THE ECOLOGY OF YIKES! ENVIRONMENTAL FORCES ALTER PREY PERCEPTION OF PREDATORS

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THE ECOLOGY OF YIKES! ENVIRONMENTAL FORCES ALTER PREY PERCEPTION OF PREDATORS

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Summary

Hard clams are slow even by clam standards (Doering 1982), and when found by predators, they are often injured and/or consumed (Irlandi and Peterson, Nakaoka 2000). Thus, their best survival strategy is to avoid predators since they lack the ability to escape or defend themselves against consumers. Results from these experiments indicate that clams detect blue crabs using chemical signals and react by reducing their feeding time. Clams were unresponsive to crabs that were starved, but displayed similar reactions to crabs whether they had eaten fish vs. clams. Since blue crabs are generalist predators, their diet is unrelated to the risk level they pose to clams, and it is perhaps unsurprising that clams responded similarly to crabs regardless of their diet. Starved predators may actually pose a greater threat to clams than those recently fed, as hungry predators tend to search longer and more frequently for food and decrease the threshold for detecting potential food before initiating a search (Zimmer-Faust and Case 1982). Thus, an inability to detect starved predators may actually increase clam vulnerability in hungry consumers. Clams did react to injured conspecifics, which may be a mechanism that allows them to respond to risk when predators are undetectable.

In addition to blue crabs and injured conspecifics, clams also reacted to knobbed whelks by reducing their feeding behavior. When clams were placed in the field with knobbed whelks or blue crabs caged nearby, clam survival was much higher than in controls with empty cages. This suggests that predator-induced feeding reductions are beneficial to clams, and by minimizing the amount of attractive chemicals they release into the environment, clams may avoid detection by predators.

Using a laboratory flume, we examined clam reactions to blue crabs and knobbed whelks in slow and fast flows to determine the impact of hydrodynamics on clam ability to respond to predators. Clam reactions to knobbed whelks were consistent regardless of flow speed, but clam responses to blue crab predators decreased in the high velocity flow. Interestingly, whelks are more likely to locate clams in fast and/or turbulent flows than are crabs (Ferner and Weissburg 2005), and clams were more responsive to whelks in these conditions.

Since hydrodynamics reduced clam reactions to blue crabs in laboratory assays, we conducted a follow-up field study to verify that turbulent flows indeed diminished clam ability to detect blue crabs. We established clam plots in the field, and compared clam survival when caged blue crabs were placed either 0.5 m or 2.0 m away in low and high levels of turbulence to survival in plots near empty cage controls. As in the earlier field study, clam survival was much greater than controls when blue crabs were caged near clam beds, even when crabs were caged 2.0 m away. However, when blue crabs were 2.0 m away in turbulent flows, clam survival was almost identical to that measured in controls, indicating that the reactive distance of clams to blue crabs had been diminished by turbulence, and validating the earlier behavioral results obtained in the flume.

Since turbulence diminished clam responses to crabs, and had previously been shown to reduce blue crab ability to find clams (e.g., Weissburg and Zimmer-Faust 1993), the role of turbulence in modulating the outcomes of clam-crab predatory interactions was unclear. We predicted that the animal (clam or crab) whose perceptive abilities were least affected by increasing turbulence levels would have a sensory

advantage and prevail in turbulent flows. We established pairs of clam plots in four field sites that differed in their mean flow velocities and turbulence levels. One member of each pair was surrounded with a ring of sun-bleached oyster shells to increase turbulence, and we compared crab predation levels both within and between sites. By increasing turbulence levels within sites, we were able to expose clams to similar conditions and isolate the effects of turbulence from other factors specific to any one site. Our results indicated that crab predation intensity was highest in sites with intermediate turbulence levels, suggesting that crabs are best able to forage in these conditions. In sites with low turbulence levels, increasing turbulence by adding shells tended to increase crab predation in low turbulent areas, possibly by elevating the turbulence level into the intermediate range that provides a sensory advantage to crabs. In sites with intermediate turbulence levels, increasing turbulence via shell additions decreased crab predation, and we attributed this result to an increase in turbulence beyond the range beneficial to crabs and into a range where clams regain the sensory advantage. Although more work is required to establish the precise flow conditions where sensory advantages switch between these organisms, it is clear that turbulence can have profound and multifaceted effects on the outcome of these predatory interactions.

In a final study, we wanted to determine if clam behaviors differed across their geographic range. It is generally accepted that prey living in environments with more intense consumer pressure will possess stronger defenses against consumers (e.g., Vermeij 1978, Bertness et al. 1981, Fawcett 1984). We tested whether prey sensitivity to risk and the likelihood of initiating predator avoidance behaviors would be affected by predation pressure. We compared predation pressure between clam populations in

Georgia and Maine, finding that predation levels were nearly an order of magnitude higher in Georgia. Clams in Maine were much less reactive to risk than were their Georgia counterparts, suggesting that predation levels can influence the thresholds prey require before reacting to risk.

Chapter 1

Hard Clams (*Mercenaria mercenaria*) evaluate predation risk using chemical signals from predators and injured conspecifics

Abstract- Hard clams, *Mercenaria mercenaria*, are sessile, filter-feeding organisms that are heavily preyed upon by blue crabs, which find their clam prey using chemical cues. Clams may evade detection by blue crabs by reducing their pumping (feeding) behavior when a threat is perceived. The purpose of this study was to determine the type of signals that clams use to detect consumers. Clams decreased their pumping time in response to blue crabs and blue crab effluent, but not to crab shells, indicating that chemical signals, and not mechanical cues mediated the response of clams to distant predators. Since predator diet can influence prey evaluation of predatory threats, we compared clam responses to blue crabs fed a steady diet of fish, clams, or were starved prior to our experiment. In addition, we used injured clams as a stimulus because many organisms detect predators by sensing the odor of injured con- or heterospecifics. Clams reduced feeding in response to injured conspecifics and to blue crabs that had recently fed. Clams reacted similarly to fed crabs, regardless of their diet, but did not respond to starved blue crabs. Since blue crabs are generalist predators and the threat posed by these consumers is unrelated to the crab's diet, we should expect clam reactions to blue crabs to be independent of the crab's diet. The failure of clams to react to starved blue crabs likely increases their vulnerability to these consumers, but clam responses to injured

conspecifics may constitute a strategy that allows animals to detect an imminent threat when signals emanating from blue crabs are not detectable.

Introduction

Predators often have profound impacts on prey populations and on the organization and function of communities in general (Paine, 1966; Carpenter et al., 1985; Schmitz et al., 1997; Schmitz, 1998; Menge, 2000). The overall effect of predators on communities is determined by interactions between individual predators and prey (Lima, 1998, 2002). Therefore, the ability of predators to forage and the ability of prey to avoid consumers influences the magnitude of top-down forces in a given system (Menge, 2000; Werner and Peacor, 2003). Since decisions made by prey under the risk of predation have profound consequences for both prey populations as well as entire communities, it is important to understand how prey evaluate and respond to predation risk (Lima and Dill, 1990; Werner and Peacor, 2003)

Although avoiding consumers is of great importance to prey, predator avoidance is often costly and results in decreased growth or fecundity (e.g., Lima and Dill, 1990; Peckarsky, 1996; Katz and Dill, 1998; Leonard et al., 1999; Nakaoka, 2000). Prey may minimize predator avoidance costs by using flexible avoidance strategies that balance the frequency or magnitude of predator avoidance responses with a perceived level of risk (Sih et al., 1985; Schmitz et al., 1997; Schmitz, 1998; Chivers and Smith, 1998; Katz and Dill, 1998; McIntosh and Peckarsky, 1999). Thus, prey require stimuli that accurately reveal the level of risk to determine when and how predator avoidance strategies should be employed.

Prey commonly use chemical signals to evaluate risk (Chivers and Smith, 1998; Katz and Dill, 1998) because chemical cues typically provide prey with accurate information concerning the location and intentions of predators (Chivers and Smith, 1998; Katz and Dill, 1998; Brown et al. 2000). This is particularly true in aquatic environments where visual or mechanical cues are often unavailable (Zimmer and Butman, 2000; Weissburg et al., 2002). Additionally, predators can more easily manipulate their posture or behavior to appear less threatening to prey than change their chemical signature (Katz and Dill, 1998; Brown et al., 2000).

Chemical cues indicative of danger may emanate from predators, from injured conspecifics, and sometimes from sympatric species (Petranka, 1987; Mathis and Smith, 1993; Katz and Dill, 1998; Chivers and Smith, 1998). Prey may use one or combinations of these signals to evaluate risk (Chivers and Smith, 1998; Katz and Dill, 1998; Bryer et al., 2001; Smith and Belk, 2001) and respond differently to chemical signals depending upon other factors such as time of day (e.g., Peckarsky, 1996). Signals released from predators provide the most accurate indication of a predatory threat, and, prey may minimize their predator avoidance costs by exclusively responding to these signals (reviewed by Katz and Dill, 1998). Although cost effective, an avoidance strategy in which prey only respond to predator odors may increase their vulnerability when these chemicals are difficult to detect or when predators reach prey prior to the arrival of their chemical signals (e.g. when olfactory predators find prey by searching upstream). In contrast, chemical cues released by injured conspecifics may provide a stronger, but less reliable indication of danger (reviewed by Katz and Dill, 1998). Yet, prey may over

utilize predator avoidance tactics and incur high costs if they depend on less reliable signals (Lima and Dill, 1990; Katz and Dill, 1998).

Some prey limit their responses to predators that have eaten conspecifics or closely related species (e.g., Chivers and Smith, 1998; Katz and Dill, 1998; Chivers and Mirza, 2001; Mirza and Chivers, 2001; Smith and Belk, 2001; Brown and Dreier, 2002; Madison et al., 2002), and this predator detection strategy has been hypothesized to minimize predator avoidance costs. However, prey that depend on predator diet cues before initiating anti-predator measures may be vulnerable to generalist predators that switch diets frequently (Bryer et al., 2001; Chivers and Mirza, 2001). Bryer et al. (2001) and Chivers and Mirza (2001) hypothesized that prey responses that are dependent on predator diets should only occur in systems where the threat posed by a predator is directly related to that predator's most recent foraging activity.

In this study, we examined the effects of a generalist predator's diet on the response of a common prey organism using blue crabs, *Callinectes sapidus*, and hard clams, *Mercenaria mercenaria*, as model organisms. Blue crabs, *Callinectes sapidus*, are important predators and scavengers in southeastern estuaries (Eggleston et al., 1992; Micheli, 1997) and are the primary consumer of juvenile hard clams, *Mercenaria mercenaria* in these areas (Micheli, 1995, 1997). Blue crabs are also a threat to adult clams as they can nip their siphons and decrease their feeding efficiency, growth, and fecundity (Peterson, 1986; Coen and Heck, 1991; Irlandi, 1994). Clams release attractive chemicals into the water as they feed, and blue crabs follow these waterborne chemical odor plumes to locate their clam prey (Weissburg and Zimmer-Faust, 1993; Weissburg et al., 2002). Irlandi and Peterson (1991) found that clams responded to the presence of

predators by reducing their feeding time and hypothesized that feeding reductions would make clams less apparent to consumers. Indeed, caging predators near clam beds decreases clam mortality (Smee and Weissburg, 2003), but clam growth and reproductive output are diminished by long-term exposure to predators (Nakaoka, 2000). Thus, clam responses to predators are adaptive and costly.

We hypothesized that clams detect to approaching blue crab predators using chemical signals, hydrodynamic signals, or both. We exposed clams to both chemical and hydrodynamic signals from blue crabs to verify the type of cue clams use to detect blue crabs. Our results indicated that clams were responding to chemical cues emanating from blue crabs and we conducted a second experiment to determine the nature of these signals. In the second experiment, we compared changes in clam behavior when exposed to blue crabs that had been fed different diets as well as to injured conspecifics. Our results suggest that prey respond to a generalist predator regardless of diet, presumably because the dietary history of such a predator does not predict the risk to its potential prey. Further, there are limits to prey perceptual abilities that may result in increased predation risk. For example, prey may be unable to detect starved predators, even though a highly motivated consumer increases the chance that potential prey may be attacked.

Materials and Methods

Animal Capture and Maintenance. Animals were collected from Wassaw Sound, GA and associated tributaries. Hard clams, *Mercenaria mercenaria*, were hand dug with clam rakes and fingers in the intertidal zone, and blue crabs, *Callinectes sapidus*, were captured with commercially purchased crab pots. After capture, animals were returned to

the Skidaway Institute of Oceanography (SkIO) near Savannah, GA and housed in flow-through sea tables supplied by water pumped from the Skidaway River. Sea table water was filtered through both gravel and sand filters, and the water temperature and salinity in the sea tables ranged from 25-30^{o} C and 25-30 ppt respectively. Clams acclimated in the sea tables for at least 6 hours prior to behavioral assays (see below) and were used in behavioral assays within 48 hours after removal from the field. Blue crabs were kept in the sea tables for at least one week prior to use in the behavioral assays. Crabs were fed a daily diet of either fish (*Menhaden* sp.) or clams (*M. mercenaria*), or were starved during the one week acclimation period. We returned each clam or crab to the field after a single use (except for a few clams that were injured as part of the experiment or used for food, see below).

Experimental Arena. Experiments were conducted in a paddle-driven racetrack flume at SkIO (4.8 m long working section x 1 m wide x 0.33 m water depth). The upstream bend of the flume is divided into five 23-cm channels to reduce secondary circulation. Flow is further conditioned by honeycomb baffling (5 cm thick with 7 mm openings) at the downstream end of this bend and by a PVC flow straightener (10 cm x 4.5 cm openings) placed at the end of the working section to prevent backflow. The working section contains a false bottom (0.30 m dia. x 0.15 m deep) located 2.3 meters downstream from the entrance point of the working section and is in the center of the flume to minimize wall effects. Both the working section and false bottom of the flume were filled to a uniform depth of one cm with commercially purchased sand (grain size 0.04 $\pm$ 0.04 cm). The flume was supplied by the same water source as the sea tables and had similar temperature and salinity. Flume water passed through both gravel and sand filters as well

as a 10-micron filter bag. Flow speed was maintained at 3 cm/s in all experiments. This flume produces stable and reproducible boundary layers at current speeds ranging from 1-15 cm s^{-1}. See Ferner and Weissburg (2005) for a detailed flume description and characterization of the flow environment boundary.

Behavioral Assays. Our experiments utilized changes in clam pumping (feeding) behavior as assays for the ability of clams to detect predation risk. Although previous investigators have assumed that clams are actively pumping only when their siphons are extended (e.g., Irlandi and Peterson, 1991), we performed preliminary experiments to verify this supposition. We visualized the excurrent from clams by carefully pippetting a 0.1% solution of fluoroscein dye above the excurrent siphon of a clam. Thirty-six clams that had their siphons extended were tested in this manner and all were releasing an excurrent. We tested 15 clams with open shells but withdrawn siphons, and only three were pumping. Thus, we concluded that siphon extension was indicative of pumping.

Behavioral trials consisted of challenging clams to detect and respond to blue crab predators, injured clams, predator-conditioned water, and predator shells. We judged clam responses to predation risk by determining if clam feeding (# siphon extension observations) was significantly less in response to these treatments when compared to a control that lacked predators or injured conspecifics. In each assay, we placed five clams in the false bottom of the flume and allowed them to acclimate for 30 minutes. Clam density in these experiments was five clams per 0.07 m^2 and mimics densities observed in natural habitats (Walker, 1987, Smee and Weissburg, unpublished data). We introduced predators, crushed conspecifics, or predator conditioned water at the conclusion of the 30-minute acclimation period by placing a tethered crab, injured clam, or the nozzle (see

below) from our delivery system 0.5 m upstream from the clam bed. We recorded the siphon position of each clam (extended or not) prior to introduction of the predator treatments and at five-minute intervals after introduction for 30 minutes. Thus, each clam could have been observed feeding (pumping) a maximum of seven times, and we used the total number of observations in which clams were pumping as our measure of clam pumping time. That is, the response of each clam in a trial was measured by a single number between 0 and 7, which indicated how many times we observed an individual clam pumping.

The order of treatments and controls in these experiments was randomly assigned each day, and each treatment and the control were replicated at least five times (5 trials x 5 clams per trial = 25 clams for each treatment and control). Each clam and predator was used only once. Clams that neither pumped nor burrowed were excluded from analysis, and we excluded approximately 25% of the clams from the experiment using this criterion. Including inactive clams in our analysis would have enhanced our results, but we excluded them since we could not clearly determine the causes of clam inactivity.

Characterization of Predator Cues. Preliminary observations suggested that clams pumped significantly less when tethered blue crabs were placed upstream. We hypothesized that potential predators created hydrodynamic signals, chemical signals, or both, that mediated the response of clam prey. Therefore, we conducted two experiments to determine the cue that clams use to detect predators. We tested responses of clams to hydrodynamic cues by placing an empty predator shell 0.1 m upstream from the clams and comparing clam pumping between this treatment and the control. Qualitative flow visualization with dye indicated that the turbulence created by the predator shells

dissipated within the first 0.25 m downstream, although we could not exclude the possibility that a more exacting analysis of flow would reveal that perturbations induced by the shell extended farther downstream. Thus, we placed the predator shell 0.1 m upstream from the clam bed to ensure that the clams were in its turbulent wake.

To determine if clams were detecting chemical signals from predators, we designed a chemical delivery system to transport blue crab effluent to the experimental clams. The delivery system pumped water out of the flume and into a container (0.31 m x 0.24 m x 0.36 m) that was left empty (control) or that housed a blue crab that had recently eaten clams. The water from the container was released into the flume 0.5 m upstream from the clam bed via a 0.076 m diameter PVC pipe oriented parallel to the flow. Water moved through the delivery system at a velocity of 3 cm s^{-1}, which matched the free stream flow velocity in the flume. The large diameter pipe was selected because it was of similar size to a blue crab, which allowed us to simulate water passing over the crab at a rate similar to that occurring in experiments with clams exposed to a live predator.

We realize that the flow diversion method may only crudely approximate the flux of chemicals experienced by a prey organism directly upstream of a crab predator. Even though our approach replicates the rate of water movement over the animal, mixing in the delivery system and introduction through a pipe will probably change the chemical signal dynamics relative to that produced by water flowing over an individual crab. However, the flow diversion method we employed is a more realistic alternative than prey soaks or body washes because the volumetric rate at which water passes over the crabs in the diversion system is roughly equal to that passing over a crab in the flume. In contrast, soaks or body washes methods concentrate predator metabolites using arbitrarily

determined volumes and time periods, and so produce unknown metabolite concentrations that will not be experienced by naturally foraging animals.

Effects of Predator Diet and Response of Clams to Injured Conspecifics. In this experiment, we measured the responses of clams when presented with odors from injured conspecifics as well as crabs that were fed different diets prior to behavioral assays. The injured clam treatment was prepared by striking a clam with the blunt edge of a kitchen knife, removing the top valve, and making multiple lacerations on the visceral mass of the clam. This treatment mimicked crab feeding and insured clam metabolites were released into the water. To measure the impact of crab diet on clam responses, collected blue crabs were fed a daily diet of fish or clams for one week, or were starved for one week prior to our experiment. We allowed clams to acclimate in the flume using the same methodology previously described, and then placed a tethered blue crab or injured clam 0.5 m upstream from the clam bed and monitored clam feeding.

Data Analysis. We initially examined the percentage of times that adjacent clams were feeding simultaneously to determine if interactions occurred between clams. Clams in control trials pumped during 87% of our observations. Thus, the proportion of time that two adjacent clams should be pumping simultaneously is 0.87^2 (0.76) assuming that adjacent animals do not influence each other. Adjacent clams (n=25 pairs) in control trials pumped simultaneously in 70% of our assays, a value not significantly different from the random expectation (Rohlf and Sokal, 1995).

Since clams were not influencing each other, observations of pumping behavior of individual clams (number of siphon extensions observed for each clam) were arcsine transformed to meet ANOVA assumptions and then compared using a nested ANOVA

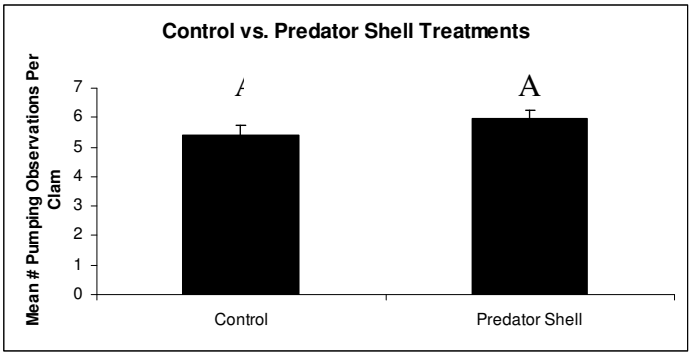
that examined the effects of predator treatment and trial nested within treatment (Sokal and Rohlf, 1995). Using a nested ANOVA allowed us to determine if variation in clam responses were affected by variability in cue quality or quantity across replicate treatments, which is a source of uncontrolled variation in our experiments. The *P* value for the nest effect was greater than 0.20 in all experiments, indicating that clams in different groups were reacting similarly to the same treatments. The lack of a significant nest effect permitted us to lump trials within treatments and test the significance of the main effect using the pooled error variance (Sokal and Rohlf, 1995). The absence of a nest effect suggests that cues from predators and injured conspecifics were roughly similar between replicate trials.

Experiments using tethered predators, predator effluents, and predator shells were conducted at different times over a period of several months. Therefore, each experiment was analyzed separately, since it would be inappropriate to compare treatments to one another under these conditions. Note that separate control experiments were performed for each experiment to account for any variation in animal or general experimental conditions. Trials using different predator diets or injured conspecifics, were intermingled, and on a daily basis, presented in random order to test clams. After completing the nested ANOVA, a Tukey-Kramer *post hoc* analysis was employed to test for pair-wise differences between treatments (Sokal and Rohlf, 1995).

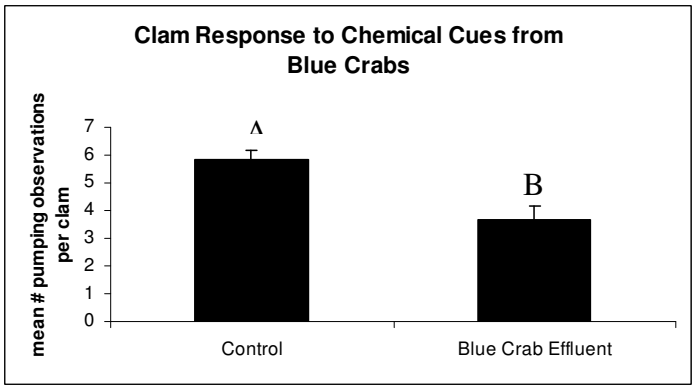
Results

Characterization of Predator Cues. Data from experiments using the predator shells indicted that there were no significant differences in clam pumping between the predator

A)



B)



C)

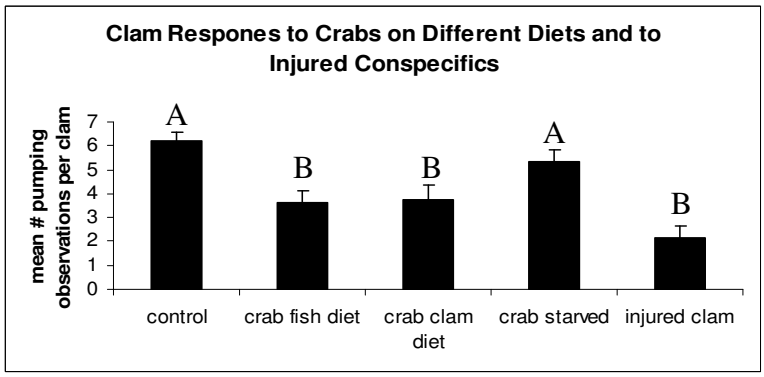


Figure 1 Mean number of pumping observations per clam (± std. err). Letters denote means that are significantly different based upon a Tukey-Kramer post hoc test. Each clam could have been observed pumping a maximum of 7 times during the 30-minute observation period. A) control vs. predator shell placed 0.1 m upstream, n = 42 and 43 clams respectively. B) control vs. blue crab effluent released from our delivery system, n = 16 and 21 clams respectively. C) clam pumping in the presence of crabs fed different diets and injured conspecifics, Sample sizes for each treatment are 24, 16, 15, 19, and 15 for the control, crab fed fish, crab fed clams, starved crab, and injured clam treatments respectively. Differences in sample size result from exclusion of inactive clams from analyses.

shell treatments and the controls ($F_{1,83} = 2.56$, $P > 0.11$, Figure 1.1A). Although not significant, we observed a higher clam-pumping rate in trials with predator shells. Thus, turbulence generated by the predator shell did not alter clam pumping, which suggested that clams were not using a hydrodynamic cue to detect predators.

In contrast to the results obtained with empty predator shells, clam pumping was significantly reduced (≈ 40%) when clams were exposed to water released from our delivery system that had passed over a blue crab as compared to water passing through the empty system without blue crabs present ($F_{1,35} = 8.69$, $P < 0.01$, Figure 1.1B). Additionally, clam feeding was affected similarly by predator-conditioned water and (non-starved) predators placed directly in the flume (see below). The failure of clams to cease pumping in response to hydrodynamic signals, combined with the positive response to predator-conditioned water delivered at environmentally realistic conditions strongly suggested that clam responses to predators were chemically mediated.

Effects of Predator Diet and Response of Clams to Injured Conspecifics. Our data revealed that clam feeding decreased by 40% when exposed to blue crabs that had recently been fed, and by 65% in the presence of injured conspecifics as compared to controls that lacked predators or injured clams ($F_{4,84} = 10.28$, $P < 0.001$, Figure 1.1C). Starved blue crabs did cause a slight (15%), but insignificant reduction in clam feeding. Additionally, post hoc analysis revealed that clams pumped significantly more in the presence of starved blue crabs than those that were recently fed, and clam responses to crab predators were similar regardless of their diet. Although not significantly different from responses to fed crabs, clams reduced their feeding time almost 40% more after detecting an injured conspecific than a crab that had recently eaten (Figure 1.1C). Thus,

clam feeding was affected more by the presence of injured clams than by the odors of fed predators, although both caused significant reductions in clam feeding as compared to controls and starved crab treatments.

Discussion

Our results indicate that clams use chemical signals to detect upstream blue crabs and respond to these predators by reducing their feeding (pumping) behavior. Other bivalves (e.g., mussels) also use chemical cues to detect predators and respond by changing their morphology (e.g., Leonard et al., 1999) or behavior (e.g., Cote' and Jelnikar, 1999). Previous studies have shown that blue crabs depend on chemical cues to locate clam prey (Weissburg and Zimmer-Faust, 1993; Finelli et al., 2000; Weissburg et al., 2002). The modulation of the blue crab-clam predatory interaction by chemicals is perhaps unsurprising given that the water in our study area is extremely turbid, and chemical cues are likely the only signals that can be detected from a distance in this habitat. Both blue crabs and their prey use the same sensory modality to detect each other, so that the conditions that affect the transmission of chemical signals will affect the sensory abilities of both organisms. Thus, the outcome of interactions between these organisms may differ considerably between areas that enhance chemical signaling as compared to those that impede it.

In nature, clam feeding rates may be influenced by other factors (e.g., food availability, temperature) that were not considered in the present study. Clearly, reactions to predators may change in the field depending on a variety of factors besides the perceived level of risk. Still, long term exposure to predators has been shown to

significantly decrease clam growth in the field (Nakaoka 2000), and in a related field study, Smee and Weissburg (in press) found that clam survival was significantly higher in clam plots with predators caged nearby as compared to control plots with empty cages. These studies indicate that clam reactions to predators, while costly, reduce mortality and suggest that clams react to predators across a range of natural conditions. Therefore, the clam reactions to predators and injured conspecifics observed in the present study should be indicative of the cues used by clams to avoid predation in the field.

Clams only responded to cues released by blue crabs if the crabs had recently fed and not if they had been starved for one week. Clams responded similarly to fed crabs regardless of whether the crab's diet consisted of fish or clams prior to behavioral assays. In addition, clams altered their feeding behavior in the presence of injured conspecifics, suggesting that they use also use these signals to detect predatory threats.

The ability of clams to react to injured conspecifics may compensate for their inability to detect hungry blue crabs. Prey organisms may benefit from living in close proximity to conspecifics or related species, as neighbors can provide for shared vigilance against consumers or early warnings of danger (Hamilton, 1971; Powell, 1974; Sullivan, 1984; Fitzgibbon, 1990; Aukema and Raffa, 2004). The benefit provided by neighbors is particularly strong in organisms that respond to the odors of injured conspecifics or heterospecifics as consumption of neighbors reveals a predatory threat (e.g., Mathis and Smith, 1993). Hard clams are commonly found in dense beds, and can reach densities in excess of 50 clams m^{-2} in our study area (Walker 1987, Smee and Weissburg unpublished data). Clams living in dense beds may be better able to avoid unapparent predators as neighbors that are eaten may warn of imminent peril.

Responses of prey that are dependent on predator diets have been found in many predator-prey systems (Crowl and Covich, 1990; Chivers et al.; 1996, Stabell and Lwin, 1997; Chivers and Mirza; 2001, Smith and Belk, 2001), but are notably absent from others (Petranka and Hays, 1998; Bryer et al., 2001). The existence of diet-dependent responses may be contingent on whether the recent dietary history of the predator is correlated with risk to a given prey species. For instance, seasonally hunting predators may pose a risk for a given prey species only at certain times, so that predator diet may predict the potential threat level to a given organism (Chivers and Mirza, 2001). Alternatively, Bryer et al. (2001) suggest that diet-dependent responses to predators may not be beneficial when prey are hunted by generalist predators, such that the risk level posed by the predator is unrelated to its foraging habits. Bryer et al. (2001) observed that slimy sculpin responses to brook trout predators were unaffected by the trout's diet. They reasoned that the threat posed by brook trout to sculpins is unrelated to the trout's foraging habits, and thus, it would not be advantageous for the sculpin to base risk evaluation on predator diet cues.

The response of clams to blue crabs in our experiment was not dependent on the crab's diet. Since blue crabs are generalist consumers and eat almost anything alive or dead (Virnstein, 1977; Eggleston et al., 1992; Micheli, 1995, 1997), the threat of predation by crabs is unrelated to the crab's recent foraging activity. As in the previous example with slimy sculpins, knowledge of a blue crab's diet provides no valuable information for their prey, suggesting that it is not advantageous for clams to rely on diet cues as their sole means of evaluating risk.

Cost-benefit analyses are often used to explain the variability in responses to predators across predator-prey systems. However, cost-benefit explanations currently are focused on response specificity as opposed to response sensitivity, and may be inadequate when prey fail to detect actual predatory threats because predators have not recently fed (Howe and Harris, 1978). If predators stop releasing chemical signals or release chemicals that are difficult to detect, then organisms may not adequately perceive the true risk level. Starved predators often show enhanced search responses relative to those that are well fed, as revealed by increases in the duration and frequency of search bouts in response to a given stimulus level, or decreases in the threshold stimulus levels that are required to initiate or maintain search (Mackie and Shelton, 1972; Zimmer-Faust and Case, 1982;). Therefore, the threat posed by a starved crab is equal to, or possibly greater than, that posed by a crab that has recently foraged. Thus, it would be prudent for clams to respond to starved crabs, and their failure to do so suggests that starvation renders blue crabs less detectable by clam prey. Prey may be more likely to depend on the odors of injured con- or heterospecifics to detect consumers when predators are commonly undetectable, although this hypothesis has not been empirically tested.

We have yet to develop risk-based models for the sensitivity of potential prey to cues derived from their consumers, and research that attempts to quantify the stimulus levels necessary to elicit prey reactions is lacking. Clearly, prey with low sensitivity thresholds may experience large costs (e.g. reductions in the opportunity to feed), particularly if prey use general metabolites to detect their predators, as these substances may come from a variety of sources. In contrast, prey with higher sensitivity thresholds may decrease predator avoidance costs, but may also be more vulnerable to their

enemies. Recent technological advances in our ability to characterize and identify chemical signals (e.g. Millar and Haynes, 1998), as well as our ability to examine chemical signal transport in aquatic systems (e.g. Webster and Weissburg 2001, Weissburg et al. 2002), may allow us to investigate threshold sensitivity and its relationship to predation risk in a more thorough manner than has been previously attempted.

Chapter 2

Clamming Up: Environmental Forces Diminish the Perceptive Ability of Bivalve Prey

Abstract

The lethal and nonlethal impacts of predators in marine systems are often mediated via reciprocal detection of waterborne chemical signals between consumers and prey. Local flow environments can enhance or impair the chemoreception ability of consumers, but the effect of hydrodynamics on detection of predation risk by prey has not been investigated. Using clams as our model organism, we investigated two specific questions: 1) Can clams decrease their mortality by responding to predators? and 2) Do fluid forces affect the ability of clams to detect approaching predators?

Previous research has documented a decrease in clam feeding (pumping) in response to a neighboring predator. We determined the benefits of this behavior to survivorship by placing clams in the field with knobbed whelk or blue crab predators caged nearby and compared mortality between these clams and clams near a cage-only control. Significantly more clams survived in areas containing a caged predator, suggesting that predator-induced alterations in feeding reduce clam mortality in the field

We ascertained the effect of fluid forces on clam perception of predators in a laboratory flume by comparing the feeding (pumping) behavior of clams in response to crabs and whelks in flows of 3 and 11 cm s^{-1}. Clams pumped significantly less in the presence of predators, but their reaction to blue crabs diminished in the higher velocity flow, while their response to whelks remained constant in both flows. Thus, clam reactive

distance to blue crabs was affected by fluid forces, but hydrodynamic effects on clam perceptive distance was predator-specific. After predators were removed, clams exposed to whelks took significantly longer to resume feeding than those exposed to blue crabs.

Our results suggest that prey perception of predators can be altered by physical forces. Prey detection of predators is the underlying mechanism for trait-mediated indirect interactions (TMIIs), and recent research has documented the importance of TMIIs to community structure. Since physical forces can influence prey perception, the prevalence of TMIIs in communities may, in part, be related to the sensory ability of prey, physical forces in the environment that impact sensory performance, and the type of predator detected.

Introduction

Predators commonly have profound impacts on prey populations and on the organization and function of communities (Paine 1966, Carpenter et al. 1985, Menge 2000). Predators in marine (Estes and Palmisano 1974, Estes et al. 1998, Menge 2000), freshwater (Carpenter et al. 1985, McQueen et al. 1989), and terrestrial (Schmitz et al. 1997, Schmitz 1998) environments affect communities through lethal predation (Sih et al. 1985) and by nonlethal mechanisms in which consumers alter characteristics of prey such as behavior or morphology (Turner and Mittlebach 1990, Katz and Dill 1998, Nakaoka 2000). These and many other studies indicate that interactions between predators and prey alter patterns of energy flow, community diversity and composition, and the importance of competitive interactions.

Studies examining the impact of predators on communities traditionally have focused on lethal effects (Sih et al. 1985) and have led to important conceptual developments such as the trophic cascade (Carpenter et al. 1985). Current studies have shown that nonlethal effects of predators can affect communities in ways that rival or mimic effects stemming from prey consumption, such as by generating trophic cascades through changes in prey behavior (Turner and Mittlebach 1990, Schmitz et al. 1997, Trussell et al. 2003). Indirect effects of predators such as those previously described are termed trait-mediated indirect interactions (TMIIs, Abrams et al. 1996).

Although predation is a strong community structuring force in many areas, the effect of predators is often minimal in habitats that experience substantial physical stress (Menge 1976, Menge and Sutherland 1987). Predators in these systems are unable to forage, and communities consist of organisms that can withstand constant disturbance (Menge 1976, Menge 2000). Such examples provide a clear demonstration of the important role physical forces play in structuring communities by limiting predator mobility or foraging activity. Recent research, however, suggests that physical forces may affect communities in less obvious ways. In these instances, physical forces diminish the ability of consumers to locate prey and reduce predation intensity in communities (Weissburg and Zimmer-Faust 1993, Leonard et al. 1998).

Predator-prey interactions in marine systems are often chemically mediated. Several studies have shown that hydrodynamics influence the structure of waterborne chemical plumes as well as the perception of chemical signals by consumers (Weissburg and Zimmer-Faust 1993, Finelli et al. 2000, Webster and Weissburg 2001, Weissburg et al. 2002, 2003). For example, the ability of blue crabs to locate prey by chemoreception

decreases as turbulence increases (Weissburg and Zimmer-Faust 1993), but knobbed whelks successfully follow odor plumes in more turbulent flow conditions than do blue crabs (Ferner and Weissburg 2005, Powers and Kittinger 2002).

Bivalves also use chemical signals and alter their morphology (Leonard et al. 1999) or behavior (Cote' and Jelnikar 1999) after detecting chemicals emanating from predators or injured conspecifics (Katz and Dill 1998). In particular, clams reduce pumping in response to chemical cues from predators or crushed conspecifics (Smee and Weissburg submitted). Although studies have examined the impact of physical forces (e.g., hydrodynamics) on predator perceptual abilities, the effects of physical forces on the sensory ability of prey have not been conducted. The lack of research on the effects of environmental factors on prey perception is surprising given that studies of predator-induced prey behavior and risk assessment are commonplace (Lima and Dill 1990, Katz and Dill 1998) and that changes in olfactory-mediated prey behavior can significantly impact communities (Turner and Mittlebach 1990, Schmitz et al. 1997, Trussell et al. 2003, Werner & Peacor 2003). In addition, recent studies indicate prey responses to danger may vary across spatial and temporal scales (Lima 1998, Lima and Bednekoff 1999, Rohr et al. 2003, Tuner and Montgomery 2003).

Since physical forces can alter the perceptive ability of consumers, we hypothesized that environmental forces might affect prey perception and alter the spatial and temporal scales at which prey perceive threats. In this study, we examined the impact of hydrodynamic forces on the ability of hard clams, *Mercenaria mercenaria*, to detect blue crab and knobbed whelk predators. This system was selected due to the ecological importance of these predator-prey interactions (e.g., Micheli 1995, 1997, Nakaoka 2000),

prior knowledge regarding sensory biology of blue crab and knobbed whelk predators (e.g., Weissburg and Zimmer-Faust 1993, Powers and Kittinger 2002, Weissburg et al. 2002, Ferner and Weissburg 2005), and because previous work has shown that flow environments can affect the perceptive ability of predators and change predation intensity in natural systems (Weissburg and Zimmer-Faust 1993, Leonard et al. 1998, Moore and Grills 1999, Finelli et al. 2000, Webster and Weissburg 2001, Ferner and Weissburg 2005). In particular, we hoped to complement the existing understanding of how hydrodynamics affect predator ability with a similar analysis of its effects on prey. Understanding both predator and prey sensory abilities may ultimately lead to an appreciation for how the physical environment affects predatory interactions. We asked two specific questions in order to further this goal: 1) Do clam responses to predators increase their survival in natural habitats? and 2) How do physical forces affect the ability of clams to detect predators from a distance? Our results suggest that physical forces alter the sensory ability of prey and may influence the prevalence of both lethal and nonlethal predator effects in communities.

Methods and Materials

Animal Capture and Maintenance

Animals used in this study were collected from Wassaw Sound, GA and associated tributaries. Hard clams, *Mercenaria mercenaria,* were dug from intertidal habitats, and knobbed whelks, *Busycon carica,* were collected from intertidal mudflats. Blue crabs, *Callinectes sapidus,* were collected in the Skidaway, Wilmington, and Herb Rivers using crab pots. Animals were returned to the Skidaway Institute of Oceanography

(SkIO) near Savannah, GA and housed in flow-through sea tables supplied by water pumped from the Skidaway River and filtered through both gravel and sand filters. Water temperature and salinity in the sea tables ranged from 25-30^{o} C and 29-32 ppt respectively. Clams were allowed to acclimate for at least 6 hours prior to behavioral assays (see below) and were not used in behavioral experiments if they had remained in the sea tables for longer than 48 hours. Knobbed whelks and blue crabs were fed an *ad libitum* diet of clams for at least one week prior to use in the field experiment or behavioral assays. Each crab, clam or whelk was used once and then returned to the field.

Effects of Clam Behavioral Changes on Mortality

Previous research has shown that clams reduce their feeding time after detecting predators (Irlandi and Peterson 1991, Smee and Weissburg submitted) and grow more slowly in the presence of consumers (Nakaoka 2000). Presumably, these feeding reductions reduce the amount of attractive chemicals clams liberate into the environment and decrease their apparency to consumers. We used a simple field experiment to determine if predator-induced changes in clam behavior increase clam survival in natural environments. These experiments consisted of establishing clam plots in the field and determining whether clam survivorship increased when predators were placed in close proximity to clams but incapable of directly interacting with them. The objective of this experiment was to alter clam feeding rates in response to predators and measure subsequent changes in clam mortality.

Clam plots contained 15 clams, 10 juveniles (shell size < 30 mm) and 5 adults (shell size > 35 mm), in a 0.25 m^2 area (0.5 m x 0.5 m), and this density mimics naturally

occurring populations in the study area (Walker 1987). Clams were individually tethered with monofilament line (15 cm long) glued to the shell and tied to ropes strung between 2 lengths of PVC pipe (50 cm long x 1.25 cm diameter). Three ropes were spaced equidistant along the PVC pipe with 5 clams tethered per rope. This rope-PVC frame allowed for easy transportation of clams to the field site and facilitated the eventual sampling of clam mortality by allowing us to recover both live clams and shells of clams that were eaten.

These experiments were performed in Herb River, a tributary of the Wilmington River, which is one of 2 main rivers flowing into Wassaw Sound, GA. Herb River is tidally driven with little freshwater input beyond runoff, an average salinity of 20-26 ppt, a tidal range of 2-3 m, and bedded with mostly fine grain mud. Wave action in Herb River is nearly non-existent except during periods of severe weather. Potential clam predators were identified using commercially purchased crab traps baited with either fish (to attract blue crabs) or with live clams (to attract knobbed whelks as they rarely recruit to traps baited with carrion; Ferner unpublished observation). We placed 20 pairs of traps in our field site for 48-hour periods from July-Nov 2003. Clam and fish-baited traps caught an average of approximately 1 (range 0-5) and 4 (range 0-13) crabs respectively. Although we commonly catch knobbed whelks in other areas using these trapping methods, we did not catch any whelks in Herb River. Additionally, whelks leave distinctive marks on bivalves (Micheli 1997, Nakaoka 2000), and clams recovered from our clam plots did not show evidence of whelk predation.

Plots were placed in the field in groups consisting of 3 predator treatments. Within each group, plots were approximately 5 m apart, and groups of 3 were spaced at

least 100 m apart. The treatments consisted of crab or whelk predators and no-predator controls. Predators were placed individually in vexar mesh cages (0.30 m diameter x 0.30 m tall, mesh size 1.0 cm^2) anchored to PVC poles pushed into the sediment, and a 2 lb weight was placed in each cage to hold it firmly on the substrate. The cage perimeter was approximately 35 cm from the center of the plot, and cages were placed on either side of the plot along the predominant current direction to insure that clams were always downstream of predators regardless of tidal flow direction. Controls were alike in every way, except cages did not contain predators. Clam plots were placed in the intertidal zone (ca. 0.0 to + 0.15 m relative to mean low water) at low tide from August to October 2003, and all plots in each grouping of 3 were placed at the same tidal height. Clam plots were recovered after 48 hrs in the field, and the number of clams recovered alive in each plot recorded. The number of surviving clams in each plot type (crab, whelk, or control) was compared using a single factor ANOVA (Sokal and Rohlf 1995).

We established predator exclusion plots early during our studies to assess clam survivorship and recovery in the absence of predation. These exclusion plots were constructed using the methods above, but one member of each pair was covered by vexar mesh to exclude predators while the other was left uncovered (control). Plots were placed in the field in pairs (n = 10 pairs). All of the clams were recovered from the exclusion plots alive, while almost 60% of the clams in the uncovered plots were crushed by crab predators or missing. Thus, we counted both empty shells and clams missing from experimental plots as having been eaten by crabs. Other investigators have followed a similar logic (Micheli 1997, Nakaoka 2000).

Hydrodynamic Environment

The Flume- Clam behavioral assays were conducted in a paddle-driven racetrack flume at SkIO (4.8 m long working section x 1 m wide x 0.33 m water depth). This flume produces stable and reproducible boundary layers at current speeds ranging from 1-15 cm s^{-1}. See Ferner and Weissburg (2005) and Smee and Weissburg (in review) for a more detailed flume description and characterization of the flow environment.

Hydrodynamic Methods –Shear velocity (u*), roughness Reynolds number (Re*), and the degree of turbulent velocity fluctuations are useful measurements of benthic boundary layer flows and are frequently used to characterize odor plume structure (e.g., Denny 1988, Weissburg and Zimmer-Faust 1993, 1994, Weissburg 2000). Flow velocities in the flume were measured with an acoustic Doppler velocimeter (ADV; SonTek® MicroADV field probe) and vendor-supplied software. ADV measurements were made at 15 heights within the log layer region of the boundary layer (i.e., the first 30%, or 10 cm extending from the substrate) as well as a free stream measurement 15 cm above the substratum. Each height was sampled for five minutes at a frequency of 10 Hz.

Shear velocity (u*) is a measure of momentum transfer in the boundary layer and is related to the strength of velocity fluctuations (turbulence) near the substrate (Schlichting 1987, Denny 1988, Weissburg 2000). Shear velocity was calculated by regression fit using the Karman-Prandtl equation ("law of the wall") from the ADV data collected at different heights (Schlichting 1987, Denny 1988). All regressions (r^2) used to calculate shear velocities exceeded 0.95.

Turbulence was determined by calculating the root mean square (RMS) of velocity fluctuations over the five-minute velocity time series measured with the ADV

0.05 m above the substrate. This height was selected because it is within the region sampled by blue crab and knobbed whelk olfactory appendages.

Roughness Reynolds Number (Re*) is a coarse fluid calculation that provides an estimation of turbulent eddy penetration into the boundary layer in non-rippled substrates. This "rule-of-thumb" parameter is less precise than measurements of u* or RMS, but may nonetheless convey a reasonable intuitive sense of the flow environment. Turbulence begins to enter the boundary layer at $3.5 < Re^* < 6$, and boundary layers are considered fully turbulent at $75 < Re^* < 100$ (Schlichting 1979, Denny 1988, Weissburg 2000). Roughness Reynolds Number was calculated by:

$$Re^* = (u^*D)/\nu$$

where u* is the shear velocity, D is the hydraulic roughness length (the diameter of grains forming the bed in nonrippled substrates; mean sand grain size was 0.11 cm in our assays), and ν is the kinematic viscosity of the fluid.

We measured and calculated free stream velocity (u), shear velocity (u*), RMS, and Re* at two locations in the flume: over the clam bed and 1.0 m upstream from the clam bed to insure that the flow was relatively uniform throughout our experimental area (Table 2.1).

We also measured flow in the field to insure that flume flows were similar to those in our field site. We continuously recorded flow velocity at 10 Hz during a full tidal cycle with the ADV measuring velocity 0.05 m above the substrate and placed 0.15 m above the mean low water line. Flow velocity ranged from 0.1 cm s^{-1} to 15 cm s^{-1} and RMS ranged from 0.3 to 7.7. Flow properties used in the flume experiments were within the range of those measured in our field site (Table 2.1) and mimic flows used in other

flume studies with blue crabs and knobbed whelks (Weissburg and Zimmer-Faust 1993, 1994, Weissburg et al. 2002, Ferner and Weissburg 2005).

Table 2.1. Summary of hydrodynamic parameters for flow regimes used. U = free-stream velocity, u* = shear velocity, RMS is the root mean square of fluctuations over the velocity time series measurements, and Re* is roughness Reynolds number. Flow conditions were measured 1.0 m upstream from the clam bed and directly over the clam bed to insure that flow conditions were generally uniform throughout the working area of the flume.

Location in Flume	U (cm s^{-1})	U* (cm s^{-1})	RMS (Turbulence)	Re*
1.0 m upstream from clam bed	3	0.22	0.38	2.1
Over clam bed	3	0.18	0.42	1.7
1.0 m upstream from clam bed	11	0.57	1.21	5.2
Over clam bed	11	0.63	1.32	5.8

Behavioral Assays

General Methods – Our experiments utilized changes in clam pumping (feeding) behavior as assays for the ability of clams to detect predation risk. Although previous investigators have assumed that clams are actively pumping only when their siphons are extended (e.g., Irlandi and Peterson 1991), we performed preliminary experiments to verify this supposition. We visualized the excurrent from clams by carefully pipetting a 0.1% solution of fluorescein dye above the excurrent siphon of a clam. Thirty-six clams that had their siphons extended were tested in this manner, and all were releasing an excurrent. We tested 15 clams with open shells but withdrawn siphons, and only three were pumping. Thus, we concluded that siphon extension was indicative of pumping.

In each trial, five clams were placed in the false bottom of the flume and were allowed to acclimate for 30 minutes. Clam density in these experiments was 5 clams per 0.07 m^2 and mimics densities observed in natural habitats (Walker 1987, Smee and Weissburg unpublished data). Predators were introduced at the conclusion of the acclimation period. We recorded the siphon position of each clam (extended or not) prior to introduction of the predator treatments and then at five-minute intervals for 30 minutes. Thus, each clam could have been observed pumping seven times (once prior to predator introduction and 6 in the presence of the predator), and the number of observations in which clams were pumping was our measure of pumping time (i.e., the response of each clam was characterized by a single value from 0-7). The burrowing depth of each clam was measured with calipers at the conclusion of the experiment. Clam burrowing depth results are not presented because they were highly variable and presented no evidence of a significant treatment effect.

The order of treatments and controls in these experiments was randomly assigned each day, and behavioral assays with each treatment and the control were replicated at least five times (5 trials x 5 clams per trial = 25 clams for each treatment and control). To insure independence, each clam and predator were used only once. Clams that neither pumped nor burrowed during the acclimation period were excluded from analysis, and we excluded approximately 25% of the clams from the experiment using this criterion.

Effects of flow on clam responses to predators – Flow environment and distance from chemical sources affect predator chemoreception ability (e.g., Weissburg and Zimmer-Faust 1993, 1994, Powers and Kittinger 2002). To evaluate the effects of hydrodynamics on prey perception, we conducted behavioral experiments at two flow

speeds (3 cm s^{-1}, 11 cm s^{-1}) and placed blue crab or knobbed whelk predators 0.5 m or 1.0 m upstream from the clams. These flow velocities were selected because they were within the range of those measured in our field site.

Duration of predator effects on clam pumping - We measured the duration of time that clam pumping was affected by predators by removing predators at the conclusion of the 30 minute behavioral assay and measuring the amount of time needed for clams to resume pumping. Clam pumping was monitored five minutes after predator removal and again after an additional 25 minutes. In this experiment, predators were placed 0.5 m upstream from the clams in a flow of 3 cm s^{-1}.

Data Analysis –We define a flow condition as an experiment conducted at one particular flow velocity with treatments placed at a fixed distance (0.5 or 1.0 m) upstream. We performed behavioral assays in four separate flow conditions: 1) 3 cm s^{-1} flow velocity with predators placed 0.5 m upstream, 2) 3 cm s^{-1} flow velocity with predators placed 1.0 m upstream, 3) 11 cm s^{-1} flow velocity with predators placed 0.5 m upstream, and 4) 11 cm s-1 flow velocity with predators placed 1.0 m upstream. Within each flow condition, separate trials were performed. Each trial involved measuring feeding responses of a group of five clams in the presence of a whelk, crab, or in a no-predator control. We completed at least 5 trials (5 clams x 5 trials = 25 clams) for each treatment (whelk, crab, or control) in each flow condition.

In our behavioral experiments, the pumping activity of each clam was monitored in the presence of either an individual blue crab or knobbed whelk or in a control without predators. Previous research has shown that adjacent clams behave independently of one

another (Smee and Weissburg 2006), so that these results are not biased by interactions between neighbors.

Replicate trials were used to collect data on clam responses, so we employed a nested ANOVA to determine if responses of clams in groups were significantly different across replicates of the same treatment in a given flow condition (i.e. an effect of the nest, Sokal and Rohlf 1995). Observations of clam pumping behavior (number of siphon extensions observed for each clam) were arcsine transformed to meet ANOVA assumptions (Sokal and Rohlf 1995). The nested ANOVA did not detect a significant nest effect as all P values for the nest effect were greater than 0.2. Thus, we lumped trials within treatments and tested the significance of the main effect using the pooled error variance (Sokal and Rohlf 1995). A Tukey-Kramer *post hoc* analysis was employed to test for pair-wise differences between treatments (Sokal and Rohlf 1995) where necessary. Experiments examining clam responses to predators in differing flow conditions were not interspersed and were conducted at different times over a period of several months. To insure that temporal changes in clam behavior were not affecting our results, we performed control trials for each flow condition interspersed between predator treatments and compared changes in clam behavior to these corresponding controls.

Our results suggested that flow velocity and predator distance affected clam responses, and we examined these effects on clam reactions to predators using a two-way ANOVA. To reduce residual variation in clam behavior between flow conditions, we normalized the number of clam pumping observations by dividing the number of pumping observations of each clam in the presence of a crab or whelk by the mean number of pumping observations of clams in corresponding controls. We then ran

separate two-way ANOVAs for clams exposed to either blue crabs or knobbed whelks and examined the effects of flow speed and distance on clam reactions to these predators.

The duration of predator effects on clam pumping was analyzed with a repeated measures ANOVA (Sokal and Rohlf 1995). For this test, we compared the percentages of clams with their siphons extended per group before predators were added, while predators were in the flume, and after predators were removed. We used the repeated measures ANOVA to detect the effects of predator type, time, and an interaction of these factors on clam pumping.

Results

Results from the Field Experiment

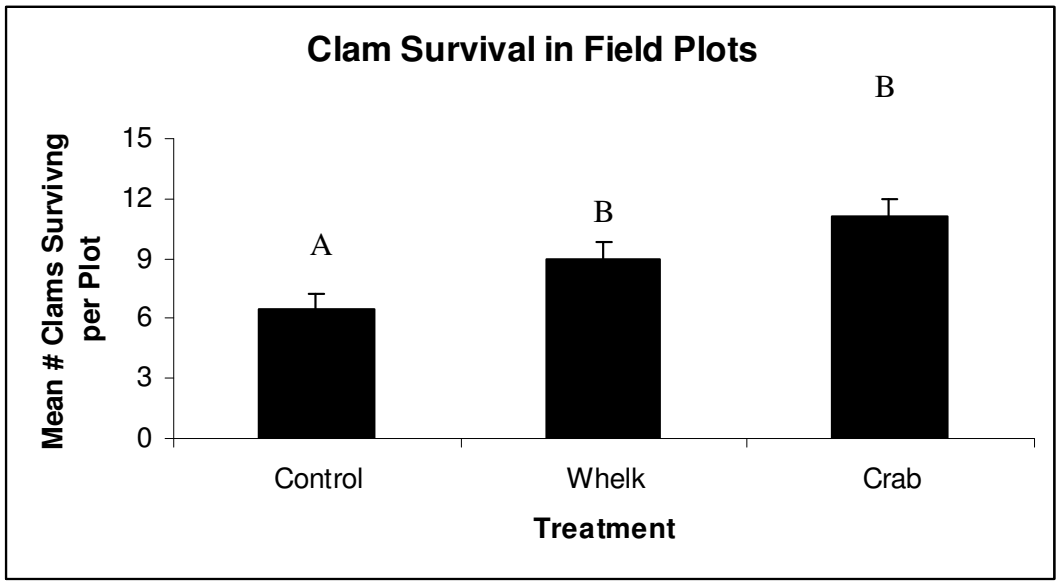
Clam survivorship increased when predators were caged next to potential prey but not allowed to interact directly with them (Fig 2.1, $F_{2,47} = 9.17$, $P < 0.001$). Survivorship was 37% to almost 75% higher in whelk and crab treatments respectively, relative to survival in the no-predator controls. These values correspond to survivorships ranging from less than 50% in controls to a nearly 80% in the crab treatment. Because clam predators leave distinctive marks on clam shells (Micheli 1997, Nakaoka 2000), we determined that all clams eaten in the experiment were consumed by crabs.

Characterization of Flow Regimes in the Behavioral Trials

The flow velocities used in our flume experiments were within the range of those measured in the field (Table 2.1). Vertical velocity profiles indicated turbulent boundary layers in the flume, and u*, turbulence magnitude (RMS), and Re* at the most upstream

predator location (1 m) were similar to those occurring in the middle of the clam bed, suggesting that our experimental arena was relatively free of flow artifacts. As expected, u*, RMS, and Re* increased with flow velocity (Table 2.1).

Fig. 2.1 Clam survivorship in the field in the presence and absence of predators. Fig shows mean number of clams surviving per plot (± SE) as a function of predator treatment. Initial clam density was 15 clams per plot. Sample sizes were 19, 16, and 15 plots from no-predator controls, whelk and crab treatments, respectively. Letters denote means that are significantly different based upon a Tukey-Kramer post hoc test and reveal that both caged crabs and whelks increased clam survival in the field.



Behavioral Assays

Clam responses to predators – Clams reacted to the presence of blue crabs and knobbed whelks by significantly reducing their feeding time ≈ 20-50% relative to no-predator controls (Fig. 2.2). When U = 3 cm s^{-1}, clams pumped significantly less in the presence of knobbed whelks and blue crabs placed 0.5 m ($F_{2,51} = 9.69$, $P < 0.005$) and 1.0 m ($F_{2,110} = 23.22$, $P < 0.005$) upstream (Fig. 2.2). Similarly, at U = 11 cm s^{-1}, clam pumping was significantly less in the presence of predators placed at 0.5 m ($F_{2,57} = 9.85$, $P < 0.0005$)

and at 1.0 m upstream ($F_{2,111} = 17.37$, $P < 0.005$, Fig. 2.2). Clams reduced their feeding time by ≈ 20% in the presence of blue crabs placed 1.0 m upstream in the 11 cm s^{-1} flow but responded to blue crabs with a ≈ 40% feeding reduction in all other flow conditions. Clam pumping was ≈ 50% less in the presence of whelks in all flume experiments. Post hoc analysis revealed that clam reactions to knobbed whelks and blue crabs were significantly different from each other only when these predators were placed 1.0 m upstream in the 11 cm s^{-1} flow. Clam responses to predators were similar when predators were placed 0.5 m upstream at this same flow velocity or when the flow velocity was 3 cm s^{-1} (Fig. 2.2).

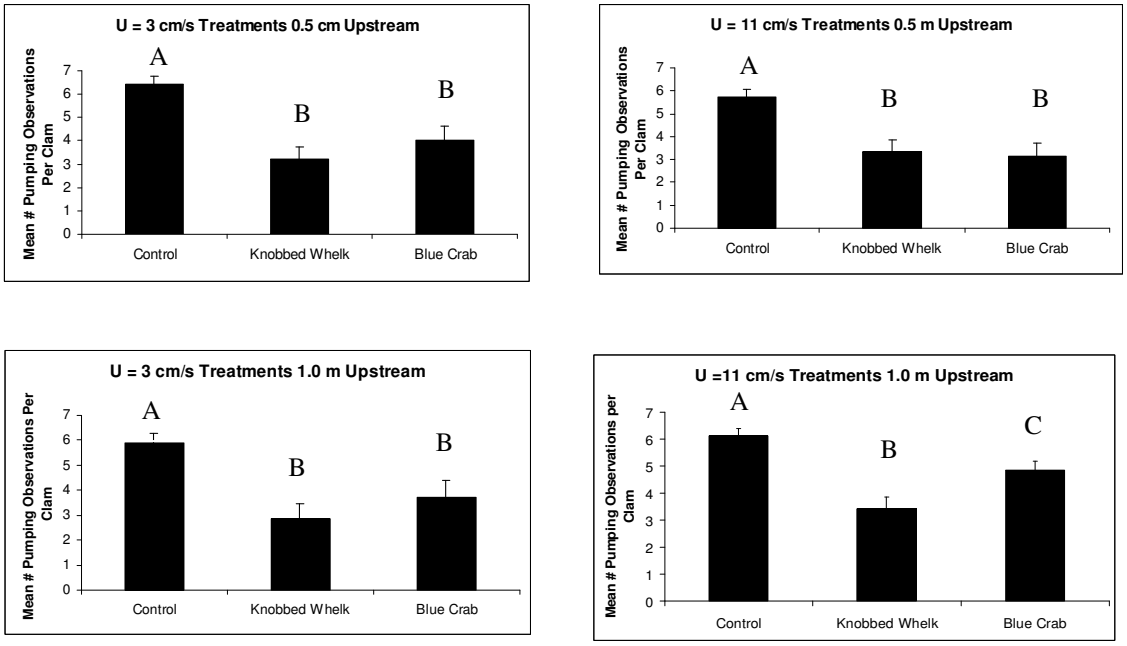


Fig. 2.2. Mean number of pumping observations per clam (± SE) with treatments at 0.5 m and 1.0 m upstream in two flow regimes (U = 3 cm s^{-1} and U = 11 cm s^{-1} over sand). Letters denote means that are significantly different based upon a Tukey-Kramer post hoc test. At an upstream distance of 0.5 m, sample sizes (# clams) consisted of 17, 20, 17 and 23, 21, 16 for control, whelk and crab treatments at 3 cm s^{-1} and 11 cm s^{-1}, respectively. At an upstream distance of 1.0 m, sample sizes (# clams) consisted of 38, 37, 38 and 38, 41, 35 for control, whelk and crab treatments at 3 cm s^{-1} and 11 cm s^{-1}, respectively. Different sample sizes result from exclusion of inactive clams from analyses. Each clam could have been pumping seven times during the 30-minute observation period.

A two-way ANOVA comparing percentages of clam pumping time in the presence of crabs in all flow conditions found a significant interactive effect between flow and distance ($P < 0.05$) but did not find a significant effect of either flow or distance separately ($P > 0.35$). This suggests that the reactive distance of clams to blue crabs diminishes in higher velocity flows (Fig 2.3). That is, the effect of blue crabs on clam pumping is controlled by both the flow environment and distance the crab is upstream. Similar analysis comparing the effects of whelks on clam pumping across all tested flow conditions did not detect an effect of flow, distance, or an interaction between these factors, indicating clam reactions to whelks are similar regardless of flow velocity or distance upstream (Fig. 2.3).

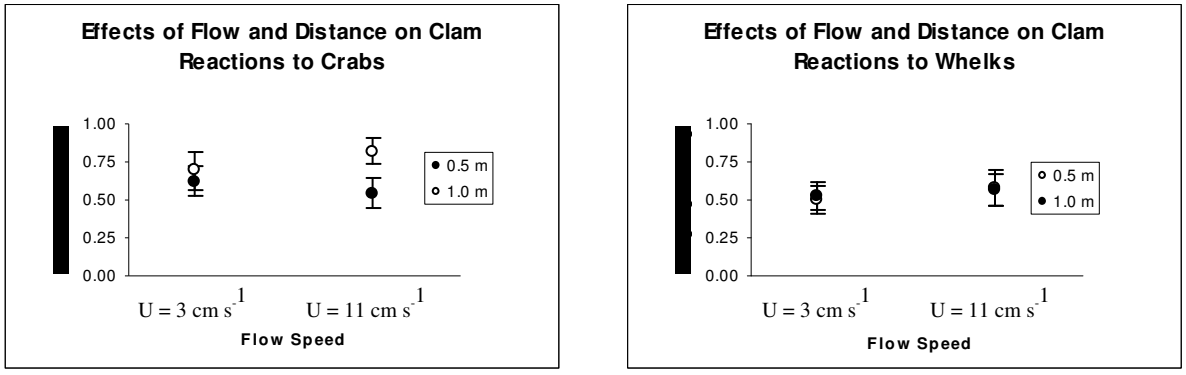


Fig. 2.3. Effects of flow speed and predator distance upstream on clam pumping. Dots are the mean percentage of pumping observations per clam observed in the presence of crabs and whelks (± SE). Clam pumping was standardized between flow conditions by dividing the number of observations in which clams were pumping by the mean number of pumping observations in corresponding controls. Thus, clams in experimental treatments that were observed to pump the same number of times as the control were assigned a value of 1. Clams responded similarly to blue crabs regardless of upstream distance when $U = 3$ cm s^{-1}, but clams showed a greater decrease in pumping when crabs were 0.5 m upstream than 1.0 m upstream when $U = 11$ cm s^{-1}. Clams responded similarly to whelks in all tested flow conditions. Therefore, clam reactive distance to blue crabs is affected by an interaction between flow speed and distance upstream.

Duration of predator effects on clam pumping – We determined the length of time clam pumping was affected by exposure to each predator by comparing the pumping behavior of clams during a 60-minute period. During the first 30 minutes, clams were exposed to knobbed whelk and blue crab predators, but the predators were removed during the final 30-minute observation period. In this experiment, the flow velocity was 3 cm s^{-1}, and the treatments were placed 0.5 m upstream.

Clam pumping in the predator removal experiments was significantly affected by predator treatment, time, and the time*treatment interaction (Fig. 2.4, $F_{2,12}$= 11.25, P < 0.01; $F_{4,9}$ = 16.25, P < 0.001; $F_{8,18}$ = 2.67, P < 0.05, respectively). Five minutes after the addition of predators, the percentage of clams pumping was less in the predator treatments when compared to controls, and the decrease in pumping lasted for 30 minutes until predators were removed (Fig. 2.4). Five minutes after predators were removed, clams exposed to whelks showed a dramatic decrease in pumping, but clams exposed to blue crabs had resumed pumping similar to those in controls. Thirty minutes after predator removal, there were no noticeable differences in clam pumping between clams exposed to crabs or whelks as compared to clams in controls. These results suggest that clams continue to respond to whelks even after the whelks have been removed, but the effects of blue crabs on clams dissipate within 5 minutes after crab removal.

Discussion

Results from our field study indicated that clam responses to nearby predators reduce their apparency to consumers and increase their survival (Fig. 2.1). These results also indicated that the predator avoidance behavior of clams measured in our lab assays

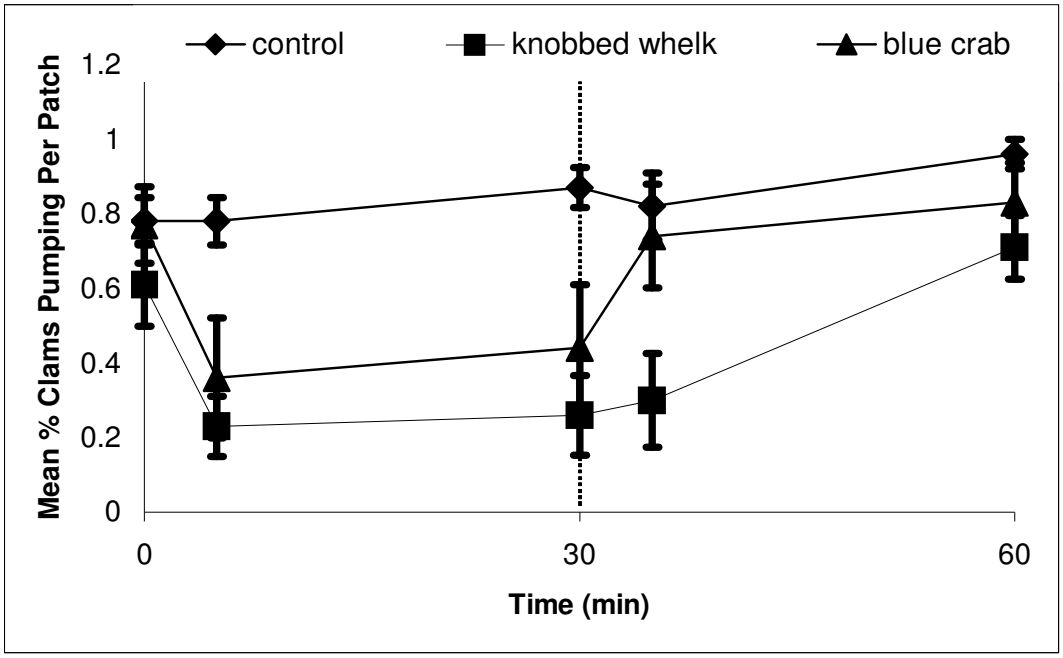


Fig. 2.4. Duration of time clam pumping was affected by predators. Data points represent mean percentage of clams pumping (± SE.) per trial. There were 5 trials for each treatment. Observations on clam pumping were made prior to addition of predators (t= 0), 5 and 30 minutes after predator addition (t = 5, 30 minutes), and 5, and 25 minutes after predator removal (t = 35, 60 minutes). The dotted line represents predator removal.

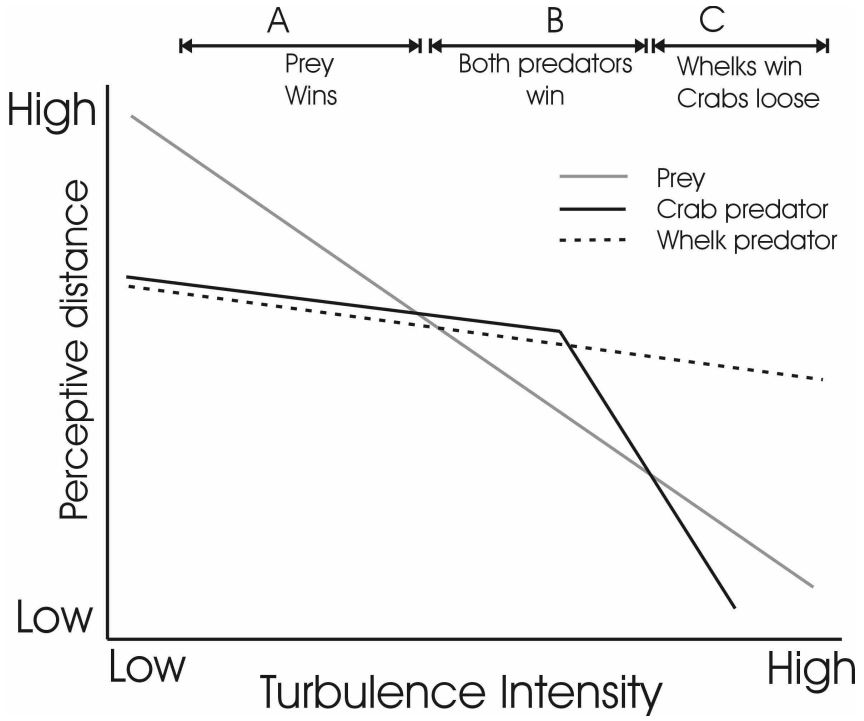


Fig. 2.5. Relationship of sensory ability of clams and two of their common predators: blue crabs and knobbed whelks. The figure represents idealized (linear) relationships between perceptive distance and turbulence. Future work is required to clarify the function relating perceptive distance to turbulence for these organisms, as well as identifying the precise boundaries where sensory advantages shift in the field. In Region A, both predators and prey have high perceptual ranges, but clams have a relative sensory advantage over their predators. The relative sensory advantage shifts to predators in Region B because of an unequal rate of decline of perceptive range between predators and prey as turbulence increases. The further decline in perception (Region C) again shifts sensory advantages, but in this region, the rate of decline is less for whelks than for crabs and clams. In this scenario, a sensory refuge may exist for crab prey, although whelks are likely to be highly successful forgers. We predict that nonlethal effects of predation are high where prey have a sensory advantage (Region A) and lethal effects predominate where predators have an advantage (Region B). In region C, predator identity becomes important as the type of predator in this situation determines whether lethal or nonlethal effects predominate.

was ecologically meaningful. We found that clams in the flume reacted to both knobbed whelk and blue crab predators by reducing their feeding behavior, but distance and flow interacted to determine clam responses to blue crabs (Figs. 2.2 and 2.3). Clams responded similarly to blue crabs that were placed 0.5 and 1.0 m upstream when flow velocity was 3 cm s^{-1}. However, increasing the flow velocity to 11 cm s^{-1} resulted in a significant reduction in clam reactions to blue crabs placed 1.0 m upstream (Fig. 2.3). We attribute this change in clam reactions to blue crabs to a roughly three-fold increase in both u* and RMS turbulence, which are indicative of the amount of turbulence in the boundary layer (Table 2.1 Denny 1988, Weissburg 2000). Re* calculations also indicated a more turbulent boundary layer in the 11 cm s^{-1} flow. Clams responded similarly to knobbed whelks in all tested flow conditions, suggesting that the strength or quality of the chemical cue renders it highly detectable under the conditions of our trials. In addition, the duration of anti-predator behavior of clams was longer in response to whelks than to blue crabs (Fig. 2.4).

Clam survival in the field was significantly higher in the presence of whelk and crab predators as compared to controls, indicating that clam responses to predators decrease clam mortality (Fig 2.1). We attribute the increased clam survival to predator-induced reductions in clam feeding that were observed in flume experiments. The cessation of clam pumping in response to predator odors appears to make clams more cryptic to predators. These results are consistent with the findings of Nakaoka (2000), who observed long-term exposure to caged whelks reduced clam growth rate, presumably because clams fed less in the presence of a potential threat.

An alternate explanation for our field results is that antagonistic interactions between caged and foraging predators reduced clam predation, but we feel that this is unlikely for several reasons. First, blue crabs readily approach and attempt to consume whelks in our sea tables, and blue crabs will enter crab traps baited with only live whelks (Smee and Weissburg unpublished data). Thus, whelks do not inhibit crab predators, and increased survival does not result from predator interference between caged whelks and crabs. Secondly, although blue crabs are known as a bellicose species, antagonistic interactions between conspecifics seem to occur during crab feeding. Blue crabs release large quantities of prey metabolites into the water when they feed, which attracts additional crabs and often leads to aggressive interactions between competitors (Clark et al. 1999). Blue crabs housed in our sea tables often engaged in combat during feeding but rarely at other times. Recall that our caged predators could not consume potential clam prey and, thus should not interfere with ambient crab predators in our study site. Additionally, when monitoring predator density in our field site we found that multiple blue crabs commonly recruit into baited traps (roughly 85% of our traps contained multiple animals), and Ferner et al. (in press) found that the presence of a live crab did not deter conspecifics from entering baited traps.

Previous studies indicate that fluid forces alter the structure of chemical odor plumes and change the ability of consumers to find prey (Webster and Weissburg 2001, Weissburg et al. 2002). Enhanced turbulence is detrimental to prey finding by some predators, as shown by decreasing foraging success and efficiency when blue crabs track prey in turbulent flows (Weissburg and Zimmer-Faust 1993, Finelli et al. 2000, Weissburg et al. 2003). In contrast, knobbed whelks can successfully follow odor plumes

in turbulent flows that severely diminish the perception of blue crabs (Powers and Kittinger 2002, Weissburg et al. 2002, Ferner and Weissburg 2005). Our results suggest that hydrodynamic forces may influence perceptual ability of prey as well as predators. Interestingly, differences in sensory ability of organisms suggest that an environment in which one organism is ineffective does not necessarily compromise its foe or competitor. For example, blue crabs show substantial reduction in their ability to locate bivalves in flow conditions where bivalves still can detect crabs upstream (Weissburg and Zimmer-Faust 1993, Powers and Kittinger 2002, Weissburg et al. 2003).

Importance of Prey Perception to Lethal Predator Effects

Ecologists have assumed that predators forage at optimal times or in conditions that maximize their perceptive ability (e.g., Weissburg and Zimmer-Faust 1993). This assumption has ignored both the sensory capability of prey and the effect physical forces have on sensory perception. Our results, along with others (reviewed by Weissburg et al. 2002), have clearly shown that physical forces influence sensory performance. Therefore, it is important to understand how environmental forces affect the sensory abilities of both predators and prey to appreciate how environmental forces might change the outcome of predatory interactions. We illustrate how physical forces (e.g., hydrodynamics) might simultaneously affect both predators and prey using the hypothetical case in Fig. 2.5. This figure is based roughly on our current understanding of blue crab-whelk-clam interactions, although further efforts are necessary to precisely characterize the relationship between perceptive ability and turbulence and to define the turbulence levels where these effects may be important in the field (as opposed to the lab).

Blue crabs challenged to locate dense patches of actively pumping bivalves in flumes have generally moderate success rates from distances of 0.5-1.0 m and show a peak performance at $u^* = 0.1$ cm s^{-1} and $Re^* = 1.0$ (Weissburg and Zimmer-Faust 1993, 1994). Blue crab prey-finding ability further declines as boundary layer turbulence increases, and successful chemical navigation is rare when crabs are 1.0 – 1.5 m from their prey in fully turbulent boundary layers ($u^* > 4$ cm s^{-1}, $Re^*>100$, Jackson et al. 2004). The results presented here suggest that clam reactive distance to blue crabs also diminishes when boundary layer turbulence increases. We represent these relationships in Fig. 2.5, where clams initially have a sensory advantage, followed by a region of turbulence levels where predators have the upper hand, and finally, a region where high levels of turbulence diminish the perceptive ability of clams and crabs but not whelks. Whelks experience little to no decline in chemosensory perception of prey over a large range of flow conditions from nearly laminar to fully turbulent flows (Ferner and Weissburg 2005), and other slow moving foragers are predicted to operate similarly (Weissburg 2000). Thus, whelk foraging performance is substantially unaffected by high levels of turbulence, even though turbulence erodes perception of competitors and prey (Ferner and Weissburg 2005).

These idealized relationships between predator and prey sensory performance suggest multiple and shifting outcomes of predation that depend on the physical environment. For example, environmental conditions that maximize the perceptive ability of predators may also maximize the perceptive and predator avoidance abilities of prey, placing the predators at a disadvantage (Fig. 2.5). Thus, prey may thrive in areas that are ideal for foraging predators because these areas maximize their own sensory capabilities.

Perhaps predators elect to forage (or are most effective) in non-optimal conditions if those conditions are more detrimental to their prey than they are to them.

Joint consideration of predator and prey perception suggests the appropriate strategy for an organism may not be to occupy areas that maximize sensory capability but instead to occupy areas that give an organism the largest sensory advantage over predators, competitors, or prey. Interactions between echolocating bats and their insect prey may be such a case. Some insects detect bat ultrasounds and respond using defensive maneuvers during flight (Surlykke 1988). To counter the insects' acoustic detection of their calls, whispering bats use a lower sound intensity that insects cannot detect, but that also reduces their perceptive distance (Dusenbery 1992).

Unfortunately, the importance of absolute vs. relative performance is difficult to evaluate, in part because environmental effects on prey perception of predation risk seem to be unknown even when constraints on predator perceptual abilities have been recognized. Some studies have identified rather coarse environmental variables, such as time of day, that affect prey evaluation of predation risk (e.g., Peckarsky 1996, McIntosh and Peckarsky 1999). However, these effects may be more related to diurnal patterns in the activity of particular predators than to specific environmental constraints on prey evaluation of predation risk. We do suggest, however, that strategies based on relative performance may be more easily identified in systems where predator and prey rely on the same sensory modality, since both participants in such a duet are likely to be affected by the same environmental features. Mutual detection of predators and prey occurs using acoustic (e.g., Surlykke 1988), visual (e.g., Blaxter 1988, Ens et al. 1993, Brown 1997, Layne et al. 1997, Skov et al. 2002) and mechanosensory (e.g., Yen and Strickler 1996,

Wilcox 1988, McIntosh and Townsend 1996, Peckarsky 1996) modalities, and systems in which animals select environments to maximize their relative rather than absolute level of sensory performance may be widespread.

We define a sensory refuge as an environment in which predator perceptual abilities are insufficient to reliably detect prey before prey have the ability to engage in anti-predator behavior (e.g., decreasing their apparency, initiating escape). The existence of such a refuge will depend on how each species responds to environmental forces (e.g., turbulence). Sensory refugia are more likely to exist when increasing levels of environmental forces affect predator sensory ability more than its prey (prey advantage) (Fig. 5). In contrast, refugia are unlikely to exist if physical forces cause greater deterioration of prey vs. predator sensory ability.

Field tests are clearly necessary to examine how turbulence impacts predation rate of prey and predators with different sensory capabilities. However, our results suggest that turbulent habitats may reduce prey perception of risk and not provide a sanctuary from consumers, as has been suggested (e.g., Weissburg and Zimmer-Faust 1993), and the role of turbulence in altering community structure via this refuge effect (e.g., Leonard et al. 1998) remains unclear. Still, for prey that lack chemoreception, turbulent environments may well provide a refuge from consumers when these habitats negatively affect consumer chemoreceptive ability.

Importance of Prey Perception to Nonlethal Predator Effects

Prey alter their behavior or morphology in the presence of predators (Katz and Dill 1998) to minimize predation risk, and predator-induced changes in prey behavior or

morphology (TMIIs; Abrams et al. 1996) can have profound effects on competitive interactions and community structure (Turner and Mittlebach 1990, Schmitz et al. 1997, Schmitz 1998, Trussell et al. 2003, Werner and Peacor 2003). Predicting when and where TMIIs should occur remains an important, but elusive goal (Werner and Peacor 2003). Clearly, the impact of TMIIs will be minimal if prey cannot perceive their predators, but the role of animal perceptual abilities or limits has not received much attention when examining the role of behavioral changes in determining community structure (e.g., Werner and Peacor 2003, but see Turner and Montgomery 2003).

Turner and Montgomery (2003) hypothesize that mobile predators moving through a habitat create a "behavioral landscape" by inducing reversible trait shifts in prey. Our results suggest the temporal and spatial grain of this landscape will vary with predator identity and environmental properties. Clam prey reduced their reaction distance for blue crabs in turbulent flows and resumed pumping quickly after brief exposure to blue crabs but more slowly when exposed to whelks. A knobbed whelk moving through a clam bed in turbulent flow conditions would create a vastly different behavioral landscape than a blue crab. Only clams close to the crab might reduce pumping and would resume pumping shortly after the crab passes. In contrast, whelks should affect clams at greater distances and for longer times. In essence, crabs might create a highly variable landscape relative to that induced by a whelk due to the differential ability of clams to detect each consumer. The behavioral landscape might not differ when turbulence is minimal because clams appear to respond equally well to both predators under these conditions, although the effects of the whelk will linger longer after this predator has left the habitat.

In chemically mediated predator-prey interactions, areas of slow flow or reduced turbulence may allow for a greater role of TMIIs because prey are more liable to sense their predators, even though predators may sense prey efficiently as well (Fig. 2.5). Alternately, if reduced turbulence indeed favors the predator, then direct lethal effects should outweigh TMIIs. As before, field tests are required to resolve the ambiguity created by simultaneous shifts in perceptual ability of predators and prey with changes in flow properties. In any case, the importance of physical factors in mediating the intensity or occurrence of TMIIs is likely to be important in many aquatic systems, given the widespread occurrence of chemically-mediated predator perception in these environments (Katz and Dill 1998). Indeed, the response of pulmonate snails to predator odor exhibits substantial variation that may be linked to variations in flow environment (Turner *pers. com.*), suggesting that the community-level changes stemming from predator-induced changes in snail behavior (Turner et al. 2000) may be under environmental control.

Predicting when TMIIs should be prevalent in communities and evaluating the scales on which TMIIs occur requires a careful examination of the environmental impacts on perceptive abilities of interacting organisms. Appropriately controlled and quantified laboratory environments may prove useful in determining the environmental conditions favoring predators vs. prey sensory systems. Although field studies are ultimately needed to document the effects of TMIIs on communities, they may be incomplete or ambiguous where heterogeneous environments that affect sensory performance have not been characterized.

Chapter 3

Environmental Conditions Alter Prey Perception of Risk and the Scales of Nonlethal Predator Effects in Natural Systems

Abstract

Recent research has documented the importance of trait-mediated interactions (TMIs) to community structure, but predicting their occurrence and magnitude remains an elusive goal. Prey must perceive and react to predatory threats for TMIs to take place, but environmental conditions that affect prey perception of risk have not been carefully studied. Using blue crabs (*Callinectes sapidus*) and hard clams (*Mercenaria mercenaria*) as a model predator-prey system, we investigated the role environmental forces play in controlling the prevalence of TMIs by altering prey reactions to risk. Crabs find their clam prey by following waterborne chemical cues released by feeding clams. Clams reduce their feeding time after detecting approaching crabs, which makes them less apparent to crabs, increases their survival, but compromises growth. However, laboratory assays suggest that clams are less responsive to crabs in turbulent flows, and we hypothesized that the reactive distance of clams to crabs in the field would diminish with increased turbulence. We measured the effects of turbulence on clam reactive distance to crabs in the field by establishing clam plots and comparing clam survival in the absence of crab predators and in the presence of caged blue crabs at two distances (0.5m, 2m) away in low and high turbulent flows. Distance and the flow environment interacted to determine clam responses; clams reacted to blue crabs 2.0 m away in low turbulence, but

their reactive distance decreased with increased turbulence. Results from this study show that environmental forces influence the reactive distance of prey to predators in the field. Therefore, the prevalence of TMIs in a given system may be strongly related to environmental conditions that affect the scales over which prey perceive and react to risk.

Introduction

Predators commonly have substantial impacts on prey populations and on the organization and function of communities (Paine 1966, Carpenter et al. 1985, Schmitz et al. 1997, Menge 2000). Predators affect prey through lethal predation (Sih et al. 1985), and by nonlethal mechanisms in which consumers alter prey characteristics or traits such as behavior or morphology (Turner and Mittlebach 1990, Katz and Dill 1998, Nakaoka 2000). Changes in prey traits in response to predators are referred to as trait-mediated interactions (TMIs, Abrams et al. 1996). Both lethal and nonlethal predator effects can cascade through communities, affecting multiple trophic levels (Sih et al. 1985, Carpenter et al. 1985, Schmitz et al. 1997, Trussell et al. 2003). Traditionally, community ecology research focused on lethal effects of predators (Paine 1966, Sih et al. 1985), but current studies have shown that TMIs can affect communities in ways that rival or mimic effects stemming from prey consumption (Turner and Mittlebach 1990, Schmitz et al. 1997, Trussell et al. 2003, Grabowski 2004). For example, behavioral responses to predators may suppress prey foraging and increase the abundance of primary producers (Schmitz et al. 1997, Schmitz 1998, Trussell et al. 2003, Werner and Peacor 2003). Nonlethal effects of predators on communities that result from TMIs such as those previously described, are termed trait-mediated indirect interactions (TMIIs, Abrams et al. 1996).

Prey must perceive their predators and initiate antipredator measures for TMIs, and subsequently TMIIs to occur (Schmitz et al. 1997, Werner and Peacor 2003). Yet, environmental forces can change the ability of prey to detect and respond to predators (Dusenbery 1992, Smee and Weissburg in press), and by affecting prey perception, environmental conditions may determine the magnitude and extent of TMIS in a given system. That is, TMIs should be prevalent when conditions favor prey perceptive abilities because this will maximize the distance a predator can induce changes in prey traits (Smee and Weissburg in press). Likewise, when environmental forces diminish prey perception, the nonlethal effects of predators should be minimized, but this hypothesis has not been empirically tested in the field.

Predator-prey interactions in aquatic systems often are mediated by water borne chemical cues (Katz and Dill 1998, Zimmer and Butman 2000). The delivery and subsequent detection of chemical signals in aquatic environments is affected by fluid forces (Weissburg and Zimmer-Faust 1993, Finelli et al. 2000, Zimmer and Butman 2000, Webster and Weissburg 2001, Powers and Kittinger 2002, Weissburg et al. 2002, 2003). By altering the transmission of chemical signals, hydrodynamic forces have been shown to reduce successful prey finding by predators (Weissburg and Zimmer-Faust 1993), alter the outcome of predator-prey interactions (Powers and Kittinger 2002), and lower predation intensity in communities (Leonard et al. 1998). Smee and Weissburg (in press) found that hydrodynamic forces changed prey responses to predators in a laboratory study and suggested that TMIs and TMIIs might vary as a result of changes in the ability of prey to sense chemical cues from potential consumers.

The purpose of this study was to determine if changes in environmental conditions (e.g., flow) affect prey reactions to risk, and by altering prey responses to consumers, affect the spatial scales by which nonlethal predator effects occur. Specifically, we wanted to determine if turbulent flows would affect the distance that prey respond to predators in the field. We selected hard clams (*Mercenaria mercenaria*) and one of their common predators, blue crabs (*Callinectes sapidus*) as model organisms for this study. Clams cease pumping when presented with chemical cues emanating from blue crabs (Smee and Weissburg 2006), and the reduction in pumping renders clams less apparent to consumers and increases their survival (Smee and Weissburg in press). Responding to predators is costly to clams as lost feeding time reduces clam growth and fecundity in the field (Nakaoka 2000). Thus, crab predators may exert either lethal or nonlethal effects on clam populations depending on whether clams react to and avoid predators. We reasoned that the decrease in clam reactions to crabs caused by turbulence would reduce the reactive distance of clams to crabs in the field, and thus, alter the spatial scales of nonlethal predator effects in this system. The results of our manipulative field experiments suggest that the scales and magnitude of nonlethal predator effects are dependent on local flow environments that affect prey reactions to risk.

Methods and Materials

Animal Capture and Maintenance

Adult clams were hand dug from intertidal habitats in Wassaw Sound, GA. Juvenile clams were purchased from a local supplier to assure a consistent supply of

juvenile clams for these experiments. Clams were maintained in flow-through sea tables at the Skidaway Institute of Oceanography (SkIO) near Savannah GA. Blue crabs were collected in the Skidaway, Herb, and Wilmington Rivers using commercially purchased crab pots. After capture, blue crabs were housed at SkIO in sea tables and fed a daily diet of clams for 72-96 hours before use in the field experiment.

Site Description

Experiments were performed in the Skidaway River, a tributary of the Wilmington River, which is one of 2 main rivers flowing into Wassaw Sound, Georgia. The Skidaway River is tidally driven and experiences long periods of unidirectional flow. It receives little freshwater input beyond runoff, has an average salinity of 20-26 ppt, a tidal range of 2-3 m, and the substrate is composed mostly of fine grain mud. Wave action in this field site is nearly non-existent except during periods of severe weather. Potential clam predators in this site included blue crabs, stone crabs (*Menippe mercenaria*), and knobbed whelks (*Busycon carica*). Monitoring of predator densities in the Skidaway River from 2002-2005 indicated that blue crabs are the most common clam predator in this study area, comprising > 95% of our collections (Smee, unpublished data) and virtually all predation in our experiment was attributed to crabs (see below).

General Protocol

Turbulence affects both the delivery and detection of chemical signals (Weissburg and Zimmer-Faust 1993, Webster and Weissburg 2001, Rahman and Webster 2005), and we examined whether increased turbulence would reduce the reactive distance of clams

to blue crabs in the field. Since caging blue crabs near clam beds increases clam survival in the field, and clam reactions to blue crabs vary with flow regime (Smee and Weissburg in press), we measured changes in clam survival when caged blue crabs were different distances from clams and in 'low' and 'high' turbulent conditions. These experiments consisted of establishing clam plots in the field and determining whether clam survivorship changed with predator proximity and turbulence level, which was locally changed by altering the substrate near clam plots. An increase in clam survival when predators are caged nearby indicates that clams are reacting to these consumers.

Clam plots contained 15 clams, 10 juveniles (shell size < 30 mm) and 5 adults (shell size > 35 mm), in a 0.25 m^2 area (0.5 m x 0.5 m), and this density mimics naturally occurring populations in the study area (Walker 1987). Clams were individually tethered with monofilament line (15 cm long) glued to the shell and tied to ropes strung between 2 lengths of PVC pipe (50 cm long x 1.25 cm diameter). Three ropes were spaced equidistant along the PVC pipe, with 5 clams tethered per rope. The rope-PVC frame was placed in the field flush with the sediment, and each clam was gently pressed into the sediment to facilitate clam burrowing. Clam tethering allowed for easy transportation of clams to the field site and aided in the eventual sampling of clam mortality by allowing us to recover both live clams and shells of clams that were eaten.

Clam plots were placed in the field in a block of 6 plots consisting of 3 levels of predation risk (no predators, caged predators 0.5 m away, caged predators 2 m away), and two turbulence levels (low, high, Fig. 3.1). Within each block, plots were approximately 10 m apart, and blocks were spaced at least 100 m apart. Vexar mesh cages (0.30 m diameter x 0.30 m tall, mesh size 1.0 cm^2) were placed on both sides of each clam plot

parallel to the predominant current direction to insure that plots were downstream from a cage regardless of tidal flow direction. Cages were anchored to PVC poles pushed into the sediment and contained a 1 kg weight to keep the cage firmly on the substrate.

We increased turbulence by surrounding the predator cages of one plot of each risk level with oyster shell hash in a ring approximately 0.30 m wide (Fig. 3.1). Using shells allowed us to increase the local turbulence level and measure the effects of turbulence on clam perception of caged crabs. Other authors have shown that increasing substrate particle size increases turbulence and affects the transmission of chemical signals (e.g., Weissburg and Zimmer-Faust 1993, Rahman and Webster 2005). The advantage of using this design was that it allowed us to expose each group of six plots to similar field conditions but also to generate higher levels of turbulence in a specific area to identify the effects of turbulence on clam detection of blue crabs. We placed the shell rings around the predator cages of one plot in each of our treatments to create 6 treatment types: Control (no predation risk; empty cage), control with shells, crabs 0.5 m away, crabs 0.5 m away with shells, crabs 2.0 m away, and crabs 2.0 m away with shells. Fifteen groups (90 plots) were used in this study. Clam plots were recovered from the field after 48 hours, and the number of clams that were alive, missing or eaten was recorded. Smee and Weissburg (in press) determined that missing clams were taken and consumed by crabs, and in this study, we counted missing clams as being eaten by crabs, as have other authors (e.g., Micheli 1997, Nakaoka 2000).

We compared clam survival between these treatments to determine the effects on risk treatment, and turbulence, which allowed us evaluate how the scale over which clams react to distant crabs is modulated by turbulence. We analyzed the number of

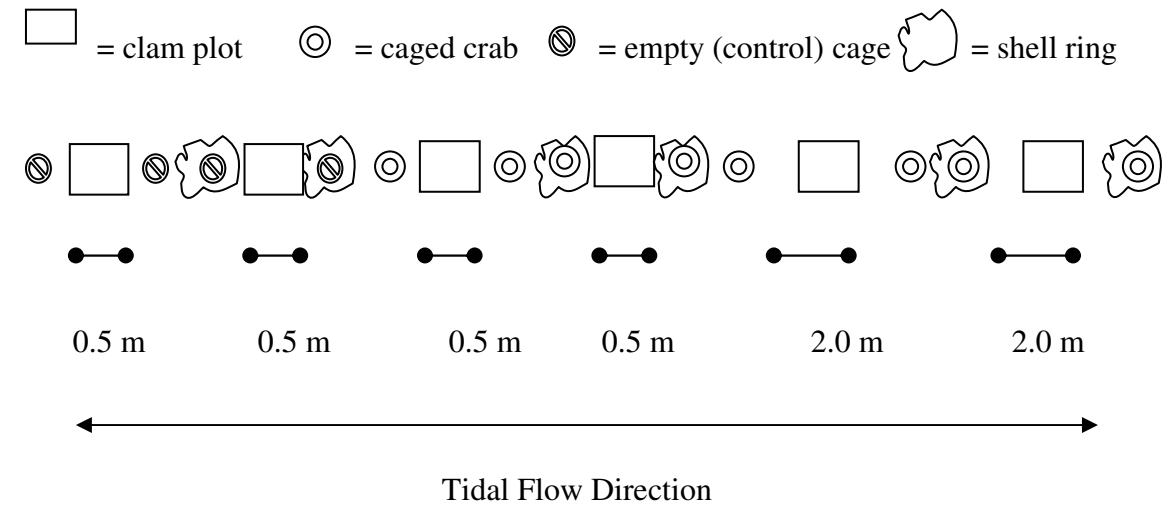


Fig. 3.1. Description of field experiment. An experimental block is pictured below, and 15 blocks were used in the experiment. Each block contained six treatments: control, control with shells, crabs 0.5 m away, crabs 0.5 m away with shells, crabs 2.0 m away, crabs 2.0 m away with shells. Within blocks, individual clam plots and cages were 10.0 m apart, and blocks were at least 100 m apart. Each block was placed in the low intertidal zone along the predominant current direction in the Skidaway River. Using cages on either side of each clam plot insured that clams were always downstream of predators regardless of tidal flow direction. The order of treatments within each block was randomized between replicates.

clams recovered alive in each plot using a blocked two factor ANOVA and a Tukey-Kramer post hoc test (Sokal and Rohlf 1995). We used the ANOVA to test for significant effects of risk distance (absent, 0.5 m, and 2.0 m), turbulence (sand vs. shells), and an interactive effect between the two. The blocking factor was significant and included in the statistical model.

Flow Measurements

We measured changes in turbulence caused by the shell rings using two acoustic Doppler velocimeters (ADVs, SonTek™) and vendor supplied software. The ADVs were different models, one a 16 MHz unit and the other a 10 MHz unit, and preliminary measurements in the SkIO flume and in the field revealed that flow velocity and turbulence intensity were measured similarly between the two instruments. The root mean square (RMS) of a velocity time series is a useful means of measuring turbulence, and we compared RMS between our ADVs to insure they measured turbulence similarly. Both ADVs were allowed to collect data simultaneously in a steady flow of 5.5 cm s^{-1} in the SkIO flume continuously for 10 minutes using a 10 Hz sampling frequency. The RMS from the velocity time series was nearly identical between the two instruments, with the 10 MHz ADV having an RMS of 0.582 and the 16 MHz ADV having an RMS of 0.578. The near equality in RMS indicated that our instruments were measuring similarly, and allowed us to compare flow at two points in the field simultaneously using both instruments. Additional results from calibrations in the field further indicated that these instruments measured flow velocity and RMS similarly. Despite preliminary data indicating that the ADVs were making similar flow measurements, we biased these measurements in favor of not detecting a difference in turbulence over the shells by measuring flow over the shells with the 10 MHz unit, which measures flow over a slightly larger sampling volume (0.25 cm^3 vs. 0.1 cm^3), and thus may underestimate the degree of small scale turbulence.

Flow measurements were made in the field over the natural substrate and over our shell treatments during one tidal cycle to ascertain the changes in turbulence caused by

the shells. Recall that the shell rings surrounding each predator cage were 0.30 m wide, and we wanted to measure changes in turbulence on the downstream edge of the shells to see the full shell effect. This was accomplished by placing oyster shell hash in a 0.60 m circle under the 10 MHz ADV and making flow measurements in the center of the circle. Measuring in the center of the shells insured that regardless of flow direction, we could measure changes in flow after passing over 0.30 m of shells, which was the width of our shell rings surrounding the predator cages. We placed the second ADV 10 m from the shell plot (which was the same distance between clam plots in the field study) and measured flow over the normal sand/mud substrate for comparison. Both ADVs were mounted so that they were measuring flow 0.05 m above their respective substrates at the same tidal height. Flow velocity was sampled in 4-minute bursts at a frequency of 10 Hz every 15 minutes for 24 hours, and we discarded all data collected when the ADVs were out of water.

Turbulence intensity (TI) is the magnitude of velocity fluctuations normalized to mean flow velocity, and was calculated using the standard formula

$$TI = 100 * \sqrt{(RMS_u^2 + RMS_v^2 + RMS_w^2)} / \sqrt{u^2 + v^2 + w^2}$$

where u, v, and w are the velocity components in the x, y, and z dimensions respectively, and RMS_u, RMS_v, RMS_w are the root mean square (std. dev) of each velocity component taken during each 4 minute sampling period (Denny 1988). Ninety-four sampling bursts from each treatment (sand and shell) were made with the ADVs, and we TI from both treatments using t-tests (Sokal and Rohlf 1995).

Results

Turbulence intensity was significantly higher over the shells as compared to the natural sand substrate in our field site (N=94, t stat = 4.91, $P < 0.001$, Fig 3.2). The 2-fold increase in turbulence intensity over shells greatly exceeded the relatively small change in average flow velocity over each substrate throughout the tidal cycle; mean flow velocities were 7.2 cm s-1 and 6.9 cm s -1 over sand and shells respectively, (Fig. 3.3). The significantly higher TIs over shells resulted more from an increase in (turbulent) velocity fluctuations associated with substrate coarseness, as opposed to increased bulk flow over shells.

The 2 way ANOVA revealed that clam survival was significantly different in our treatments ($F_{19,70} = 4.38$, $P << 0.001$, Fig. 3.4). Risk and turbulence level had significant effects on clam mortality ($F_{19,70} = 15.98$, $P << 0.001$; $F_{19,70} = 4.65$, $P < 0.05$) for risk and turbulence, respectively. We also noted a significant interactive term between these factors ($F_{19,70} = 3.58$, $P < 0.05$). Post hoc analysis revealed that survival was greater in 3 of the 4 treatments containing caged blue crabs when compared to controls, indicating that clams in these treatments detected the caged blue crabs, reacted to them, and consequently were less apparent to ambient consumers. We were initially concerned that the presence of shells might interfere with interactions between our experimental clams and naturally occurring predators, and we controlled for this effect by using control plots (with empty predator cages) both with and without shell rings. Clam survival was nearly identical in control plots with and without shells (Fig. 3.4), indicating that the presence of shells did not directly affect clam detection of ambient consumers nor did they influence the foraging success of naturally occurring blue crabs. Therefore, changes in clam

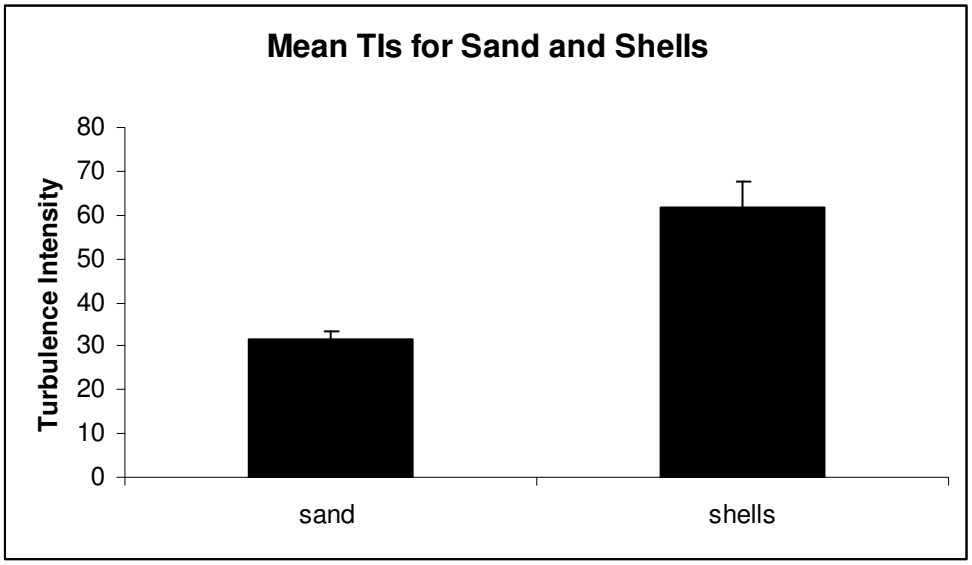


Fig. 3.2 Comparison of mean TI values (N = 94) calculated from ADV measurements made over the natural sand substrate and the shell substrate used in our field experiments. Turbulence intensity is significantly higher over shells, as expected ($P < 0.001$).

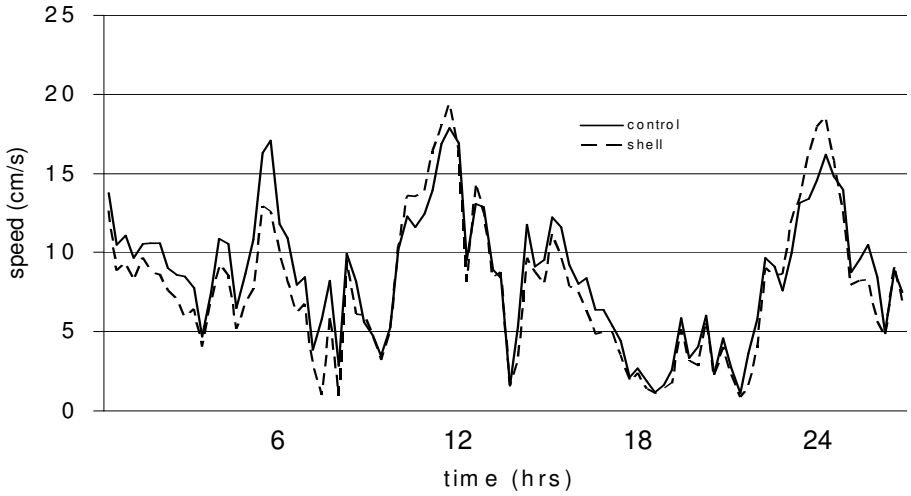


Fig. 3.3. Flow velocity over sand and shells measured with the ADV. Velocity in x,y,z directions was combined into a single value for each 4-minute sampling interval. Mean flow velocity during each sampling interval is similar over sand and shells, indicating that differences in turbulence between sand and shells results from substrate coarseness and not from differences in bulk flow.

survival among our caged crab treatments are caused by an increase in turbulence from the shells, which decreases clam reactive distance to blue crabs.

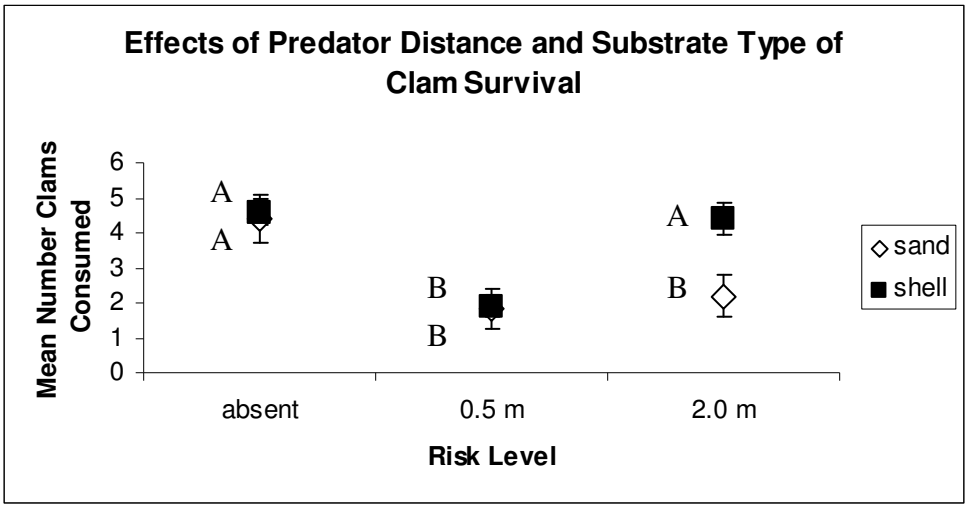


Fig. 3.4. Effects of predator distance and turbulence level (substrate type) on clam survival in the field. Dots represent mean number of clams consumed in each treatment (± SE). Letters beside each dot denote a significantly different mean based upon a Tukey-Kramer post hoc test. The risk levels included empty cage controls (absent) and caged crabs 0.5 m or 2.0 m away. Note the significant interactive effect of distance and substrate type as increased turbulence diminishes the distance clams can detect caged predators and increases their vulnerability to ambient consumers. Plots were put in the field in blocks of six (n =15 blocks with 90 replicate plots).

The treatment with crabs 2.0 m away surrounded by shells was the only predator treatment that did not increase clam survival, and clam survival in this condition was nearly identical to controls (Fig. 3.4). Clam survival was significantly higher than controls when caged crabs were 2.0 m away but not surrounded by shells (Fig. 3.4), indicating that clams can detect and react to blue crabs from this distance when natural turbulence levels were not enhanced. Differential clam survival in plots with and without shells surrounding caged crabs at different distances confirmed that clam reactive

distance to blue crabs was reduced by shell-enhanced turbulence. Clam survivorship in the presence of crab predators placed 0.5 m away was higher than in controls, regardless of the presence of shells. The lack of a shell effect when predators were 0.5 m away indicates that the level of turbulence used in our study did not affect clam reactions to crabs from this distance, although it did reduce clam reactions crabs 2.0 m away.

Discussion

Prey alter their behavior or morphology in the presence of predators to minimize predation risk (reviewed by Katz and Dill 1998), and predator-induced changes in prey characteristics (e.g., foraging) can have profound effects on competitive interactions and community structure (Turner and Mittlebach 1990, Schmitz et al. 1997, Schmitz 1998, Trussell et al. 2003). Prey must perceive and react to predators for TMIs to occur (Schmitz et al. 1997, Smee and Weissburg in press). Therefore, the magnitude of TMIs in a given system should be minimal if prey cannot sense their predators, but the effect of animal perceptual abilities or limits has not received much attention when examining the role of behavioral changes in determining community structure (e.g., Werner and Peacor 2003). A better understanding of conditions that favor TMIs in nature can be attained by determining how prey gather information about their environment and the effects physical forces have on prey perception.

Clams, like other aquatic organisms, utilize chemical signals to detect predators (Doering 1982, Irlandi and Peterson 1991, Smee and Weissburg 2006). Clams react to predators by decreasing their feeding time, but predator avoidance is costly for clams, reducing growth and fecundity (Nakaoka 2000). Although potentially costly, reducing

feeding time decreases the amount of attractive chemicals clams liberate into the environment, makes them less conspicuous to predators, and increases their survival. We found that clam survival increased in the presence of caged blue crabs and this finding is consistent with earlier results that demonstrate increased clam survival in the field when predators are caged nearby (Smee and Weissburg in press). Results from our experiment revealed that clams detect and respond to blue crabs that are 2.0 m away, but that their reactive distance to crabs decreases when turbulence is enhanced. Previously, in a laboratory study, Smee and Weissburg (in press) noted that clam responses to blue crabs decreased in turbulent flows, and the increased turbulence caused by the shells in our experiment likely mixed the chemical cue emanating from the crab and made it less detectable to clams 2.0 m away. Thus, clams in this treatment were unable to detect the caged crab, continued feeding, and were more vulnerable to ambient consumers as compared to clams in treatments were crabs were 2.0 m away but not surrounded by shells. The reactive distance of clams to crabs was reduced by turbulence, demonstrating that environmental conditions can affect the spatial scales of prey ability to detect and respond to risk.

Our results clearly show that the physical environment mediates clam responses to blue crab predators. We attribute the increased clam survival to predator-induced reductions in clam feeding that make clams more cryptic to consumers. An alternate hypothesis is that agonistic interactions between caged and foraging predators reduced clam predation, but we feel that this explanation is unlikely for two reasons. First, Ferner et al. (2005) found that the presence of a live crab did not deter crabs from entering baited crab traps, and while trapping crabs for this study we usually collected multiple crabs

from each crab trap. Secondly, Smee and Weissburg (in press) found that placing caged knobbed whelks (*Busycon carica*) near clam beds increased clam survival similarly to placing caged blue crabs near clams in the field, even though knobbed whelks are attractive to crab predators. The similarity in clam survival when crabs and whelks are caged near clams in the field suggests that predator interference cannot account for the reduction in clam mortality when predators are caged near clams.

The spatial scales over which prey can respond to predators will presumably alter the importance of TMI and TMIIs in a given system, and any attempts to model nonlethal predator effects must consider the distance an individual predator can induce trait shifts in prey (Turner and Montgomery 2003). Turner and Montgomery (2003) describe changes in prey characteristics as they respond to mobile predators as a "behavioral landscape." The appearance of the behavioral landscape is a function of predator movement, the latency of predator cues in the habitat, the distance over which a predator can affect prey traits, and predator density (Turner and Montgomery 2003). Yet, the scales by which predators affect prey as well as the latency of predator signals should be controlled by physical forces that influence prey perception (i.e. hydrodynamics), in addition to endogenous predator properties. Consider the behavioral landscape created as blue crabs traverse a clam bed. The appearance of the behavioral landscape will differ drastically depending on the turbulence level over the clams, such that crab-induced changes in clam behavior will be more prevalent in flows with low turbulence. Therefore, predator density and movement may contribute less to the degree of TMIs when environmental conditions are favorable for prey perception such that a few stationary predators are detected throughout the system. Predator density and/or movement likely

has a greater affect on the magnitude of TMIs in environments that diminish prey perception such that prey only perceive threats that are nearby.

Chemical signaling is widespread in aquatic systems (Katz and Dill 1998, Zimmer and Butman 2000), and TMIs in other aquatic systems may be similarly affected by hydrodynamics. Thus, the spatial and temporal patterns of TMIs are likely quite different in freshwater lentic and lotic systems. In ponds or lakes were flow conditions are fairly static, TMIs may extend from a predator in all directions and linger for extended periods. In contrast, TMIs in flowing water should occur largely downstream from a predator and be carried away from prey so that their latency is short. Variation in flow regimes in lotic systems may diminish prey perception in some areas while enhancing it in others and create a spatial pattern of TMIs. In marine systems where flow differs both spatially and temporally with the tides, the sphere of influence of predators on prey and the prevalence of TMIs likely vary both with location and with time. That is, nonlethal predator effects are likely greatest in areas with flows that enhance prey perception, but nonlethal effects may also abound during slack water times in all areas and diminish with changes in tidal flow.

Although we have focused our research and much of this discussion on chemical signaling in aquatic systems, terrestrial systems may be similarly affected by airflows that deliver airborne chemical cues. For example, some terrestrial plants manufacture chemical defenses after detecting that neighbors are under attack from herbivores (Karban and Baldwin 1997), and differential levels of chemical defenses can affect herbivore susceptibility as well as plant competitive ability and fecundity (Baldwin 1998). Although many studies have shown that plants utilize cues from neighbors that are

attacked, a considerable amount of research has failed to detect consistent differences in plant defensive chemistry when surrounded by conspecifics that are being grazed (Karban and Baldwin 1997). Perhaps some of the variability in these experiments may be explained by differential delivery of chemical signals by wind currents. Additionally, parasitic insects and other arthropod predators follow chemical cues to locate prey or find plants on which to forage (Murlis et al, 1992, Vickers 2000). Changes in vegetation density or wind velocity or direction may change the perception of chemical cues by both plants and animals and alter the magnitude of top-down forces in terrestrial systems.

The change in reactive distance of clams to crabs under different environmental conditions highlights the necessity for understanding how physical conditions affect prey perception of risk in developing models that explain the role of TMIs and TMIIs in communities. Many studies have shown that hydrodynamics can affect chemical signal delivery and chemoreceptive ability of organisms (Weissburg and Zimmer-Faust 1993, Atema 1995, Finelli et al. 2000, Weissburg et al. 2002, 2003, Smee and Weissburg in press). Other environmental conditions such as varying light levels or vegetation density may also affect prey ability to detect predators by interfering with visual or acoustic signals (Dusenbery 1992) and in turn, control the prevalence of TMIs. In short, predicting both the occurrence and magnitude of TMIs requires a careful understanding of mechanisms prey use to evaluate risk and environmental conditions that affect that ability.

Chapter 4

Turbulence Alters the Outcomes of Predatory Interactions in the Field

Abstract

Predator-prey interactions in marine systems are often mediated by reciprocal detection of waterborne chemical cues. When these chemical signals travel through water, hydrodynamic forces (e.g., turbulence) can alter chemical signal structure and the chemoreceptive abilities of both consumers and prey. Using hard clams and blue crabs as model organisms, we examined how changes in turbulence influence the outcome of clam-crab predatory interactions. We established pairs of clam plots in four field sites, increased turbulence around one member of each pair by adding a ring of shells around the clams, and compared clam survival between control and treatment plots. Changes in turbulence affected clam survival, but these effects depended on the mean flow velocities and ambient turbulence levels in each study site. Increasing turbulence decreased clam survival in our two sites with low turbulence levels, whereas clam survival increased in plots with shells in our two highest turbulent sites. Previous studies have shown that turbulence decreases the chemoreceptive ability of clams and crabs, and we attribute the different effects of turbulence on crab-clam interactions in this study to differential changes in sensory ability. Although both clam and crab sensory abilities decline as turbulence increases, our results suggest that they decline at different rates, creating sensory advantages that alternate between clams and crabs depending on the level of turbulence in the environment. Changes in sensory advantages caused by variation in

environmental conditions may alter the outcomes of predatory interactions in other systems, particularly when both predator and prey detect each other using the same sensory modality. By understanding how environmental conditions modulate predator-prey interactions, ecologists may be able to predict the importance of top-down forces in a given community and the relative extent that lethal and nonlethal predator effects act to structure that community.

Introduction

Predators commonly have large impacts on the structure and function of communities (Paine 1966, Carpenter et al. 1985, Schmitz et al. 1997, Pace et al. 1999, Menge 2000) through consumption of lower trophic levels (lethal effect, Paine 1966, Sih et al. 1985, Menge 2000) and by altering the characteristics of prey such as behavior or habitat selection (nonlethal effect, Turner and Mittlebach 1990, Schmitz 1998, Trussell et al. 2003). Although both lethal and nonlethal effects of predators can affect prey populations and communities (Paine 1966, Schmitz et al. 1997, Pace et al. 1999, Trussell et al. 2003), predicting and modeling their separate influence in many systems has proven to be an elusive goal (Werner and Peacor 2003, but see Turner and Montgomery 2003, Grabowski 2004). The purpose of this study was to explore how environmental context, via its effects on sensory abilities, influences the outcomes of predatory interactions and ultimately controls the magnitude of lethal predator effects.

Sensory capabilities play a pivotal role in the outcome of predatory encounters as organisms must acquire and interpret information from their environment to both forage and avoid predators (Dusenbery 1992). The ability to perceive a potential consumer or

prey organism before being detected provides a key advantage, and thus slight perceptive advantages likely determine whether predators or prey prevail in a given encounter (Powers and Kittinger 2002). Sensory advantages may also control the scale of which prey are able to respond to predators (Smee and Weissburg in press), and hence, influence the magnitude of lethal and nonlethal predator effects in communities. Lethal predator effects should be prevalent when predators are successful foragers, and this should occur when predators possess a sensory advantage over prey. Likewise, , prey can successfully avoid predators when prey have a sensory advantage, but responding to predators frequently increases the potential for nonlethal predator effects (Turner and Montgomery 2003, Smee and Weissburg in press). The magnitude of lethal and nonlethal predator effects in a given system may depend on the frequency that predators consume prey vs. how often prey detect, react to, and avoid consumers (Smee and Weissburg in press). By understanding how the environment affects sensory advantages, ecologists may be better able to predict the outcomes of predatory interactions and the relative contribution of both types of predator effects to community structure.

Regardless of the sensory modality employed by organisms for information gathering, environmental factors can affect the perceptual ability of animals and modify their performance in these ecologically critical activities (Dusenbery 1992, Weissburg et al. 2002, Smee and Weissburg in press). For example, the distance a sound can be transmitted is affected by temperature, wind, and the presence of sound-reflecting objects (Wiley and Richards 1978, Dusenbery 1992), which alters the perceptive space of acoustic detection. Consequently, the echolocation frequency of bats is related to the environment in which they forage (Surlykke 1988). Bats that forage near foliage or detect

prey at close distances produce higher frequency sounds than other bat species that forage in open areas (Surlykke 1988, Dusenbery 1992). Similarly, the visual ability of organisms can be compromised or enhanced by differences in transmission of light between targets of different size or color (Spaethe et al. 2001), and physical forces that block or bend light impact the visual acuity of organisms (Dusenbery 1992). As with light and sound, chemoreception is influenced by physical forces such as fluid velocity and turbulence levels that change the delivery of chemical cues (Weissburg 2000, Vickers 2000, Webster and Weissburg 2001).

Predator-prey interactions in marine systems are often chemically mediated, and these chemical signals are transported via moving fluids over a scale of centimeters to meters (Weissburg 2000, Zimmer and Butman 2000). Several studies have shown that hydrodynamics (e.g., flow velocity, turbulence) influence the delivery of waterborne chemical cues as well as the perception of these signals by organisms (Weissburg and Zimmer-Faust 1993, Finelli et al. 2000, Webster and Weissburg 2001, Weissburg et al. 2002, 2003, Rahman and Webster 2005). For example, blue crabs (*Callinectes sapidus*) find bivalve prey by following waterborne chemical cues released by feeding bivalves. The ability of blue crabs to locate prey by chemoreception decreases as both flow velocity (Powers and Kittinger 2002, Weissburg et al. 2003) and substrate roughness (Weissburg and Zimmer-Faust 1993) increase because these factors increase turbulent mixing and dilute chemical signals. Previous authors have hypothesized that habitats with turbulent or high velocity flows could offer prey a refuge from crab predators (e.g., Weissburg and Zimmer-Faust 1993). This supposition is supported by studies from Leonard et al. (1998) and Bertness et al. (2004), both of whom found that crab predation

decreased as flow velocity increased in the Damariscotta River, a rocky intertidal estuary in Maine.

In contrast to blue crabs, knobbed whelks (*Busycon carica*) can successfully follow chemical odor plumes in more turbulent flow conditions than can blue crabs, and levels of turbulence that diminish blue crab prey-finding ability actually seem to enhance knobbed whelk foraging success (Powers and Kittinger 2002, Ferner and Weissburg 2005). Knobbed whelks and blue crabs are both common bivalve predators in soft-sediment communities of the SE United States. Since turbulence has opposite effects on crab and whelk foraging, some authors (e.g., Powers and Kittinger 2002, Weissburg et al. 2002, Ferner and Weissburg 2005) have speculated that flow might serve as a niche dimension between crabs and whelks.

Although many studies have shown that flow conditions influence predator foraging ability, until recently, none has addressed similar effects of flow on the ability of prey to detect and avoid consumers. Smee and Weissburg (in press) found that hard clams (*Mercenaria mercenaria*), a common prey item for blue crabs and knobbed whelks, reacted to these predators by reducing their feeding behavior. Reductions in feeding clams minimized the amount of attractive chemical signals released into the environment and made them less vulnerable to consumers (Smee and Weissburg in press). Clams were less responsive to blue crabs in higher velocity flows, which may increase their vulnerability to these predators (Smee and Weissburg in press). Smee and Weissburg (in press) questioned whether turbulence actually provides a refuge for prey, especially in situations where prey ability to detect and avoid consumers is diminished.

Behavioral studies of predator-prey interactions, like those previously mentioned (e.g., Weissburg and Zimmer-Faust 1993, Powers and Kittinger 2002, Smee and Weissburg in press), are often incomplete however because they only examine the behavior of prey in response to a static predator (Lima 2002) or treat prey as unresponsive organisms that predators find and devour (Weissburg et al. 2002). That is, they examine individually how flow affects a predator or its prey, but do not empirically test how changes in perception affect the outcome of predatory interactions.

Changes in sensory performance caused by environmental forces like turbulence may switch perceptive advantages between predators and prey and alter the outcomes of these interactions (Powers and Kittinger 2002). Since turbulence affects the perceptive abilities of both hard clams and their predators, we elected to use this model system to investigate how environmental forces affect predator foraging success and the ability of prey to detect and avoid consumers. Specifically, we manipulated turbulence levels in the field to ascertain how the physical environment modulates the outcome of clam-crab encounters. Our results show that changes in environmental conditions, in this case turbulence, may alter sensory performance and the outcomes of predator-prey interactions. Ultimately, the magnitude of lethal and nonlethal predator effects in a given system may depend on the frequency that predators consume prey vs. how often prey detect, react to, and avoid consumers (Smee and Weissburg in press).

Methods and Materials

Site Description

Experiments were performed in Moon, Herb, Skidaway, and Wilmington Rivers, all of which are estuarine rivers associated with Wassaw Sound and are adjacent to the Skidaway Institute of Oceanography (SkIO) near Savannah, GA (Fig. 4.1). These rivers are tidally driven and experience long periods of unidirectional flow. Wave action in these estuaries is limited to periods of severe weather or is caused by boat traffic. All sites are bordered by marsh (*Spartina alterniflora*), receive little freshwater input beyond runoff, have an average salinity of 20-28 ppt, a tidal range of 2-3 m, and the substrate in each consists primarily of fine grain sand and mud. These sites were selected because they are typical of soft-sediment habitats common throughout much of the SE U.S and are natural habitats of clams and their predators. Mean flow velocities and turbulence levels are different in each site and span a range of hydrodynamic conditions (Table 4.1).

Predator Density

The most common clam predators in coastal Geogia are blue crabs (*Callinectes sapidus*), stone crabs (*Menippe mercenaria*), and knobbed whelks (*Busycon carica*).We used commercially purchased crab traps (pots) to estimate the number of predators in each field site. Traps were baited with dead fish (*Menhaden* sp.) and ten traps were deployed in each field site during May and September of 2004. Traps remained in the field for 24 hours, and the number of clam predators captured was recorded. Crab traps have been successfully used to collect blue crabs, stone crabs, and knobbed whelks in Wassaw Sound and its tributaries (A. Power *personal communication*) and are a useful means to compare predator numbers between sites. We compared the number of clam

predators collected per trap in each site using a single factor ANOVA (Sokal and Rohlf 1995).

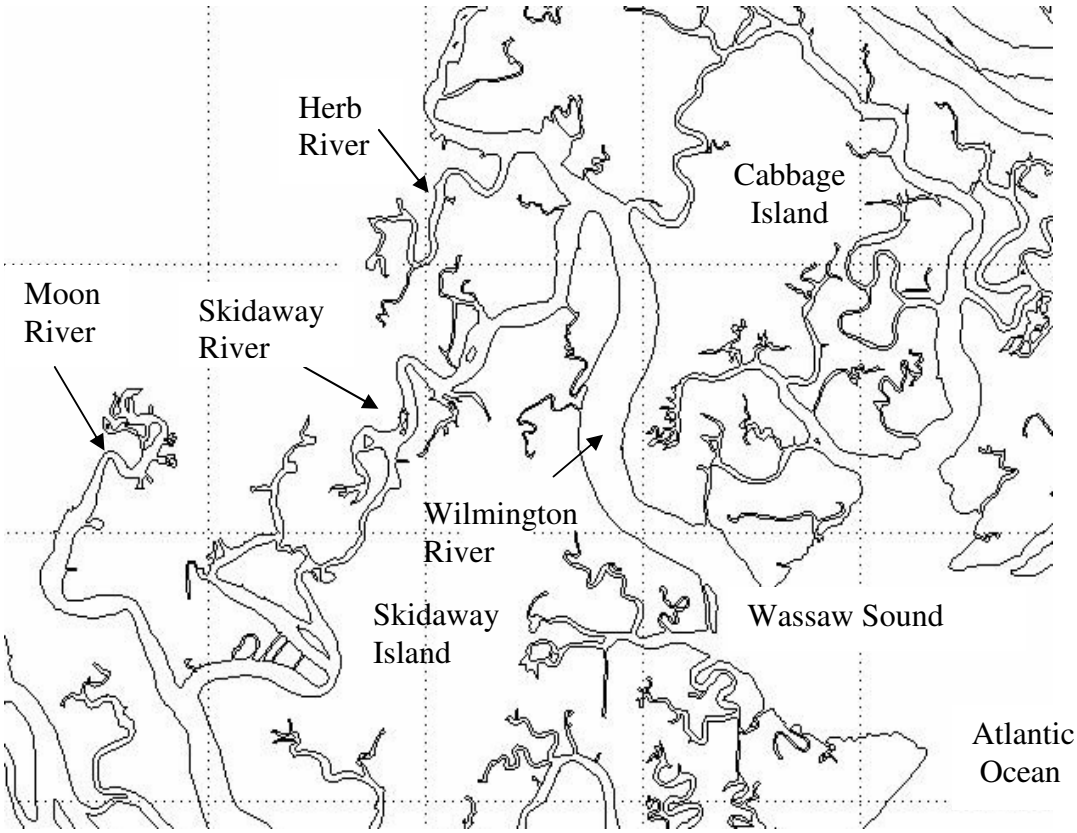


Fig. 4.1 Map of study area.

Table 4.1. Flow conditions measured with the ADVs in each study site.

Site	N =	Mean Velocity (cm s^{-1})	Mean RMS	Mean TI
Wilmington River	82	7.6	2.6	33
Skidaway River	87	8.6	2.45	33
Herb River	74	9.6	2.8	36
Moon River	81	11.0	2.98	31

Experimental Rationale

Since turbulence affects clams, crabs, and whelks differently, we wanted to determine how the outcomes of these predatory interactions would be affected by changing the turbulence level. These experiments consisted of establishing clam plots in the field, manipulating local turbulence levels, and determining the effect increased turbulence would have on clam survivorship.

General Protocol

Animal capture and maintenance- Adult clams were hand dug from intertidal habitats in Wassaw Sound, GA, and juvenile clams were purchased from a local supplier to assure a consistent supply of juvenile clams for these experiments. Clams were maintained in flow-through sea tables at SkIO prior to use in the field experiment.

Mark and Recapture Experiment- We tethered clams to a PVC-rope frame to facilitate deployment and recovery of clams in the field. Clams were individually tethered with monofilament line (15 cm long) glued to the shell and tied to ropes strung between 2 lengths of PVC pipe (50 cm long x 1.25 cm diameter). Three ropes were spaced equidistant along the PVC pipe, with 5 clams tethered per rope. Clam plots contained 15 clams, 10 juveniles (shell size < 30 mm) and 5 adults (shell size > 35 mm), in a 0.25 m^2 area (0.5 m x 0.5 m), and this density mimics naturally occurring populations in the study area (Walker 1987, Smee unpublished data). The PVC rope frame was placed in the field so that it was flush with the sediment, and each clam was gently pressed into the sediment to facilitate burrowing. This clam tethering technique has previously been used successfully (Smee and Weissburg in press) and allowed us to sample clam mortality by recovering both live clams and shells of clams that were eaten.

Clam plots were placed in the field in pairs, each clam plot was approximately 5 m from its counterpart, and each pair was at least 100 m from any other pair. Clam plots were placed in the intertidal zone (ca. 0.0 to + 1.0 m relative to mean low water) during low tide, and each pair was placed at the same tidal height. We increased turbulence by surrounding one member of each pair with a ring of sun-bleached oyster shells approximately 0.30 m wide. The shells did not cover the clams, but rather changed the turbulence level over the clam plot so that clams would have to detect approaching predators in more turbulent flow conditions than those in control plots. Similarly, predators would have to find clams in conditions that are more turbulent when they were surrounded by shells as compared to controls. The advantage to this experimental design was that it allowed us to expose each pair of clam plots to similar field conditions and generate higher turbulence levels in specific areas to identify the effects of turbulence on clam survivorship.

Clam plots were recovered from the field after 48 hours, and the number of clams that were alive, missing, or eaten was recorded. Crushed clam shells are indicative of crab predation (Micheli 1997, Nakaoka 2000), and Smee and Weissburg (in press) determined that missing clams were taken and consumed by crabs. In this study, we counted crushed clam shells as well as missing clams as being eaten by crabs, as have other authors (Micheli 1997, Nakaoka 2000).

These experiments were conducted from May through August in the summers of 2003 and 2004. In 2003, we placed 20 pairs of clam plots in the Wilmington River and another 20 pairs in Herb River, and then in the summer of 2004 we placed 25 pairs of

clam plots in each of our four sites (Wilmington, Herb, Moon, and Skidaway Rivers). We were unable to recover some plots after 48 hour and these were excluded from analysis.

We compared predation intensity between sites by comparing the number of clams consumed in control plots using a single factor ANOVA and a Tukey-Kramer post hoc test (Sokal and Rohlf 1995). We then used a single factor ANOVA to compare shell effects between our four study sites. Predation intensity was extremely variable between pairs of clam plots, and so we standardized clam consumption in each shell plot by the total predation intensity in each control-shell plot pair. The resulting measure of predation (% clams consumed in each shell plot) preserved the pairwise nature of the experiment and minimized variability in predation intensity. In this test, the percentage of clams consumed within shell plots was arcsine transformed to meet ANOVA assumptions (Sokal and Rohlf 1995). Tukey-Kramer post hoc tests were used to compare differences in the percentages of clams eaten in plots surrounded by shells across our study sites.

Flow measurements

We measured flow in each field site as well as changes in turbulence caused by the shell rings using two acoustic Doppler velocimeters (ADVs, SonTek™) and vendor supplied software. These ADVs measure flow velocity and turbulence similarly (Smee, Ferner, and Weissburg unpublished data), and we used them to make simultaneous measurements over the shell rings and natural substrate in each of our field sites during one tidal cycle. Recall that the shell rings surrounding the treatment clam plots were 0.30 m wide, and we wanted to measure changes in turbulence on the downstream edge of the shells to see the full shell effect. This was accomplished by placing oyster shell hash in a

0.60 m circle under one ADV and making flow measurements in the center of the circle. Measuring in the center of the shells insured that regardless of flow direction, we could measure changes in flow after passing over 0.30 m of shells, which was the width of our shell rings. We placed the second ADV ~ 5.0 m from the shell plot (which was the same distance between clam plots in the study) and measured flow over the normal sand/mud substrate for comparison. Both ADVs were mounted so that they were measuring flow 0.05 m above their respective substrates and were placed just above the mean low water line in each study area. Flow velocity was sampled in 4-minute bursts at a frequency of 10 Hz every 15 minutes for 24 hours, and we discarded all data collected when the ADVs were out of water. We made these comparative flow measurements in all four of our study sites on four consecutive days midway between spring and neap tides when predicted tidal heights were similar.

The root mean square (RMS) of a velocity time series is a useful means of quantifying turbulence, and we calculated RMS as well as mean flow velocities with the ADVs. By sampling at 10 Hz, the ADVs were able to make 2400 measurements during each 4 minute sampling interval. Since the ADVs measure flow in 3 dimensions, we integrated flow in the x, y, and z directions into one value for each measurement and then calculated the mean flow velocity and standard deviation (RMS) of the integrated values for each sampling burst. The mean velocity and RMS for each site were calculated by averaging the mean velocities and RMS levels from each sampling period.

Although RMS is a useful parameter for quantifying turbulence, it is generally higher in faster flows. Turbulence intensity (TI) is the magnitude of velocity fluctuations normalized to mean flow velocity, and was calculated using the standard formula

$$TI = 100 * \sqrt{(RMS_u^2 + RMS_v^2 + RMS_w^2)} / \sqrt{u^2 + v^2 + w^2}$$

where u, v, and w are the velocity components in the x, y, and z dimensions respectively, and RMS_u, RMS_v, RMS_w are the root mean square (std. dev) of each velocity component taken during each 4 minute sampling period (Denny 1988). TI was calculated for each sampling period, and the TI calculations over sand and shells were compared using a single factor ANOVA and a Tukey-Kramer post hoc test (Sokal and Rohlf 1995). TI values more than three standard deviations from the mean were excluded from analyses. Nearly all such high TI values were from measurements made over shells and including them in analysis would have shown even greater differences in the TI level over sand and shells.

Results

Predator Density- Blue crabs comprised over 97% of the animals collected in each field site, and ~100% of predation in the field experiment was attributed to blue crabs. The number of crabs collected per trap in each site was not significantly different ($F_{3, 76} = 1.41$, $P = .25$, Fig. 4.2), suggesting that predator densities are similar throughout the study area.

Flow measurements- We characterized the flow conditions in each field site using data collected with the ADV over the natural substrate and present this data in Table 4.1. Adding shells significantly increased mean TI values in all field sites as compared to the natural substrate ($F_{7, 627} = 5.07$, $P < 0.01$, Fig. 4.3). Mean TI values increased ~ 25% with the addition of shells, changing from ~33 over the natural substrate to ~ 44 over the shells (Fig. 4.3). Flow velocities within sites were similar over sand and shell plots throughout

the tidal cycle, indicating that differences in TI were caused by the addition of shells and not by differences in bulk flow (Fig. 4.4). Significant increases in TI were found over the shells, but the TI levels in the four control plots were similar in each study site as were the TI levels over the four shells plots (Fig. 4.3).

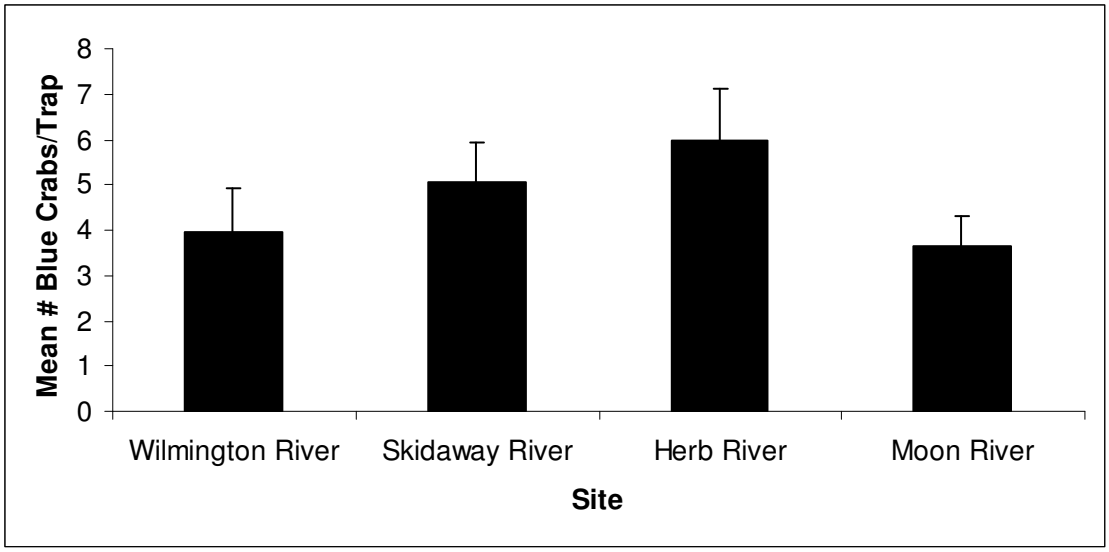


Fig. 4.2. Mean number (± SE) of blue crabs caught per trap (n=20 trap days/site) in each field site. Significant differences in crab catch were not found.

Mark Recovery Experiment Results- Predation intensity was significantly different between field sites ($F_{3, 105} = 5.26$, $P < 0.01$, Fig. 4.5), and was 50% greater in sites with intermediate levels of turbulence. Increasing turbulence levels by adding shells affected clam survival differently in our field sites. The variability in the shell effect was apparent in the ANOVA comparing percentages of clams eaten in the shell plots in each study area, which revealed significant differences in clam survival in the shell plots between sites ($F_{3, 105} = 3.64$, $P < 0.05$, Fig. 4.6). Post hoc analysis showed that significantly more clams survived in shell plots in Moon and Herb Rivers than in the Wilmington River. The

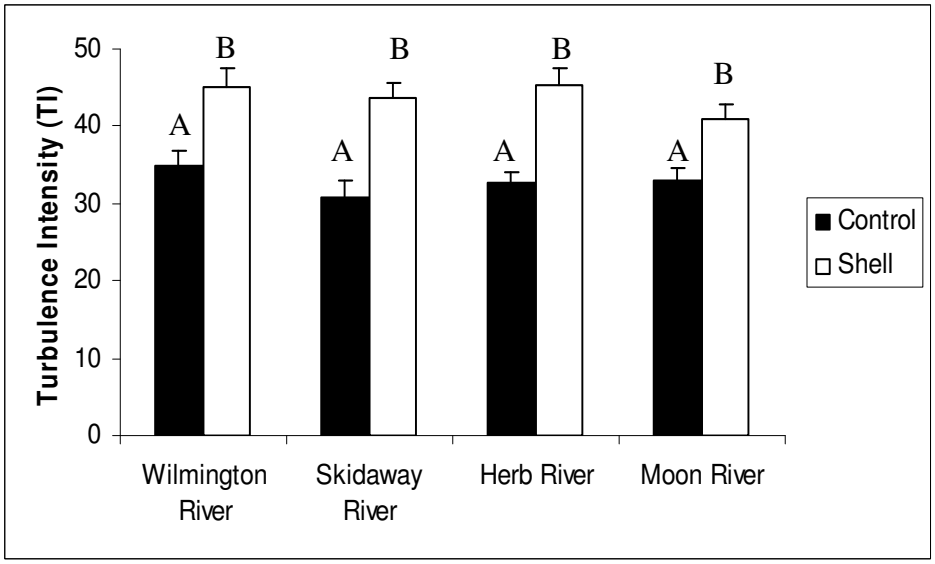


Fig. 4.3. Mean TIs (±SE) measured over the natural substrate and shell additions in each study site. Letters denote significantly different means based upon a Tukey-Kramer post hoc test. The addition of shells causes ~ 25% increase in TI, which is a significant increase as compared to the natural substrate in each site.

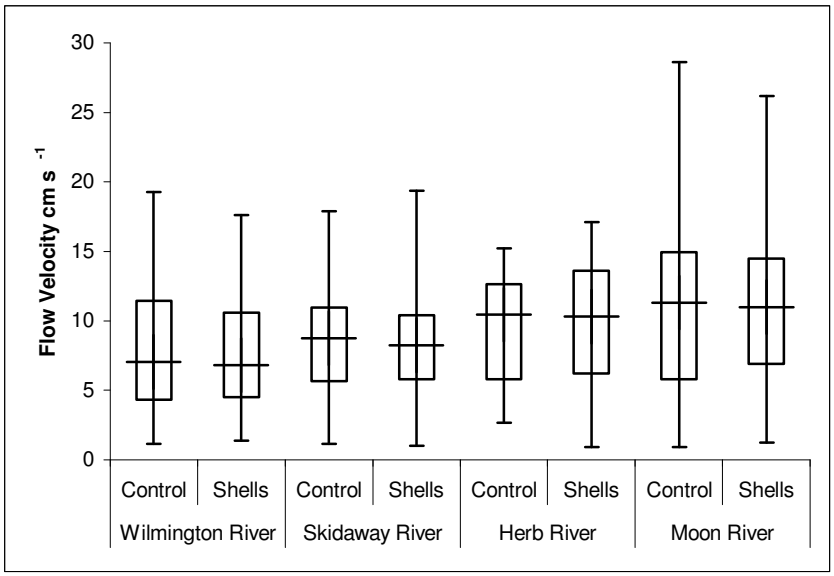


Fig. 4.4. Box plot of flow velocity measured over sand and shell plots in each field site. Error bars represent the range, centerlines represent median, boxes represent 1st and 3rd quartiles. Velocity in x,y,z directions was combined into a single value for each 4-minute sampling interval and termed speed. Flow velocity was similar over sand and shells within sites, indicating that differences in turbulence between sand and shells results from substrate coarseness and not from differences in bulk flow.

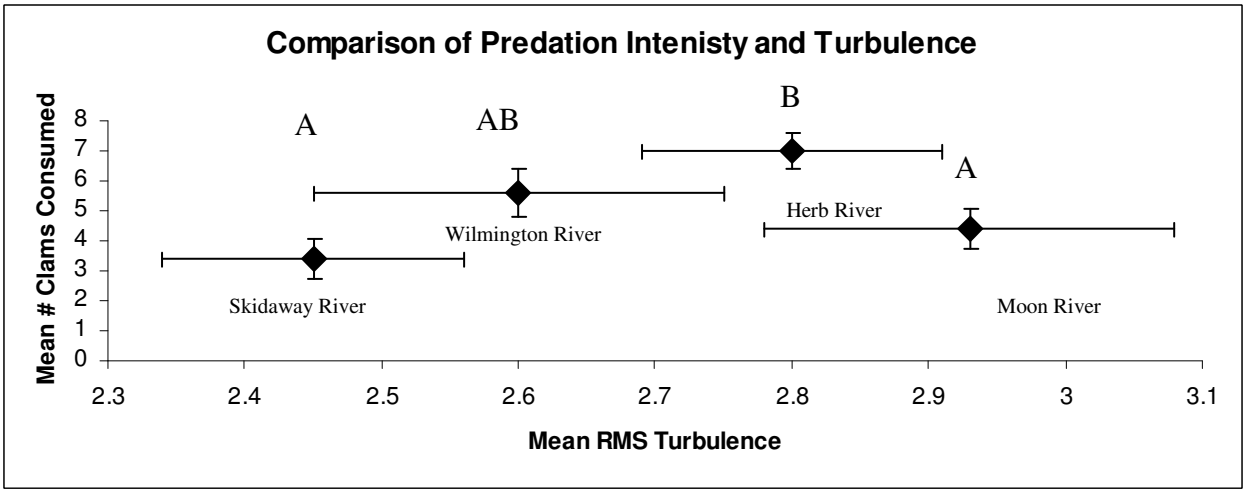


Figure 4.5. Comparison of predation levels and turbulence (RMS) in each field site. Dots represent mean # of clams consumed (+SE), and letters denote significantly different predation intensities based upon a Tukey-Kramer post hoc test. Sample sizes are 22, 30, 39, and 15 for the Skidaway, Wilmington, Herb, and Moon River sites respectively. We also show SE bars on the x axis to show RMS variability. Note that predation is highest at intermediate levels of turbulence.

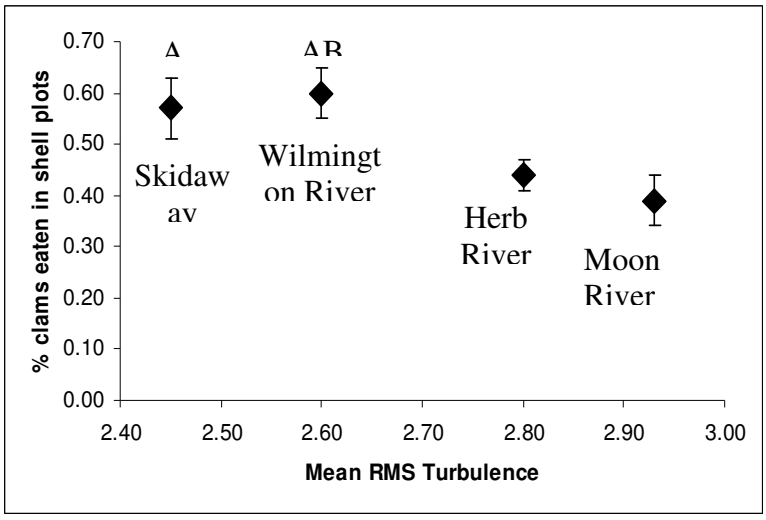


Figure 4.6. Relationship of mean RMS and mean % clams consumed in the shell plots in each study site. In slow flows, shells increase clam mortality and in faster flows, they decrease clam mortality. Letters denote significantly different means (±SE) of % of clams eaten in shell plots in our four study sties based upon a Tukey-Kramer post hoc test. Sample sizes are 22, 30, 39, and 15 for the Skidaway, Wilmington, Herb, and Moon River sites respectively.

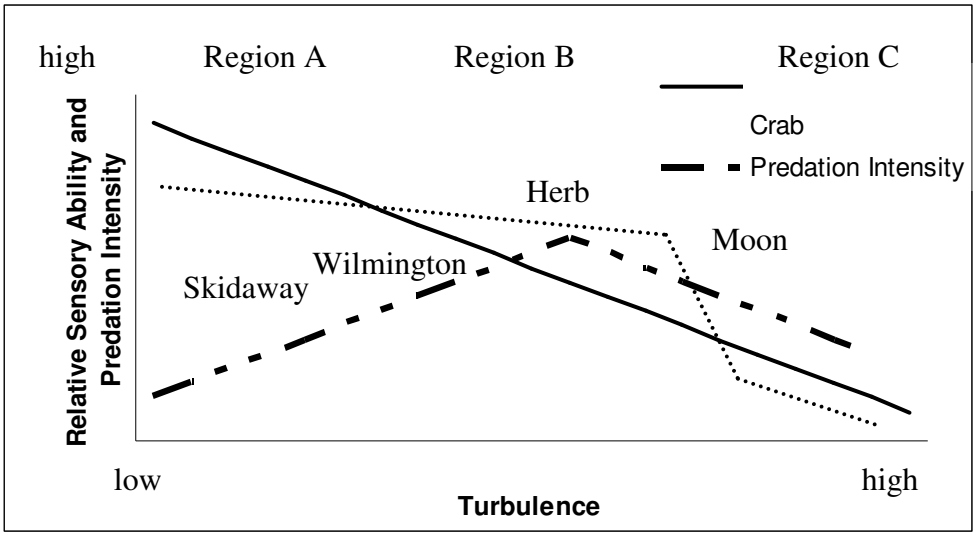


Fig. 4.7. Changes in clam and crab sensory ability caused by turbulence. A similar model was presented in Smee and Weissburg (in press). In Region A, both predators and prey have high perceptual ranges, but clams have a relative sensory advantage over crabs. The relative sensory advantage shifts to crabs in Region B because of an unequal rate of decline of perception between predators and prey as turbulence increases. The further decline in perception (Region C) again shifts sensory advantages, but in this region, the rate of decline for crabs is much greater than for clams, and again clams gain a sensory advantage. We have modified this figure by adding a line showing how predation intensity may vary as sensory advantages change and by placing our study sites into the model based upon their turbulence levels and predation intensities. This figure represents idealized (linear relationships) between turbulence, predation intensity, and relative sensory advantages based upon our field study. By adding shells, we increased the turbulence levels in each site, and shifted each into a region where sensory advantages between clams and crabs change. That is, adding shells to clam plots in the Wilmington and Skidaway Rivers increased their turbulence level into region B, provided crabs with a sensory advantage, and caused higher levels of predation than in controls. In Herb and Moon Rivers, adding shells pushed their turbulence levels into region C where clams have a sensory advantage, and caused a decline in clam mortality.

addition of shells in site decreased clam predation in our two most turbulent sites, Moon and Herb River (Fig. 4.6). Unlike the results for Moon and Herb Rivers, we found that shells increased clam predation in the Wilmington River and Skidaway Rivers (Fig. 4.5). Thus, the addition of shells tended to increase the proportion of clams consumed from

shell plots in the two sites with lowest turbulence levels, but, adding shells decreased relative predation intensity on shell plots in the more turbulent sites.

Discussion

Chemical signaling is ubiquitous in marine environments (Zimmer and Butman 2000), and the transmission of chemicals is strongly affected by hydrodynamics (Webster and Weissburg 2001, Rahman and Webster 2005). Previous studies have shown that blue crab foraging success declines as flow velocity (Powers and Kittinger 2002, Weissburg et al. 2003) and turbulence increase (Weissburg and Zimmer-Faust 1993), as does the ability of clams to detect and respond to approaching blue crab predators (Smee and Weissburg in press). Since turbulence diminishes the perceptive abilities of both clams and crabs, Smee and Weissburg (in press) predicted that the organism (clam or crab) whose sensory abilities were least affected by turbulence would prevail in these predatory interactions. By manipulating turbulence levels in the field, we were able to demonstrate that hydrodynamics simultaneously affect clams and their crab predators and may alter the outcomes of these interactions.

Our results indicate that there is not a simple monotonic relationship between turbulence magnitude and predation intensity. Clam survivorship in control plots was lowest at intermediate turbulence levels (Fig. 4.5) indicating that relatively low and high turbulence magnitudes are detrimental to predator foraging and enhance clam survival. In Herb and Moon Rivers, our sites with the highest flow velocities and turbulence levels (Table 4.1), adding shells increased clam survival, suggesting that in these areas, added turbulence served as a predation refuge. The sites with the lowest flow velocities and

turbulence levels (Wilmington and Skidaway Rivers) differed substantially, as clam mortality increased with the addition of shells. The highest predation levels were measured in Herb and Wilmington Rivers (Fig. 4.5), but shell additions produced opposite effects on clam predation. This may be explained by the level of turbulence achieved in each site by the addition of shells.

The differential effect of shells on clam survivorship is presented in Fig. 6 and illustrates a relationship between the effect of shell additions on clam survival and ambient RMS levels. Smee and Weissburg (in press) proposed a hypothetical model where the sensory abilities of clams and their predators declined at different rates. The unequal rates of decline created sensory advantages that alternated between these predators and prey. Based on the results from this study, we offer a companion model comparing relative sensory advantages, predation intensity, and turbulence levels (Fig. 4.7). In the model, we assume that changes in predation intensity result from changes in relative sensory advantages that are altered by increasing levels of turbulence.

The sensory abilities of both crabs and clams are greatest in flows with low turbulence levels (Weissburg and Zimmer-Faust 1993, Smee and Weissburg in press). In Fig. 7., although the perceptive abilities of both clams and crabs are maximal in region A where turbulence levels are low, clams have a perceptive advantage over their crab predators and should prevail under these conditions. We base this prediction on the measured predation intensity in the Skidaway River, our lowest turbulent site and subsequently, the site with the lowest predation intensity (Fig. 4.5). Dawkins and Krebs (1979) predicted that prey should be under greater selection pressure to avoid consumers than are consumers to find prey as the cost for prey is much higher. Therefore, it is

perhaps unsurprising that clams have a greater perceptive ability than that of their predators.

In region B of Fig. 4.7, blue crabs become more effective foragers, due to a faster rate of decline in sensory performance by their prey. The advantage gained in region B is returned to clams in region C due to a sharp decline in blue crab sensory ability. The rapid decline may be explained by the combined affect of flow velocity and turbulence. Weissburg et al. (2003) found that fast flows were disadvantageous for blue crab foraging not only because of greater turbulent mixing, but also because blue crabs adopted a drag-minimizing posture in fast flows that hindered their chemoreceptive ability. In fast flows, blue crabs turn so that their bodies are parallel to the current to reduce drag (i.e. walk sideways). Blue crab antennules mediate upstream movement after detecting concentrated odor filaments and chemosensors on the legs aid blue crabs in spatial sampling (Keller et al. 2003), and thus the ideal orientation for a blue crab to detect and follow a waterborne chemical plume is by facing the source of the plume. By turning sideways to lessen drag, blue crabs compromise their ability to follow chemical odor plumes because their chemoreceptors are not in an ideal alignment for chemical navigation (Weissburg et al. 2003). In short, flows that are fast and turbulent are doubly problematic for blue crabs, and in our study, appear to shift the sensory advantage back to clams.

The differential effects of increased turbulence in the shell plots may be related to the initial turbulence level and subsequent predation intensity in each field site. Turbulence levels in the Skidaway River were low, and this site had the lowest measured predation intensity. Adding shells in this site increased predation by shifting the

turbulence level toward region B where crabs forage most effectively. The Wilmington River was more turbulent than the Skidaway River and had a higher predation intensity. Increasing turbulence in this sight also shifted the turbulence level into region B and increased clam mortality. Increasing turbulence in the more turbulent Herb and Moon River sites decreases predation intensity by shifting the turbulence level to a level where crab predators are no longer effective. Seemingly, there should be a level of turbulence that sufficiently decreases perception of both predator and prey. We did not observe this situation in our study, perhaps because our turbulence levels were too low. Alternatively, clams may retain some ability to detect crabs even in the most turbulent flows.

These results suggest that turbulence can offer a predation refuge, but only at levels that diminish predator foraging ability more than the ability of prey to detect and avoid consumers. Powers and Kittinger (2002) found that increasing flow velocity in the field using wooden channels decreased blue crab foraging success. Although this study was important in demonstrating that field conditions could reduce blue crab foraging efficiency, by preventing crabs from leaving their narrow channel, Powers and Kittinger (2002), may have elevated the likelihood that clams would be found in slower flows. Previous studies by Leonard et al. (1998) and Bertness et al. (2004) document low levels of crab predation in environments with high mean flow velocities. Their results may also be explained using the proposed model in Fig. 4.7. In their system, flows are much faster than we measured in Georgia. The mean flow speed reported by Leonard et al. (1998) is over 20 cm/s in their slow flow site, and may be sufficient to be placed in region B of our model, where crabs have an advantage over their prey. The high flow sites in these areas may then return the advantage to prey and be placed in region C. It is important to note

that the levels of turbulence where sensory advantages switch in our system may differ in other systems, but there should be boundaries that mark when flow conditions favor predators and prey.

Sensory advantages not only may contribute to the outcome of predatory interactions, but may also affect the prevalence of lethal and nonlethal predator effects in natural systems (Smee and Weissburg in press, Smee et al. submitted ms). In environments where prey have a sensory advantage over predators (Regions A&C, Fig. 4.7), prey should be able to successfully avoid predators, and the magnitude of lethal predator effects should be minimal. However, frequent detection of predators and initiation of predator avoidance responses likely increases the magnitude of nonlethal predator effects. Likewise, when predators have a sensory advantage over prey (Region B, Fig. 4.7), they should be successful foragers and exert a strong lethal effect. In Fig. 4.7, the sensory advantage switches back to prey in region C, and nonlethal predator effects should again be more prevalent than lethal effects in these conditions. However, the magnitude of nonlethal predator effects on this prey population will be greater in region A than region C due to a greater perceptive ability of prey in low turbulence levels.

Predicting and modeling the prevalence of nonlethal predator effects in natural systems and discerning the relative contributions of lethal and nonlethal predator effects in communities remains an important goal in contemporary ecology (Werner and Peacor 2003). Advantages in perception may determine the outcome of predator-prey interactions (Dusenbery 1992, Powers and Kittinger 2002, Smee and Weissburg in press). Careful examination of how environmental conditions affect the sensory abilities of both

predators and prey should permit ecologists to predict the outcomes of predatory interactions as well as the relative roles of lethal and nonlethal predator effects in a given system when environmental conditions that affect sensory ability have been quantified. Furthermore, the overall importance of top-down forces in a given system may be reduced or strengthen by environmental conditions. That is, top-down forces (lethal and nonlethal) may be minimal in environments that diminish the perceptive abilities of both predators and prey and be greater when perception of both is heightened.

Chapter 5

Heightened Prey Responses in Risky Habitats: Does predation pressure affect prey sensitivity to predation risk?

Abstract

Biogeographical studies have shown that predation and herbivory are greater in lower latitudes, and prey living under intense consumer pressure possess stronger defenses against consumers than related species in habitats where consumer pressure is low. We tested whether prey sensitivity to risk would be heightened in habitats with elevated predation pressure and whether prey living in these areas would be more likely to initiate predator avoidance behaviors. Using hard clams, *Mercenaria mercenaria*, as a model organism, we compared predation intensity on clams as well as their responses to predators from a population in Georgia to one in Maine. Predation was significantly greater in Georgia, and previous studies have shown that Georgia clams react intensely to risk. Predation rates in Maine were extremely low, and clams there reacted less dramatically to risk. Our results suggest that prey sensitivity to risk, and the intensity of their response to predators may be related to local consumer pressure. Prey sensitivity to risk may subsequently display a geographical pattern, as predation is generally more intense in lower latitudes.

Introduction

Prey decrease their vulnerability to predators through a variety of responses (Katz and Dill 1998) including changing their morphologies (e.g., Leonard et al. 1999, Nakaoka 2000, Relyea 2001), levels of chemical defense (e.g., Hay 1996, Bolser and Hay 1996), behavior (e.g., Lima and Dill 1990, Schmitz et al. 1997, Trussell et al. 2003), or habitat (Turner and Mittlebach 1990). Predator avoidance is costly to prey and the degree to which prey respond to predators is related to the perceived level of risk (Harvell 1986, Katz and Dill 1998). Thus, changes in prey traits are often greater in environments with intense consumer pressure (Vermeij 1978, Menge and Lubchenco 1981, Bolser and Hay 1996, Pennings et al. 2001).

Many studies have shown that consumer pressure exhibits a biogeographical pattern, where both predation and herbivory are greater at lower latitudes (Vermeij 1978, Jeanne 1979, Bertness et al. 1981, Menge and Lubchenco 1981, Gaines and Lubchenco 1982, Fawcett 1984, Heck and Wilson 1987, Bolser and Hay 1996, Pennings et al. 2001). Prey living in lower latitudes that experience higher levels of consumer pressure often display stronger morphological (Vermeij 1978, Bertness et al. 1981) or chemical (Coley and Aide 1991, Bolser and Hay 1996, Pennings et al. 2001) defenses than congeners or conspecifics living in temperate habitats where consumer pressure is lower. Like morphological and chemical defenses, behavioral responses to predators can also differ between prey populations across geographic areas that experience different predation rates (Bertness et al. 1981, Fawcett 1984).

Bertness et al. (1981) compared the foraging activity of herbivorous snails in tropic and temperate waters. In New England, snails foraged during high tide to avoid

physical stress caused by exposure during low tide. In contrast, snails in Panama typically foraged during low tide, even though conditions at low tide were more stressful than in New England. Bertness et al. (1981) attributed differences in snail foraging to predation pressure from snail-crushing fish, which are common in tropical waters and not found in New England. Thus, tropical snails elected to forage in a more stressful (exposed) environment to negate predation risk by fish.

Similarly, Fawcett (1984) compared predation intensity and habitat choice by a trochid snail, *Tegula funebralis*, along the coast of California and found both higher predation rates in lower latitudes and differential predator responses in habitats with low vs. high predation pressure. Fawcett (1984) found that the lower limits of *Tegula* in the intertidal zone are higher in habitats with intense consumer pressure. *Tegula* migrated further up the shore and into a less suitable habitat to reduce predation risk despite lower resource availability in the high intertidal zone. *Tegula* transplanted between northern and southern sites exhibited similar behaviors, and regardless of their original location, moved faster and further up the shore in habitats where predation pressure was greater (Fawcett 1984). Fawcett (1984) attributed the greater predation rates in the south to the presence of the octopuses, which were not present in northern study sites.

Since morphological, chemical, and behavioral defenses can vary between prey populations under different consumer pressure, we tested whether prey sensitivity to risk and their likelihood of initiating avoidance behaviors would be greater in habitats with more intense predation. Although Bertness et al. (1981) and Fawcett (1984) demonstrate geographical differences in predation pressure and predator avoidance responses, the increase in consumer pressure and change in prey responses between regions results from

a guild of predators that are present in lower latitudes and absent in higher ones. Therefore, neither study directly addresses how predation pressure affects prey thresholds for initiating avoidance behaviors because it is unclear whether prey detect these different predators using similar mechanisms.

Using hard clams, *Mercenaria mercenaria*, as our model organism, we compared predation intensity on clams, as well as their predator detection and avoidance responses, between populations in Georgia and Maine. *M. mercenaria* are found in the intertidal zone from the Gulf of Mexico into the Gulf of St. Lawrence. They provide an excellent model organism for this study because they experience different predation levels from the same guild of predators, which allowed us to determine if predation pressure changes clam reactions to risk. Previous research has shown that clams in Georgia alter their feeding behavior in response to predators as well as injured conspecifics (Smee and Weissburg 2006, in press), which decreases their mortality (Smee and Weissburg in press). Our results suggest that predation intensity was greater in Georgia than Maine, that Georgia clams were more likely to respond to predation risk than conspecifics in Maine. These observations support the hypothesis that prey react to lower risk levels when living in areas with intense consumer pressure.

Methods and Materials

Study Sites

Surveys of clam densities and measurements of predation intensity on clams were conducted in the Damariscotta River in Maine and in the Wilmington River and two of its tributaries (Skidaway and Herb Rivers) near Wassaw Sound in Georgia. Both sites are

inland estuaries with minimal wave action, lack significant freshwater input, have a large tidal range (2-4 m), and are natural habitats of clams and their predators. In Georgia, our field site was bordered by marsh (*Spartina alterniflora*) and is typical of other soft-sediment habitats in the SE U.S. The Damariscotta River contains both rocky intertidal and soft-sediment habitats. Clams are soft-sediment animals, and we conducted our experiments in these areas of the Damariscotta River, which are similar to other northern soft-sediment communities.

Clam Density Survey

We conducted a survey of hard clam population densities in both study areas to establish a known clam density for use in our predation intensity comparison experiments (see below). Clam densities were measured by placing a 1.0-m^2 grid in the intertidal zone, digging for clams using rakes and fingers, and counting the total number of clams within the grid. Hard clams are commonly aggregated in the field, and we wanted to assess the density of clams found within established beds. Naturally occurring clam beds were located by haphazardly digging 0.25-m^2 areas using a clam rake. Whenever a clam was found, we would then place the 1.0-m^2 grid over the clam and sample the surrounding area. We counted all clams within the grid and determined if each clam was a juvenile or adult by measuring its shell length (adult clams > 30 mm). A one-way ANOVA was used to compare clam densities between Maine and Georgia.

Comparison of Predation Intensity

Predation intensity on clams was measured in Georgia and Maine using a simple mark and recovery experiment. Clams collected from the field were individually tethered with monofilament line (0.15 m long) glued to the shell and tied to ropes (0.50 m long) strung between two lengths of PVC pipe (0.50 m long x 0.125 m diameter). The area of the PVC-rope frame was 0.25 m^2, and it provided easy transportation of clams to the field site and facilitated the eventual sampling of clam mortality by allowing us to recover both live clams and shells of clams that were eaten. Clam plots were placed in the field and recovered after 48 hours in Georgia and one week in Maine. Preliminary data indicated that predation rarely occurred in ME after 48 hours, and we allowed clam plots to remain in the field longer in Maine to insure measurable predation rates. After clam plot retrieval, we recorded the status of each clam as alive, missing, or eaten. Previous research has shown that missing clams are taken by crabs (Smee and Weissburg in press), and we counted missing clams as being consumed in this study. Other investigators have followed a similar logic and have attributed missing clams to crustacean predators (Micheli 1997, Nakaoka 2000).

To measure predation rates in Georgia, we attached 15 clams, 10 juveniles (< 20 mm) and 5 adults (> 35 mm), to the rope-PVC frame and haphazardly placed them at least 100m apart in the Wilmington River and associated tributaries near Savannah, GA. This created a clam density of 60 clams m^{-2} inside the plots and was within the range of naturally occurring clam densities in Wassaw Sound (Walker 1987). We also placed plots containing 15 clams (10 juveniles and 5 adults, density 60 clams m^{-2}) haphazardly in the Damariscotta River, ME, near naturally occurring clam populations. Since clam plots

were left in the field for different intervals in each study area, we converted each measurement of predation intensity into a rate of clams eaten per day in order to compare results across regions. This data was arcsine transformed to meet ANOVA assumptions and compared using a t test (Sokal and Rohlf 1995).

Clam densities in Georgia was almost double that measured than Maine (see results), and using 15 clams per plot in Maine may have caused us to report higher predation rates than naturally occur in this habitat. Thus, we conducted an additional experiment to determine if changes in clam density affected predation rates in Maine, and to establish predation levels on patches that more closely resembled clam density of populations in Maine. In this experiment, we created clam plots with 5 clams per plot, 3 juveniles and 2 adults, creating a clam density of 20 clams per m^{-2}. This density more closely resembled the naturally occurring density of clams in ME. We then placed them in the Damariscotta River alongside plots with 15 clams. Low and high-density plots were placed 10 m apart and at least 100 m from any other pair. Ten pairs of these plots wee used, and they plots remained in the field for one week. We compared the number of clams found alive in the low and high-density plots using a paired-*t* test (Sokal and Rohlf 1995).

Behavioral Assays

Animal Capture and Maintenance

Animals used in the study were collected from the Damariscotta River near the Darling Marine Center (DMC), Walpole, ME. Clams were collected by digging in the intertidal zone, and clam predators including American lobsters (*Homarus americanus*),

rock crabs (*Cancer irroratus*), Jonah crabs (*Cancer borealis*), green crabs (*Carcinus maenas*), and northern starfish (*Asterias forbesi*) were collected in the Damariscotta River using baited lobster traps and by hand using SCUBA. After capture, animals were returned to the DMC and housed in flow-through sea tables. Clams were allowed to acclimate for at least 6 hours prior to behavioral and were not used in experiments if they had remained in the sea tables for longer than 48 hours. All clam predators were fed an *ad libitum* diet of clams for at least one week prior to use in the behavioral assays. Each clam and predator were used only once and then returned to the field.

Blue crabs (*Callinectes sapidus*) and knobbed whelks (*Busycon carica*) are the primary clam predators in Georgia, and clams from Maine also were exposed to these exotic predators in behavioral assays. These animals were collected in Wassaw Sound, GA; blue crabs using crab pots, and knobbed whelks by hand collecting in the intertidal zone. After collection, blue crabs and knobbed whelks were shipped to the DMC and placed in isolated aquaria to prevent introduction of nonnative organisms into Maine waters. Water in these aquaria was changed daily, and both blue crabs and knobbed whelks were the fed same clam diet as local predators before use in experiments.

Hydrodynamic Environment for Behavioral Studies

Behavioral experiments were conducted in a laboratory flume at the DMC. The flume was 2.2 m long, 0.53 m wide, and had a false bottom (diameter 0.13 m) located 1.4 m downstream, which permitted clams to burrow. Free-stream flow velocity and water depth were maintained at 3 cm s^{-1} and 0.10 m respectively during all behavioral assays. The flume was supplied by water pumped directly from the Damariscotta River and was

discharged back into the river after a single pass through the flume. Water used in trials with exotic animals was first captured in a large tank and treated with bleach prior to release in the field.

Clam Reactions to Predators

These experiments utilized changes in clam pumping (feeding) behavior as assays for the ability of clams to detect and respond to predation risk. Previous research using hard clams from Georgia has shown that clams are actively feeding when their siphons are extended (Smee and Weissburg 2006, in press), and other authors have used siphon extension as an indicator of clam feeding (Irlandi and Peterson 1991). Unlike GA clams, we found that siphon extension was not indicative of pumping for clams in Maine. Maine clams would respond to certain predators by closing their excurrent siphon and stop releasing an excurrent, while leaving their siphons extended beyond their shells. We carefully pipetted dye near the excurrent siphon of each clam to visualize the excurrent jet and verify clam pumping activity in each treatment. In some cases, Maine clams would 'clam up' as clams in Georgia do, and we reported occurrences of both siphon withdrawal as well as instances when clams stopped pumping but left their siphons extended and shells open (hereafter referred to as feeding cessation).

Behavioral assays were conducted in the DMC flume and consisted of challenging clams to detect and respond to predators, injured conspecifics, and in some cases, combinations of these cues. We judged clam responses to predation risk by determining if clam feeding was significantly less in response to these treatments when compared to a control that lacked predators or injured conspecifics. In each assay, we

placed three clams in the false bottom of the flume and allowed them to acclimate for 20 minutes. Preliminary observations indicated that this time was sufficient for clams to burrow and begin pumping. After the 20-minute acclimation period, we introduced predators, crushed conspecifics, or a combination of these cues by placing a caged predator, injured clam, or both 0.5 m upstream from the clams. The predator cage was made from vexar mesh, containing 1.0 cm^2 openings, and was cylindrical in shape with a height of 0.10 m and a diameter of 0.15 m.

We recorded the siphon position (extended or not) and feeding activity (feeding or not) of each clam prior to introduction of the predator treatments and at four-minute intervals after introduction for 20 minutes. Thus, each clam could have been observed feeding (pumping) a maximum of six times, and we used the number of observations in which clams were pumping as a measure of clam pumping time. We tested clam responses to a variety of sympatric predators including American lobsters (*Homarus americanus*), rock crabs (*Cancer irroratus*), Jonah crabs (*Cancer borealis*), green crabs (*Carcinus maenas*), and northern starfish (*Asterias forbesi*) as well as injured conspecifics. We also exposed clams in Maine to knobbed whelks (*Busycon carica*) and blue crabs (*Callinectes sapidus*) from Georgia, since Georgia clams react to these predators (Smee and Weissburg 2006, in press). Responses of all clams were compared to controls in which clam feeding behavior was examined in the absence of upstream predators or injured conspecifics.

Results from initial experiments indicated that clams did not react to rock, Jonah, or green crabs, so we examined the responses of clams when rock, Jonah, and green crabs were allowed to actively consume clams during the behavioral trials. In these tests, we

placed a single clam (0.03 to 0.04 m shell length) into the vexar cage along with a caged crab. We removed the top valve of the clam so that each crab could readily consume the clam during the experiment. Clams reduced their feeding time when rock crabs were feeding on clams during these assays, but we did not observe any clam reaction when Jonah or green crabs were eating clams during the experiments. These responses indicated that the crab's feeding manner exerted a significant effect. Rock crabs are messy eaters, and clam fluids and flesh pieces could be easily seen during as rock crabs ate clams in the flume. In contrast, we did not observe clam fluids being released when Jonah or green crabs were eating clams. This suggested that the amount of clam fluid (degree of clam injury) being released might influence clam reactions, and we conducted two final experiments to examine this supposition. First, we removed the top valve of a clam and made a single laceration to its visceral mass using a kitchen knife. We then placed a caged rock crab upstream from the clams as before and placed the injured clam outside the cage so that the crab could not consume the clam. This allowed the clams to receive odors from an injured clam and a rock crab simultaneously, even though the crab was not feeding. Clam fluids were not visible during in this trial, and thus, the quantity of injured clam cue more closely represented that observed in trials when Jonah or green crabs were feeding. In the second experiment, we placed only an injured clam upstream from the experimental clams. In this trial, we made multiple lacerations on the visceral mass of the clam with a knife immediately before placing it into the flume and again after it had been in the flume for 10 minutes to insure clam metabolites were released into the water and simulate feeding by a rock crab. That is, we injured the clam until its fluids could easily be seen leaking into the flume.

The order of treatments and controls in these experiments was randomly assigned each day, and each treatment and the control were replicated at least five times (5 trials x 3 clams per trial = 15 clams for each treatment and control). Additional control trials were conducted each day to establish a baseline of clam feeding in the absence of predators. Each clam and predator were used only once and then returned to the field. Clams that neither pumped nor burrowed were excluded from analysis, and approximately 20% of the clams were excluded using this criterion.

Smee and Weissburg (2006) found that neighboring clams behave independently of one another in a similar flume study, and thus, interactions between clams are not biasing our results. Since clams do not influence each other, the behavior of a single clam is an appropriate unit of measurement. Observations of pumping behavior of individual clams (number of siphon extensions observed for each clam) were arcsine transformed to meet ANOVA assumptions and then compared using a nested ANOVA that examined the effects of predator treatment and trial nested within treatment (Sokal and Rohlf, 1995). Using a nested ANOVA allowed us to determine if variations in clam responses were affected by variability in cue quality or quantity across replicate treatments, which is a source of uncontrolled variation in our experiments. The *P* value for the nested effect was greater than 0.25 in all experiments, indicating that clams in different groups were not reacting significantly different to the same treatments, and suggests that cues from predators and injured conspecifics were roughly similar between replicate trials. Nest effects have not been found in other studies using similar assays, (Smee and Weissburg 2006, in press).

The lack of a significant nesting effect permitted us to lump trials within treatments and test the significance of the main effect using the pooled error variance (Sokal and Rohlf, 1995). A Tukey-Kramer post hoc analysis was employed to test for pair-wise differences between treatments and controls (Sokal and Rohlf 1995). Recall that clams reacted to predators in two ways: by withdrawing their siphons and by leaving their siphons extended but ceasing to pump (cessation of feeding). We employed 3 separate ANOVAs to examine changes in clam behavior caused by risk. In the first ANOVA, we compared the number of clam reactions between treatments and controls by combining responses of shell closure with responses of feeding cessation. We ran two other ANOVAs in which we looked separately at each clam response to predators (siphon withdrawal vs. feeding cessation). These additional tests allowed us to compare the types of responses clams were having to each treatment tested. We deemed feeding cessation as a less intense reaction to risk than siphon withdrawal and examining these behaviors separately allowed us to determine the level of response of Maine clams to each treatment.

Results

Density Survey

Clam densities were significantly higher in Georgia than in Maine (Figure 5.1, p120, $t=9.1$ $P < 0.001$), with densities measuring 26.1 (SE $\pm$ 1.5) and 9.1 (SE $\pm$ 1.2) clams m^{-2} respectively. In Maine, 35 out of 50 clam beds had < 10 clams m^{-2}, and only 4 had more than 20 clams m^{-2}. By comparison, 36 clam beds in Georgia had densities > 20 clams m^{-2}, and only 2 had densities < 10 clams m^{-2}. The sizes of clams collected were

noticeably different between these areas, as juveniles were rarely collected in Georgia but accounted for nearly 30% of total clam collections in Maine (Figure 5.1).

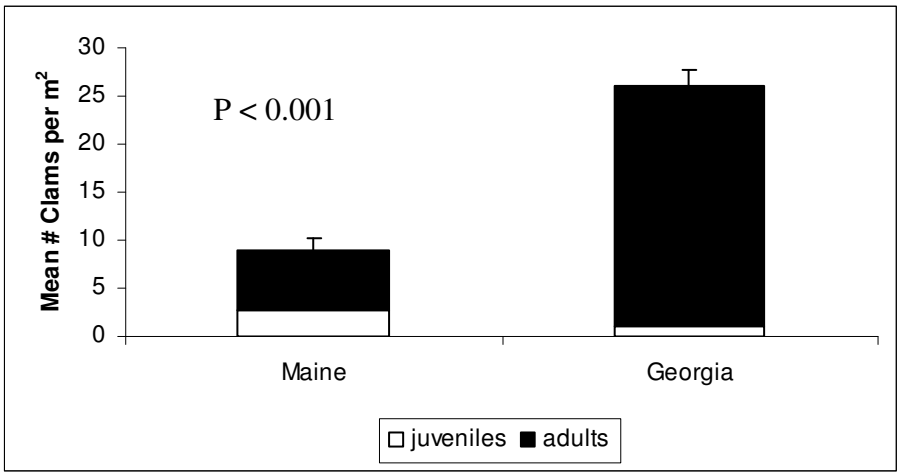


Figure 5.1. Mean density (± std. err.) of clams per m^{-2} (*n*=50 plots per site) in Georgia and Maine. Mean density of juvenile and adult clams collected in each area are also shown. Clam densities between these areas were significantly different ($P < 0.001$)

Predation Intensity

Predation intensity on clams in Maine vs. Georgia was compared using clam plots with 15 clams per 0.25 m^2. The rate of clam predation was significantly higher in Georgia compared to Maine (Figure 5.2 $t = 8.1$, $n = 20$, $P < 0.001$), and the total number of clams eaten in Georgia was much higher than in Maine even though plots in Maine were in the field for one week and those in Georgia were recovered after 48 hrs. Recall that clam plots in Maine had a significantly higher density than naturally occurring clam beds, which may have artificially elevated these measured predation rates. On average, 0.3 clams were eaten per day in Maine as compared to 2.7 in Georgia. Despite the additional time in the field and artificially high clam density in Maine, the mean number of clams consumed per plot in Georgia was still more than twice that measured in Maine A

preliminary study in both locations revealed that predation was nearly nonexistent in Maine after 48 hrs, but > 95% of clams were consumed in Georgia after 1 week., Roughly 90% of clam mortality was attributed to crustaceans in both Georgia and Maine, and juvenile clams were more commonly eaten than adults (Figure 5.2).

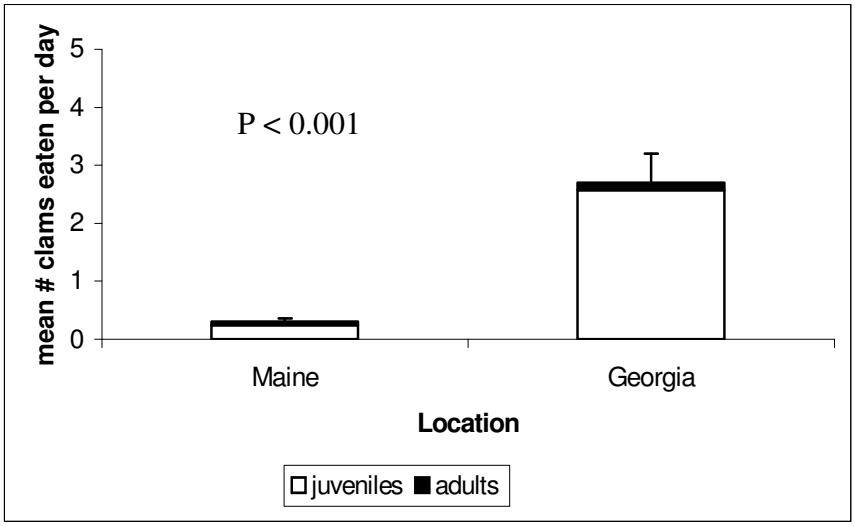


Figure 5.2. Mean number (± std. err.) of adult and juvenile clams consumed per day in the field plots in Georgia and Maine. 20 clam plots were placed in both Maine and Georgia, and predation rates were significantly different ($P < 0.001$). Nearly 100% of juvenile clam mortality was attributed to crustacean predators.

Clams in Maine were placed in the field in low (5 clams per 0.25 m^{-2}) and high (15 clams per 0.25 m^{-2}) density plots. The low-density plots more closely resembled the naturally occurring density of Maine clams, whereas the high-density plot reflected naturally occurring densities in Georgia. Significantly more clams were consumed in the higher density plots (Figure 5.3, $t = 4.02$, $n = 20$, $P < 0.05$). Eight out of the ten low-density plots were recovered with 100% of the clams alive, whereas only two out of then high-density plots displayed zero mortality. Thus, the high-density plots were four times

more likely to be discovered by predators suggesting that Maine clams may gain a spatial refuge from predators by existing in low densities.

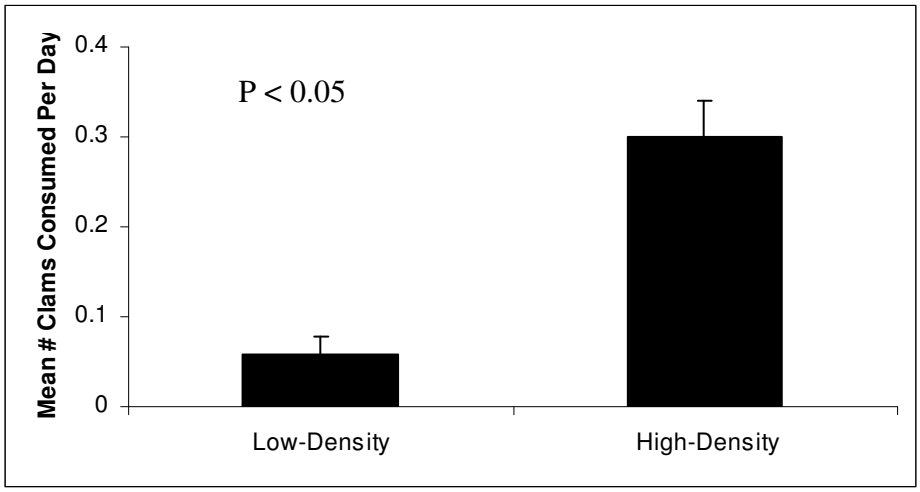


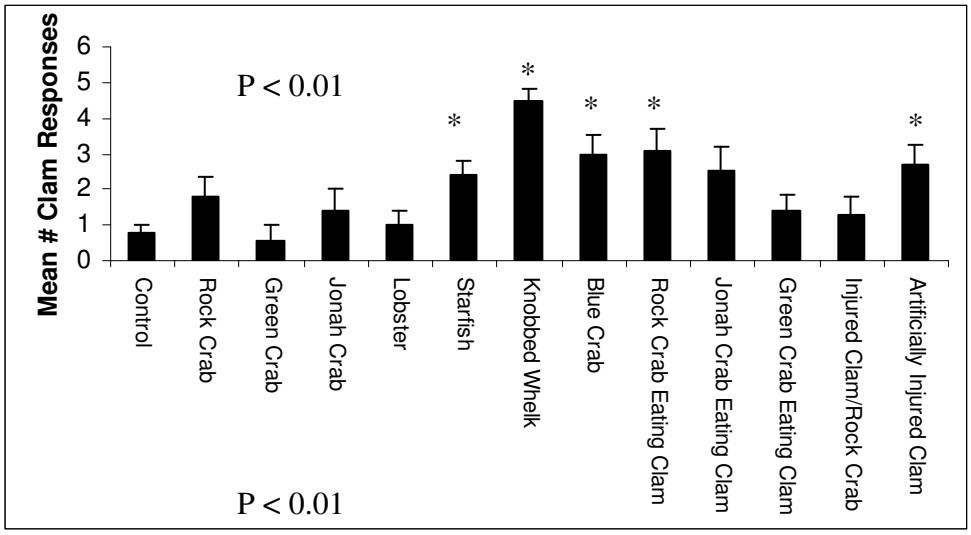
Figure 3. Mean number of clams consumed per day (± std. err.) in low and high density plots in Maine. Plots (n=10 pairs) remained in the field in Maine for one week, low-density plots contained 5 clams and high-density plots contained 15 clams per 0.25 m^{-2}. High densities plots had significantly higher predation rates ($P < 0.05$).

Behavioral Assays

Smee and Weissburg (2006, in press) found that clams in Georgia respond to predation risk (i.e., predator cues) by withdrawing their siphons and closing their shells (siphon withdrawal). We found that clams in Maine showed two different responses to risk. Clams sometimes would cease pumping by closing their excurrent siphon, but would leave their siphons extended and shells open (feeding cessation). In other cases, clams would withdraw their siphons and close their shells. Thus, we documented occurrences of both siphon withdrawal and feeding cessation for clams in Maine.

In the DMC flume, we examined changes in clam behavior caused by the presence of sympatric and exotic predators, injured conspecifics, and combinations of

A)



B)

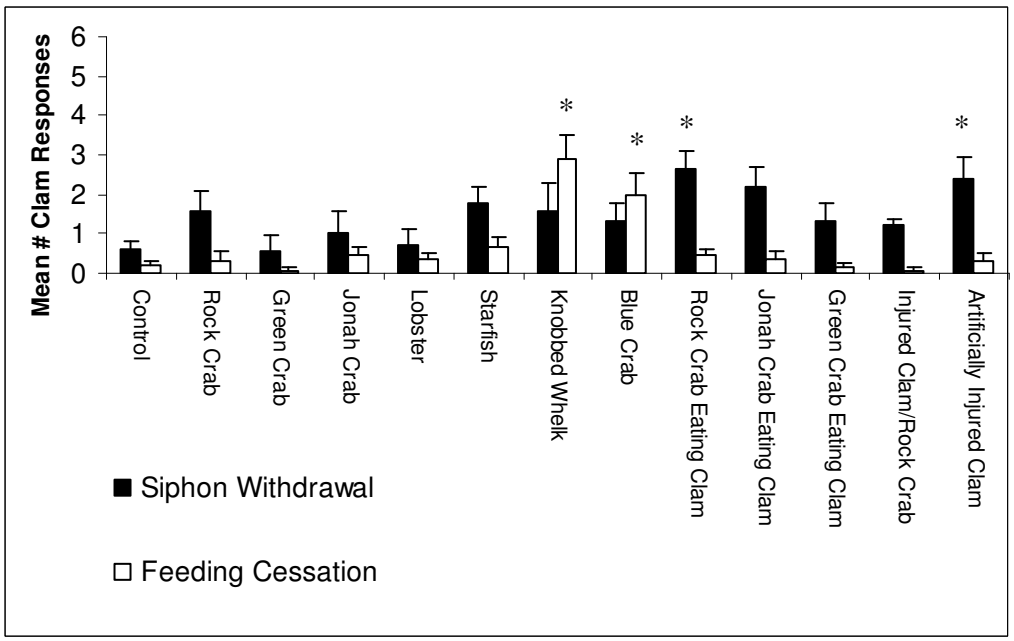


Figure 4. Mean number (± std. err.) of responses of Maine clams exposed to all treatments in the flume behavioral assays. Responses range from 0 (clams observed pumping in all 6 observations) to 6 (clam reacted in all 6 observations). Part A shows the total number of clam responses, and part B displays the number of each type of response (siphon withdrawal & feeding cessation) separately. * denote means significantly different from the control based on a Tukey-Kramer post hoc test. Treatments marked rock, Jonah, or green crabs eating clams are trials in which the crab was consuming a clam during the trial. The treatment marked Injured Clam/Rock Crab was performed with both an injured clam and a rock crab, but the crab was not allowed to contact the clam, and the clam was punctured only once. The artificially injured clam was lacerated with a knife multiple times to mimic rock crab feeding. The following Sample sizes (listed in the same order as in the figure) are 44, 14, 13, 11, 15, 16, 14, 13, 16, 13, 14, 14, and 15.

these cues. The first analysis compared all changes in clam pumping behavior caused by predators (siphon withdrawal and feeding cessation), and we found that clams reduced their feeding time by 40%-75% after detecting starfish, rock crabs eating clams, injured (lacerated) clams, blue crabs, and knobbed whelks (Figure 5.4 $F_{12,209} = 6.2$ $P < 0.01$). The second analysis indicated that a significant siphon withdrawal response occurred for clams exposed to rock crabs eating clams and to the severely injured clams wounded with a knife (Figure 5.4, $F_{12,209} = 2.54$ $P < 0.01$). The final ANOVA revealed that clams responded to exotic blue crab and knobbed whelk predators with feeding cessations (Figure 5.4, $F_{12,209} = 6.04$ $P < 0.01$). Note that the clam reactions to starfish were not significantly different than controls when we examined each response component (withdrawal and feeding cessation) separately. Clams exposed to starfish would display both responses and would sometimes close, whereas other times they would stop feeding but leave their siphons extended. Thus, there was a significant behavioral change in this treatment, but examining these behaviors separately does not reveal that either occurred at a level different than the control.

Clam feeding was unaffected by lobsters, rock crabs, green crabs, and Jonah crabs, even when green and Jonah crabs were feeding on clams during the assays. Qualitative observations revealed that the feeding behavior of rock, Jonah, and green crabs differed substantially. Rock crabs were extremely aggressive eaters, piercing clams with their claws and releasing clam fluids into the water. In contrast, green and Jonah crabs primarily consumed the clam using only their mouthparts, and any clam fluids released into the water were not visible. Clams also reacted to the artificially injured clams that we pierced repeatedly with a knife until clam fluids were leaking into the

water, but they did not respond to the injured clam with a single wound placed in the flume next to a rock crab.

In summary, Maine clams reduced their feeding time after detecting starfish, blue crabs, and knobbed whelks, but did not withdraw their siphons in response to these predators. Maine clams did withdraw their siphons after detecting rock crabs eating clams and artificially injured clams, but showed no response to lobsters, rock crabs, green crabs, or Jonah crabs, even when the latter two crab predators were feeding on conspecifics.

Discussion

Prey utilize many tactics to minimize their susceptibility to consumers including changing their morphology (Vermeij 1978, Leonard et al. 1999, Nakaoka 2000), increasing their levels of chemical defenses (Bolser and Hay 1996, Pennings et al. 2001), or altering their behavior or habitat selection (Bertness et al. 1981, Fawcett 1984, Turner and Mittlebach 1990). As a general trend, consumer pressure is greater in lower latitudes, and prey possess heightened consumer defenses in these areas (Vermeij 1978, Jeanne 1979, Bertness et al. 1981, Menge and Lubchenco 1981, Gaines and Lubchenco 1982, Fawcett 1984, Heck and Wilson 1987, Bolser and Hay 1996, Pennings et al. 2001). Our results suggest that like other defensive adaptations, predator perception and the likelihood of initiating predator avoidance behaviors are related to local predation pressure and exhibit a geographical pattern that is seemingly produced by variation in predation intensity. Clams from Maine (low predation) respond to few predators, respond with intermediate behaviors (feeding cessation) even to predators that evoke dramatic responses from Georgia clams, and require cues indicative of immediate, potential threat

(cues from injured conspecifics) before initiating their most protective behavior (shell closure).

Clam Density and Predation Pressure

We found that clam densities were three fold higher in Georgia than in Maine (Fig. 1) and predation on clams was significantly higher in Wassaw Sound, GA than in the Damariscotta River, ME (Fig. 5.2). The higher level of clam predation measured in Georgia was especially striking considering that predation rates in Maine were likely elevated by placing clams in the field at unnaturally high densities (Fig. 5.3). The rate of clam predation per day was nine times higher in Georgia (2.7 clams eaten per day vs. 0.3 in ME).

Crushed clam shells and missing clams are indicative of predation by crustaceans (Micheli 1997, Nakaoka 2000), and almost all clam mortality in our plots in both Georgia and Maine was caused by crustacean predators. Hard clams reach a size refuge from blue crab predators when their shells reach 30 mm across (Micheli 1995, 1997), and we found that juvenile clams were more readily eaten than adults in both study sites. The fraction of mortality attributable to predation on juveniles was 95% and 80% in Georgia and Maine respectively, and juvenile clams were rarely collected in Georgia but were much more common in Maine (Fig. 5.1, ratios of adults to juveniles in GA 25:1, in ME 3:1). Walker et al. (1980) monitored predation on clams in experimental field plots in Wassaw Sound and found that predation was much more common on juveniles than adults, and that juvenile clams were rare in Wassaw Sound. Our results are consistent with these findings.

Despite the more intense predation (Fig. 5.2), clam densities were nearly three-fold higher in Georgia than Maine (Fig. 5.1). The high clam density in Georgia, coupled with the heavy predation on juvenile clams, suggests that clams in this population experience a bottleneck caused by predation from crustaceans, insuring that any surviving clams in this area possess keen predator avoidance capabilities. That is, predation pressure in Georgia has likely selected for heightened predator detection and avoidance responses whereas the low predation pressure in Maine has not. The greater sensitivity to predators may allow Georgia clams to reach high densities, despite the intense consumer pressure.

Clam Behaviors in Georgia and Maine

Clams in Georgia and Maine experienced vastly differently levels of predation pressure, and we were able to use these clam populations as a natural experiments to test whether prey perception of and reaction to risk are heightened in habitats with intense consumer pressure. Previous research by Doering (1982), Irlandi and Peterson (1991), and Smee and Weissburg (2006, in press) found that hard clams reacted to predators by withdrawing their siphons and closing their shells. Clams that close their shells in response to risk reduce their growth rate (Irlandi and Peterson 1991, Nakaoka 2000) but improve their chances of survival (Smee and Weissburg in press). This study is the first to document a reaction of hard clams to predators where they cease pumping but leave their siphons extended. The costs and benefits of this behavior are unknown, but clams likely remain vulnerable to consumers (Burnett 1960) by not withdrawing their siphons. Thus, we interpreted this behavior as an intermediate response to risk and complete shell

closure as a stronger reaction to predators., Predation pressure is high in southern clam populations that have not been observed to employ this intermediate behavior; southern clams close up completely after detecting predators or injured conspecifics (Irlandi and Peterson 1991, Smee and Weissburg 2006, in press).

Smee and Weissburg (2006, in press) have shown that hard clams in Georgia reduce their feeding time by 40%-50% and withdraw into their shells after detecting injured conspecifics as well as blue crab and knobbed whelk predators. Results from our behavioral assays indicated that Maine clams reduced their feeding time when exposed to starfish, but did not react to any other sympatric predators tested including lobsters, rock crabs, green crabs, and Jonah crabs (Fig. 5.4). We were surprised that Maine clams did not react to any of their crustacean predators because these predators were responsible for the majority of clam mortality in our field experiment (Fig. 5.2). Although Maine clams did reduce their feeding time in the presence of starfish, they did not withdraw their siphons into their shells in some of the assays. Doering (1982) found that hard clams from Rhode Island withdrew into their shells when placed downstream from starfish, and he did not observe intermediate risk responses. The response to starfish in this study differed from that of conspecifics further south where predation is more intense (Doering 1982).

Reactions of Maine clams to their sympatric predators were generally much lower than reactions of Georgia clams to their primary consumers. Georgia clams stop feeding and close their shells in the presence of injured conspecifics as well as sympatric knobbed whelks and blue crab predators (Smee and Weissburg 2006, in press). We exposed clams in Maine to these Georgia predators, which allowed us to compare clam responses to the

same predators from two geographically different populations that experienced vastly different levels of consumer pressure. Maine clams responded to knobbed whelks and blue crabs by reducing their feeding time but not closing their shells. Thus clams in Maine reacted less intensely than Georgia clams to the same predator signals. We were somewhat surprised that Maine clams reacted to these exotic predators, especially since they did not react to local crustacean consumers, and we offer possible explanations for these results. First, blue crabs and knobbed whelks may exude larger quantities of metabolites than Maine crustaceans and are thus easier for clams to detect. This explanation may also account for the response of Maine clams to starfish, if starfish are releasing larger quantities of warning cues than crustaceans. Second, Maine clams reacted to blue crabs and knobbed whelks similarly as to starfish (by not feeding but leaving their siphons extended), and the chemical signature of these exotic predators may be similar. Finally, Maine clams may be descendents from clam populations further south where blue crabs and knobbed whelks commonly occur. Although we are uncertain of the mechanisms that allow Maine clams to detect knobbed whelks and blue crabs, it is clear that clams in Maine do not respond to blue crabs and knobbed whelks as strongly as do Georgia clams.

We observed the most intense reactions of Maine clams when they were presented with odors from conspecifics that were being consumed by rock crabs or that had been repeated injured. These treatments were the only ones in which we observed Maine clams withdrawing into their shells. Metabolites leaking from injured clams are both necessary and sufficient to evoke shell closure. Clams closed their shells in response to rock crabs that were consuming clams, did not respond when exposed to a clam with a single

puncture wound alongside a caged rock crab, but did withdraw into their shells when exposed to a conspecific that was repeatedly lacerated before and during the experiment. These results suggest that Maine clams primarily use cues from injured conspecifics to evaluate risk, but require a high level of cue before predator avoidance behaviors are initiated. In contrast, clams from Georgia react to injured conspecifics regardless of the degree of injury (Smee and Weissburg 2006, Smee unpublished data). We did not observe any clam reactions when Jonah or green crabs were eating clams during the experiments. These responses may be explained by the crab's feeding manner. Rock crabs are messy eaters, and clam fluids and flesh pieces could be easily seen during as rock crabs ate clams in the flume.

Environmental Differences vs. Predation Pressure

Clams in Maine were less responsive to local predators, reacted less strongly to knobbed whelks and blue crabs, and required a higher level of injured clam signal than southern conspecifics before reacting to risk. Combined with our data showing the significantly greater level of predation pressure in Georgia, these results suggest that clam sensitivity to cues indicative of predation risk is related to predation intensity. Two possible mechanisms may explain this phenomenon. First, intense predation pressure has selected for heightened sensitivity in southern clam populations. Intense predation by crabs in Georgia may create a bottleneck, insuring that clams reaching adulthood be capable of reacting to consumers. Although crustacean predators accounted for the majority of clam mortality in Maine, the consumer pressure there was insufficient to select for heightened predator detection and avoidance capabilities.

Alternatively, differences in environmental conditions (e.g., temperature, food availability) affect the costs of clamming up, and Maine clams continue to pump in the presence of predators to meet basic energetic requirements. In this situation, low predation pressure in Maine enables clams to survive without reacting to predators, but in Georgia, intense predation pressure eliminates clams that fail to respond to predatory threats. Regardless of the mechanism, predators play a key role in modulating clam behavior.

Clearly environmental differences (e.g., temperature, food availability) between Georgia and Maine exist, and many studies have shown that bivalve pumping and growth are affected by these factors (reviewed in Grant 1996). We suggest that environmental differences are unlikely to be the primary cause of dissimilarities in clam reactions to predators between these populations for several reasons. First, several species of bivalves exhibit some degree of temperature acclimation (reviewed in Grant 1996) and adjust both their feeding behavior and energetic needs to maximize feeding efficiency in response to changes in temperature and/or food availability. Assuming that, like other bivalves, clams in Maine have acclimated to the colder temperatures, predator avoidance costs between these clam populations should be similar. Bivalves close up and stop feeding (Grant 1996) when they are not within a range of tolerable temperatures. Clams pumped in our behavioral assays and in sea tables prior to experimentation, which suggests that they were within their acclimated temperature range. Secondly, clams that are found by predators are nearly always consumed, and thus, failure to respond to predators should outweigh any costs associated with clamming up. Hard clams can live in excess of 40 years, and while short-term growth and fitness losses can occur through frequent

reactions to predators (Nakaoka 2000), the long term cost on overall fitness is unknown. Smee and Weissburg (in press) demonstrated that clam survival increased when clams reacted to predators, and thus, costs incurred by clams are not unrewarded. Even if there is a greater cost associated with predator avoidance in Maine, the cost of failure to avoid predators is likely still less than the alternative (death), suggesting that costs differences alone are inadequate to account for differential responses from clams in these populations.

Biogeographical Effect

Maine clams clearly react less intensely to predation risk than conspecifics from Georgia, North Carolina, and Rhode Island (Doering 1982, Irlandi and Peterson 1991, Smee and Weissburg 2006, in press). Many studies, including ours, have found that consumer pressure is inversely related to latitude, being greatest in low latitudes and decreasing in higher ones (e.g., Jeanne 1979, Bertness et al. 1981, Menge and Lubchenco 1982, Heck and Wilson 1987). This study, along with Bertness et al. (1981) and Fawcett (1984) indicate that predator avoidance behaviors differ between populations that experience different levels of consumer pressure. Furthermore, our results suggest that heightened predator awareness may exhibit a biogeographical pattern, like other prey responses to consumers (Vermeij 1978, Bertness et al. 1981, Bolser and Hay 1996, Pennings et al. 2001). However, additional clam populations must be sampled to determine if this is a robust biogeographical pattern and does not result from local variation in predation.

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