the field. Special thanks go to Jenna Malek and Linsey Haram for their help in the field; thanks for staying through the lightning. Additional thanks go to Martha Sanderson for her aid in crab "pee" collection, thanks for facing you fear of crabs to give me a hand. Jeb Byers deserves special acknowledgement for his loan of equipment and facilities without which this research would not have been possible.

I would also like to thank Buddy and Judy Sharpton for their amazing help and support. Thank you for the dinners, the housing, and letting me a part of your family. My research in Savannah would not have been possible without your kind help and advice.

Thanks go to my fellow student Larisa Pender-Healy. Your advice and friendship was essential to my survival. Thank you for all the laughter that helped me through the tough times. Your help studying and editing over the years was indispensable. I'll never forget "Animal Hoarders" or copepods drinking coffee. I am glad to count you as my friend.

Special thanks go to my good friends Randi and Asa Wilson. You've fed me, you've housed me, and you've helped me at my lowest. You are the best kind of people and I don't know what I would have done without you. Special thanks to Randi for all the conversations about science, advice about graduate school, and advice about writing. I never would have made it without you.

Finally, I would like to thank my parents. I know you wished I would stay when I said I was headed to Georgia but you supported me anyways. You have been every step of the way and helped me however you could. You listened to me rant about science even when you didn't understand what I was talking about and even came and glued some clams. Thank you for all your love and support.

TABLE OF CONTENTS

LIST OF FIGURES

SUMMARY

The ability of prey to detect and respond appropriately to predator risk is important to overall prey fitness. Many aquatic organisms assess risk through the use of chemical cues that can change with predator diet. Two variable characteristics of diet are: 1. prey type and 2. prey mass. To assess the effect of these two characteristics on the assessment of risk by the mud crab *Panopeus herbstii*, I exposed mud crabs to the urine of the blue crab *Callinectes sapidus* fed one of 5 diet treatments: 10g of oyster shell free wet mass, 5g of oyster shell free wet mass, 10g crushed mud crabs, 5g crushed mud crabs, and a mix of 5g of oyster shell free wet mass and 5g crushed mud crab. Effects on *P. herbstii* foraging were tested in a previously developed bioassay by measuring shrimp consumption over a 4 hour period. I hypothesized that *P. herbstii* would have a larger magnitude response to urine from *C. sapidus* fed a diet of crushed mud crabs than to urine from *C. sapidus* fed a diet of oysters. I further hypothesized that *P. herbstii* would have a larger magnitude response to urine from *C. sapidus* fed a high mass diet relative to a lower mass diet. Contrary to expectations there was no observed effect of urine on *P. herbstii* foraging in any of the treatments. Results suggest that bioassay protocol may be unreliable suggesting further replication to determine the difference between this study and previous results. Future studies examining how *P. herbstii* varies with urine concentration will aid in understanding the ecological scale of this predator cue system. Determining the role of other potential cue sources will improve the predictive abilities of these studies.

CHAPTER 1

EFFECT OF PREDATOR DIET ON FORAGING BEHAVIOR OF *PANOPEUS HERBSTII* IN RESPONSE TO PREDATOR URINE CUES

1.1 Introduction

The ability of prey to assess predation risk and respond appropriately has a significant impact on survival and fitness. Prey utilize a range of defenses against predators. These defenses, however, are generally costly; whether it is due to lost opportunities (e.g. mating), lost resources (e.g. foraging) or metabolic expenditure (Lively 1986). It follows, therefore, that in an unpredictable biological landscape, prey would evolve to detect predators and respond with an appropriate defense only when a predator is present (Harvell 1990). Such inducible defenses are a common prey strategy and encompasses a broad spectrum of defensive forms including morphological, chemical, and behavioral (Harvell 1990, Padilla and Savedo 2013). For example, *Daphnia* respond to chemical cues from predators with changes in their morphology that reduce susceptibility to predators (Dodson 1989, Spitze 1992). Other organisms induce the production of unique metabolites that are toxic or otherwise harmful to predators to deter predation. The brown algae *Ascophyllum nodosum* induces production of phlorotannins—a known herbivore deterrent—in response to physical damage from the grazer *Littorina obtusata* (Pavia and Toth 2000). Changes in behavior of prey in the response to predator presence is increasingly recognized as an important and impactful form of prey defense (Peckarsky et al. 2008). Predation risk can cause a number of

changes in prey behavior including reduced foraging, decreased activity, missed mating events and changes in habitat use (Lima and Dill 1990, Lima 1998). Changes in behavior affect not only the prey species, but can also have farther reaching impacts in the community. For instance, suppression in foraging of the intertidal snail *Littorina littorea* in response to cues from the green crab *Carcinus maenus* results in changes in the abundance and diversity of the algal community (Trussell et al. 2002). Inducible defenses thus have important consequences both for the individual prey species and the community as a whole.

To employ inducible defenses, prey must detect predators and assess predation risk. Many prey species detect predators through chemical cues (Ferrari et al. 2010). This is particularly true in marine environments where chemical cues may be more efficient than other forms of detection because they allow prey to detect predators from a distance and may be more reliable in turbid environments where vision is limited. This extended range is of particular interest because it allows prey to react before they come in contact with a predator. Chemical cues will also expand the interaction over time as cues can persist in the environment for hours (Ferner et al. 2005). This allows prey to detect and react to predation events that have occurred in the past. However, just as prey species are selected to avoid detection by predators, predators are selected to avoid detection by prey. Predators are unlikely to evolve to release cues that make them easily detectable by prey. However, metabolites are necessarily released by predators as part of the process of being alive (breathing, excreting etc.) and these metabolites are subsequently dispersed to the environment by diffusion and bulk flow. Metabolites or combinations of metabolites that uniquely identify the releaser can be used by prey to

assess risk. In this case, prey are essentially "spying" on predators by evolving to recognize these necessary but unique releases. These metabolites may be particularly reliable cues for prey because the necessity of their release may reduce the level of control predators have over these cues. Alternatively, prey can also evolve to respond to cues released by conspecifics. When an organism is damaged, chemical substances are leaked to the environment. Many organisms have evolved to detect these chemicals as sign of risk (Ferrari et al. 2010). In some cases, it is proposed that prey species have evolved to release specific alarm substances to signal risk to others (Mathis and Smith 1993, Chivers and Smith 1998, Kats and Dill 1998, Bryer et al. 2001). However, this should be rare as the alarm signal must provide a benefit to the sender in order to evolve (Smith 1992). Evolution of alarm signals, therefore, has typically been proposed to occur in closely related communities through kin selection (Chivers and Smith 1998). Alternatively, it has been hypothesized that alarm signals may provide benefit to senders by calling in the predators of their predator (Mathis et al. 1995). Overall, there are a multiple sources of metabolites available to prey species that may signal potential risk.

Metabolites are released by all organisms at all trophic levels. Competitors, prey, and non-threatening heterospecifics in the environment will all be releasing metabolites to the environment along with predators. The question becomes how do prey recognize a particular metabolite or metabolites as a sign of predation risk? Some prey appear to have evolved to recognize predator cues from birth, predator recognition is innate (Vilhunen and Hirvonen 2003, Hawkins et al. 2007, Dixson et al. 2012, Mogali et al. 2012). In a two channel flume assay naïve larval reef fishes significantly avoided predator cues (Dixson et al. 2012). This effect was also evident in the field where the

application of these predator diet cues to coral reefs resulted in decreased juvenile recruitment. Other organisms appear to learn to recognize predators by associating cues from crushed prey or digested alarm cues with a novel predator (Brown and Godin 1999, Mirza and Chivers 2003a, Ferrari et al. 2010). In this case, predator experience with the prey species provides an accurate assessment of predation risk.

One factor that can aid prey in assessing which cues are associated with risk is predator diet. Changes in predator diet have been shown to invoke changes in prey assessment of risk. Predators that consume conspecifics are likely riskier than those that consume unrelated heterospecifics. In fact, a number of prey species increase their anti-predator responses in response to cues from the consumption of conspecifics or closely related heterospecifics (Brodin et al. 2006, Ferland-Raymond and Murray 2008, Ferrari et al. 2010, Manassa and McCormick 2012). These stronger magnitude responses suggests that changes in diet can change the chemical signature of a predator in such a way that prey recognize them as riskier when consuming conspecifics. In some cases, this dietary assessment is directly related to alarm chemicals produced in conspecifics. The predators become "labeled" by digested alarm chemicals that pass through the digestive system (Mathis and Smith 1993, Brown et al. 1995, Ferrari et al. 2010). These increased assessments of risk can be reflected in prey defenses in two ways: (1) increased magnitude of responses, and/or (2) induction of new or additional responses (Schoeppner and Relyea 2009). In the case of increased magnitude, prey species will induce a more extreme phenotypic response in response to riskier cues. Damselfly larvae showed decreased activity and predator avoidance when exposed to cues from a dragonfly predator fed both zooplankton and damselfly larvae (Brodin et al. 2006). However, the

response to dragonflies fed damselfly larvae was significantly stronger, exhibiting the lowest activity and the strongest avoidance of the predator area (Brodin et al. 2006). In the case of new or additional responses, the information in the cues about perceived risk changes allow prey to change or adjust to a more appropriate defensive strategy. This adjustment in strategy may result because the kairomones from predators consuming conspecifics imply greater risk and therefore justify more costly defensive strategy or it may be that these kairomones signify a different type of risk, which requires the use of a different defensive strategy. For example, a general kairomone from a predator might indicate that predator abundance is high suggesting long term risk and induce long term strategies such as adjustment of life history traits, while kairomones that include chemicals from conspecifics might suggest a more immediate risk and induce a short term strategy such as decreased foraging.

Conversely some prey do not respond to changes in predator diet. The hard-clam, *Mercenaria mercenaria*, reduces pumping in response to chemical cues from the blue crab *Callinectes sapidus* regardless of diet type (Smee and Weissburg 2006). *Nucella* decreased movement in response to green crabs *Carcinus maenus* that consumed both mussels and conspecifics and the intensity of their behavior reflected an equivalent risk assessment (Large and Smee 2010). In this case, past diet may not provide specific information about predation risk. Both *Callinectes sapidus* and *Carcinus maenus* are generalist predators. Information about past diet likely does not provide accurate data about predation risk when predators feed opportunistically on a wide variety of prey (Smee and Weissburg 2006). However, many species that lack diet specific responses retain the ability to respond to injured conspecifics which may allow for a more accurate

assessment of risk (Smee and Weissburg 2006, Large and Smee 2010). These cues from damaged conspecifics are known as alarm cues. Alone, alarm cues are generally weaker than those from feeding predators (Alexander and Covich 1991, Ferrari et al. 2010). However, the addition of these alarm cues to predator cues can enhance the response of prey (Schoeppner and Relyea 2005, 2009). This illustrates one feature of how multiple cues can potentially interact. When prey obtain cues from multiple sources that can influence the same trait they can interact in one of three ways: (1) additive in which the net phenotype is simply the sum of the phenotype effects when prey are exposed to the cues separately; (2) interference in which the phenotype is less than the separate phenotype effects; or (3) synergistic in which the phenotype is greater than the sum of the separate effects. Both additive and synergistic effects have been shown in prey defenses when prey receive cues from multiple sources (Schoeppner and Relyea 2005, 2009). This can make it difficult to predict prey phenotypes in the community, as prey phenotype cannot be determined by simply summing individual reactions to cues and signals. The introduction of new cues either from other individuals of the same species or other predator species could fundamentally alter the anti-predator responses of prey (DeWitt and Langerhans 2003). Additionally, the response of prey to one predator can alter its vulnerability to other predators (Carey and Wahl 2011).

Another way prey might perceive risk is through concentration of metabolites (Marcus and Brown 2003, Kusch et al. 2004). Fathead minnows respond to cues from pike only once a threshold concentration of cue was reached (Kusch et al. 2004). Larger concentrations of metabolites may indicate predator proximity as cues will be at higher concentration nearest their source and closer predators represent higher risk.

Alternatively, larger concentrations of metabolites may indicate higher risk because larger predators will produce higher volumes of metabolites. The concentration of metabolites could also be influenced by the mass of prey eaten. McCoy et al. (2012) found that amount of prey consumed impacted the strength of the anti-predator responses. Red-eyed tree frog tadpoles that were exposed to cues from a dragonfly predator fed varying masses of tadpoles decreased growth as a function of the biomass of prey consumed (McCoy et al. 2012). In this case, stronger non-consumptive effects result because increased prey mass appears to leads to higher output of the metabolites associated with predator risk and therefore an increase in the perceived predation risk by prey. Prey mass may also be important because increased output of metabolites could result in a larger perceptive distance for potential prey. Chemical cues are degraded over distance by turbulent mixing (Keller and Weissburg 2004). Increasing chemical concentrations may allow chemicals to be detected over longer distances because it will take longer for cues to degrade below detectable levels allowing the cue to travel farther. Increased prey perceptive distance will increase the number of prey influenced by chemical cues from a single predator. The relative importance of these non-consumptive effects compared to consumptive effects is important to understanding how predator impacts will reverberate through communities (Preisser et al. 2005). If increased prey mass causes increased prey perceptive distance it suggests the relative magnitude of non-consumptive effects may be a function of the magnitude of consumptive effects.

Understanding where cues associated with these diet specific responses are released allows for better assessment of prey behavior and assessment of risk. One potential source of cues is urine. Urine is a necessary metabolic release that is a frequent

cue for prey, and induces a range of anti-predator behaviors in both aquatic and terrestrial systems (Nolte et al. 1994, Brown et al. 1995, Apfelbach et al. 2005, Shrader et al. 2008). The widespread use of urine as a predator cue suggests that it is a particularly reliable indicator of predation risk. Urine as an end product of digestion has a potential to provide information about past diet and allow prey to make accurate decisions about predation risk. However, whether diet cues result from predator cues in urine alone, or from a combination of predator cues in urine and external conspecific cues released while feeding is unclear in most cases.

Mud crabs, *Panopeus herbstii*, are important members of the oyster reef communities and respond to chemical cues from predators with decreased foraging and increased refuge use (Grabowski 2004, Grabowski and Kimbro 2005, Grabowski et al. 2008, Hill and Weissburg 2013a, b). These foraging effects are of particular interest because mud crabs consume significant amounts of juvenile oysters and have the potential to impact overall community health (O'Connor et al. 2008). Foraging by mud crabs is more strongly suppressed in response to blue crabs that have consumed conspecifics than to either blue crabs fed oysters or cues from crushed conspecifics alone (Hill 2011). However, since mud crabs were exposed to both cues from feeding blue crabs and those from crushed conspecifics in the treatment where blue crabs were fed crushed mud crabs, it is unclear if this stronger effect results from dietary cues alone or is the result of a combination of feeding cues and crushed conspecific cues. *P. herbstii* responds similarly to large predators and multiple small predators of the same biomass (Hill and Weissburg 2013a, b). This suggests that *P. herbstii* will assess risk based on concentration of cues in the absence of other cues (Hill and Weissburg 2013b). If mud

crabs are already sensitive to changes in cue concentration from predator cues to assess predator body size, they further might be able to detect changes in cue concentration concurrent with changes in prey mass in the predator diet. Here we explore two questions related to the chemically mediated non-consumptive effect in the mud crab, *P. herbstii*: (1) does prey mass in a predator diet effect the magnitude of the non-consumptive effect; and (2) does prey type in a predator diet effect the magnitude of the non-consumptive effect? To answer these questions I explored the mud crab response to blue crab urine alone, to separate the potential effects of predator diet cues, from external conspecific alarm cues that might result at the site of predation event. I hypothesized that *P. herbstii* would have a larger magnitude response to urine from *C. sapidus* fed a diet of crushed mud crabs than to urine from *C. sapidus* fed a diet of crushed oysters. I further hypothesized that *P. herbstii* would have a larger magnitude response to urine from *C. sapidus* fed a high mass diet relative to a lower mass diet.

1.2 Methods and Materials

1.2.1 Animal Collection and Maintenance

Blue crabs (*Callinectes sapidus*) were collected using commercial crab traps or purchased from local fishermen from Wassaw Sound. Mud crabs (*Panopeus herbstii*) were collected by hand from oyster reefs in the Savannah River Estuary. After collection, all crabs were housed in outdoor flow-through seawater tanks at the Skidaway Institute of Oceanography. All crabs were maintained on a diet of crushed bivalves ad libitum, fed every other day until the experimental time period. Blue crabs were starved for 24 hours before beginning of feeding for diet treatments to empty the stomach of any past diet (McGaw and Reiber 2000). Mud crabs were starved for 48 hours prior to the bioassay to ensure a hunger level that would result in significant consumption (Hill and Weissburg 2013a, b).

1.2.2 Experimental Design

I employed a factorial design to answer our two questions: (1) does prey type affect the magnitude of non-consumptive effects in response to predator diet cues; and (2) does prey mass affect the magnitude of non-consumptive effects in response to predator diet cues? Adult *C. sapidus* were fed one of 5 dietary treatments: (1) 10g of oyster shell free wet mass, (2) 5g of oyster shell free wet mass, (3) 10g of crushed mud crab, (4) 5g of crushed mud crabs and (5) a mix of 5g of oyster shell free wet mass and 5g of crushed mud crabs. The inclusion of the mixed diet treatment allows assessment of whether these mechanisms (prey type and prey mass) can affect NCEs independently and if so whether these affects are additive or synergistic. Diet masses were determined based on initial feeding trials to ensure complete consumption over the feeding period (unpublished observation). In addition to these treatments, a small group of adult blue crabs was fed an ad libitum diet of shrimp for comparison with the initial urine exposure bioassay conducted at Georgia Tech (Poulin, unpublished).

1.2.3 Experimental Feeding

Blue crab feeding was conducted in runs with each run consisting of 5-7 adult blue crabs randomly assigned to each of the diet treatments. Blue crab predators were placed in isolation boxes within larger flow-through tanks. Crabs were initially starved for 24hours to empty the digestive and excretory systems of any previous material. Each crab was fed the appropriate diet type and mass for 3 days. For all mud crab diets, live mud crabs were crushed with a blunt instrument directly on the carapace to ensure rapid death. The walking legs were removed, as ratio of wet mass:carapace is low in the legs and we wanted the measured mass to be as close to the wet mass (consumable) as possible. For all oyster diets the ratio of shell free wet mass:total mass (shell + internal wet mass) was

first determined by constructing a linear regression based on measurements of the total mass (g) and corresponding wet mass (g), n=235. The relationship between total mass and shell free wet mass was determined to follow the following equation:

Shell Free Wet Mass (g) = 0.211 (Total Mass (g)) – 0.085 (R^2=0.95, F=4562.9, p<0.001)

Based on this equation *C. sapidus* predators were fed either 47.8g (H) or 24.1g (L) of crushed oysters.

1.2.4 Urine Collection

Urine was collected 8 hours post-feeding on the third day of feeding. *C. sapidus* were placed in an ice bath for 5-10 minutes to slow their metabolism and aid in handling. A small needle (23-25 gauge) connected to a vacuum was inserted into the nephropore at the opening to the atennal gland for urine collection. All tubing was Teflon coated to avoid absorption and loss of any chemical signals. Urine was removed via low pressure vacuum and collected in a glass vial. Samples were filtered through a 0.2 micron filter and stored at -20°C until the time of the experiment. All urine samples in a run were collected on the same day(s) to ensure the relative magnitudes between treatments was unaffected by any potential degradation of the signal while in storage. Urine was collected from each diet treatment unless precluded by animal deaths. Urine was collected using identical methods from blue crabs fed an ad libitum shrimp diet.

1.2.5 Bioassay

Due to restrictions of space and urine supply, replicates were conducted in blocks over the course of May-August 2013. Mud crab (*P. herbstii*) consumption in response to *C. sapidus* urine from the 5 different diet treatments was tested in a four hour bioassay. Mud crab consumption in response to *C. sapidus* urine from an ad libitum shrimp diet was also tested in four hour bioassay. Bioassay conditions were modeled on a previous study that

showed a significant response in *P.herbstii* foraging when exposed to urine from *C. sapidus* (Poulin unpublished data). Bioassays were performed in glass tanks (0.25x0.25cm) to minimize absorption of chemical signals from the urine. The sides of each tank were covered in black paper to eliminate the influence of visual cues from the outside environment. Each bioassay tank was filled with 2L of seawater collected from the flow-through systems at the Skidaway Institute of Oceanography (16-34‰). Each tank was provided with a central shell refuge as refuge seeking is a common antipredator behavior in mud crabs (Hill and Weissburg 2013b). Four mud crabs (carapace width 25-35mm) were placed in each bioassay tank, and allowed to acclimate for 15 minutes. After acclimations, 5mL of *C. sapidus* urine (or seawater control) was added to each tank. Each tank was lightly agitated to ensure mixing of the urine throughout the bioassay area. Each tank enclosure was provided with approximately 4g of pre-soaked shrimp cut into four approximately 1g pieces. Mud crabs were allowed to forage freely for 4 hours. At the end of 4 hours, the shrimp was recollected, blotted on a paper towel to remove excess moisture and re-weighed. For each block an additional bioassay tank with shrimp only (no *P. herbstii*) was set-up to measure change in shrimp mass over the course of the experiment. The amount of shrimp consumed was calculated in grams and as a percentage of the original amount provided. Mass was adjusted by the no *P. herbstii* control tank for each block to account for mass gain or loss unrelated to consumption over the course of experiment. The mass (g) and percentage of shrimp eaten from the 5 diet treatments were analyzed using a Generalized Linear Model in Systat 10.2. The mass (g) and percentage of shrimp eaten from the shrimp ad libitum diet were analyzed using a t-test in Systat 10.2.

1.3 Results

All mud crab consumption in treatments exposed to urine was similar to consumption in seawater controls (mean 47.2 ± 26.7%). Mud crab consumption in bioassays did change over time (GLM, BLOCK, $F_{(16, 41)}$=5.543, p<.001) but overall consumption in bioassays was equivalent regardless of treatment (GLM, $F_{(5, 41)}$=1.601, p=0.182). Results based on percent consumption were similar (GLM, $F_{(5, 41)}$=1.453, p=0.226).

Mud crabs consumed similar amounts of shrimp in bioassays regardless of prey mass. Mud crabs exposed to urine from blue crabs fed 10g of mud crabs (HMC) consumed a mean of 52.8 ± 24.7%, while those exposed to urine from blue crabs fed 5g of mud crabs consumed 49.4 ± 13.3 (Figure 1). This pattern was repeated in oyster diet treatments, where mud crabs exposed to urine from blue crabs fed 10g of oysters consumed a mean of 53.6 ± 20.2%, while those exposed to urine from blue crabs fed 5g of oysters consumed a mean of 56.9 ± 7.1% (Figure 1).

Comparing consumption across diets but within equivalent prey mass treatments, mud crabs consumed similar amounts of shrimp regardless of predator diet (Figure 1). Further, mud crabs exposed to urine from blue crabs fed a mixed diet of 5g mud crabs and 5g of oysters consumed a mean of 54.0 ± 16.1%, comparable to both the 10g of oysters (mean 53.6 ± 20.2%) and 10g of crushed mud crab (mean 52.8 ± 24.7%) treatments. There was no significant difference in mud crab consumption between mud crabs exposed to urine from blue crab fed an ad libitum shrimp diet (35.8±17.9%) and mud crabs exposed to a seawater control (64.7±30.6%) (t=1.415, d.f.=3.2, p=0.246).

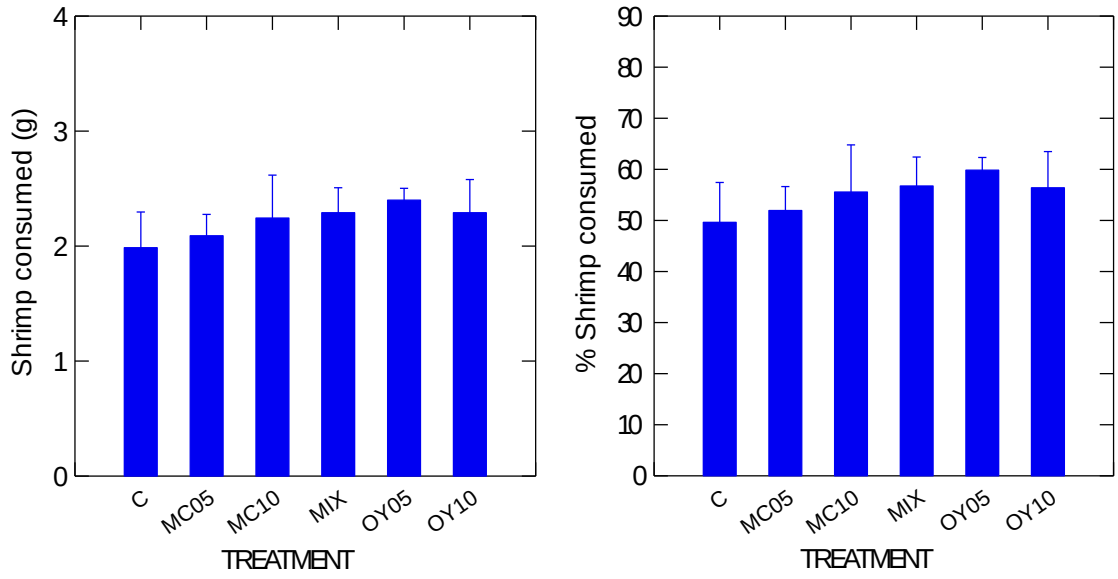

Figure 1. Shrimp Consumed by Mud Crabs in a Four Hour Bioassay by Diet Treatment. Left: Amount Eaten in grams; Right: Amount Eaten as a Percent of Total Provided. Mud crabs were exposed to 5mL of urine from blue crabs fed one of five different diets (MC10=10G crushed mud crab; MC05=5G crushed mud crab, OY10=10G of oyster wet mass, OY05=5G of oyster wet mass, MIX=5G crushed mud crab and 5G of oyster shell free wet mass) or 5mL of seawater control in 2L bioassay tanks. Mud crabs in each tank were given ~4g of shrimp. At the end of 4 hours the shrimp was re-weighed and a percentage of the original mass consumed was calculated. There was no significant difference between treatments (Left: GLM, F=1.601, P=0.182; Right: GLM, F=1.453, P=0.223)

1.4 Discussion

I detected no alteration in consumption by *Panopeus herbstii* due to urine from *Callinectes sapidus* predator regardless of predator diet. This was surprising as previous research indicated that *P. herbstii* respond to the presence of chemical cues from a blue crab predator with suppressed foraging and decreased activity (Hill and Weissburg 2013a, b). Urine was selected as the likely source of those cues because urine is the source of a number of bioactive molecules in crustaceans (Atema 1995, Shabani et al.

2009, Hardege et al. 2011, Anderson and Behringer 2013) and urine in general is a frequent source of predator signals (Nolte et al. 1994, Ferrari et al. 2010). Initial bioassays with blue crab urine showed a significant decrease in consumption associated with identical concentrations (5mL urine in 2L of seawater) as employed here (Poulin unpublished data). Contrary to these initial findings, all 5 diet treatments failed to show suppression relative to the control.

There are several potential explanations for the discrepancy between the findings here and the initial bioassay assessing the effect of blue crab urine on mud crab foraging. Initial bioassays used shrimp as a diet for blue crabs while we used oysters or mud crabs. One might expect that the 10g of crushed mud crabs used here should have a stronger magnitude affect than that initiated by a shrimp diet. Non-consumptive effects are frequently strongest when prey are exposed to predators fed conspecifics (Mirza and Chivers 2003b, Wirsing et al. 2005, Hoefler et al. 2012, Manassa and McCormick 2012). However, when prey recognize conspecifics through substances that are not alarm signals, the substances they use to identify conspecifics may be more general. Mammalian prey were shown to have a stronger reaction to urine from coyotes fed meat than those fed a diet of fruit (Nolte et al. 1994). In this case, recognition of increased predator risk appears to be largely mediated by presence of sulphurous compounds that are common in the urine after consumption of meat. This effect is also seen in marine systems, where larval fish recognized a non-piscivore "predator" as risky through the consumption of an artificial diet containing fish products (Dixson et al. 2012). The ability to label a non-piscivore as risky by introducing fish products to its diet suggests prey are responding to a general cue that is present in fish prey. In both the marine and

terrestrial examples, riskiness is associated with a general cue that is associated with consumption of a range of prey types rather than consumption of a specific species. If *P. herbstii* are cueing on a more generalized cue it is possible that this cue is present in larger concentrations in digested shrimp diet than those produced by digested mud crabs or oysters. If this is the case, it may require more urine to produce an equivalent concentration of cue. Prey should not evolve to respond to cues that are stronger in heterospecifics than conspecifics if they do not accurately reflect risk magnitude. Blue crabs, however, are generalist predators increasing the likelihood that a general cue could be an appropriate measure of risk.

The shrimp diet could also have produced a stronger response because of diet mass. As previously suggested the biomass of prey consumed can potentially affect the magnitude of a consumptive response by producing higher concentrations of cues signaling risk (McCoy et al. 2012). Blue crabs in the initial bioassay were fed a shrimp diet ad libitum. While high diet masses were chosen to be close to ad libitum, they were purposely kept slightly below ad libitum in order to ensure predator would consistently consume the entire biomass of interest. It is possible that *C. sapidus* only produce high enough concentrations of predator cues in their urine to have a significant impact on *P. herbstii* consumption when feeding ad libitum. While this might seem unlikely to evolve--as a predator is likely less risky when it is full and therefore would not provide a strong selective pressure--it is important to consider the time period of the bioassay. The bioassay is conducted for a four hour period, and it is possible that lower magnitude predator reactions instigated by lower prey masses might become apparent if consumption was assayed for a longer time period. Initial whole body experiments in the

field and in mesocosms demonstrating the suppression of *P.herbstii* were conducted over a period of 24 to 48 hours (Hill and Weissburg 2013a, b). Longer assay times may be necessary to discern weaker but still ecologically significant effects.

The results may also have been influenced by the use of natural seawater systems. Blue crab predators were housed in flow-through seawater tanks that were subject to natural fluctuations in salinity and temperature. *C. sapidus* are osmoconformers at high salinity (>27)‰, but osmoregulators at low salinity and urine production will fluctuate with changing salinity (Robinson 1982). Dietary cues should be a function of food consumption and therefore remain constant regardless of salinity. Urine production increases 2X in 50% seawater relative to that produced in 100% seawater (Robinson 1982). If urine production is fluctuating with salinity but dietary cues production is constant, diet cue concentration may have been variable. The variable cue concentration may have decreased cues levels below those detectable by *P. herbstii*. The likelihood that this affected our results is small. Fluctuations in urine production due to osmoregulation were likely small given that the bulk of osmoregulation occurs across the gills (Robinson 1982, Kinsey et al. 2003). However, it should not be dismissed entirely because it is unclear how close the concentration used in this study is to the minimum detectable concentration by *P. herbstii* for predator diet cues. Initial bioassays were only conducted at two cue levels (2.5mL of urine in 2L; 5mL in 2L) and those at the lower cue level were insignificant (Poulin unpublished data). If the current cue concentration is close to the minimum detectable concentration under bioassay conditions, very small variations in cue concentration could potentially lower the concentration enough to eliminate any significant behavioral reaction. Future studies should aim to construct a

concentration reaction curve so to better understand how the anti-predator behavior of *P. herbstii* varies with concentration of cue.

Natural seawater may also have affected the stability of the cue. Bioassays were conducted in 2L aliquot of natural seawater taken from flow through seawater system at the time of the experiment. This seawater passes through a sand filter designed to remove large particles of suspended materials but otherwise contains natural communities of microbes. Microbes contained in seawater may have broken down the cue limiting the ability of *P. herbstii* to detect and react to cues in the urine. Preliminary data suggests that the use of artificial vs. natural seawater may affect consumption by *P. herbstii* when exposed to *C. sapidus* urine (N=3, p=0.056, unpublished data). This effect however is unlikely to extend to field. Alarm cues in the field have been shown to persist for up to 18 hours (Ferner et al. 2005). The bioassays are performed in 2L of standing water with a single chemical release. In the field, flow will likely have a stronger influence on the detection and reaction to the cue than microbial interactions. Additionally, *C. sapidus* will be present in the field producing urine over time and likely have multiple releases. While it might not be possible to separate out the effects of the various potential mechanisms of signal degradation, future studies can examine the active area of the cue to determine the overall importance of potential cue degradation to the interaction.

Considering all five treatments failed to exhibit suppression relative to control, the results suggest it is possible that predator cues from *C. sapidus* are not located in the urine. While a number of bioactive molecules are found in crustacean urine (Atema 1995, Shabani et al. 2009, Hardege et al. 2011, Anderson and Behringer 2013) they are typically functional at much lower concentrations of urine than used in this study (Kamio

et al. 2008). Although, this may simply be because these studies are primarily examining pheromones in which the sender and receiver have evolved to participate in communication. Predator kairomones such as we studied here likely require higher concentrations for detection as only the prey has evolved to participate in the interaction. However, blue crab urine is produced at a rate of .09 to .18mL per 100g per hour (Robinson 1982), suggesting it would take 12-24 hours for the average adult blue crab to accumulate enough urine to match the output necessary to produce the effective concentrations in the initial bioassay.

The field and laboratory evidence with whole body *C. sapidus*, however, strongly supports that there are in fact chemical cues indicating predator risk to *P. herbstii* (Hill and Weissburg 2013a, b). This raises the question: where is this signal coming from if not from urine? Metabolites are constantly being released from living organisms as they eliminate wastes and release necessary signals; indicating that there are multiple alternative cue sources for prey attempting to detect predation risk. Similar to urine, feces is commonly known to act as a source of predator cues (Brown et al. 1995, Slusarczyk and Rygielska 2004, Griffiths and Richardson 2006, Ferrari et al. 2007, Shrader et al. 2008, Manassa and McCormick 2012). Like urine, feces have the potential to provide additional information about predator risk given that it is an after product of digestion and can therefore provide information about past-diet. Predator cues in feces may provide more consistent signal of predator risk because they will dissolve over time, creating a slow continual release of cues to the environment. Another potential source of anti-predator cues are substances excreted across the gills. During the breathing process, *C. sapidus* can release ions across the gills in order to excrete them from the body.

Anuran tadpoles and larval dragonflies appear to assess predation risk through the detection of a negative ion (Ferland-Raymond et al. 2010), opening the possibility that ions excreted across the gills could contribute to predator detection. This, however, is unlikely as crabs are primarily excreting mono-ions and substances must be actively transported across the surface of the gills (Weihrauch et al. 1999). If the gills are acting in predator cueing one candidate for the predator cue might be ammonia. Gills are one of the primary sites for excretion of ammonia in crabs (Weihrauch et al. 2004). Ammonia has previously been implicated in alarm cueing suggesting it could potentially be involved in predator cues as well (Hazlett 1990, Kiesecker et al. 1999).

Another possibility is that *P. herbstii* are not responding to a single cue substance but to a mixture of chemicals that it associates with predation risk. As previously stated, all organisms are producing metabolites as a part of the process of being alive. This might suggest that many of the potential cues available to prey are likely not exclusively produced by predators. In this case, it might not be a particular chemical that encodes predation risk but a particular combination of cues or ratio of concentrations that are particular to predator species that indicates risk. In this scenario, prey might need both cues from the urine and the feces to recognize a predator as risky. Hill and Weissburg (2013a,b) exposed prey to water flowing through a tank or cage containing a live predator. In this case, prey would be exposed to both urine and dissolved fecal cues. The burrowing bivalve *Macoma balthica* exhibits anti-predator responses to both fecal and urine cues both independently and in combination (Griffiths and Richardson 2006). In this case, both cues are not necessary to induce anti-predator behavior but that does not preclude the necessity in other systems. Combination cues may also be context

dependent. Since they rely on the sum of information from different cues, prey must evaluate the sum signal and respond with an appropriate behavior. External crushed conspecific cues are known to enhance predator cues in some systems (Schoeppner and Relyea 2005, 2009) and the combination of these cues could be necessary to the induction of anti-predator behavior in some systems. This, however, is unlikely in this system as *P. herbstii* have been shown to react to have significant reactions to *C. sapidus* fed un-related heterospecifics from the community (Hill and Weissburg 2013a, b). Further study into the potential response of *P. herbstii* to faecal cues both independently and in combination with urine cues might provide better insight into *P. herbstii* anti-predator behavior.

P. herbstii foraging behavior can have important impacts on oyster reef health. Mud crabs consume juvenile oysters and can therefore, have a significant impact on recruitment to reefs. Oyster reefs are ecologically important communities that have declined 94% worldwide (Jackson 2008). The reduced foraging seen in *P. herbstii* in response to chemical cues from *C. sapidus* (Hill and Weissburg 2013a, b), might suggest that the abundance of top predators could have a significant impact on oyster reef recruitment. As *C. sapidus* is a commercially important species that is subject to population declines due to overfishing, understanding the importance of this predator-prey interaction may be particularly important to assessing future impacts on reef health. The results of this study suggest that more needs to be understood about predator cue sources and effective cue concentrations to evaluate the ecological importance of this chemically mediated interaction.

1.4.1 Future Directions

Replication of the urine exposure bioassay under Georgia Tech laboratory and Savannah laboratory conditions is important to future studies evaluating the importance of urine

based predator cues in the *C. sapidus* (predator) – *P. herbstii* (prey). Replication under these two conditions should serve to clarify whether there is a systematic difference between the results of this study and that of the initial bioassay performed at Georgia Tech or if the bioassay itself is unreliable. Replication can likely be combined with an assessment of the difference between shrimp diet and the natural diets used here. This factor should be addressed as initial replication under conditions used in this study did show a non-significant drop in *P. herbstii* consumption when exposed to urine from *C. sapidus* fed shrimp (N=3, P=0.233) which could become significant with further replication. Future studies should examine how *P.herbstii* anti-predator behavior varies with urine concentration to better understand the ecological scale of this predator cue system. Examination of other potential cue sources independently and in combination with urine cues, will further the predictive ability of our studies.

REFERENCES

Alexander, J. E. and A. P. Covich. 1991. Predator avoidance by the fresh-water snail *Physella-virgata* in response to the crayfish *Procambarus-simulans*. Oecologia **87**:435-442.

Anderson, J. R. and D. C. Behringer. 2013. Spatial dynamics in the social lobster Panulirus argus in response to diseased conspecifics. Marine Ecology Progress Series **474**:191-200.

Apfelbach, R., C. D. Blanchard, R. J. Blanchard, R. A. Hayes, and I. S. McGregor. 2005. The effects of predator odors in mammalian prey species: A review of field and laboratory studies. Neuroscience and Biobehavioral Reviews **29**:1123-1144.

Atema, J. 1995. Chemical signals in the marine environment: dispersal, detection, and temporal signal analysis. Proceedings of the National Academy of Sciences of the United States of America **92**:62-66.

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., D. P. Chivers, and R. J. F. Smith. 1995. Localized defecation by pike: a response to labeling by cyprinid alarm pheromone? Behavioral Ecology and Sociobiology **36**:105-110.

Brown, G. E. and J. G. J. Godin. 1999. Who dares, learns: chemical inspection behaviour and acquired predator recognition in a characin fish. Animal Behaviour **57**:475-481.

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.

Carey, M. P. and D. H. Wahl. 2011. Foraging modes of predators and behaviors of prey determine the outcome of multiple predator interactions. Transactions of the American Fisheries Society **140**:1015-1022.

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

DeWitt, T. J. and R. B. Langerhans. 2003. Multiple prey traits, multiple predators: keys to understanding complex community dynamics. Journal of Sea Research **49**:143-155.

Dixson, D. L., M. S. Pratchett, and P. L. Munday. 2012. Reef fishes innately distinguish predators based on olfactory cues associated with recent prey items rather than individual species. Animal Behaviour **84**:45-51.

Dodson, S. I. 1989. The ecological role of chemical stimuli for the zooplankton: predator-induced morphology in *Daphnia*. Oecologia **78**:361-367.

Ferland-Raymond, B., R. E. March, C. D. Metcalfe, and D. L. Murray. 2010. Prey detection of aquatic predators: Assessing the identity of chemical cues eliciting prey behavioral plasticity. Biochemical Systematics and Ecology **38**:169-177.

Ferland-Raymond, B. and D. L. Murray. 2008. Predator diet and prey adaptive responses: Can tadpoles distinguish between predators feeding on congeneric vs. conspecific prey? Canadian Journal of Zoology-Revue Canadienne De Zoologie **86**:1329-1336.

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.

Ferrari, M. C. O., M. R. Brown, M. S. Pollock, and D. P. Chivers. 2007. The paradox of risk assessment: comparing responses of fathead minnows to capture-released and diet-released alarm cues from two different predators. Chemoecology **17**:157-161.

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-Revue Canadienne De Zoologie **88**:698-724.

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

Grabowski, J. H., A. R. Hughes, and D. L. Kimbro. 2008. Habitat complexity influences cascading effects of multiple predators. Ecology **89**:3413-3422.

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

Griffiths, C. L. and C. A. Richardson. 2006. Chemically induced predator avoidance behaviour in the burrowing bivalve *Macoma balthica*. Journal of Experimental Marine Biology and Ecology **331**:91-98.

Hardege, J. D., H. D. Bartels-Hardege, N. Fletcher, J. A. Terschak, M. Harley, M. A. Smith, L. Davidson, D. Hayden, C. T. Muller, M. Lorch, K. Welham, T. Walther, and R. Bublitz. 2011. Identification of a female sex pheromone in Carcinus maenas. Marine Ecology Progress Series **436**:177-189.

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

Hawkins, L. A., A. E. Magurran, and J. D. Armstrong. 2007. Innate abilities to distinguish between predator species and cue concentration in Atlantic salmon. Animal Behaviour **73**:1051-1057.

Hazlett, B. A. 1990. Source and nature of disturbance-chemical system in crayfish. Journal of Chemical Ecology **16**:2263-2275.

Hill, J. M. 2011. One crab, many crabs, small crab, large crab: cue concentration, diet, and sensory context determine how prey perceive the risk associated with predator body size and density. Georgia Institute of Technology, SMARTech.

Hill, J. M. and M. J. Weissburg. 2013a. Habitat complexity and predator size mediate interactions between intraguild blue crab predators and mud crab prey in oyster reefs. Marine Ecology Progress Series **488**:209-219.

Hill, J. M. and M. J. Weissburg. 2013b. Predator biomass determines the magnitude of non-consumptive effects (NCEs) in both laboratory and field environments. Oecologia **172**:79-91.

Hoefler, C. D., L. C. Durso, and K. D. McIntyre. 2012. Chemical-mediated predator avoidance in the european house cricket (*Acheta domesticus*) is modulated by predator diet. Ethology **118**:431-437.

Jackson, J. B. C. 2008. Ecological extinction and evolution in the brave new ocean. Proceedings of the National Academy of Sciences of the United States of America **105**:11458-11465.

Kamio, M., M. A. Reidenbach, and C. D. Derby. 2008. To paddle or not: context dependent courtship display by male blue crabs, Callinectes sapidus. Journal of Experimental Biology **211**:1243-1248.

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

Keller, T. A. and M. J. Weissburg. 2004. Effects of odor flux and pulse rate on chemosensory tracking in turbulent odor plumes by the blue crab, Callinectes sapidus. Biological Bulletin **207**:44-55.

Kiesecker, J. M., D. P. Chivers, A. Marco, C. Quilchano, M. T. Anderson, and A. R. Blaustein. 1999. Identification of a disturbance signal in larval red-legged frogs, Rana aurora. Animal Behaviour **57**:1295-1300.

Kinsey, S. T., E. Buda, and J. Nordeen. 2003. Scaling of gill metabolic potential as a function of salinity in the euryhaline crab, Callinectes sapidus rathbun. Physiological and Biochemical Zoology **76**:105-114.

Kusch, R. C., R. S. Mirza, and D. P. Chivers. 2004. Making sense of predator scents: investigating the sophistication of predator assessment abilities of fathead minnows. Behavioral Ecology and Sociobiology **55**:551-555.

Large, S. I. and D. L. 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.

Lima, S. L. 1998. Nonlethal effects in the ecology of predator-prey interactions - What are the ecological effects of anti-predator decision-making? Bioscience **48**:25-34.

Lima, S. L. and L. M. Dill. 1990. Behavioral decisions made under the risk of predation: a review and prospectus. Canadian Journal of Zoology-Revue Canadienne De Zoologie **68**:619-640.

Lively, C. M. 1986. Canalization versus developmental conversion in a spatially variable environment. American Naturalist **128**:561-572.

Manassa, R. P. and M. I. McCormick. 2012. Risk assessment via predator diet cues in a coral reef goby. Journal of Experimental Marine Biology and Ecology **426**:48-52.

Marcus, J. P. and G. E. Brown. 2003. Response of pumpkinseed sunfish to conspecific chemical alarm cues: an interaction between ontogeny and stimulus concentration. Canadian Journal of Zoology-Revue Canadienne De Zoologie **81**:1671-1677.

Mathis, A., D. P. Chivers, and R. J. F. Smith. 1995. Chemical alarm signals: predator deterrents or predator attractants? American Naturalist **145**:994-1005.

Mathis, A. and R. J. F. Smith. 1993. Chemical labeling of northern pike (*Esox lucius*) by the alarm pheromone of fathead minnows (*Pimephales promelas*). Journal of Chemical Ecology **19**:1967-1979.

McCoy, M. W., J. C. Touchon, T. Landberg, K. M. Warkentin, and J. R. Vonesh. 2012. Prey responses to predator chemical cues: disentangling the importance of the number and biomass of prey consumed. Plos One **7**:e47495.

McGaw, I. J. and C. L. Reiber. 2000. Integrated physiological responses to feeding in the blue crab Callinectes sapidus. Journal of Experimental Biology **203**:359-368.

Mirza, R. S. and D. P. Chivers. 2003a. Fathead minnows learn to recognize heterospecific alarm cues they detect in the diet of a known predator. Behaviour **140**:1359-1369.
Mirza, R. S. and D. P. Chivers. 2003b. Predator diet cues and the assessment of predation risk by juvenile brook charr: do diet cues enhance survival? Canadian Journal of Zoology-Revue Canadienne De Zoologie **81**:126-132.
Mogali, S. M., S. K. Saidapur, and B. A. Shanbhag. 2012. Tadpoles of the bronze frog (*Rana temporalis*) assess predation risk before evoking antipredator defense behavior. Journal of Ethology **30**:379-386.
Nolte, D. L., J. R. Mason, G. Epple, E. Aronov, and D. L. Campbell. 1994. Why are predator urines aversive to prey? Journal of Chemical Ecology **20**:1505-1516.
O'Connor, N. E., J. H. Grabowski, L. M. Ladwig, and J. F. Bruno. 2008. Simulated predator extinctions: Predator identity affects survival and recruitment of oysters. Ecology **89**:428-438.
Padilla, D. K. and M. M. Savedo. 2013. A systematic review of phenotypic plasticity in marine invertebrate and plant systems. Pages 67-94 *in* M. Lesser, editor. Advances in Marine Biology, Vol 65.
Pavia, H. and G. B. Toth. 2000. Inducible chemical resistance to herbivory in the brown seaweed Ascophyllum nodosum. Ecology **81**:3212-3225.
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, O. J. Schmitz, and G. C. Trussell. 2008. Revisiting the classics: Considering nonconsumptive effects in textbook examples of predator-prey interactions. Ecology **89**:2416-2425.
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.
Robinson, G. D. 1982. Water fluxes and urine production in blue crabs (*Callinectes sapidus*) as a function of environmental salinity. Comparative Biochemistry and Physiology a-Physiology **71**:407-412.
Schoeppner, N. M. and R. A. Relyea. 2005. Damage, digestion, and defence: the roles of alarm cues and kairomones for inducing prey defences. Ecology Letters **8**:505-512.
Schoeppner, N. M. and R. A. Relyea. 2009. Interpreting the smells of predation: how alarm cues and kairomones induce different prey defences. Functional Ecology **23**:1114-1121.
Shabani, S., M. Kamio, and C. D. Derby. 2009. Spiny lobsters use urine-borne olfactory signaling and physical aggressive behaviors to influence social status of conspecifics. Journal of Experimental Biology **212**:2464-2474.
Shrader, A. M., J. S. Brown, G. I. H. Kerley, and B. P. Kotler. 2008. Do free-ranging domestic goats show 'landscapes of fear'? Patch use in response to habitat features and predator cues. Journal of Arid Environments **72**:1811-1819.
Slusarczyk, M. and E. Rygielska. 2004. Fish faeces as the primary source of chemical cues inducing fish avoidance diapause in Daphnia magna. Hydrobiologia **526**:231-234.
Smee, D. L. and M. J. Weissburg. 2006. Hard clams (*Mercenaria mercenaria*) evaluate predation risk using chemical signals from predators and injured conspecifics. Journal of Chemical Ecology **32**:605-619.

Smith, R. J. F. 1992. Alarm signals in fishes. Reviews in Fish Biology and Fisheries **2**:33-63.

Spitze, K. 1992. Predator-mediated plasticity of prey life history and morphology: *Chaoborus americanus* predation on *Daphnia pulex*. American Naturalist **139**:229-247.

Trussell, G. C., P. J. Ewanchuk, and M. D. Bertness. 2002. Field evidence of trait-mediated indirect interactions in a rocky intertidal food web. Ecology Letters **5**:241-245.

Vilhunen, S. and H. Hirvonen. 2003. Innate antipredator responses of Arctic charr (Salvelinus alpinus) depend on predator species and their diet. Behavioral Ecology and Sociobiology **55**:1-10.

Weihrauch, D., W. Becker, U. Postel, S. Luck-Kopp, and D. Siebers. 1999. Potential of active excretion of ammonia in three different haline species of crabs. Journal of Comparative Physiology B-Biochemical Systemic and Environmental Physiology **169**:25-37.

Weihrauch, D., S. Morris, and D. W. Towle. 2004. Ammonia excretion in aquatic and terrestrial crabs. Journal of Experimental Biology **207**:4491-4504.

Wirsing, A. J., J. D. Roth, and D. L. Murray. 2005. Can prey use dietary cues to distinguish predators? A test involving three terrestrial amphibians. Herpetologica **61**:104-110.