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THE ROLE OF BEHAVIORAL TEMPERAMENTS ON THE ECOLOGY OF THE CARIBBEAN REEF OCTOPUS, OCTOPUS BRIAREUS

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THE ROLE OF BEHAVIORAL TEMPERAMENTS ON THE ECOLOGY OF THE CARIBBEAN REEF OCTOPUS, OCTOPUS BRIAREUS.

A Thesis
Presented to
the Graduate School of
Clemson University

In Partial Fulfillment
of the Requirements for the Degree
Master of Science in
Biological Sciences

by
Timothy Scott Jordan
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Accepted by:
Dr. Michael J. Childress, Committee Chair
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ABSTRACT

Traditional studies of predator-prey interactions have primarily focused on direct consumption as the most important effect on prey. Recent studies, however, have illustrated that nonlethal, risk effects may have an even greater impact. In this study, I evaluated the role of direct consumption and risk effects of the Caribbean reef octopus, *Octopus briareus*, on multiple species of crab and spiny lobster prey in Florida Bay, Florida. I conducted 13 monthly censuses of 8 nearshore field sites and observed the density and distribution of octopuses, crabs and lobsters. I found a significant negative correlation between the density of octopus predators and crabs, but not with lobsters. A manipulative tethering study found no correlation between crab mortality and the density of octopuses. To examine risk effects, I also quantified traits of individual octopuses, such as size, sex, and behavioral temperament (boldness). Average octopus weight was negatively correlated with crab density, but not lobster density. I also analyzed the frequency of crab and lobster prey co-denning with each octopus in shelter blocks. Shelter block use was significantly reduced for both lobsters and crabs when a resident octopus was present. Neither size nor boldness of the octopus, however, influenced the frequency of co-denning. Finally, to examine if octopus behavioral temperaments were due to adaptive phenotypic plasticity or a fixed behavioral syndrome, I captured a subset of octopuses for further behavioral observations in the laboratory. I found repeatability of behavior within individuals in response to boldness tests in the field and in the laboratory, but no correlations in temperament across situations (boldness in the field vs. boldness in the laboratory) or contexts (boldness in the field vs. activity in the laboratory). My results
suggest that (1) direct consumption effects are more important on crabs than on spiny lobsters but may be relatively weak overall, (2) octopus risk effects influence den use of both lobsters and crabs, reducing availability of protective shelters and increasing risk of predation from other predators, and (3) octopuses have consistent behavioral temperaments that are expressed variably in each new situation and context. I found no significant relationship between the temperament of octopuses and co-denning of either prey species, suggesting prey limitations in assessing risk effects associated with predators of varying boldness.
DEDICATION

I would like to dedicate this thesis to all those people who believed in me, supported me, and became the strength I drew upon during times when I needed them. I also give thanks to God for his many blessings and providing this serendipitous life experience, without which I would not be the person I am today.

I have always had a passion for the outdoors. As a child, my uncle Dennis would take me fishing quite often and I would play and explore until the sun went down. Later in life my venue changed from my hometown freshwater lake to the ocean, with all its spectacular inhabitants. I never dreamed I would one day live among and study them as I have so many times while pursuing this degree. Words cannot express my gratitude to my uncle for those experiences as a child that ignited my passion for the outdoors and inspired me to become the naturalist I now am.
ACKNOWLEDGMENTS

First and foremost, I would like to thank my advisor, Dr. Michael Childress for his patience, guidance, and support throughout my graduate career at Clemson. I am appreciative that he chose me over many other qualified applicants for the position and allowed me the opportunity to join his laboratory and become part of the marine science community. I would also like to thank my committee members: Dr. Margaret Ptacek, Dr. Robert Kosinski, and the late Dr. Jerry Waldvogel for their contributions to both my project and this document. I remain indebted to Dr. Peter Bouwma, Kirk Parmenter, Adrianna Zito-Livingston, Sara Tappan, Jenn Seda, Jamie Dowd, Kaighn Morlock, and all those in the Childress and Ptacek labs at Clemson University for all their help in the field and otherwise. In addition I would like to thank Cynthia Lewis and all the staff at the Marathon office of FWC and the Keys Marine Lab for their assistance in the field. Finally, I wish to thank Erin Cooke for her input and assistance in proofreading and editing this document.
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CHAPTER ONE

RISK EFFECTS AND BEHAVIORAL TEMPERAMENTS

Project Summary

Predator density can influence prey density by either direct consumption (i.e. density-mediated interaction, or DMI) or by intimidation (i.e. trait-mediated interaction, or TMI). Consideration of TMI effects of predators upon prey is important, for studies have suggested that these are often equal to or even substantially larger than direct effects. These indirect influences can cause changes in the behavior, morphology, or physiology of prey organisms, which may in turn alter fitness in a variety of ways.

The Caribbean reef octopus, *Octopus briareus*, is an important predator found in the shallow-water seagrass communities throughout the Caribbean. These animals are notorious predators of crabs, shrimp, lobsters, and a variety of mollusks. Previous studies have found that juvenile spiny lobsters avoid shelters containing resident octopuses, however, it is unclear if this decrease in local prey density is due to direct predation or risk effect (i.e. TMI) on prey distribution. It is also unclear if this effect is similar for other prey species such as stone crabs and blue crabs.

Octopuses, like many species of vertebrates, show remarkable variation among individuals that potentially influences their predatory efficiency and/or intimidation of prey. Therefore, I am interested in how variation in octopus size and temperament may influence their efficiency as a predator and wanted to examine intimidation effects upon prey.
In this thesis, I examined four questions related to these topics: (1) How does octopus density influence the density of juvenile crabs and lobsters at regional and local scales? (2) How does octopus size influence the spatial distribution of juvenile crabs and lobsters at regional and local scales? (3) How does octopus behavioral temperament influence the spatial distribution of juvenile crabs and lobsters at regional and local scales? (4) Are octopus behavioral temperaments the result of adaptive plasticity or behavioral syndromes?

To answer these questions I conducted monthly censuses of eight sites across Florida Bay, Florida for a period of three years. Censuses included capture and identification of all crabs, lobster, and octopuses. Octopuses were subjected to a simulated threat test from which they were scored according to a boldness scale ranging from -1 to +3. Following this test, some octopuses were collected, weighed, sexed, and returned to a temporary outdoor laboratory facility, where behavioral observations were conducted. I also conducted a tethering experiment to determine the mortality rate of crabs on sites in relation to octopus density.

Results from these experiments will provide a better understanding of the impact of octopuses upon nearshore communities in the Florida Keys allowing me to quantify the effects of trait-mediated interactions in this system and separate them from effects of direct predation alone. Specifically, I hope to examine how varying degrees of temperament (boldness in this case) in octopuses influence the distribution of prey within this community. Results of this project can then be used to guide future studies of other important fisheries where trait-mediated predator effects may be important.
Project Description

Density- vs. Trait-Mediated Interactions

Predators play an important role in shaping prey communities. Not only are they able to remove prey directly by means of consumptive predation, but they are also capable of intimidation of prey species (Lima 1998). Direct consumption is a type of density-mediated interaction (DMI) in which an increase in the density of predators causes a reduction in the population of prey as a result of direct killing. Traditionally, ecologists have chosen this interaction as the focus for empirical studies and theoretical models (Abrams 1995). Although the effect of direct predation can be great in some circumstances, there is another process by which predators influence their prey. Predators may induce defensive strategies in prey by means of intimidation, or risk effects (Figure 1.1; Creel & Christianson 2008).

Intimidation occurs when the mere presence of predators causes compensatory responses in prey, whose outcome can be costly and lead to reduced numbers in populations. Costs may occur from development of defensive strategies, including reduced energy budget, greater energetic investment (in defensive morphology, etc.), lower mating success rates, increased vulnerability to secondary predators, emigration, as well as other costs accrued in anti-predatory behavior (Preisser et al. 2005). This phenomenon of intimidation upon

Figure 1.1 Predators influence prey either by direct killing or by risk effects. From Creel & Christianson, 2008.
prey by predators has also been referred to as “trait-mediated interactions”, or TMI (Abrams 1995), “non-lethal effects” (Lima 1998), “risk effects” (Creel & Christianson 2008), and “non-consumptive effects” (Peckarsky et al. 2008). These have been the subject of recent studies set on reshaping existing models of predator-prey dynamics to include such effects for a more accurate representation of natural systems.

Effects of trait-mediated interactions can be quite large, and even outweigh those of density-mediated interactions (Creel & Christianson 2008). A meta-analysis by Preisser et al. (2005) reviewed 166 studies in order to determine the importance of TMIs in relation to DMIs, using standard examples of predator-prey interactions. Although classical formulation of trophic cascades has depended mostly upon DMIs, the authors elucidated the importance of TMI influences upon these systems. By examining the literature, they found that altering phenotypic traits by prey organisms in order to reduce the risk of mortality also led to altered growth (Kohler & McPeek 1989), development (Werner et al. 1983), fecundity (Creel et al. 2007), emigration rate (Crowl & Covich 1994), or survival (Preisser et al. 2005). Their analysis also concluded that TMIs were generally as strong as, or stronger than DMIs, with a profound effect of intimidation in many cases. The authors caution that such strong TMIs are likely to complicate empirical assessments of predator-prey interactions, making it even more difficult for experimental designs to separate intimidation from direct consumption.

Given this knowledge that TMIs may be widespread and important, predator-prey models can no longer assume that consumption rates are a good predictor of a predator’s impact upon prey numbers in a population (Abrams 1995). Thus both DMIs and TMIs
must be considered when analyzing changes in prey densities in response to predators. Better experimental designs will be needed to differentiate risk-mediated effects from those of direct consumption, but this no easy task.

Effects of TMIs have been shown to be considerably stronger in aquatic systems than in terrestrial systems (Preisser et al. 2005). One possible explanation for this pattern is that aquatic organisms are better able to assess their level of risk due to more effective transmission of chemical cues through the water medium. This finding makes our study system ideal in examining the effects of TMIs upon organisms with keen chemosensory abilities, such as crabs and lobsters.

Measuring the impact of TMIs on prey population structure is often very difficult. Frequently, studies interpret a decrease in prey density in response to increased predator density as evidence for DMIs, but this may not always be the case (Peckarsky et al. 2008). Often there are alternative explanations that, on the surface, may seem to be direct predatory effects, yet actually turn out to be something else. For example, if predators cause prey to emigrate from an area, this change in local density may be a TMI that appears to be the result of consumption (Sih & Wooster 1994). Disregard of TMIs in this instance, could lead to an overestimation of the effects of direct predation upon this system. Thus, loss by predation must be distinguished from loss by emigration in order to develop models and make accurate predictions about a system.
Role of Predation on the Crustacean Community of Florida Bay

Florida Bay is a shallow-water nursery habitat for many species of marine fishes and invertebrates. This area is part of the Florida Keys National Marine Sanctuary and is composed of mostly hard-bottom, reef, or sand and seagrass habitat located in primarily shallow waters (~ 1 to 3 meters). The bay itself is a mosaic of soft- and hard-bottomed communities that exhibit extreme variability in terms of species richness. Nearshore live-bottom communities are characterized by a variety of low-relief corals, octocorals, sponges, and an abundance of algal cover (Chiappone & Sullivan 1994), while the shallow banks are covered with dense meadows of seagrasses such as *Thalassia testudinum, Halodule wrightii*, and *Syringodium filiforme* (Holmquist et al. 1989), all of which provide shelter and forage for a variety of organisms. An assessment of crustaceans by Holmquist et al. (1989) found 51 decapod and 2 stomatopod species, with many of these in great abundance. This same study showed a broad distribution of species, with many species spread across the bay. However, this community is susceptible to rapid habitat changes in the form of dredging, thermal stress, and natural destructive events that may affect populations of many species. In addition, waters in this area are prone to high turbidity and occasional eutrophic runoff from the Everglades National Park, which can cause widespread algal blooms (Fourquarean & Robblee 1999). Such destructive environmental events can be harmful to many species, but are especially difficult for host-specific species such as the anemone shrimp, *Periclimenes yucatanicus*, which may be more susceptible to rapid environmental change (Silbiger & Childress 2008). Though fragile and unstable, this unique ecosystem harbors a fascinating
assemblage of vertebrates, invertebrates, and plants that is unlike any other area in the southeastern United States. This diversity makes Florida Bay an ideal, as well as important, site for studying a variety of marine organisms that are subjected to a wide range of biotic pressures and abiotic conditions.

Several important commercial species, such as Caribbean spiny lobsters, *Panulirus argus*, utilize Florida Bay as their primary settlement habitat (Butler et al. 2006). Phyllosome larvae metamorphose offshore into puerulus larvae and eventually migrate shoreward to settle in the nearshore algae. These juveniles then grow and reach adult stage to migrate offshore themselves and spawn. Thanks to their abundance and widespread distribution, harvesting of lobsters has become the most economically valuable fishery in Florida today (Hunt 2001).

Previous studies have identified predation on early juvenile lobsters as a critical factor limiting the productivity of the Florida Bay nursery (Smith & Herrnkind 1992; Butler & Herrnkind 1997). These animals are preyed upon by a multitude of organisms including octopus, portunid crabs, bonnethead sharks, nurse sharks, sting rays, snapper, toadfish, bonefish, permit, and others (Smith & Herrnkind 1992). Studies have shown that pueruli use conspecific odor cues to choose settlement sites (Goldstein & Butler 2009). If juveniles are preyed upon too heavily, there may be less odor cues and, therefore, less likelihood of larvae settling upon a site (Zito-Livingston & Childress 2009). This can create a cascading effect of decreasing recruitment over time, limiting the nursery’s productivity. Early transitional phase lobsters must rely on anti-predatory
strategies in order to ensure their survival, thereby, eliminating a potential bottleneck to recruitment in juvenile populations.

One important predator of juvenile spiny lobsters is the Caribbean reef octopus, *Octopus briareus*, a common predator often found occupying the same type of crevice shelters as those used by juvenile lobsters and crabs (Bouwma & Herrnkind 2009). One study of *O. briareus* in a lagoon lake demonstrated that these animals preferred darker dens, with non-restrictive entrances, which they often close by constructing middens around the opening (Aronson 1986). Octopuses use their dens as refuges, points of ambush, and starting points from which they roam about and forage (Aronson 1986; Anderson 1997). Choice of shelter may likely increase interactions with those prey organisms that also prefer darker crevices, while decreasing interactions with those organisms that inhabit illuminated shelters. Both lobsters and many species of crabs also tend to inhabit these same types of shelters and are found to co-occur with octopuses throughout the tropics (Berger & Butler 2001; Butler & Lear 2009). This may cause conflict between predator and prey attempting to occupy the same shelters, especially in those areas where shelters are scarce. Exacerbating this effect is the fact that population densities of both octopuses and lobsters have been shown to be limited by shelter abundance (Aronson 1986; Butler & Herrnkind 1997). Although octopuses can occupy a variety of dens, den fidelity is usually low and rarely is any particular den occupied for very long (Mather 2008). This means that octopuses move around quite frequently, increasing the likelihood of encountering suitable dens that are already occupied by other octopuses or perhaps, prey. Generally, octopuses have a tendency to avoid cohabitation
with conspecifics, but residing in close proximity to prey could be beneficial. Since octopuses are known predators of a variety of crustaceans (Bouwma & Herrnkind 2009), these encounters will likely end in the consumption or displacement of prey species (Butler & Lear 2009). Lobsters and crabs, on the other hand, often share residences with conspecifics as well as a variety of other species. This is thought to be mostly attributed to reduced predation risk due to cooperative defense (Butler et al. 2001), but is also influenced by the availability of suitable shelters (Childress & Herrnkind 1997; 2001). For these animals, living in close proximity to their prey is also beneficial. In fact, both lobsters and octopuses are known to seek similar mollusk prey (Berger & Butler 2001). However, how does predator abundance influence den sharing behavior in prey species? Both lobsters and crabs use their acute chemosensory abilities to avoid those shelters that contain octopuses, both in the field (Bouwma & Herrnkind 2009) and in mesocosm experiments (Berger & Butler 2001; Butler & Lear 2009). Therefore, while there is a constant shifting of dens by octopuses, there also tends to be a redistribution of prey in accordance with octopus presence and potential increased predation risk. This can lead to a number of behavioral responses in prey, such as delayed emergence of algal-phase lobsters, higher aggregation densities of juvenile lobsters in areas of low shelter densities, abandonment of shelters by crabs, as well as many other possibilities. Therefore, a more complete understanding of the tradeoffs for prey in response to predation risk by octopuses is necessary for a more accurate depiction of the dynamics driving this community.
A recent manipulative experiment using artificial shelter blocks (Zito-Livingston & Childress 2009) provided an opportunity for detailed observations of the interaction between Caribbean reef octopuses and their potential juvenile lobster and crab prey. This experiment, originally intended for analyzing spiny lobster recruitment, added artificial shelter blocks to several nearshore sites across Florida Bay, providing additional refuges for octopuses as well as lobster. Since shelters are a limiting factor on both octopus and lobster density (Aronson 1986; Butler & Herrnkind 1997) and both animals are known to occupy the same shelter types (Berger & Butler 2001), these shelters likely increased populations of both types of organisms in the immediate area, therefore, increasing the amount of data that could be collected. The current study was carried out across these same eight sites, allowing for data accumulated during the original study to be combined with data from the current study.

Individual Variation in Octopus Temperament

Behavioral ecologists have recognized for many years that individuals in a population often show distinct behavioral phenotypes that are consistent across contexts and situations (Wilson et al. 1994). This has been well studied in, but not limited to, human behavioral research (Dall et al. 2004). In fact, heterogeneity of behavior among individuals of a population appears quite ubiquitously throughout the animal kingdom. These distinct behavioral phenotypes have been called “coping styles” (Koolhaas et al. 1999), “personalities” (Gosling 2001; Dall et al. 2004), “behavioral syndromes” (Sih et al. 2004), or “behavioral temperaments” (Reale et al. 2007). Past research in animal
behavior has often written off these individual differences as noise around a behavioral mean that has been shaped by selection as the optimal phenotype (Sih et al. 2004). However, recent research has begun to examine the importance of such individual differences and decided to view behavior in a more holistic manner, considering impacts of individual variation in behaviors upon life history traits and population level influences (Stamps 2007). Such individual differences can be advantageous, for they produce variation in fitness (Sneddon 2003, as cited in Frost et al. 2007), providing the raw material for natural selection to act upon (Clarke & Boinski 1995, as cited in Sinn et al. 2001). We realize the importance of this inherent variation when we consider that every important situation affecting survival requires a potentially different adaptive response. Such heterogeneity in behavior may allow populations to persist in rapidly changing conditions, whereas homogeneous behavioral phenotypes may lead to extinction when the environment changes markedly. It is important that we analyze these potential inter-individual differences in behavior that occur and evaluate the differential effects that each level or type may have upon a community. This will allow variability in behavior to be incorporated into current models, which will help develop more accurate analyses regarding behavior and its role in shaping a community.

In order for a behavior to be considered a “temperament” it must meet two requirements: (1) individuals of a population must differ in such behaviors, and (2) specific behaviors must be consistent and repeatable for individuals through time (Bell 2007). As one can imagine, these simple requirements can lead to many possible types of temperaments (Reale et al. 2007). One commonly accepted measure is the bold/shy
continuum. For example, Coleman and Wilson (1998) found repeatability and consistency of boldness in pumpkinseed sunfish, *Lepomis gibbosus*, while exposing fish to a threat stimulus. However, many temperaments other than boldness exist. Riechert and Hedrick (1993) identified the temperament of aggression in desert grass spiders, *Agelenopsis aperta*, under reproductive conditions. Individuals may also have correlated temperaments, which have been called behavioral syndromes (Sih et al. 2004). Huntingford (1976) found that temperaments governing activity and aggression in stickleback fish, *Gasterosteus aculeatus*, were positively correlated. In contrast to behavioral syndromes, studies supporting behavioral plasticity of temperaments are more numerous and such plasticity in behavioral response has been shown in a variety of species: pumpkinseed sunfish, *Lepomis gibbosus*, (Coleman & Wilson 1998), swamp sparrows, *Melospiza georgiana*, (Greenberg 1989), dumpling squid, *Euprymna tasmanica*, (Sinn & Moltschaniwskyj 2005), and rainbow trout, *Oncorhynchus mykiss*, (Wilson & Stevens 2005). These studies identified many temperaments, most of which appear to be context-specific. Still other studies provide mixed results. For example, Bell and Stamps (2004) found evidence for correlated temperaments between activity and boldness in one population of stickleback, *G. aculeatus*, but not another. All these studies have one thing in common; they demonstrate consistent individual variation in behavioral temperaments. This suggests that temperaments do exist and may constitute a considerable source of variation in natural populations.

So why are temperaments so important? It has been suggested that certain temperaments may be beneficial, advantageous, and possibly influence life history traits.
A study by Stamps (2007) found that temperament affects growth and mortality rate, with distinct temperaments having significantly different effects. Temperament differences could explain findings across a wide range of taxa, including fish, Salvelinus alpines, (Bjorklund et al. 2003), amphibians, Ambystoma macrodactylum, (Ragland & Carter 2004), reptiles, Eulamprus heatwolei, (Stapley & Keogh 2005), birds, Parus major, (Godin & Dugatkin 1996), and mammals, Mus musculus (Kirkpatrick & Lofsvold 1992), which have shown that different individuals have varying growth rates, despite optimal conditions for all.

Several studies have specifically considered the temperament of boldness; bold male guppies, Poecilia reticulata, were more attractive to females (Godkin and Dugatkin 1996), while bold killifish, Rivulus hartii, dispersed farther in the field (Fraser et al. 2001). Another study on fishing spiders, Dolomedes triton, identified a positive correlation between boldness and growth rate (Johnson & Sih 2005). Finally, increased boldness in bighorn sheep, Ovis canadensis, was discovered to be correlated with higher weaning success and better survival (Reale et al. 2000). These results across a wide variety of taxa suggest that boldness may be a preferred temperament. So then, if bold individuals seem to acquire the greatest benefit from their temperament, how are shy individuals maintained in populations? One explanation may be that both temperaments have equal payoffs. While bold individuals were found to learn more quickly (Frost et al. 2007), they were also more active and took more risks, which may have a negative effect on survival and fitness. It has also been proposed that temperamental variation can be good for populations in combating predation (Sterrer 1992), suggesting this variation may
provide potential prey with a protean search image that makes them less easily identifiable by predators. If potential prey behave variably, then predators cannot easily key on specific behavioral characteristics in their predatory search. Other plausible explanations exist concerning temperamental variation within populations, many of which deserve further investigation.

In this study I concentrated on the specific temperament of boldness, for it is widespread and easily identifiable. Although boldness, along with its consequences, has previously been identified in several species, including octopus, this has most often been analyzed from the prey’s perspective (except for the spider examples mentioned above). To my knowledge, no study has adequately assessed the influence of a predator’s temperament on its demographic impact upon prey. This top-down approach to examining intimidation effects within this community may help to identify relationships and consequences that would otherwise remain unclear. Moreover, analyzing behavioral discrepancies between individual predators and the consequences thereof may help to explain their role within the ecosystem more completely. Building upon this knowledge, existing models of predator/prey interactions can be revised to incorporate my findings so that a more accurate portrayal of the system is developed.

Cephalopods, like many other animals, have remarkable behavioral variation among individuals (Hanlon & Messenger 1996). These variations, or temperaments, tend to manifest themselves early during development, may be modified by experience, and can have a profound effects upon life history traits (Sinn et al. 2001). This pattern has been well-documented in the dumpling squid, Euprymna tasmanica. Studies have shown
high degrees of variation between individual dumpling squid across a wide array of contexts. Moreover, consistent, individual variation in shy versus bold behavior has been identified in multiple studies (Sinn & Moltschaniwskyj 2005; Sinn et al. 2006, 2007), with squid often showing context-specific behaviors and no across-context correlations between treatments (Sinn & Moltschaniwskyj 2005; Sinn et al. 2007). Sinn et al. (2007) found consistent, individual variation between squid concerning boldness in both threat and feeding tests, yet these behaviors were not correlated across the two experimental contexts. These same studies also discovered that mature adults displayed the most consistent behaviors, which is not surprising. However, a unique pattern was discovered between contexts. As individuals matured they displayed more boldness in threat tests while becoming shyer in feeding tests. This finding illustrates behavioral plasticity in squid and may provide supporting evidence that learning is important in the development of these animals. As animals mature they gain experience, which may shape behaviors appropriately according to context. Since not all animals have the same life experiences, their uniqueness may result in part from these differences. Some studies also suggest a genetic component of temperament, as well as, an influence of temperament on life history traits. Sinn et al. (2006) found significant heritability of behaviors in one context (threat test), but not in another (feeding test). Therefore, they concluded that the genetic contribution to behavior in squid varied across contexts. This same study made some interesting observations involving temperament and life history traits. The authors found that shyer females in the feeding test tended to be larger and this size increase resulted in increased brood hatching success. In addition they also discovered that fertilization
success increased between males and females with similar foraging shy/bold phenotypes (Sinn et al. 2006). These findings provide evidence that behavioral variation in squid may have consequences that can affect life history and may be heritable. This may provide some explanation for how different temperaments can be maintained in populations by natural selection.

For over a decade, studies of octopus behavior have provided evidence for the existence of behavioral temperaments. Mather and Anderson (1993) found large, individual differences in Octopus rubescens across alert, threat, and feeding contexts that were consistent over a two week period. A separate study by Sinn et al. (2001) found that discrete behaviors of O. bimaculoides could be reliably grouped into dimensions of temperament over the first nine weeks of life. Therefore, both of these studies provide some support for the establishment of temperaments in octopuses.

Another possible characteristic of individuals possessing distinct temperaments is the presence of learning (Fiorito & Scotto 1992). Studies in squid suggest that learning may be a key component in shaping the temperaments of individuals (Sinn et al. 2007). Evidence has also shown variation in behavior among octopuses to be highly dependent upon learning (Mather & Anderson 1993), and this may be caused by exploration and play (Mather & Anderson 1999). Octopuses are known to be curious organisms that often explore. This exploratory behavior is a natural way of creating situations in which learning is likely to take place. Since octopuses are said to be highly dependent on learning, it follows that experience should be a driving force behind individual differences (Mather & Anderson 1993).
Octopuses are incredibly sophisticated, highly intelligent, generalist predators equipped with an arsenal of prey-capturing abilities; making them a top predator in many marine benthic communities (Budelmann 1994). These animals have great dexterity in their arms, a sharp and powerful beak, an amazing cryptic ability, powerful cephalotoxins, and outstanding visual acuity, with an eye structure similar to that of vertebrates (Hanlon & Messenger 1996). Quite possibly their greatest asset, however, is their superior intelligence (Mather 2008). It is this extreme intelligence that continues to intrigue scientists and has allowed the octopus to thrive in marine communities primarily dominated by piscine predators. The cognitive abilities of octopods allow these animals to solve problems and determine plans of action that overcome possible shortcomings of their anatomy or physiology (Mather 2008). This ability has also allowed them to persist over time and establish their role as a keystone predator in a variety of marine ecosystems. These attributes may make them prime candidates for possessing varying temperaments, which may have far-reaching impacts upon the communities in which they live.

The ability to learn, especially in relation to capturing and handling different types of prey, may lead to individual variation in the predatory ability of octopuses that feed on lobsters and crabs in Florida Bay. Octopuses are predators with many options when it comes to methods of prey capture. They may ambush prey by pouncing upon and subduing it, using their superior quickness and dexterity; they may stalk prey out in the open by incorporating camouflage, or may simply crawl across the bottom while reaching into nearby crevices in search of unsuspecting prey items. With such a variety of
techniques at their disposal, it seems only reasonable that they would choose which one to use according to the situation. For example, one observation showed that foraging tactics of octopuses varied according to substrate type, and that octopuses tended to use methods and approaches most suitable for each type (Forsythe & Hanlon 1997). Since octopuses live in such diverse habitats, this could lead to variation between individuals in preferred methods of prey capture. When it comes to prey handling octopuses have just as many choices. These generalist foragers often exhibit considerable individual variation in choice of prey (Mather 1991), which may actually drive the need for diverse capture techniques. This has often been found to depend upon the size of prey in relation to the octopus. Anderson and Mather (2007) found that prey selection of several types of mollusk prey by octopus, *Enteroctopus dofleini*, depended on the ease of removal from their shells. A similar decision was made by octopuses in a separate study when presented with crabs of different sizes (Grisley et al. 1999). Smaller octopuses tended to puncture the eyes of larger crabs for easier penetration and manipulation, whereas larger octopuses simply punctured the carapace of smaller crabs by boring. This is most likely due to some perceived energy tradeoff between energy intake and reduced risk of injury during prey handling (Anderson & Mather 2007). These observations suggest that octopuses make decisions according to the situation, possibly using trial and error learning. New discoveries in this area will undoubtedly increase our understanding of octopuses’ capabilities in relation to temperament, so that we may then apply this knowledge to analyze the potential impacts of temperament on predation strategies.
Research Objectives

For this Master’s thesis, I examined DMIs and TMIs between Caribbean reef octopuses and their prey; juvenile spiny lobsters and crabs. My first objective was to measure the response of lobsters and crabs to variation in octopus density, size and boldness. My second objective was to identify whether octopuses possess distinct behavioral temperaments via adaptive plasticity or fixed behavioral syndromes in order to better understand the role of predator behavior in these interactions with their prey.
CHAPTER TWO

DENSITY VS. TRAIT-MEDIATED EFFECTS OF OCTOPUSES UPON PREY

Introduction

Traditional studies of predator-prey relationships have taken the approach of analyzing the interaction from the perspective of the prey species involved (Abrams 1995). This has led to better understanding of what happens within predator-prey dynamics, but does not answer the question why. Much of a prey’s behavior is influenced by the behavior of their predators (Lima 1998). Prey often make drastic changes in behavior to evade, hide from, or even avoid certain predators altogether. Alternatively, they may tolerate predators in exchange for favorable living conditions among valuable, limiting resources. In addition to behavioral changes, physiological changes in prey may also occur in response to predation pressures. In fact, cascading effects of predator behavior may be far-reaching, affecting multiple species of prey across a wide range of trophic levels (Creel & Christianson 2008). Examination of these behavioral interactions, including variation in predator behavior, is essential for understanding the full impact of predation on community structure (Peckarsky et al. 2008).

The Caribbean reef octopus, Octopus briareus, is an important predator of juvenile lobsters, Panulirus argus, and crabs, Callinectes sapidus, Callinectes similis, Menippe mercenaria, Mithrax spinosissimus, Portunus ordwayi, and Portunus sebae throughout the Caribbean (Aronson 1986; Childress 1995; Weiss et al. 2008). In Florida Bay, juvenile lobsters, crabs and octopuses compete for crevice shelters in the sponge-soft coral hardbottom community (Childress & Herrnkind 1997). Previous studies have
found that juvenile lobster density is negatively correlated with octopus density (Berger & Butler 2001). Lobsters actively avoid shelters with the odor of octopus predators (Berger & Butler 2001). Furthermore, lobster survival was negatively correlated with octopus density (Butler & Lear 2009). These studies conclude that the major impact of octopus predators on juvenile spiny lobster prey is a direct effect rather than a risk effect. However, there are several reasons why this conclusion might be premature. First, another study conducted in Florida Bay failed to find a negative correlation between octopus density and lobster density (Childress & Herrnkind 1997), providing conflicting results. Second, previous studies (Berger & Butler 2001; Butler & Lear 2009) only estimated lobster and octopus density from artificial structures, disregarding natural ones, which could conceal true effects. If lobsters avoid blocks with octopus odors, they may be utilizing sponges, corals or solution holes, not censused during these studies. Finally, no previous study has examined either the direct or risk effects on alternative prey species such as crabs.

If Caribbean reef octopuses have a non-lethal effect on their prey, it has the potential to influence prey density, distribution and willingness to share crevice shelters. Furthermore, this trait-mediated interaction may also be influenced by octopus size or boldness. Previous studies have linked boldness to risk-taking behavior in guppies (Godin & Dugatkin 1996), spiders (Johnson & Sih 2005), rainbow trout (Wilson & Stevens 2005) and sticklebacks (Bell 2007), but to date, no study has measured the potential risk effect of predator boldness on prey behavior and demography.
In this study, I tested four specific hypotheses regarding the influence of octopuses on lobster and crab prey density and survival to determine if there is a direct effect, a risk effect or both (Table 2.1). For each hypothesis, I evaluated the impact for eight replicate study sites, each 25 m X 25 m (regional scale) with 10 artificial blocks (local scale), totaling 80 artificial shelter blocks, with and without an octopus. Evaluation of prey response at these two scales allowed me to distinguish between predator density-mediated direct effects, which should operate at regional scales, and trait-mediated risk effects that operate at local scales (Table 2.1).

Table 2.1 Hypotheses and predictions supporting direct effect and risk effects of octopuses on lobster and crab prey.

<table>
<thead>
<tr>
<th>Hypothesis</th>
<th>Prediction</th>
<th>Support for direct effect</th>
<th>Support for risk effect</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>H₁</strong>: Prey density decreases with increasing octopus density</td>
<td>Negative correlation at regional scale</td>
<td>√</td>
<td>√</td>
</tr>
<tr>
<td></td>
<td>Prey density depends on octopus presence</td>
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<td></td>
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<tr>
<td><strong>H₂</strong>: Prey survival decreases with increasing octopus density</td>
<td>Negative correlation at regional scale</td>
<td>√</td>
<td>√</td>
</tr>
<tr>
<td></td>
<td>Prey survival depends on octopus presence</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>H₃</strong>: Prey density decreases with increasing octopus size</td>
<td>Negative correlation at regional scale</td>
<td>√</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Den sharing depends on octopus size</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>H₄</strong>: Prey density decreases with increasing octopus boldness</td>
<td>Negative correlation at regional scale</td>
<td>√</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Den sharing depends on octopus boldness</td>
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</tbody>
</table>
Methods

Study Site

My study was carried out in Florida Bay, located in the middle Florida Keys in part of the Florida Keys National Marine Sanctuary (Figure 2.1A). Across Florida Bay, eight study sites (25 m x 25 m) were selected in shallow (~ 1 to 3 m depth), nearshore, hardbottom habitats with plentiful sponges and soft corals (Figure 2.1B). Each site received ten concrete blocks (39.5 cm x 19.5 cm x 29.0 cm), each with four openings (13.5 cm x 19.5 cm x 7.0 cm), which served as artificial shelters and were randomly distributed across the site. Blocks housed a variety of animals including octopuses < 275 g, lobsters < 70 mm carapace length, and several species of crabs < 135 mm carapace width. Surveys were conducted in Feb-July 2006, June 2007, Oct 2007, Mar 2008, and May-July 2008. Water temperatures ranged from 15 to 30 °C and salinity fluctuated from 34 to 37 ppt.

Population Census

In each monthly survey, the number of crabs, lobsters, and octopuses present in natural and artificial shelters were visually censused by divers and recorded underwater on data sheets, following the protocol of Livingston-Zito and Childress (2009). Crabs were separated into three categories: spider crabs, *M. spinosissimus*, swimming crabs, *Callinectes* spp. & *Portunus* spp., and stone crabs, *M. mercenaria*. All lobsters were captured and returned to the boat where they were measured, sexed, examined for signs of injury and disease, and then released back upon the site. Octopuses were located and
dens were marked, so that divers could return and perform a field threat test. Immediately following the threat test, octopuses were captured, sexed, weighed, and either kept for lab experiments or returned to their dens upon the site.

Figure 2.1 Location and diagram of field sites. Field data were collected in Florida Bay, part of the Florida Keys National Marine Sanctuary (A). Sites (indicated by circles) were spread across nearshore habitats at eight different locations (B), sites were located 250-750 m from shore at 1.5-2.5 m depth (C). Each site (25 m X 25 m) was further subdivided into 5m X 5m cells by perpendicular transect lines. This grid allowed for accurate mapping of all available natural and artificial shelters. Artificial shelter blocks were randomly placed in 10 of the 25 cells of each site (D).
Crab Tethering Experiment

To determine if octopus density had a direct impact on the survival of potential prey species (i.e. direct predation), I conducted a tethering experiment using a novel prey type, the fiddler crab, *Uca pugilator*. This novel prey species was chosen because octopuses quickly learned to subdue this prey in the lab, but were not likely to have differences in previous experience handling fiddler crabs across my sites. Only adult crabs were used, both male and female. To reduce potential predation by piscine predators, I tethered crabs inside a 30 cm length of 5 cm diameter PVC pipe glued to a smooth brick. This device excluded larger predators, while allowing access by octopuses. Clear, monofilament fishing line (50 lb. test) was threaded through the PVC and loops tied on each end, with a swivel in the center to connect the two ends of the fishing line. Fiddler crabs were then tethered to a separate, short section of clear, 4 lb. test fishing line (approximately 10 cm long) by gluing one end of the line to the crab’s carapace and tying a loop in the opposite end. This assembly was then fastened to the swivel of the tethering device, allowing the crabs approximately 10 cm of movement, yet anchoring them to the middle of the pipe. Lastly, the swivel was positioned inside the PVC pipe, approximately mid-pipe, to necessitate octopus entry into the device in order to attack the crab.

I placed a tethered crab one meter away from each artificial shelter block (N = 80) in June 2007 and again in June 2008. Each device remained in place for two days, upon which divers returned and examined each tether. Tethered crabs were assigned one of four possible fates: eaten (the crab had been removed, with only a small piece of carapace still glued to the line), dead (the crab remained intact, yet was no longer living), alive (the
crab remained attached to the tether and was still alive), or missing (the crab was no longer present and no portion of the carapace remained glued to the line or the tether appeared to have come undone, with the crab no longer present). Tethered crab status for each artificial shelter block was recorded along with the presence or absence of an octopus predator.

Field Threat Test

To assess variation in octopus behavioral temperament, simulated threat tests were conducted in June-July 2006, June 2007, Oct 2007, Mar 2008, May-July 2008, and Oct 2008. The threat tests were implemented by a diver making contact with an octopus using a fiberglass rod and observing the response of each animal (N = 157). These tests were videotaped underwater and later analyzed. Fiberglass rods consisted of yellow, 7 mm diameter, 450 mm long, cylindrical rods, typically used in lobster capture. During experiments, a fiberglass rod was inserted into the portion of a den containing an octopus until contact was made. Threat tests were then scored on a five point scale of boldness (Table 2.2) modeled after a previous study by Mather and Anderson (1993). The scored responses ranged from rapid escape (-1) to vigorous pulling (+3).

Field threat tests were administered in replicates of 5 contacts, with a rest period of 10 seconds in between. This allowed me to analyze both the initial response and the repeatability of response for each individual. Once the in situ test was complete, the number and type of any co-residents in the crevice shelter with the octopus were recorded. The position and type of shelter was also noted on a detailed map of the site.
Beginning in June 2007, octopuses were collected by hand net and returned to the boat. I recorded the gender and weight (to the nearest 0.1g) for each individual and then returned it to the crevice shelter from which it was captured (N = 70).

Table 2.2 Ethogram of Threat Test for Boldness

<table>
<thead>
<tr>
<th>Boldness score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>-1</td>
<td>Quickly jets or crawls away from shelter. Avoids contact with fiberglass rod.</td>
</tr>
<tr>
<td>0</td>
<td>Slowly leaves shelter. Crawls into a nearby shelter. Avoids contact with fiberglass rod.</td>
</tr>
<tr>
<td>+1</td>
<td>Holds fiberglass rod without pulling it. Moves within shelter but does not leave shelter. Allows fiberglass rod to contact body.</td>
</tr>
<tr>
<td>+2</td>
<td>Grasps fiberglass rod. Pulls slightly (only 1-3 pulls).</td>
</tr>
<tr>
<td>+3</td>
<td>Grasps fiberglass rod. Pulls vigorously (3+ pulls). Reaches outside of shelter.</td>
</tr>
</tbody>
</table>

Statistical Analyses

To analyze the regional scale relationship between lobster and crab density vs. octopus density, size, and boldness, and crab survival vs. octopus density, I conducted linear regression analyses. Both lobster and crab count data were squared-root-transformed in order to meet the assumption of normality of residuals. To analyze the local scale relationship between lobster and crab density vs. octopus presence/absence per shelter block, I conducted a one-way ANOVA. To analyze the local scale relationship between lobster and crab presence/absence vs. octopus presence/absence, octopus size class (S,M,L), and octopus boldness (-1,0,1,2,3), and crab survival vs. octopus
presence/absence, I conducted log likelihood ratio tests. All analyses were performed using JMP (version 5, Cary, NC, USA).

Results

Influence of Octopus Density on Prey Density

If predators are directly consuming significant numbers of prey, the density of predators and prey should be inversely related (Preisser et al. 2005). To assess whether this was the case in Florida Bay, census data from 8 sites sampled 13 times were used to determine whether or not a correlation between the densities of lobsters and crabs and the density of their octopus predators at a regional scale existed (Figure 2.2). Results of linear regression analysis found no significant negative relationship between octopus density and lobster density (F = 2.66, df = 1, 102, p = 0.106, r² = 0.016). However, there was a significant negative relationship between octopus density and crab density (F = 24.75, df = 1, 102, p < 0.0001, r² = 0.187).

To determine the risk effect of octopuses on lobsters and crabs at the local scale, the presence of an octopus and the density of crabs and lobsters were analyzed per individual shelter block. The average number of crabs inhabiting shelter blocks containing octopuses was 0.26/block (± 0.089) versus shelter blocks without octopuses 0.70/block (± 0.29), a statistically significant difference (t = 6.56, df = 118, p < 0.0001). This same trend occurred for lobsters, with blocks containing octopuses having fewer lobsters, 1.15/block (± 0.192), than those without octopuses 1.55/block (± 0.063). This difference was also statistically significant (t = 2.65, df = 102, p = 0.0083).
Another way to estimate the risk effect of octopus presence on lobster and crab presence is by a comparison of shelter block occupancy (Figure 2.3). In the absence of an octopus, over 95% of all blocks were either occupied by a lobster, a crab or both. These proportions decreased dramatically (< 60%) when an octopus was present. Log likelihood ratio tests found both lobsters ($\chi^2 = 9.168$, df = 1, p = 0.0025) and crabs ($\chi^2 = 24.883$, df = 1, p < 0.0001) significantly reduced their use of shelter blocks when an octopus was present.

**Figure 2.2 Influence of octopus density on prey density (regional scale).** Lobster density (open squares) was not related to octopus density but crab density (gray diamonds and gray solid line) was significantly and negatively related to octopus density.
Influence of Octopus Density on Prey Survival

Eight sets of 10 fiddler crabs each, were individually tethered one meter from each artificial shelter block in June 2007 and again in June 2008. After 48 hours, the fate of each individual crab was classified as alive, missing, dead on tether, or eaten (Figure 2.4). Minimum survival rate was determined by combining eaten, missing, and dead crabs categories, illustrating the most conservative estimate of crab survival. Maximum survival rate was determined by using only those tethered crabs that had been eaten, illustrating a less conservative estimate of crab survival. Therefore, the actual survival rate is likely some value in between these two estimates. Linear regression analysis found neither the maximum survival estimate ($F = 1.44$, df = 1, 14, $p = 0.250$, $r^2 = 0.028$) nor the minimum survival estimate ($F = 0.46$, df = 1, 14, $p = 0.507$, $r^2 = 0.000$) were related to octopus density.

![Figure 2.3 Influence of octopus presence on prey presence (local scale).](image-url)
I also examined the tethering results for all 160 fiddler crabs on the basis of each individual shelter block. The same four categories: eaten, missing, alive, and dead on tether, were used, with data separated categorically according to the presence/absence of a resident octopus in each particular shelter block. The log likelihood ratio test of tethering outcomes did not differ between blocks with an octopus present vs. blocks with an octopus absent ($\chi^2 = 1.482, \text{df} = 3, p = 0.686$).

Figure 2.4 Influence of octopus density on tethered crab survival (regional scale). Maximum estimate of % survival (open squares) were eaten crabs only. Minimum estimate of % survival (gray diamonds) were eaten, missing or dead crabs. Neither measure of survival was found to be related to octopus density.
Influence of Octopus Size on Prey Distribution

If octopus presence influences prey presence, so might octopus size. Presumably larger octopuses are more dangerous predators and may potentially have greater risk effects. To examine this prediction, I compared the average octopus weight per site vs. the density of lobsters and crabs per site. Results of linear regression analysis found no significant relationship between octopus weight and lobster density ($F = 0.51$, $df = 1, 18$, $p = 0.483, r^2 = 0.000$). However, there was a significant negative relationship between octopus weight and crab density ($F = 5.89$, $df = 1, 18$, $p = 0.026, r^2 = 0.205$).

Figure 2.5 Influence of octopus density on tethered crab survival (local scale). Each tethered fiddler crab was classified as alive, eaten (evidence of carapace still present on tether), missing, or dead on tether.
To determine the risk effect of octopus size on lobsters and crabs at the local scale, I examined the co-denning of lobsters and crabs with octopus residents in the shelter blocks. Each octopus was categorized into one of three size classes: small = 0 to 50 g; medium = 50 to 100 g; large = >100 g. The ratio of blocks occupied by crabs and lobsters was examined according to size class of the resident octopus (Figure 2.7). Log likelihood ratio tests found neither lobster ($\chi^2 = 0.95$, df = 2, $p = 0.953$) nor crab ($\chi^2 = 0.61$, df = 2, $p = 0.736$) occupancy rates were influenced by the size of the octopus present in the shelter block.
Influence of Temperament on Prey Distribution

If octopus presence influences prey presence, so might octopus temperament. Bolder octopuses could be more dangerous predators and, therefore, could potentially have greater risk effects. To examine this prediction, I measured the boldness of each octopus resident on a categorical scale from -1 (shy) to +3 (bold). I then compared the average octopus boldness per site vs. the density of lobsters and crabs per site. Results of linear regression analysis found no significant relationship either between octopus boldness and lobster density ($F = 0.04, \text{df} = 1, 39, p = 0.846, r^2 = 0.000$), or octopus boldness and crab density ($F = 0.41, \text{df} = 1, 39, p = 0.527, r^2 = 0.000$).

Figure 2.7 Influence of octopus size on prey distribution (local scale). Resident octopus predators were classified into small (< 50 g), medium (50-100 g), or large (>100 g). The ratios of blocks occupied by lobsters or crabs were not influenced by the size class of the resident octopus.
To determine the risk effect of octopus boldness on lobsters and crabs at the local scale, I examined the co-denning of lobsters and crabs with octopus residents in the shelter blocks. Each octopus was categorized into one of five boldness categories: -1, 0, 1, 2, or 3. The ratio of blocks occupied by crabs and lobsters was examined according to boldness category of the resident octopus (Figure 2.9). Log likelihood ratio tests found neither lobster ($\chi^2 = 4.04$, df = 4, $p = 0.401$) nor crab ($\chi^2 = 1.59$, df = 4, $p = 0.811$) occupancy rates were influenced by the boldness of the octopus present in the shelter block.

Figure 2.8 Influence of octopus boldness on prey distribution (regional scale). Average octopus boldness across sites was calculated according to threat test boldness score. Neither lobster (open squares) nor crab densities (gray diamonds) were affected by octopus boldness across sites.
Overall, I found evidence in support of both direct effects and risk effects of octopuses on their prey, particularly for crabs (Table 2.3). Direct effects were only found to influence prey density, not prey survival, as no effect of octopus density was found on fiddler crab survival in my tethering experiment (Figure 2.4). The influence of octopus predators on crabs and lobsters differed in significant and important ways. Crab density decreased in response to octopus density and size, whereas lobster density did not. However, both lobsters and crabs showed local scale effects, such as reduced use of shelters containing octopus predators, a predictable result of predator risk effects (Table 2.3).

Figure 2.9 Influence of octopus boldness on prey distribution (local scale).
Resident octopus predators were characterized on an increasing boldness scale from -1 (shy) to +3 (bold). The ratios of blocks occupied by lobsters or crabs were not influenced by the boldness of the resident octopus.
Table 2.3 Summary of results in support of direct effect and risk effects of octopuses on lobster and crab prey. Checks indicate statistically significant support, Xs indicate no support.

<table>
<thead>
<tr>
<th>Hypothesis</th>
<th>Prediction</th>
<th>Support for direct effect</th>
<th>Support for risk effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>H1: Prey density decreases with increasing octopus density</td>
<td>Negative correlation at regional scale</td>
<td>X-Lobsters</td>
<td>√-Lobsters</td>
</tr>
<tr>
<td></td>
<td>Prey density depends on octopus presence</td>
<td>√-Crabs</td>
<td>√-Crabs</td>
</tr>
<tr>
<td>H2: Prey survival decreases with increasing octopus density</td>
<td>Negative correlation at regional scale</td>
<td>X-Crabs</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Prey survival depends on octopus presence</td>
<td>X-Crabs</td>
<td></td>
</tr>
<tr>
<td>H3: Prey density decreases with increasing octopus size</td>
<td>Negative correlation at regional scale</td>
<td>X-Lobsters</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Den sharing depends on octopus size</td>
<td>√-Crabs</td>
<td></td>
</tr>
<tr>
<td>H4: Prey density decreases with increasing octopus boldness</td>
<td>Negative correlation at regional scale</td>
<td>X-Lobsters</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Den sharing depends on octopus boldness</td>
<td>X-Crabs</td>
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</table>

Influence of Octopus Density on Prey Density

Crab density decreased on sites containing a higher number of octopuses, whereas lobster density did not. A negative correlation in density between predator and prey may be the result of direct consumption or may be the result of a risk effect leading to emigration or increased predation by another predator species (Creel & Christianson 2008). Clearly, crabs appear to be more strongly influenced by octopuses than are juvenile spiny lobsters. Octopus will prey on both crabs and lobsters and have been videotaped hunting and killing them at night (Weiss et al. 2008). Previous studies of the
density relationship between octopuses and spiny lobsters in Florida Bay have found conflicting results. Childress and Herrnkind (1997) found no correlation between octopus density and juvenile spiny lobster density. However, Butler and Lear (2009) did find a significant negative correlation. One possible explanation for this discrepancy may have to do with how prey density was determined in different studies. My methods followed those of Childress and Herrnkind (1997) in which crab, lobster and octopus densities were calculated by a fixed area searched (625 m$^2$). In contrast, Butler and Lear (2009) estimated density using an average number of lobsters and octopuses per artificial shelter block, regardless of area searched or the total number of shelter blocks per site, as shelter block density varied from site to site. As a result of this method, lobsters and octopuses occupying natural structures (sponges, octocorals, solution holes, etc.) were not included in the Butler and Lear (2009) density estimates.

I also found that the average number of crabs and lobsters occupying an individual shelter block decreased when a resident octopus was present. This supports the observations of Berger and Butler (2001) that shelter blocks containing octopuses housed significantly fewer juvenile lobsters. Traditionally this would have been considered evidence for a density-mediated interaction (DMI) as the most parsimonious explanation (Creel & Christianson 2008). However, it is possible that a risk effect could also produce these same results. Berger and Butler (2001) experimentally demonstrated that given a choice, juvenile lobsters avoid choosing shelter blocks that contain the odor of a resident octopus.
To distinguish between direct predation effects and risk effects, I compared regional scale data with that of local scale and looked for patterns that emerged. This separation between regional and local scales allowed me to identify effects of octopuses upon prey that would otherwise be masked by averaging site data. Comparison of the local scale data with that of the regional scale showed some similarities; the density of crabs decreased as octopus density increased on both scales. Therefore, octopus may exert both DMIs and TMIs upon crabs. However, this does not seem to be the case for lobsters, especially since there was no significant effect of octopus density on lobster density at the regional scale. This comparison suggests that although local scale patterns show fewer lobsters cohabitating with resident octopuses, octopus density did not influence their overall density upon a site. This suggests that lobsters excluded from artificial shelter blocks may have sought alternative natural crevice shelters such as solution holes, sponges or octocorals (Livingston-Zito & Childress 2009), providing possible support for a risk effect of octopus predators on lobsters.

Influence of Octopus Density on Prey Survival

To separate the effects of direct predation versus risk, a tethering study was performed. By determining the amount of predation due to octopus predators upon sites, I could identify whether the decreased crab abundance on those sites with greatest octopus density was a direct predation effect. This study attempted to limit predation by other predator species by designing a tethering device that could restrict access to the prey. My intent was to limit access by any predator other than octopuses, but to do so in such a way
that octopuses had easy entry. Results were partitioned categorically so that different
styles of predation could be assessed. For example, octopuses typically removed the
entire crab from the tether (personal observation from laboratory specimens), whereas
other predator species (e.g. fish, stomatopods, other crabs, etc.) tended to destroy or crush
crabs, leaving portions of the carapace and appendages behind. From the four possible
outcome categories, I constructed a maximum estimate (consisting of any death category)
and a minimum estimate (consisting of the eaten category only). Neither the maximum
nor the minimum estimate of crab predation per site was related to average octopus
density. Furthermore, the frequency of outcomes did not vary among those crabs tethered
in front of shelter blocks with or without a resident octopus. This suggests that direct
predation by octopuses may not fully explain the negative correlation observed between
crab density and octopus density, substantiating the need to consider risk effects.

Previous studies conducted in Florida Bay have also found conflicting results.
Childress and Herrnkind (2001) found no correlation between octopus density and
tethered lobster survival, but Butler and Lear (2009) found a negative correlation. My
study followed the methods of Childress and Herrnkind (2001) where tethered crabs were
hidden inside of crevice shelters, whereas Butler and Lear (2009) tethered juvenile
lobsters without shelter. Since it is not possible to know for certain if a tethered lobster
was killed by an octopus or fish predator, and previous studies have shown relative
predation rates to be much higher when lobsters are tethered without shelter (Smith and
Herrnkind 1992), these differences between my results and those of Butler and Lear
(2009) may be due to differences in experimental design.
With direct predation unrelated to octopus density, any decreased crab abundance on sites containing high numbers of octopuses may potentially be the result of risk effects of octopus predators. The use of fiddler crabs to estimate direct predation may have been an inappropriate surrogate for actual stone crabs, swimming crabs, spider crabs and juvenile lobsters. However, given that octopuses consistently eat fiddler crabs in the laboratory, it is unlikely that this is the case. Alternatively, the negative correlation between octopus density and crab density might be due to competition for shelter blocks and the increased risk of predation by other predators that crabs experience if displaced from crevice shelters. This is likely to have a greater impact on crabs than on juvenile lobsters that regularly share shelters with conspecifics and utilize more shelter types than do most crabs (Childress 1995).

Influence of Octopus Size and Boldness on Prey Distribution

Local scale effects suggest risk effects of octopus predators on crabs and lobsters. How might individual characteristics of octopus predators (i.e., size or boldness) influence prey density or shelter occupancy? If intimidation by octopus predators is an important influence upon prey density or shelter occupancy, then octopus size should play an important role. My predictions were that octopus size would have a negative correlation with prey density at both regional and local scales.

At the regional scale, there was a significant negative correlation between the average octopus size and crab density but no relationship with lobster density (Figure 2.6). These results indicate that average octopus size is a good predictor of the number of
crabs inhabiting those sites. However, the precise reason for octopus size influencing crab density is not known. It could be that larger octopuses eat more crabs or produce more odor cues, leading to greater intimidation effects. Since average octopus size was not a significant indicator of lobster density, the relationship between octopus size and lobster density across sites is different than that between octopuses and crabs. Lobsters are known to avoid octopus odor cues (Berger & Butler 2001), and larger octopuses should leave more odor cues, which may suggest a risk effect. However, since average octopus size was not a significant indicator of lobster density, the risk effect explanation may not explain these results.

Despite finding a significant negative correlation between octopus size and crab density on the regional scale, I did not find any significant influence of octopus size on crab co-dennning at the local scale. Perhaps this was due to the fact that so few crabs were willing to share dens with octopuses of any size (N = 20) and those that did share shelters were at similar risk regardless of the size of the resident octopus. The same may be true for lobsters, as the likelihood of co-dennning was similar for all sizes of octopus residents (N = 41). My results again differ from those of Berger and Butler (2001) and Butler and Lear (2009), whose studies were conducted in the same general region, but did not observe lobsters co-dennning with any octopus. Contrary to these reports, I found the frequency of co-dennning with an octopus to be 48.2% for lobsters and 23.5% for crabs (N = 85). These frequencies seem rather high if lobsters and crabs are actively avoiding octopus predators. However, co-dennning lobsters and crabs may be making the best of a bad situation if the there are no alternative crevice shelters available in the nearby
vicinity. Lobsters without a crevice shelter have been shown to be 3-10 times more likely to be killed by a predator than those in crevice shelters (Smith & Herrnkind 1992; Eggleston and Lipcius 1992).

Given the lack of an octopus size effect on lobster and crab co-denning, it is not surprising that octopus boldness was also not significant. Lobsters and crabs are probably at risk from both shy and bold octopuses; especially those in such close proximity as the same shelter block. Although some previous studies have examined the influence of octopus boldness on predatory behaviors (Sinn et al. 2001; Anderson & Mather 2007), this may be the first study to attempt to quantify this effect in the field.

On both regional and local scales, octopus boldness was not a significant effect on prey density or co-denning. Although boldness has been suggested to be important to fitness in general (Godkin & Dugatkin 1996; Reale et al. 2000) and predatory ability specifically (Fraser et al. 2001; Johnson & Sih 2005), it may be that octopus boldness is not easily detected by prey and, therefore, is not a reliable indicator of predation risk.

Conclusions

This study provides evidence that octopus density does in fact influence prey density in the hardbottom community of Florida Bay. My results show that this effect is not likely the result of direct predation but rather the effect of intimidation, resulting in decreased use of shelter blocks and potentially an increase in predation by other predators. The risk effects of an octopus appear not to be related to either size or boldness, which may not be reliable indicators of risk to lobster and crab prey.
CHAPTER THREE
OCTOPUS BEHAVIORAL TEMPERAMENTS: ADAPTIVE PLASTICITY OR BEHAVIORAL SYNDROME?

Introduction

Evolutionary theory predicts that animals should perform behaviors that maximize their fitness for any environmental condition in which they occur. Individuals that possess such adaptive plasticity should adjust their behaviors in response to rapidly changing conditions like encountering a potential predator (Wilson et al. 1994). However, recent studies have found that many individual animals seem to have fixed rather than plastic behavioral responses across a wide range of situations (Sih et al. 2004; Bell 2007). These consistent responses have been called coping styles (Koolhaas et al. 1999), personalities (Gosling 2001), or behavioral temperaments (Reale et al. 2007). There are two characteristics that define behavioral temperaments: (1) individuals of a population differ in their response to specific stimuli, and (2) individuals are consistent in their response to the same stimuli through time (Bell 2007). Behavioral temperaments are usually defined along specific personality axes such as bold-shy (Coleman & Wilson 1998), proactive-reactive (Koolhaas et al. 1999), aggressive-passive (Dall et al. 2004), or active-inactive (Johnson & Sih 2005).

For some species, behavioral temperaments are strongly correlated across contexts such as mating, foraging and avoiding predators (sticklebacks, *Gasterosteus aculeatus*, Bell & Stamps 2004; fishing spiders, *Dolomedes triton*, Johnson & Sih 2005) and potentially show inappropriate behaviors given a particular set of environmental
conditions (Sih et al. 2004). Strongly correlated behavioral responses have been described as behavioral syndromes (Sih et al. 2001) and have the potential to evolve through linkage disequilibrium with other important life history traits such as growth rates or reproductive strategies (Reale et al. 2000; Stamps 2007).

Cephalopods are an interesting group in which to study behavioral temperaments and individual cephalopods have been described as possessing distinct personalities (Mather & Anderson 1993; Sinn & Moltschaniwskyj 2005). Previous studies have demonstrated that some cephalopods meet the criteria of possessing behavioral temperaments by showing variation among individuals in response to a specific stimuli and individual consistency with repeated exposure (Sinn et al. 2001; Sinn et al. 2006).

In this study, I assessed whether or not Caribbean reef octopuses, *Octopus briareus*, have distinct behavioral temperaments using a standardized threat test to evaluate boldness. Specifically, I tested if octopuses (1) show distinct differences among individuals that are consistent through repeated exposure to the same stimuli, (2) if these temperaments are correlated across situations, (3) if these temperaments are correlated across contexts, and (4) if these temperaments are related to sex, size or shelter type.

**Methods**

**Study Site**

My study was carried out in Florida Bay, located in the middle Florida Keys in part of the Florida Keys National Marine Sanctuary (Figure 3.1). Across Florida Bay, eight study sites (25 m x 25 m) were selected in shallow (~ 1 to 3 m depth), nearshore,
hardbottom habitats with plentiful sponges and soft corals (Figure 3.1). Each site received ten concrete blocks (39.5 cm x 19.5 cm x 29.0 cm), each with four openings (13.5 cm x 19.5 cm x 7.0 cm), which served as artificial shelters and were randomly distributed across the site. Surveys were conducted in Feb-July 2006, June 2007, Oct 2007, Mar 2008, and May-July 2008. Water temperatures ranged from 15 to 30 °C and salinity fluctuated from 34 to 37 ppt.

Field Threat Test

To assess variation in octopus behavioral temperament, simulated threat tests were conducted in June-July 2006, June 2007, Oct 2007, Mar 2008, May-July 2008, and Oct 2008. The threat tests were implemented by a diver making contact with an octopus using a fiberglass rod and observing the response of each animal (N = 157). These tests were videotaped underwater and later analyzed. Fiberglass rods consisted of yellow, 7 mm diameter, 450 mm long, cylindrical rods, typically used in lobster capture. During experiments, a fiberglass rod was inserted into the portion of a den containing an octopus until contact was made. Threat tests were then scored on a five point scale of boldness (Table 3.1) modeled after a previous study by Mather and Anderson (1993). The scored responses ranged from rapid escape (-1) to vigorous pulling (+3).
Field threat tests were administered in replicates of 5 touches, with a rest period of 10 seconds between each replicate. This allowed me to analyze both the initial response and the repeatability of response for each individual. Once the in situ test was complete, the number and type of any co-residents in the crevice shelter with the octopus were recorded. The position and type of shelter was also noted on a detailed map of the site. Beginning in June 2007, octopuses were collected by hand net and returned to the boat. I recorded the gender and weight (to the nearest 0.1 g) for each individual and then returned it to the crevice shelter from which it was captured (N = 48).

Figure 3.1 Location and diagram of field sites. Field data was collected in Florida Bay, part of the Florida Keys National Marine Sanctuary (A). Sites (indicated by circles) were spread across nearshore habitats at eight different locations (B), sites were located 250-750 m from shore at 1.5-2.5 m depth (C). Each site (25 m X 25 m) were further subdivided into 5m X 5m cells by perpendicular transect lines. This grid allowed for accurate mapping of all available natural and artificial shelters. Artificial shelter blocks were randomly placed in 10 of the 25 cells of each site (D).
Table 3.1 Ethogram of Threat Test for Boldness

<table>
<thead>
<tr>
<th><strong>Boldness score</strong></th>
<th><strong>Description</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>-1</td>
<td>Quickly jets or crawls away from shelter. Avoids contact with fiberglass rod.</td>
</tr>
<tr>
<td>0</td>
<td>Slowly leaves shelter. Crawls into a nearby shelter. Avoids contact with fiberglass rod.</td>
</tr>
<tr>
<td>+1</td>
<td>Holds fiberglass rod without pulling it. Moves within shelter but does not leave shelter. Allows fiberglass rod to contact body.</td>
</tr>
<tr>
<td>+2</td>
<td>Grasps fiberglass rod. Pulls slightly (only 1-3 pulls).</td>
</tr>
<tr>
<td>+3</td>
<td>Grasps fiberglass rod. Pulls vigorously (3+ pulls). Reaches outside of shelter.</td>
</tr>
</tbody>
</table>

**Laboratory Threat Test**

To assess if octopus boldness is consistent among individuals across situations, a sample of 13 individuals collected in Oct 2008 and 7 individuals collected in Oct 2009 were returned to the Keys Marine Laboratory for further observation and testing. Animals were individually housed in 30 gallon aquaria and kept in an environment of constant salinity (~34 ppt) and temperature (~20 °C). Each tank contained one shelter composed of either Kiln-burned brick, arranged in such a way to create a crevice that allowed octopuses to crawl underneath, or a Kiln-burned, spherical gourd with a 4 cm diameter circular opening. Aquaria were visually isolated from one another and were aerated by a high velocity submersible pump. Octopuses were fed a diet of one fiddler crab (*Uca pugilator*) per day. Illumination was provided by indirect natural light at a photoperiod of approximately 12 hour light/12 hour dark. Specimens were acclimated to laboratory conditions for a period of 24 hours prior to behavioral testing.
The laboratory threat test was identical to the field threat test. The standardized presentation of a fiberglass rod was administered in replicates of 5, with 10 second intervals between each. Tests were performed between the hours of 08:00 and 10:00. Tests were again videotaped and scored on a five point scale of boldness from rapid escape (-1) to vigorous pulling (+3) (Table 3.1). For a detailed description of this procedure, see Mather and Anderson (1993).

**Laboratory Activity Test**

To assess if octopus boldness in the threat test was correlated with boldness in activity levels, 24 octopuses were videotaped for 2-8 hours after sunset (1800 – 0600 hours). Tanks were illuminated with indirect, low-power (25 watt) red lights and videotaped using a SONY DCR-HC96 Handicam in NightShot mode. Red lights were used so as not to disturb octopuses, yet allow adequate illumination for the video camera to function properly. Animals were housed under the same conditions as described in the laboratory threat test described above. During analysis of the videotapes, I recorded the percent of time active (crawling) vs. resting, and also percent of time in shelter, on shelter, and out of shelter. Observations were averaged for each hour to determine if activity varied by time of day. No such pattern was observed so activity levels for all hours were averaged for each individual.
Statistical Analyses

To evaluate if octopuses have distinct behavioral temperaments, I compared the initial response in the field and laboratory threat tests to the average response for replicate trials 2-5 using Pearson’s correlation. To evaluate if octopus boldness shows a significant correlation across situations, I compared the initial field threat test response to the initial lab threat test response using a Pearson’s correlation. To evaluate if octopus boldness shows a significant correlation across contexts, I compared the initial field threat test response to the average time spent active and average time spent in shelter using a Pearson’s correlation. To evaluate if octopus boldness is related to sex, size class or shelter type, I compared the initial field threat test response across these categories using a one-way analysis of variance with Tukey’s HSD post hoc comparisons. All analyses were performed using JMP (version 5, Cary, NC, USA).

Results

Evidence for Behavioral Temperaments: Repeatability within Individuals

In order to be considered a temperament, behaviors must vary between individuals in a population and also remain consistent through time. To assess whether this was the case concerning Caribbean reef octopuses in Florida Bay, behavioral data were collected from individual octopuses in the field and examined for both variation and repeatability. Individual variation was analyzed by calculating the percentage of octopuses falling into each response category (boldness score = -1: 7%, 0: 39%, 1: 11%, 2: 22%, 3: 22%). These data did not follow a normal distribution, which suggests the
variation between individuals is not predicted by a single optimal boldness score.
Repeatability was analyzed by comparing an individual’s initial response in the field
threat test to its average response over the remaining 4 replicates (Figure 3.2A). From
this, I was able to evaluate the consistency of an individual’s behaviors across successive
trials. Results of linear regression analysis demonstrated a significant positive
relationship between initial field boldness scores and average field boldness scores across
the remaining 4 replicates ($F = 42.13$, $df = 1, 43$, $p < 0.0001$, $r^2 = 0.483$). A positive slope
indicated a high consistency in behavior, with initial response of an individual serving as
a good predictor of response over the next 4 replicates.

Parallel threat test experiments were also performed under laboratory conditions
and data was recorded in the same manner as that in the field (Figure 3.2B). Results of
lab threat tests were similar to those of field threat tests. Linear regression analysis again
showed a significant positive relationship between initial lab boldness scores and average
lab boldness scores across the 5 replicates ($F = 21.67$, $df = 1, 30$, $p < 0.0001$, $r^2 = 0.400$),
with a positive slope indicating that the initial response of an individual was a good
predictor of his or her responses over the next 4 replicates.
Figure 3.2 Consistency in individual octopus boldness scores between (A) initial field threat test and average field threat test boldness score and (B) initial lab threat test boldness score and average lab threat test boldness score. Initial response was significantly related to average response in both the field and laboratory over the course of following 4 replicate trials.
Evidence for Fixed Behavioral Phenotypes: Correlations Across Situations

The results for field and lab threat tests suggest existence of the distinct temperament of boldness/shyness in Caribbean reef octopuses in Florida Bay. To determine whether this behavior is dynamic or fixed regardless of situation, I compared boldness data collected from field threat tests, under natural conditions, to that of threat tests conducted in the laboratory. These two distinct sets of conditions allowed me to evaluate whether octopuses exhibit behavioral plasticity in respect to a similar threat or if expression of boldness changed according to situation. I found no correlation between initial boldness in the field and initial boldness in the lab (Figure 3.3A), nor between average boldness in the field and average boldness in the lab (Figure 3.3B). Regression analyses found neither initial boldness scores ($F = 0.74$, $df = 1, 32$, $p = 0.395$, $r^2 = 0.000$) nor average boldness scores ($F = 0.16$, $df = 1, 28$, $p = 0.688$, $r^2 = 0.000$) were correlated.
Figure 3.3 Correlation across situations for octopus boldness scores. A comparison of individual octopus behavior between (A) initial field threat test boldness score vs. initial lab threat test boldness score, and (B) average field threat test boldness score vs. average lab threat test boldness score. Neither initial nor average boldness scores were significantly associated between field and laboratory threat tests.
Evidence for Behavioral Syndromes: Correlations Across Contexts

In order for behavioral syndromes to occur, behavioral responses must be strongly correlated across contexts (Sih et al. 2004). Therefore, I analyzed the temperament of boldness in Caribbean reef octopuses across the contexts of threat test and activity level. Both field and lab threat tests yielded similar results, therefore, I chose to use boldness scores from field threat tests in this comparison. To accomplish this, I took individual octopuses’ initial threat test responses in the field and compared them to the percentage of time these same octopuses spent actively crawling around their holding tanks (Figure 3.4A). This allowed me to compare an octopus’ boldness with its activity level. In addition, I examined octopuses’ initial field threat test responses and compared them to the percentage of time the same animals spent residing within their holding tank shelters (Figure 3.4B). This comparison allowed me to compare levels of inactivity or reclusion with boldness level. Results of linear regression analysis showed no significant relationship between initial field threat boldness and activity level \( (F = 0.34, \text{df} = 1, 11, p = 0.570, r^2 = 0.000) \) or between initial field threat boldness and percentage of time spent inside shelter \( (F = 0.11, \text{df} = 1, 11, p = 0.741, r^2 = 0.000) \).
Figure 3.4 Correlation across contexts for octopus boldness scores. A comparison of individual octopus behavior between (A) initial field threat test boldness score and the percent time active during laboratory activity trials, and (B) initial field threat test boldness score and the percent of time spent in shelter during laboratory activity trials. Neither the percent of time octopuses spent actively crawling around the holding tank nor the percent of time spent inside a crevice shelter were significantly related to boldness score in the field threat tests.
Influence of Octopus Characteristics on Boldness Temperaments

My results suggest that distinct behavioral temperaments do exist within the population of octopuses in Florida Bay. My next step consisted of an effort to identify correlations with octopus boldness and physical features such as sex, size, and shelter type.

Results of a one-way ANOVA indicated that the sex of an individual was not a good predictor of its boldness ($F = 0.0008$, df = 1, 51, $p = 0.977$, $r^2 = 0.000$). Next I analyzed the size of octopuses in relation to their boldness level. Size of an octopus consisted of its wet weight, which was grouped into 3 categories: small (<50 g), medium (50-100 g), and large (>100 g). Results of a one-way ANOVA indicated the size of an octopus had a significant effect on boldness ($F = 9.83$, df = 2, 99, $p = 0.0001$, $r^2 = 0.149$). A Tukey’s post-hoc test revealed that large octopuses were significantly bolder than medium or small ones.

Since octopuses are known to inhabit a variety of shelter types within Florida Bay, I chose to analyze shelter type in relation to boldness. Shelter types were categorized into 3 types: coral, solution hole, and artificial shelter block. Results of a one-way ANOVA showed that boldness was related to shelter type ($F = 5.98$, df = 2, 99, $p = 0.004$, $r^2 = 0.090$). A Tukey’s post-hoc test revealed that octopuses inhabiting solution holes had significantly higher levels of boldness than those in other shelter types.
Discussion

My results support the prediction that octopuses indeed possess distinct behavioral temperaments (Table 3.2). However, the lack of correlation between responses in different situations and contexts suggests individuals show adaptive plasticity in boldness. Initial boldness in the field is best predicted by octopus size, which is likely to be important in the defense of preferred crevice shelters (solution holes). Smaller, shy individuals are more likely to seek shelter in artificial shelter blocks and coral heads.

Table 3.2 Summary of octopus behavioral results for each hypothesis. Checks indicate statistically significant support, Xs indicate no support.

<table>
<thead>
<tr>
<th>Hypothesis</th>
<th>Prediction</th>
<th>Evidence Supports</th>
<th>Evidence Rejects</th>
</tr>
</thead>
<tbody>
<tr>
<td>H₁: Octopuses show distinct behavioral temperaments</td>
<td>Individuals will differ in their responses to threat stimuli; Individual responses to threat stimuli will remain consistent across 5 trials</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>H₂: Octopus temperaments are correlated across situations</td>
<td>Positive correlation between field and lab boldness tests</td>
<td>✓</td>
<td>X</td>
</tr>
<tr>
<td>H₃: Octopus temperaments are correlated across contexts</td>
<td>Positive correlation between threat test boldness and activity level</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>H₄: Octopus temperaments are correlated with sex, size, or shelter type</td>
<td>Positive correlation between boldness and sex; Positive correlation between boldness and size; Positive correlation between boldness and shelter type</td>
<td>✓</td>
<td>X</td>
</tr>
</tbody>
</table>
Octopus Behavioral Temperaments

Individual octopuses differed in their responses to threat tests and each individual remained consistent across the 5 replicate trials. According to the requirements for a behavioral temperament (Bell 2007), these findings satisfy both criteria. This corresponds to results from previous studies that also found cephalopods to vary greatly between individuals (Hanlon & Messenger 1996) and exhibit consistency in behavior (Sinn et al. 2001; Sinn et al. 2006). Although my results suggest the existence of boldness within octopuses in this community, my previous findings showed no significant effect of this temperament upon prey distribution (Chapter 2). While other studies have shown effects of boldness in cephalopods (Sinn & Moltschaniwskyj 2005; Sinn et al. 2006; Sinn et al. 2007), to my knowledge none has assessed the impacts of boldness levels upon prey.

Although my results suggest the presence of the temperament of boldness in octopuses within Florida Bay, exactly why such variation exists between individuals remains unknown. It could be that these differences are the result of individual experience (Mather & Anderson 1993; Sinn et al. 2001). In such a diverse habitat as Florida Bay, circumstances can vary greatly from one area to another, allowing the development of different temperaments in individual octopuses as they mature. Furthermore, the community of Florida Bay is very susceptible to rapid habitat change and, thus, behavioral variation may assist in perpetuating populations in such environments. It may also be that temperamental variation is good in combating predation (Sterrer 1992), providing prey with a protean search image so as not to stand out to predators. This could prove most beneficial to juvenile octopuses, who themselves
may be considered prey for a wide variety of fish and invertebrate predators (Smith & Herrnkind 1992).

Correlations Across Situations

The response of an individual to a particular threat may be either fixed or dynamic (Koolhaas et al. 1999). If an octopus’ behavior is expressed similarly in two different environments it may suggest some type of behavioral constraint, while evidence for dynamic behavior may illustrate just how intelligent and adaptive these animals are. However, some studies have suggested that fixed behaviors may prevail simply because behavioral plasticity is costly (DeWitt et al. 1998). My results found no correlation between boldness of octopuses in threat tests when comparing the situations of field and laboratory. This suggests that no strong associations exist between octopus boldness in one situation and another. Previous studies of dumpling squid, *Euprymna tasmanica*, reached a similar conclusion; individuals that were consistently bold in one treatment were not any more likely to be bold in another (Sinn et al. 2006; Sinn & Moltschaniwskyj 2005). Although certain temperaments have been suggested to affect life history traits (Stamps 2007) and boldness has been identified as a preferred temperament (Reale et al. 2000; Frost et al. 2007), it may be that octopuses have the capability to discern which response is best according to the situation, and in the case of my study, interpreted the two situations differently. This conclusion seems reasonable considering the cognitive ability of octopuses; they have been shown to change methods of foraging tactics according to which approach was most suitable (Forsythe & Hanlon 1997) and are highly
dependent upon learning (Mather & Anderson 1993). Any factor influencing their perception of the situation (e.g., the novel environment of the laboratory, perturbation from handling and transport, confinement to the aquaria, etc.) could easily alter how octopuses interpret the situation and, therefore, warrant a different response.

Correlations Across Contexts

Correlations between behaviors in different contexts have previously been suggested and are referred to as behavioral syndromes (Sih et al. 2001). Previous studies have shown some support for behavioral syndromes between boldness and activity in other species such as stickleback fish (Huntingford 1976; Stamps 2004), which is why I chose these same two parameters for my analysis. In addition to the evidence supporting positive relationships between certain behaviors, other studies suggest that an individual’s range of responses may be relatively fixed and, therefore, cause them to react similarly regardless of the context (Sih et al. 2004; Bell 2007). Such indiscriminate behavior has been referred to as context-general behavior. However, more numerous are studies that support individualized behavior that is unique according to situation, termed context-specific behavior (e.g., Greenberg 1989; Coleman & Wilson 1998; Sinn & Moltschaniwskyj 2005; Wilson & Stevens 2005). My results supported the latter, with all evidence pointing to behavioral plasticity. I found no relationship when comparing initial field boldness to the level of activity in the lab. Therefore, it appears that individual octopus possess the ability to adapt to the current situation by making independent decisions concerning appropriate behavior. My results are similar to previous studies of
dumpling squid that showed context-specific behaviors and no across-context
correlations (Sinn & Moltschaniwskyj 2005; Sinn et al. 2007). The idea of behavioral
plasticity is also supported by evolutionary theory, which predicts that each unique
situation requires a potentially different response. Following this line of reasoning, it is
logical that individual octopus within a population would alter their behaviors according
to what is most advantageous for the immediate context. Despite my support of context-
specific behavior between the boldness and activity level, the possibility of other across-
context relationships in Caribbean octopus behavior remains unexplored. I have only
measured specific behaviors in explicit contexts and more studies are needed in this area
to assess other potential relationships that exist.

Octopus Correlates of Boldness

Previous research concerning a variety of species has suggested bolder individuals
are more attractive (Godkin & Dugatkin 1996), better able to disperse (Fraser et al.
2001), grow faster (Johnson & Sith 2005), and survive better (Reale et al. 2000). If in fact
boldness is a preferred temperament, then it seems logical that there would be a fitness
differential between individuals of various levels of boldness. I compared the boldness
levels of octopuses to see if sex, size, and/or shelter type were related. I predicted that the
boldness levels of males and females would differ. This seems only reasonable
considering that octopuses are highly dependent upon learning (Mather & Anderson
1993) and males and females are likely to encounter different life experiences during
ontogeny. However, results showed no relationship between the sex of individual
octopuses and their level of boldness. These results indicate that the gender of an octopus is not a good predictor of its boldness. Although boldness has been demonstrated to be advantageous, it could be that it benefits both sexes equally, thereby showing no differences between the sexes.

My next trait to compare with boldness was the size of octopuses. This wet weight analysis of individual octopuses provided an intuitive conclusion; results illustrated that size was a good indicator of boldness level, with large octopuses being the most bold. Smaller individuals are likely younger and do not have the experience level of adults. If adolescent octopuses have little or no experience in distinguishing threats from non-threats as reliably as adults, then they may err on the side of caution. Younger individuals are also physically smaller, which makes them more vulnerable to threats (hence the lower level of boldness), and may have influenced their actions. It may also be that boldness has a positive correlation with growth rate, as was the case in fishing spiders, *D. triton*, (Johnson & Sih 2005). However, previous studies of dumpling squid showed that shier females were actually larger, on average, than bold females and had increased brood hatching success (Sinn et al. 2006).

My final analysis was to evaluate boldness in relation to shelter type. In this analysis, the boldness level of an individual was compared to the shelter type they inhabited upon capture. Here I made the assumption that the type of shelter each octopus resided in upon the time of capture was its preferred choice. Results indicate that there was a difference in boldness level according to shelter type, with solution hole inhabitants having the highest levels of boldness. This finding seems relevant when considering the
many types of predators present in Florida Bay. Potential octopus predators include nurse sharks, bonnethead sharks, sting rays, snapper, toadfish, permit, grouper, and many varieties of crabs, and others that could make the correct shelter choice vital. Solution holes provide a secure refuge that is difficult to penetrate. In addition, octopuses and typical prey species such as juvenile spiny lobsters and a variety of crabs tend to inhabit the same shelter types (Bouwma & Herrnkind 2009), so it could be that solution holes offer the best of both worlds; predation defense and ample prey availability, leading to increased occupancy of solution holes by bolder octopuses.

Conclusions

Caribbean reef octopuses within Florida Bay indeed possess distinct, consistent boldness as a behavioral temperament. However, my data showed no relationship across the situations of field and lab or across the contexts of threat response and activity level. Therefore, boldness does not appear to be a fixed behavioral response. Results did show a positive correlation between boldness and characteristics such as size and shelter type, but no such relationship existed with gender. Therefore, boldness may yet constitute a preferred temperament when expressed by larger, reproductively active individuals when competing for the best available shelters. However, more experimentation is necessary in this area. This study provides a starting point for future experiments designed to ascertain precisely which parameters are important in relation to the temperament of boldness in Caribbean reef octopuses and to assess the impacts thereof upon prey.
LITERATURE CITED


