THE USE OF OLFACTORY FORAGING CUES BY INTERTIDAL HERMIT CRABS

By

Mark Van Tran

A DISSERTATION

Submitted to Michigan State University in partial fulfillment of the requirements for the degree of

Zoology – Doctor of Philosophy Ecology, Evolutionary Biology and Behavior – Dual Major

ABSTRACT

THE USE OF OLFACTORY FORAGING CUES BY INTERTIDAL HERMIT CRABS

By

Mark Van Tran

Aquatic crustaceans rely heavily on their sense of olfaction to mediate vital behaviors, such as foraging and predator avoidance. The importance of olfactory cues to the survival of aquatic crustaceans has led to the evolution of highly sensitive and elaborate olfactory organs. A plethora of research has identified the types of olfactory cues used by crustaceans during foraging, and numerous studies have demonstrated that the cues that are most abundant and easily dispersed from prey tissues elicit the strongest foraging responses. Common foraging cues for aquatic crustaceans include amino acids, nucleotides, and carbohydrates. Since each prev species or food item emits a distinct chemical signature, the finding that two sympatric species utilize different olfactory cues to forage has been suggested by previous authors to be indicative of food niche differentiation between the species (i.e., the species are attracted to different food items via the olfactory cues emitted by the food). However, few studies have demonstrated empirically that sensory divergence is an accurate measure of food niche differentiation. Furthermore, few studies have taken a comparative approach to the study of sympatric resource competitors, and thus the links between the sensory biology and the ecological niches of species remain largely unexplored.

This dissertation uses a pair of ecologically similar, sympatric hermit crab species to test hypotheses regarding the links between sensory divergence and food niche differentiation. Chapter 1 provides a general introduction, and provides a background on why hermit crab species make an excellent model system for studies of sensory driven feeding behaviors. Chapter 2 identifies a subset of the olfactory cues used by the sympatric hermit crab species, and shows that the species rely on different olfactory cues to mediate their foraging behaviors. Chapter 3 compares the food niches of the two species using a combination of field experiments and laboratory analyses of collected specimens. Chapter 4 identifies a plausible mechanism of food niche differentiation between the focal species. Chapter 5 examines how the species utilize the olfactory cues released by injured conspecifics and heterospecifics to mediate cannibalistic behaviors. Chapter 6 tests the behavioral reactions of a single hermit crab species to novel food odors under different dietary treatments.

The overall goal of this dissertation is to address gaps in the current literature regarding the ecological effects of sensory divergence among ecologically similar, sympatric species. The research methods used in this dissertation integrate techniques from the fields of animal behavior, ecology, and stable isotope chemistry. While understanding of the links between sensory biology and community ecology remains relatively poorly developed, the results presented in this dissertation suggest that sensory divergence may (1) be an important determinant of resource use differentiation among species in nature, and (2) contribute to the coexistence of ecologically similar, sympatric species.

ACKNOWLEDGEMENTS

My love for biology started when I was a kid. I went through a phase in which my main hobby was tossing ants into spider webs and watching the ensuing show unfold. From an early age I was enthralled by what many would call "the struggle for existence." During my childhood experiments with bugs and other animals, I would spend hours observing the behavior of animals and trying to understand how nature worked. Back then I never would have imagined being where I am now. When I reached high school, my interest in biology took a backseat to other, perhaps less productive, interests. When I went away to college, I chose Biology as a major largely on a whim. I did not really know what I was interested in, nor did I really know what I wanted to be when I was done with school. It was not until my sophomore year that I finally realized I wanted to be a biologist. I remember sitting in the lecture of my Evolution class when something clicked in my mind and sent me down a long and difficult path culminating in this dissertation. For all of those who helped me reach that point, and to all of those who have helped me since, I extend my sincerest gratitude.

First and foremost, I have to thank my family: Mom, Dad, and Chris. To Mom and Dad, you have always been an inspiration to me. You have motivated me to aim high and work hard to reach my goals, and you encouraged me at times when discouragement was all that I felt. Chris, you were right there with me throwing those ants into spider webs. You might not have been as interested as I was, but I always looked up to you.

To all of my friends from high school and before, I thank you for helping to shape me into the person that I am today. I extend the same thanks to all of my friends from college, especially Brian, Greg, and Nick for all of your help and guidance along the way. To all my friends in Michigan, I thank you for listening to me complain and for helping me through all of

iv

the tough times I had during my tenure at Michigan State University. This is especially true for Kevin, Dave, and Jeremy. To Aaron, it has been great sharing a lab and animal room with you. Life in the dungeon of the Natural Sciences basement would have been really boring without you around.

Thank you to everyone who has helped to make my research a success. To my committee: Weiming Li, Gary Mittelbach, and Nathaniel Ostrom, I thank you for all of your guidance throughout my studies. I thank B. Wagner and A & M Aquatics for helping me to acquire my research animals, and for giving me tons of guidance along the way. A special thank you to the staff at the Intercultural Center for the Study of Deserts and Oceans in Puerto Peñasco, Sonora, Mexico for all of their help in planning and executing my field project, and for being so welcoming during my stay in Mexico. This is especially true for E. Blake-Dyke, who volunteered to brave the cold ocean waters with me on all of those early mornings of research. Thanks to P. Ostrom and H. Gandhi for help with carrying out my isotope work.

I thank my advisor, Richard Hill, for all of his contributions to my project and professional development. Dick, your guidance has shaped me into the scientist and teacher that I am today. Your "hands off" style was a challenge and a blessing. Although at times I never thought I could figure it out on my own, I made it through and I am a better scientist because you let me figure things out for myself. I think that the most important lesson that I have learned from you is to keep my passion for science at the front of my mind, and to never lose sight of that passion while navigating the negative aspects of science.

Finally, above all else, I would like to dedicate this work to Jessica. You stood by me without wavering through all the highs and lows. I absolutely would not have made it to this point without your help and support.

v

TABLE OF CONTENTS

LIST OF TABLES	ix
LIST OF FIGURES	.xi
CHAPTER 1:	
GENERAL INTRODUCTION	1
GENERAL INTRODUCTION	2
STUDY SYSTEM	3
OPERATIONAL DEFINITIONS OF TERMINOLOGY USED IN DISSERTATION	4
SUMMARY OF CHAPTER 2	5
SUMMARY OF CHAPTER 3	6
SUMMARY OF CHAPTER 4	7
SUMMARY OF CHAPTER 5	7
SUMMARY OF CHAPTER 6	8
CHAPTER 2:	
DIVERGENT REACTIONS TO OLFACTORY FORAGING CUES BETWEEN TWO	
ECOLOGICALLY SIMILAR, SYMPATRIC HERMIT CRAB SPECIES	9
INTRODUCTION	.10
MATERIALS AND METHODS	13
ANIMAL HOUSING AND MAINTENANCE	.13
STIMULUS PREPARATION	.14
DESCRIPTION OF FEEDING BEHAVIOR	.16
STIMULUS DISCRIMINATION	16
CHEMOSENSITIVITY	.18
RESULTS	19
STIMULUS DISCRIMINATION	19
CHEMOSENSITIVITY	.23
DISCUSSION	25
CHAPTER 3:	
ANALYZING DIET OVERLAP BETWEEN SYMPATRIC HERMIT CRAB SPECIES	.28
INTRODUCTION	29
MATERIALS AND METHODS	.32
STUDY AND SPECIMEN COLLECTION SITE	32
FOOD CHOICE EXPERIMENTS IN THE FIELD	.33
ANIMAL COLLECTIONS AND PRESERVATION	.34
GUT CONTENT ANALYSES	.35
STABLE ISOTOPE ANALYSES	.36
STATISTICAL ANALYSES	37
RESULTS	37

FOOD CHOICE EXPERIMENTS IN THE FIELD	37
GUT CONTENT ANALYSES	39
STABLE ISOTOPE ANALYSES	41
DISCUSSION	43
CHAPTER 4:	
AGGRESSION AND FOOD RESOURCE COMPETITION BETWEEN SYMPATRIC	
HERMIT CRAB SPECIES.	48
INTRODUCTION.	49
MATERIALS AND METHODS	51
ANIMALS, HOUSING AND MAINTENANCE	51
TESTING APPARATUS.	55
FOOD ODOR AND FOOD PELLET PREPARATION	
INTERSPECIFIC AGGRESSION IN THE PRESENCE OF FOOD ODOR	
INTRASPECIFIC AGGRESSION IN THE PRESENCE OF FOOD ODOR	- 7
INTERSPECIFIC COMPETITION IN THE PRESENCE OF FOOD	
KEOULIS.	
INTERSPECIFIC AGGRESSION IN THE PRESENCE OF FOOD ODOR	50
UNLY	
INTRASPECIFIC AGGRESSION IN THE PRESENCE OF FOOD ODOR	60
INTERSPECIFIC COMPETITION IN THE PRESENCE OF FOOD	00
FEEDING TIMES WITHOUT COMPETITION	60
DISCUSSION	63
	05
CHAPTER 5 [.]	
THE SCENT OF CANNIBALISM: THE OLFACTORY BASIS OF CANNIBALISM IN	
HERMIT CRABS	67
INTRODUCTION	68
MATERIALS AND METHODS	72
STUDY SYSTEM	72
ANIMAL HOUSING AND MAINTENANCE	72
TESTING APPARATUS	73
BEHAVIORAL REACTIONS TO THE ODORS OF CRUSHED CONs ANI)
HETs	73
CONSUMPTION ASSAYS	76
ETHICAL NOTE	78
RESULTS	78
BEHAVIORAL REACTIONS TO THE ODORS OF CRUSHED CONs ANI)
HETs	78
CONSUMPTION ASSAYS	82
DISCUSSION	86

CHAPTER 6:

BEHAVIORAL REACTIONS TO NOVEL FOOD ODORS BY INTERTIDAL HERMIT	
CRABS	89
INTRODUCTION	90
MATERIALS AND METHODS.	91
ANIMAL HOUSING AND MAINTENANCE	91
STIMULUS PREPARATION	92
TESTING APPARATUS	93
QUANTIFYING FORAGING BEHAVIORS	93
TRACKING ANIMAL IDENTITIES	93
REINFORCED FEEDING TREATMENT	94
UNREINFORCED FEEDING TREATMENT	94
STATISTICAL ANALYSES	96
RESULTS	96
REINFORCED FEEDING TREATMENT	96
UNREINFORCED FEEDING TREATMENT	97
DISCUSSION	100
REFERENCES	103

LIST OF TABLES

TABLE 2.2. Baseline (pre-stimulus) activity levels of test animals. Data represent Mean (SEM)number of pre-stimulus feeding movements. *P*-values come from a Friedman's test comparingpre-stimulus feeding movements among stimuli within each test group. "ASW" = Artificial SaltWater, "Gly Betaine" = Glycine Betaine, "AA Mix" = Amino acid mix, "Glu Acid" = Glutamicacid, and "Nuc. Mix" = Nucleotide mix.21

TABLE 3.1. Bait sources used for field choice experiments. ¹ The exact identify of this fish was difficult to determine because the baits were purchased pre-cleaned. Identification was based on body shape and tail morphology. ² A combination of these two species was used for each
deployment. These two species were the most abundant algae in the area
TABLE 4.1. Aggressive and Submissive Behaviors Observed During Foraging
TABLE 4.2. Behaviors observed during interspecific aggression trials. Total counts of behaviors shown. Counts were summed among test animals of the same species. $N = 20$ trials
TABLE 4.3. Behaviors observed during intraspecific aggression trials. Total counts of behaviors shown. Counts were summed among test animals of the same species. $N = 17$ trials for each species.59
TABLE 5.1. Correlation analyses between the time of day trial was conducted and MovementScores. $N = 13$ for all analyses. ^a Correlation coefficients are based on Pearson's correlationanalyses (<i>C. digueti</i>) and Spearman's rank correlation analyses (<i>P. perrieri</i>)

TABLE 5.3. Latency and feeding times of C. digueti on conspecific and heterospecific baits...84

TABLE 5.4.	Latency and feeding times of <i>P. perrieri</i> on conspecific and heterospecific	
baits		1

TABLE 5.5. Correlation analyses between body size (shell length) and latency times to contabait item. $N = 20$ for all analyses	act 85
TABLE 5.6. Correlation analyses between body size (shell length) and feeding times for baititems. $N = 20$ for all analyses.	t 85
TABLE 6.1. Results of two-way repeated measures ANOVA for <i>reinforced</i> feeding treatment	97
TABLE 6.2. Results of two-way repeated measures ANOVA for <i>unreinforced</i> feeding treatment.	97

LIST OF FIGURES

FIGURE 4.2. Outcome of food competition between *Clibanarius digueti* and *Paguristes perrieri*. Mean \pm SEM time spent feeding shown. N = 26 trials. * P < 0.05......61

FIGURE 5.2. Acceptance and rejection rates for bait sources offered. $N = 20$ trials per bar.	*
denotes that acceptance and rejection rates for that bait source were significantly different in a	ι
chi-square goodness-of-fit test ($P < 0.05$)	.83

CHAPTER 1

GENERAL INTRODUCTION

GENERAL INTRODUCTION

This dissertation focuses on the interaction between the use of olfactory foraging cues and the foraging ecology of two sympatric species of intertidal hermit crabs, *Clibanarius digueti* and *Paguristes perrieri*. This work aimed to use a single study system to test how differences in the sensory biology (i.e., use of olfactory cues) of sympatric species can influence: (1) the food resources the species use, and (2) patterns of interspecific interactions between the species. Sympatric hermit crab species make an excellent study system to test hypotheses regarding the interaction between sensory biology and foraging ecology. Hermit crabs, like most decapod crustaceans, rely heavily on olfactory cues to mediate their foraging behaviors (Rittschof, 1992), and are generalist detritivores that feed opportunistically on carrion and detritus (Hazlett, 1981). Because of the opportunistic nature of their foraging, and the large variety of food items they can consume, it is widely believed that sympatric hermit crab species exhibit extensive food niche overlap in nature (Hazlett, 1981).

Recent research has demonstrated that subtle differences in the operation of sensory mechanisms between sympatric species contribute to food niche differentiation, and help permit the coexistence of ecologically similar species by reducing interspecific competition (Siemers and Schnitzler, 2004; Siemers and Swift, 2006; Safi and Siemers, 2010). However, these aspects of sensory and resource use differentiation between sympatric species have not been examined extensively in the context of olfaction. Indeed, the classic studies on olfaction in aquatic crustaceans have largely lacked a comparative angle, and thus our knowledge of how the operation of olfactory mechanisms differs among species remains scarce. In this dissertation, I used a combination of laboratory behavioral assays, field experiments, and analytical techniques to assess: (1) whether *C. digueti* and *P. perrieri* utilize the same olfactory cues to mediate their

foraging behaviors, (2) if the species have overlapping food niches in nature, and (3) plausible mechanisms of niche differentiation between the species. Chapters 2 - 5 of my dissertation are comparative, and utilize both *C. digueti* and *P. perrieri*. Chapter 6 is the only non-comparative chapter of my dissertation, and utilizes *C. digueti* to test how the species uses novel olfactory cues to forage.

STUDY SYSTEM

Clibanarius digueti and *Paguristes perrieri* are two sympatric species of intertidal hermit crabs from the Gulf of California. The species are of similar body sizes (Harvey, 1988) and exist as part of an assemblage that consists of at least 3 additional species (Personal Observation). In the Northern Gulf of California, *C. digueti* is the most abundant hermit crab species. *P. perrieri* is far less abundant than *C. digueti*, but is still relatively common (Personal Observation). Both species occupy rocky intertidal habitats. In the intertidal zone of Puerto Peñasco, Mexico, the distributions of the two species overlap over all of *P. perrieri*'s distribution (Figure 1.1). *C. digueti* occupies a wide range of water depths, ranging from the subtidal to the upper intertidal (Ayón-Parente and Hendrickx, 2010; Personal Observation). *P. perrieri* occupies a narrow band in the mid-intertidal, and is not commonly found in the upper intertidal (Ayón-Parente and Hendrickx, 2010; Personal Observation).

Both *C. digueti* and *P. perrieri* show behavioral rhythms that coincide with the tidal cycle. During low tide, the animals form large, mixed-species clusters of up to 700 individuals (Snyder-Conn, 1980) where they remain stationary and generally do not feed (Personal Observation). At the onset of flood tide (when the tide washes over the intertidal zone), *C*.

digueti and *P. perrieri* become behaviorally active and disperse to forage (Snyder-Conn, 1980; 1981).



FIGURE 1.1. Approximate distributions of *Clibanarius digueti* and *Paguristes perrieri* in the intertidal region of Puerto Peñasco, Sonora, Mexico. Distribution map is based on personal observations made during fieldwork in the area.

OPERATIONAL DEFINITIONS OF TERMINOLOGY USED IN DISSERTATION

Various technical terms are commonly and consistently used throughout this dissertation, and thus should be operationally defined. Some of the following definitions are re-stated in later chapters. In this dissertation, "sympatric" species are defined as species that coexist in the same geographical area. Species are referred to as being "ecologically similar" when they use similar resources, such as food, shells, and habitats. "Intraspecific" interactions are defined as interactions between individuals of the same species. "Interspecific" interactions are defined as interactions between individuals of different species. "Preference," in the context of foraging, is operationally defined as an animal actively choosing one food item over another when the food items are equally accessible. Thus, species exhibiting "overlapping preferences" choose the same food items when given a choice among equally accessible food items. "Fundamental food niches" refer to all of the food resources that an animal could feasibly consume, and "realized food niches" refer to the food items that the animal actually consumes.

SUMMARY OF CHAPTER 2

In Chapter 2, I tested the behavioral reactions to olfactory foraging cues by two ecologically similar, sympatric hermit crab species. Previous studies (Tierney and Atema, 1988; Janecki and Rakusa-Susczewski, 2005) have hypothesized that differences in the olfactory cues used by sympatric species to mediate foraging behaviors are indicative of food niche differentiation between the species. Sympatric hermit crab species make an excellent model system to test this hypothesis because hermit crabs are generalist scavengers that feed opportunistically on carrion and detritus (Hazlett, 1981), and rely heavily on olfaction to mediate foraging behaviors (Rittschof, 1992). Because they feed opportunistically on food resources that are spatially and temporally unpredictable in abundance (Britton and Morton, 1994), hermit crabs have evolved broad food niches, and it is believed that sympatric hermit crab species should show a high degree of food niche overlap (Hazlett, 1981). I tested the behavioral reactions of *C. digueti* and *P. perrieri* to 16 individual olfactory cues, and constructed a concentration-response curve of behavioral reactions to a known foraging cue for both species. I found that *C. digueti* and *P. perrieri* showed divergent reactions to the olfactory cues tested,

despite similarities in their behavioral chemosensitivities to foraging cues. This result suggests that the diets of the two species may be differentiated (Tierney and Atema, 1988; Janecki and Rakusa-Susczewski, 2005).

SUMMARY OF CHAPTER 3

In Chapter 3, I conducted field and laboratory analyses on the food niches of *C. digueti* and *P. perrieri*. First, I analyzed the attraction of the species to a variety of food sources in the field (Puerto Peñasco, Sonora, Mexico) in order to determine if the species differed in their preferences for food items. This difference in preference for food items would be indicative of differentiation of the fundamental food niches of the species. Second, I collected specimens from the field site, and conducted gut content and stable isotope analyses to test whether the diets of the two species differed in nature. My results showed that the preferences for food items in the field experiments were largely similar between the species. The results from my gut content analyses suggested that *C. digueti* relies more heavily on photosynthetic tissues than *P. perrieri* during feeding. I found significant differences between the δ^{13} C and δ^{15} N values of muscle tissues between *C. digueti* and *P. perrieri*, which is indicative of past differentiation of the realized food niches of the species. Differences in δ^{15} N values between the species suggest that *P. perrieri* may be feeding at a higher trophic level than *C. digueti*, which is consistent with the results of the gut content analyses.

SUMMARY OF CHAPTER 4

In Chapter 4, I studied whether interspecific food competition between *C. digueti* and *P. perrieri* could contribute to the differentiation of the realized food niches of the species. I conducted a series of competition assays testing aggressiveness and competitive abilities of the species, and found that *C. digueti* was the dominant food competitor. When foraging together, *C. digueti* gained increased access to shared food resources compared to *P. perrieri*. *C. digueti* was shown to be the more aggressive species, and this difference in aggressiveness is a plausible mechanism by which *C. digueti* gains competitive advantage over *P. perrieri* during foraging. Based on my results, I hypothesized that competitive dominance by *C. digueti* during interspecific food contests has resulted in *P. perrieri* consuming food resources that are not first choice, and thus the differentiation of food niches between the species.

SUMMARY OF CHAPTER 5

In Chapter 5 I tested the species responses to cannibalistic foraging cues in order to assess the relative importance of cannibalism in the diets of the species. I also tested whether the species fed preferentially on conspecifics or heterospecific tissues. I found that *C. digueti* and *P. perrieri* responded similarly to the cannibalistic olfactory cues, showing foraging reactions instead of fear reactions when presented with the odors of dead conspecifics and heterospecifics. *C. digueti* readily consumed both conspecific and heterospecific tissues, suggesting that this species may rely heavily on cannibalism to supplement its diet. *P. perrieri* largely rejected conspecific tissues as food sources, and consumed heterospecifics in 11 of the 20 trials performed. The results suggest that (1) cannibalism in hermit crabs is mediated by olfactory

cues emitted from dead or injured conspecifics and heterospecifics, and (2) *C. digueti* may rely more heavily on cannibalism as a dietary supplement than *P. perrieri*. The influence that this difference in cannibalistic tendencies has on the structure of hermit crab assemblage in the Gulf of California is unknown.

SUMMARY OF CHAPTER 6

In Chapter 6, I examined the behavioral reactions of *C. digueti* to novel food cues. Specifically, I tested how the species responds to a novel food odor, and how this behavior changes over repeated exposures to the odor under different feeding treatments. Animals showed strong behavioral reactions to the novel food odor upon first exposure. When the cue was reinforced by allowing the animals to feed on the novel food item, the behavioral reactions to the odor upon subsequent exposures remained strong. When the cue was not reinforced (i.e., the animals were not allowed to feed on the novel food item), the foraging reactions to the cue were rapidly lost. The results show that *C. digueti* (1) possesses sensory mechanisms to detect and use novel food odors during foraging, and (2) requires reinforcement of the novel cue to maintain its baseline level of attraction to the cue.

CHAPTER 2

DIVERGENT REACTIONS TO OLFACTORY FORAGING CUES BETWEEN TWO ECOLOGICALLY SIMILAR, SYMPATRIC HERMIT CRAB SPECIES

Tran, M.V. (2013). Divergent reactions to olfactory foraging cues between two ecologically similar, sympatric hermit crab species. *Journal of Crustacean Biology* 33(4): 512-518. Brill.

INTRODUCTION

It is often assumed that species with generalist diets and overlapping habitats have similar ecological niches and thus compete for food resources. Because food resources are often limited, differences in the competitive abilities between the competing species usually result in one species outcompeting the other (Gause, 1934; Hardin, 1960). However, this outcome may not be the case if the species differ subtly in their foraging behaviors or sensory capabilities. Such subtle behavioral or sensory differences could be enough to facilitate subtle niche shifts and permit coexistence between ecologically similar species (Siemers and Schnitzler, 2004). For example, species that locate their food using chemical cues (olfaction) may differ subtly in their sensory capabilities (i.e., the cues used or sensitivity to cues) and these differences could result in the species using different food resources. Previous research on the characteristics of echolocation signals used by bats during foraging has confirmed that subtle sensory differences between sympatric species can permit food niche differentiation and species coexistence (Siemers and Schnitzler, 2004; Siemers and Swift, 2006; Safi and Siemers, 2010). However, practically nothing is known about how subtle differences in the use of olfactory cues can permit coexistence between ecologically similar, sympatric species.

Many animals rely heavily on olfaction during foraging (Conover, 2007). Numerous authors have asserted that a strong interaction exists between an animal's ecological food niche and the identities of the chemical cues it uses during foraging (Trott and Robertson, 1984; Johnson and Atema, 1986; Janecki and Rakusa-Suszczewski, 2005). Each food item emits its own distinct signature of chemical cues into the environment (Carr et al., 1996), permitting foraging animals to use chemical cues to locate their preferred food items. Thus, it has been predicted that if two morphologically similar, sympatric species utilize different olfactory cues

while foraging, the food niches of the two species must be differentiated (Tierney and Atema, 1988; Janecki and Rakusa-Susczewski, 2005). However, empirical tests of this prediction remain lacking.

Logically, animals that rely on olfaction to forage should show preferences for the chemical cues that are both (1) abundant in their preferred food items and (2) easily dispersed and distinguished within their foraging environments. Animals that are primarily carnivorous have been shown to respond most strongly to compounds abundant in animal tissues, such as amino acids, amines, peptides, and muscle metabolites (Johnson and Atema, 1986; Tierney and Atema, 1988; Zimmer et al., 1999). Alternatively, animals that are primarily herbivorous have been shown to respond strongly to compounds abundant in plant tissues, such as carbohydrates (Trott and Robertson, 1984; Tierney and Atema, 1988). Omnivorous animals show responses to compounds abundant in both plant and animal tissues (Tierney and Atema, 1988). The identities of the individual chemical cues used by a species for foraging (i.e., the specific amino acids or carbohydrates used) are expected to vary based on the specific chemical composition of the species' preferred food items (Carr et al., 1996), as well as the chemical background of its foraging environment. Because species foraging in the same environment are likely to be subjected to the same chemical background noise, if sympatric species are found to rely on different olfactory cues to forage, the most parsimonious explanation is that the species prefer different food items with different chemical signatures. Therefore, identifying and comparing the specific chemical cues used by perceived food competitors can offer insights into the degree of food niche overlap between the species.

Based on ecological research, sympatric hermit crab species have been interpreted to have high diet overlap and to compete for food resources. Most hermit crab species exhibit a

generalist detritivore feeding strategy (Hazlett, 1981), feeding opportunistically on carrion and other detritus sources stranded by the tides. This apparent sharing of the food niche, paired with the spatial and temporal irregularity of marine carrion abundance (Britton and Morton, 1994), is believed to lead to a high degree of interspecific competition for food between sympatric hermit crab species (Kaiser et al., 1998). Although food resource competition has been studied far less than shell resource competition in hermit crabs, research has shown that competition for carrion resources is common between sympatric hermit crab species (Ramsay et al., 1996; Kaiser et al., 1998) and between hermit crabs and other carrion scavengers (Morton and Yuen, 2000). Hermit crabs, like most aquatic crustaceans, rely on chemical cues to mediate their foraging behaviors. The unique pairing of this high diet overlap with the reliance on chemical cues for foraging makes sympatric hermit crab species an attractive model for studies addressing the interaction between ecological food niches and the use of chemical foraging cues.

Clibanarius digueti and *Paguristes perrieri* are sympatric hermit crab species found in the intertidal zone of the Gulf of California. Both species predominately occupy rocky areas of shallow depth (less than 5 m) in the intertidal zone (Ayón-Parente and Hendrickx, 2010), and thus have overlapping distributions. Additionally, the two species cluster near each other during low tide and disperse onto the rock surfaces at the onset of flood tide (Personal Observation). These aspects of their species distributions, paired with their generalist detritivore feeding strategies, facilitate food competition between the species.

This study was designed to identify olfactory foraging cues used by *C. digueti* and *P. perrieri*. Specifically, my *a priori* prediction was that the two species would show positive foraging reactions to the same olfactory cues, since sympatric hermit crab species are described as relying on the same food items (Hazlett, 1981). To test this prediction, I used a series of

behavioral bioassays to (1) identify the olfactory cues used by the two species and (2) compare the olfactory sensitivities of the two species. This study demonstrates that ecologically similar, sympatric species can differ subtly in the sensory cues they rely on, with perceived food competitors relying on different olfactory cues to forage.

MATERIALS AND METHODS

ANIMAL HOUSING AND MAINTENANCE

Wild-caught *Clibanarius digueti* and *Paguristes perrieri* from the Gulf of California were obtained from a commercial distributor (A & M Aquatics, Lansing, MI) and acclimated to laboratory conditions for a minimum of 2 weeks prior to use in the experiments. Animals were held communally in 10-gallon glass aquaria containing aerated artificial salt water (ASW; Instant Ocean). This ASW and other ASW mentioned in this report was maintained at 24.7±0.9°C, specific gravity 1.021-1.024, and pH 8.2. Animals were fed a krill-meal-based pellet food (NewLife Spectrum) 2-3 times per week during acclimation. Prior to the initiation of the experiments, animals were denied food for a minimum of 48 h to ensure motivation to forage and were not fed for the duration of the experiments. On the day before the start of the experiments, animals were moved to individual housing units consisting of 8.5 cm³ glass containers sunken into a 20-gallon glass aquarium. The bottoms of the glass containers were covered with a thin layer of aquarium gravel. The animals were held in these housing units for the duration of the experiments.

STIMULUS PREPARATION

Table 2.1 lists the stimuli used in these experiments and the rationale behind the selection of each test stimulus. Stimuli were chosen for testing because they elicit positive behavioral reactions in other species of crustaceans (Field, 1974; Fuzessery and Childress, 1975; Trott and Robertson, 1984; Zimmer-Faust et al., 1984; Johnson and Atema, 1986, Tierney and Atema, 1988; Rittschof and Buswell, 1989; Weissburg and Zimmer-Faust, 1991; Corotto et al., 2007), are found in high abundance in marine animal tissues (Carr et al., 1996), or are well-known constituents of animal or plant tissues. All stimuli were prepared at $10^{-3}M$ using ASW. Mixtures were prepared by combining equal volumes of individual stimuli that were prepared at $10^{-3}M$. A liquid extract (Food Extract) from the animal's normal pellet food was made by soaking 3.2 g of food pellets (NewLife Spectrum) in 400 mL of ASW for 10 minutes. The liquid supernatant was poured off and filtered through medium filter paper. This Food Extract was used as a positive control because it consistently elicited strong behavioral reactions in both species. Plain ASW was used as a negative control. All stimuli, including controls, were prepared prior to the start of the experiment, frozen in 15 mL aliquots, and thawed at room temperature on the day of use.

TABLE 2.1. List of test stimuli used. Amino acid mix = β -Alanine, L-Taurine, and L-Histidine. Nucleotide mix = Cytosine, Thymine, and Uracil. * Shown to cause foraging reactions in other crustaceans (see text for references). + Found in high abundance in marine animal tissues (See Carr et al., 1996 for concentrations). # Known component of animal and plant tissues.

Stimulus	Reason	for Select	tion
Food Extract	Positive	Control	
ASW	Negative	e Control	
β-Alanine	*	+	
L-Alanine	*	+	
Amino Acid Mix	*	+	
L-Arginine	*	+	
D-(+)-Glucose	*		#
L-Glutamic acid	*	+	
L-Glycine	*	+	
Glycine Betaine	*	+	
L-Histidine	*	+	
L-Isoleucine	*	+	
L(+)-Lysine	*	+	
L-Methionine	*	+	
Nucleotide Mix	*		#
L-Proline		+	
Sucrose	*		
L-Taurine	*	+	

DESCRIPTION OF FEEDING BEHAVIOR

Like most hermit crab species, *Clibanarius digueti* and *Paguristes perrieri* feed on organic debris picked from the substrate using their chelipeds. The motion of feeding is characterized by repeated movements of the chelipeds between the food and the animal's mouthparts (Case and Gwilliam, 1961; Field, 1974, 1977). The dactyls of the walking legs are used to probe the substrate for food items and are routinely moved to contact the mouthparts. Together, these cheliped- and dactyl-to-mouth movements are termed "feeding movements." Feeding movements are present during normal exploratory behavior, but the frequency of these movements is enhanced upon the detection of a chemical feeding effector (feeding incitant or stimulant; Johnson and Atema, 1986). This change in the frequency of feeding movements was used as a means to quantify an animal's resulting degree of feeding stimulation after the detection of a potential feeding effector.

STIMULUS DISCRIMINATION

A total of 52 healthy, intact animals of each species (mean \pm SD shell length: *C. digueti* = 2.18 \pm 0.31 cm; *P. perrieri* = 2.30 \pm 0.32 cm) were selected at random from population tanks for study. Each animal was tested using four chemical cues, as well as the positive and negative controls (N = 13 animals per stimulus, 624 total observations). Sample sizes were determined based on a combination of factors, including results from preliminary experiments and previously published sample sizes used in similar experiments (Johnson and Atema, 1986; Tierney and Atema, 1988; Hazlett et al., 2002; Corotto et al., 2007). Animals were tested individually over a 6 day period during which they received one stimulus per day in random order. The observer did not know the identity of the stimulus being tested, ensuring a blind

protocol. The testing apparatus consisted of a 250 mL glass Erlenmeyer flask containing 250 mL ASW and gravel substrate. A single animal was placed in the apparatus and allowed to acclimate for a minimum of 20 min. Following acclimation, 2 mL of plain ASW were introduced through a glass pipette onto the animal's antennules from a vertical distance of ~4 cm over a period of ~8-10 seconds. Preliminary dye trials showed that this method of stimulus delivery produced a concentrated stream of liquid reaching the animal's antennules. The number of feeding movements was counted for a period of 3 min immediately following the cessation of ASW introduction. This count represented the pre-stimulus count. After this initial 3 min observation period, 2 mL of test stimulus (Table 2.1) were delivered in the same manner as above, and the number of feeding movements was again counted for 3 min. This count represented the post-stimulus count. A Movement Score was calculated for each animal's reaction to each stimulus by subtracting the number of pre-stimulus feeding movements from the number of post-stimulus feeding movements. Two-sided Wilcoxon Signed-Rank tests for matched pairs were used to determine if the number of post-stimulus feeding movements was significantly greater or smaller than the number of pre-stimulus feeding movements for each stimulus (Johnson and Atema, 1986). The positive control (Food Extract) was used to ensure that (1) the test animals showed positive reactions to a known foraging cue and (2) the employed methods of measuring foraging reactions were reliable. The negative control (ASW) was used to ensure that the animals would not show foraging reactions to mechanosensory inputs alone. I made the *a priori* decision to exclude any data sets that either (1) showed no significant increase in Movement Scores to the positive control or (2) showed a significant increase in Movement Scores to the negative control. However, no data sets met these criteria for exclusion.

In order to identify any possible effects that differences in baseline activity levels had on overall responses to stimuli, pre-stimulus counts of feeding movements were compared among stimuli within each test group (i.e., all animals that were tested with the same stimuli, Table 2.2) using a Friedman's test. It was not possible to use an overall ANOVA model for all stimuli at once, because the stimuli within a test group had a repeated measures component, while stimuli between test groups did not. To assess the consistency of responses across test groups within each species, Movement Scores elicited by the positive control (Food Extract) were compared using a Kruskal-Wallis test, followed by Dunn's Multiple Comparisons test (with Bonferroni α adjustment), when appropriate. Spearman's rank correlations were used to determine if there were significant associations between test day (i.e., hunger level) and mean Movement Scores for each stimulus tested. This was done to ensure that neither hunger level nor previous experience with the apparatus was associated with Movement Scores across test days. P = 0.05was used as the significance cutoff for all statistical tests used in this study. For all aforementioned statistical tests, non-parametric statistical tests were used rather than transforming the non-normally distributed data in order to ease the interpretations of the results.

CHEMOSENSITIVITY

To determine if the two species differed in their sensitivity to chemical foraging cues, concentration-response curves for the positive control (Food Extract) were constructed. The standard was serially diluted with ASW to make the following test dilutions: Full Strength (undiluted), 1:10, 1:100, 1:1000. Clean ASW was used as a negative control. Thirteen animals of each species (mean \pm SD shell length *C. digueti* = 2.24 \pm 0.37 cm; *P. perrieri* = 2.19 \pm 0.32 cm, 130 trials total) were tested using the same procedures stated in the "Stimulus

Discrimination" section. A two-way repeated measures ANOVA was used to analyze the Movement Scores, with dilution and species as the two factors. Post hoc Bonferroni multiple comparisons tests (with appropriate α adjustments) were used following significant ANOVA results. Data were rank transformed (RT-1 method; Conover and Iman, 1981) prior to using ANOVA in order to satisfy the assumptions of the tests.

RESULTS

STIMULUS DISCRIMINATION

Baseline activity levels (pre-stimulus counts of feeding movements) did not differ among stimuli within test groups (i.e., animals receiving the same stimuli) for either species (Friedman's test, P > 0.05; Table 2.2). Movement Scores elicited by the positive control (Food Extract) did not differ among test groups for *P. perrieri* (H = 2.219, df = 3, P = 0.5283), but were significantly different for *C. digueti* (H = 19.37, df = 3, P = 0.0002). In *C. digueti*, test group 1 had a significantly higher Movement Score than test group 4, and test group 2 had a significantly higher Movement Score than test group 3 and 4 (Dunn's Multiple Comparisons Tests, P < 0.05).

Of the chemical cues tested, no single stimulus or mixture (other than the positive control) elicited a significant response in both *C. digueti* and *P. perrieri* (Figure 2.1), indicating that the two species rely on different individual olfactory cues to forage. *C. digueti* showed significant increases in the number of feeding movements when presented with sucrose and arginine, but showed significant decreases in the number of feeding movements when presented when presented with sucrose and with glycine betaine and isoleucine. *P. perrieri* showed significant increases in the number of

feeding movements when tested with glucose, histidine, glutamic acid, and mixtures of nucleotides and amino acids. Except for *C. digueti*'s reactions to proline ($r_s = -0.9429$, df = 4, *P* = 0.0167) and *P. perrieri's* reactions to the nucleotide mix ($r_s = -0.9429$, df = 4, *P* = 0.0167), there were no significant correlations between test day (i.e., hunger level) and mean Movement Scores for any stimuli. Thus, differences in hunger state and prior experience with the testing apparatus among test days did not appear to be associated with the animals' reactions to the stimuli.

TABLE 2.2. Baseline (pre-stimulus) activity levels of test animals. Data represent Mean (SEM) number of pre-stimulus feeding movements. *P*-values come from a Friedman's test comparing pre-stimulus feeding movements among stimuli within each test group. "ASW" = Artificial Salt Water, "Gly Betaine" = Glycine Betaine, "AA Mix" = Amino acid mix, "Glu Acid" = Glutamic acid, and "Nuc. Mix" = Nucleotide mix.

Test					
Group	Stimulus	C. digueti	P-value	P. perrieri	P-value
1	Food Extract	19.62 (7.26))	17.69 (5.57))
	ASW	21.23 (8.22)		13.69 (4.20)	
	β – alanine	13.69 (3.45)	ļ	12.31 (4.28)	Ĺ
	Glucose	20.46 (8.99)	0.8093	18.38 (5.54)	0.5368
	Proline	18.77 (5.68)		20.15 (5.06)	
	Gly Betaine	23.38 (6.67))	24.92 (6.63))
2	Food Extract	5.54 (1.77))	15.08 (4.09))
	ASW	7.00 (3.05)		11.69 (2.76)	
	L – alanine	12.92 (4.25)	Ļ	8.54 (2.67)	>
	Taurine	10.54 (2.74)	0.4815	9.69 (3.61)	0.9144
	Histidine	12.08 (5.18)		8.77 (3.13)	
	Glycine	7.23 (3.10))	7.69 (2.24))
3	Food Extract	8.92 (2.21))	7.39 (1.38))
	ASW	12.00 (6.53)		9.62 (1.72)	
	Sucrose	7.69 (2.34)	\ \	12.15 (2.86)	5
	AA Mix	6.31 (1.68)	0.7050	9.85 (4.33)	0.3424
	Glu Acid	6.69 (2.66)		9.62 (3.81)	
	Nuc. Mix	7.15 (1.33))	9.85 (2.52))
4	Food Extract	6.15 (2.68))	8.92 (2.22))
	ASW	9.46 (2.79)		7.39 (1.79)	
	Isoleucine	8.08 (2.93)	Ş	7.23 (1.57)	Ş
	Methionine	6.08 (1.42)	0.5229	7.08 (2.46)	0.7258
	Lysine	8.77 (1.94)		4.85 (1.51)	
	Arginine	6.15 (1.28))	5.46 (1.60))
	-	× /		` '	



FIGURE 2.1. Behavioral reactions to potential chemical foraging cues by *Clibanarius digueti* and *Paguristes perrieri*. Mean + SEM shown. N = 13 for all bars. * denotes that the number of post-stimulus feeding movements was significantly greater or less than the number of prestimulus feeding movements in a Wilcoxon Signed-Rank test for matched pairs (P < 0.05). Arrows were added to clarify which species showed a significant response. Gray arrows = *P*. *perrieri*. White arrows = *C. digueti*. Movement Score = the number of post-stimulus feeding movements minus the number of pre-stimulus feeding movements.

CHEMOSENSITIVITY

Figure 2.2 shows that the responses of C. digueti and P. perrieri to the different dilution strengths of Food Extract closely paralleled one another. Table 2.3 shows the results of the twoway repeated measures ANOVA on the rank-transformed Movement Scores in the chemosensitivity tests. Dilution had a significant effect on Movement Scores (F4, 96 = 51.76, P < 0.0001), while species did not (F_{1, 96} = 0.8138, P = 0.3760). The interaction between species and dilution was not significant (F4, 96 = 1.373, P = 0.2492). Post hoc Bonferroni multiple comparisons tests ($\alpha = 0.05$) showed that, for *C. digueti*, there were no significant differences in Movement Scores between the Full Strength, 1:10, and 1:100 dilutions, but that the Movement Scores for the 1:1000 dilution were significantly smaller than the Full Strength and 1:10 dilutions. For C. digueti, ASW elicited smaller Movement Scores than all other dilutions tested. For P. perrieri, Full Strength elicited significantly larger Movement Scores than all other dilutions. Additionally, for *P. perrieri*, the 1:10 dilution elicited significantly larger Movement Scores than the 1:1000 dilution and ASW, and the 1:100 dilution elicited significantly larger Movement Scores than ASW.

Socies. But were fullit transformed prior to analysis.				
Source of Variation	Df	F	<i>P</i> -value	
Species x Stimulus	4	1.373	0.2492	
Species	1	0.8138	0.3760	
Dilution	4	51.76	< 0.0001	
Subject (matching)	24	2.428	0.0012	
Residual	96			

TABLE 2.3. Results of two-way repeated measures ANOVA on chemosensitivity Movement Scores. Data were rank transformed prior to analysis.



FIGURE 2.2. Concentration-Response curves. Mean \pm SEM shown. N = 13 for each species. Movement Score = the number of post-stimulus feeding movements minus the number of prestimulus feeding movements.
DISCUSSION

The *a priori* prediction that the two species would show foraging reactions to the same olfactory cues was not supported by the data collected in this study. While both species showed positive responses to a subset of the amino acids and carbohydrates tested, no single stimulus or mixture, other than the positive control, elicited significantly positive foraging reactions in both species (Figure 2.1). Thus, the data indicate that the species rely on different olfactory cues to mediate their foraging behaviors. These differences in stimulus responses are not likely due to differences in overall chemosensitivity to foraging cues, since comparisons of concentration-response curves show no significant differences between species (Figure 2.2). A subset of the chemical cues tested did not elicit any significant responses in either *C. digueti* or *P. perrieri*, suggesting that some of the olfactory cues tested are not reliable indicators of food availability for either test species.

The most parsimonious explanation for the results shown here is that subtle differences in the olfactory cues used by *C. digueti* and *P. perrieri* during foraging have facilitated subtle food niche shifts between the species, and thus permitted coexistence in nature. Previous authors have asserted that the utilization of different olfactory cues to mediate foraging between ecologically similar, sympatric species is indicative of food niche differentiation (Tierney and Atema, 1988; Janecki and Rakusa-Suszczewski, 2005). The finding that *C. digueti* and *P. perrieri* utilize different olfactory cues to mediate their foraging behaviors points to previously unidentified niche differentiation between the species. In speaking of hermit crabs as generalist detritivores, the term "generalist" can be misleading, because although most hermit crabs are believed to be generalist detritivores (Hazlett, 1981), previous studies have shown that preferences for certain food items do exist (Thacker, 1996). While these preferences for food items, as well as for food-

related odorants, could be influenced by ecological and physiological factors operating during the lifetimes of individuals, such as associative learning (Wight et al., 1990; Hazlett, 1994; Ristvey and Rebach, 1999), avoidance of recently eaten foods (Thacker, 1996), or nutritional imbalances (Janecki and Rakusa-Suszczewski, 2005), it is unlikely that any of these factors differentially affected the two test species enough to influence the results shown here because the animals were all acclimated to the same standard diet prior to testing. Chapter 3 of this dissertation includes detailed field analyses of the food niches of *C. digueti* and *P. perrieri*, and provides strong analytical evidence of food niche differentiation between the species.

Alternative explanations for the results shown here are less parsimonious than the hypothesis of food niche differentiation. It is possible that *C. digueti* and *P. perrieri* do indeed exhibit overlapping food niches, but utilize different foraging cues to track the same food resources. This possibility seems unlikely, since natural selection should favor the evolution of sensory systems that utilize the most reliable environmental cues. In the case of foraging, the most reliable chemical cues for an animal would be those that are found in the highest abundance within its food resources and that are easily dispersed and detected within the foraging environment. Since *C. digueti* and *P. perrieri* forage in the same environment, they are subject to the same chemical background within their foraging environments. Thus, if the two species utilized the same food resources, natural selection should favor the utilization of the same chemical cues for foraging.

Comparisons of the chemically-induced feeding responses between sympatric species could offer important clues for elucidating possible mechanisms governing the coexistence of ecologically similar species. While neither *C. digueti* nor *P. perrieri* appears to gain competitive advantages over the other based solely on chemosensitivity (i.e., the ability to detect food from

longer distances), it is plausible that the use of different environmental cues could help to alleviate interspecific competition for food resources. Siemers and Swift (2006) showed that divergence in sensory ecology facilitates niche differentiation in sympatric bat species. This divergence allows two congeneric, sympatric species to forage in the same areas without competitive displacement. Alternatively, Behmer and Joern (2008) showed that it is possible for multiple ecologically similar, sympatric grasshopper species to coexist as long as they utilize different absolute amounts of the same resources. Similarly, *C. digueti* and *P. perrieri* could have undergone subtle food niche shifts to avoid high levels of food competition. These niche differences could be reflected in their divergent responses to foraging cues, but may be too subtle to uncover by simple dietary analyses. This is especially true for studies masking the effects of interspecific competition by investigating fundamental, rather than realized food niches.

While most research on hermit crabs to date has focused on the ecological aspects of crab-shell interactions (for review see Gherardi and Tricarico, 2011), further research into the use of olfactory foraging cues in these animals is warranted. Sympatric hermit crab species provide a valuable study system for examining the ecological and evolutionary factors influencing the use of chemical cues during foraging. Because of the widespread use of chemical cues to mediate foraging in animals, studies of this sort have the potential to uncover previously unrecognized mechanisms of food niche differentiation and interspecific competition for resources between sympatric competitors.

CHAPTER 3

ANALYZING DIET OVERLAP BETWEEN SYMPATRIC HERMIT CRAB SPECIES

INTRODUCTION

Ecological communities are comprised of multiple species that compete for limited resources, and the differentiation of resource use among species is not always readily apparent. However, no two species can utilize the same resources in exactly the same way because innate differences in competitive abilities between the species will eventually result in the competitive exclusion of the inferior competitor by the superior one (Gause, 1934; Hardin, 1960). In terms of the food niche, interspecific competition for limited food resources results in either the differentiation of food niches to an extent permitting stable coexistence (Pianka, 1974; Behmer and Joern, 2008) or competitive exclusion (Gause, 1934; Hardin, 1960). Thus, ecological communities comprised of multiple species with seemingly extensive food niche overlap are particularly perplexing. In this regard, studies of resource partitioning among foraging generalists (Behmer and Joern, 2008) have the potential to further our knowledge of how much niche differentiation is needed to permit coexistence among ecologically similar species.

Sympatric hermit crab species offer a unique, but largely understudied, model system for dietary comparisons among sympatric competitors because multiple ecologically similar species often coexist in close sympatry (Vance, 1972; Bach et al., 1976; Snyder-Conn, 1980; Bertness, 1981; Barnes, 1997) and appear to overlap extensively in their diets (Hazlett, 1981; Barnes, 1997). Most hermit crab species are generalist detritivores, but research suggests that some species show distinct preferences for certain foods (Thacker, 1996; Thacker, 1998). "Preference," in the context of foraging, is operationally defined as an animal actively choosing one food item over another when the food items are equally accessible. Comparative analyses of the diets of sympatric hermit crab species are lacking, despite their potential to uncover important ecological insights on how ecologically similar, sympatric species partition their food

niches in nature. Indeed, the handful of studies that have been done addressing hermit crab feeding ecology have shown that food competition may be an important structuring force for hermit crab assemblages (Barnes, 1997; Ramsay et al., 1996; McNatty et al., 2009).

The opportunistic nature of hermit crab scavenging has necessitated the evolution of broad food niches, with food resource use spanning multiple trophic levels and including both plant and animal tissues (Hazlett, 1981; Wolcott and O'Connor, 1992). Thus, at a fundamental level, the food niches of most hermit crab species appear broad, and permit the use of a myriad of food types. However, variations in feeding ecologies, including suspension feeding, have evolved in certain lineages (Hazlett, 1981), and studies have demonstrated that species show innate preferences for certain food types (Thacker, 1996; Thacker, 1998) and the capability for rapid associative learning (Wight et al., 1990). In particular, hermit crabs seem to show preferences for protein-rich animal tissues (Hazlett, 1981; Barnes, 1997) and for food items that they have not recently eaten (Thacker, 1996; Thacker, 1998). This suggests that the fundamental food niche of hermit crabs is "generalist", but that their realized food niches may reflect either their innate preference (Thacker, 1996; 1998), learning (Wight et al., 1990), or the outcomes of interspecific competition (Ramsay et al., 1996; McNatty et al., 2009).

The coexistence of multiple ecologically similar species is particularly perplexing when viewed in light of the competitive exclusion principle (Gause, 1934; Hardin, 1960). However, it has been shown that multiple ecologically similar, sympatric generalist species can coexist if they utilize different absolute amounts and ratios of the same food resources (Behmer and Joern, 2008). Intertidal hermit crabs rely on spatially and temporally irregular carrion inputs (Britton and Morton, 1994) to obtain necessary nutrients, such as nitrogen and essential amino acids (Wolcott and O'Connor, 1992). These carrion inputs are generally contested for by multiple

species of animals (e.g., birds, crustaceans), are rapidly removed from the system (Britton and Morton, 1994), and are therefore both scarce and unpredictable resources for hermit crabs (Barnes, 1997). It appears implausible that multiple species of hermit crabs could stably coexist while relying on directly overlapping food resources (Barnes, 1997; Kaiser et al., 1996). In this regard, some differentiation of the diets of sympatric hermit crabs is likely to exist. This differentiation could occur at (1) the fundamental level, in which different species show distinct preferences for different food items, or (2) the realized level, in which ecological factors, such as competition, force species to consume different food items.

Clibanarius digueti and *Paguristes perrieri* are ecologically similar, sympatric hermit crab species that coexist in the intertidal region of the Gulf of California. C. digueti is the numerically dominant species throughout the area (Snyder-Conn, 1980), and can be found in water depths ranging from the highest intertidal area to the subtidal (Personal Observation, Ayón-Parente and Hendrickx, 2010). P. perrieri is less abundant and is generally found in a narrow band in the mid-intertidal region (Personal Observations). Thus, the distributions of the two species overlap over all of *P. perrieri's* distribution, making it unlikely that the species partition their feeding niches through geographical differences in foraging ranges (Pianka, 1974). Both species are most active and forage during flood tides (Personal Observation; Snyder-Conn, 1980; 1981), making temporal differences in foraging behaviors another unlikely mechanism of niche partitioning between the species (Pianka, 1974). Hermit crabs, like most decapod crustaceans, rely heavily on olfactory cues during foraging (Rittschof, 1992) and a previous study on C. digueti and P. perrieri has shown that the species use different olfactory cues to mediate their foraging behaviors (Tran, 2013; Chapter 2). This sensory differentiation suggests that the species may be attracted to different food items (Siemers and Schnitzer, 2004; Siemers

and Swift, 2006) and is thus a plausible mechanism of food niche differentiation between the species (Tran, 2013).

The objectives of this study were to analyze the natural diets of *C. digueti* and *P. perrieri* in order to understand whether the species (1) have overlapping preferences for food items, and (2) consume the same food resources in nature. To accomplish these objectives, I used a combination of field (bait preference experiments) and laboratory techniques (gut content and stable isotope analyses) to examine the animals' diets over different timescales. This multipronged approach allowed me to compare the fundamental and realized niches of the test species by first looking at what they prefer to eat (i.e., their fundamental niches), and then examining what they actually consumed in the past (i.e., their realized niches).

MATERIALS AND METHODS

STUDY AND SPECIMEN COLLECTION SITE

This study was conducted in the rocky intertidal region of Las Conchas, Puerto Peñasco, Sonora, Mexico. The area is situated at the Northern end of the Gulf of California, is characterized by metamorphic rock and coquina shelf substrates, and has semidiurnal mixed tides with tidal amplitudes that can exceed 7 m vertical displacement (Hofknecht, 1978). Within the intertidal zone of Puerto Peñasco, macroalgae grows in shallow tide pools created by breaks in the rock surface, but is not highly abundant (Personal Observation). Macroalgae becomes more abundant in the lower intertidal and subtidal zones. No seagrass patches or algal mats were observed in the intertidal zone.

FOOD CHOICE EXPERIMENTS IN THE FIELD

Field food choice assays were performed to assess whether the species displayed overlapping preferences for food items. Again, "preference" is operationally defined as the selection of one food item over another when an animal is given equal access to the food items. Table 3.1 lists the bait sources used in these experiments. Only locally caught baits that are native to the area were used. Baits (except for algae which was collected fresh daily) were purchased fresh from local vendors, cut to a standard size ($\sim 5 \text{ cm}^3$), and frozen individually in a standard laboratory freezer to ensure standardized freshness across trials. The testing apparatus consisted of Six 30-mL plastic Nalgene bottles that were evenly spaced along a ~ 2 m rope transect and held in place by plastic zip ties. Metal mesh plates were attached to the ends of the transect to aid handling and deployment. On the day of the trials, baits were fully thawed at room temperature and placed individually in the plastic bottles. Bait positioning along the transect was randomly assigned each day, and the bottles were thoroughly rinsed with freshwater after each trial to avoid any carryover effects of residual chemical cues. The exact position of the deployment site was varied on each test day to avoid pseudoreplication. The transect was deployed in areas of sympatry during the onset of flood tide and held in position by placing rocks on the metal plates and along the rope. The baits were observed for 30 - 90 minutes following placements. Observation times were dictated by the physical characteristics of the area on individual test days, as well as the availability of adequate natural light for observation (use of artificial light caused avoidance behaviors in hermit crabs). On some days, high wave activity made it difficult to observe the baits for long periods of time and often swept away approaching animals. During the observational period, animals that arrived within ~5 cm of the baits were collected, placed in labelled buckets, identified to the species level, and released into the water

after the days trials had ended. In order to maximize the number of trials that could be done during the time in the field, on some days the entire transect was re-deployed following the initial period of observations in an area higher in the intertidal region (lower water depth) where the flood tide had not yet reached. This allowed for up to 3 observation periods during one flood tide event, usually during periods of spring tides. A total of 30 deployments were conducted between January and February, 2013, successfully attracting 355 *C. digueti* and 37 *P. perrieri* individuals. Water temperatures ranged from 12 - 20 °C.

ANIMAL COLLECTIONS AND PRESERVATION

A total of 60 animals (30 *C. digueti* and 30 *P. perrieri*) were collected during low tide between January 10 and February 5, 2013. *P. perrieri* collections were made on 7 different days, with 2 to 8 animals collected per day. *C. digueti* collections were made on 6 different days, with 4 to 6 animals collected per day. Animals were removed from their shells and frozen for later use in gut content and stable isotope analyses. Shield lengths (mean \pm SD) were 0.36 \pm 0.06 cm for *C. digueti* and 0.36 \pm 0.07 cm for *P. perrieri*. Because animals were not allowed to consume the baits used in the field choice experiments, any incidental collection of animals previously attracted to the baits offered would not confound the results of gut content and stable isotope analyses. **TABLE 3.1.** Bait sources used for field choice experiments. ¹ The exact identify of this fish was difficult to determine because the baits were purchased pre-cleaned. Identification was based on body shape and tail morphology. ² A combination of these two species was used for each deployment. These two species were the most abundant algae in the area.

Bait	Specific Bait Used
Fish	Corvina fish (Cynoscion sp.) ¹
Bivalve Mollusk	Oyster (Ostrea sp.)
Cephalopod Mollusk	Squid (Lolliguncula panamensis)
Crustacean	Shrimp (Penaeus sp.)
Algae	Padina sp. and Sargassum sp. ²
Blank (Control)	

GUT CONTENT ANALYSES

The guts of collected specimens (*C. digueti* N = 23, *P. perrieri* N = 30) were physically removed by dissection. Gut content analyses were confined to the posterior gut contents because the anterior foregut contents (anterior to the gastric mill) could not be extracted. Gut contents were immediately mounted in a drop of distilled water (dH₂O) on a microscope slide containing an adhesive grid (VWR) made up of 1 mm squares arranged in a 10 x 10 grid. Ten randomly selected 1 mm squares were chosen for analysis and viewed under a light microscope at 10x magnification (40x used to identify objects when necessary). From the ten randomly chosen squares, I counted the total number of occurrences in the following three categories: animal, plant/algae, and cyanobacteria. Food particles in the gut were considered separate occurrences in the analysis if they were not touching (i.e., were not attached). Food categories were later combined into photosynthetic (plant, algae, and cyanobacteria) and non-photosynthetic (animal) materials for further analysis. The decision to combine the food categories into photosynthetic and non-photosynthetic materials was made *a priori*. Because of the inability to extract the anterior foregut contents and the highly digested state of the gut contents of the posterior gut, identification to more specific taxonomic units was not possible.

STABLE ISOTOPE ANALYSES

Nitrogen and carbon stable isotope analyses were conducted on specimens collected from the field. Muscle tissue was carefully dissected away from the exoskeleton of the animals using a dissecting microscope. Only muscle tissue that was free of exoskeleton fragments and any gut contents were used for isotope analyses. After dissection, muscle tissue was dried overnight in a 60 °C oven, lipid extracted, and re-dried in a vacuum oven. Dried samples (0.8 - 1.2 mg) were analyzed for carbon (N = 28 *C. digueti*, N = 30 *P. perrieri*) and nitrogen (N = 29 *C. digueti*, N = 30 *P. perrieri*) stable isotope ratios using a Eurovector elemental analyzer interfaced to an Isoprime stable isotope mass spectrometer (Elementar, Mt. Laurel, NJ). Isotope values of samples were expressed relative to those of standards using delta (δ) notation:

$$\delta X = [(R_{Sample}/R_{Standard}) - 1] \times 1,000$$

where R is the abundance ratio of the heavy to light isotope $({}^{13}C/{}^{12}C \text{ or } {}^{15}N/{}^{14}N)$ and X is the heavy isotope of element either C or N. All isotope values are reported with respect to the international standards VDPB for carbon and atmospheric N₂ for nitrogen. Analytical reproducibility is 0.2‰ for both carbon and nitrogen isotope values. Because of permitting restrictions, I was not allowed to collect potential food items or carrion sources found in the field. Thus, my analyses are restricted to the isotope values of *C. digueti* and *P. perrieri*, and I cannot estimate the percent contribution of food items to the diets of the test species.

STATISTICAL ANALYSES

All statistical tests in this report employed a significance cutoff of P = 0.05. I analyzed attraction to the baits in the field experiment both within and between species. For within species comparisons, I used chi-square goodness-of-fit tests to test whether the species showed preferences for certain bait types. For between species comparisons, I first converted the data to proportions (expressed as percentages of animals of each species attracted to each bait source) and compared the species using a Fisher's Exact Test. I also analyzed gut contents within and between species. For within species comparisons, I used chi-square goodness-of-fit tests to compare the frequency of diet objects in the guts. This tested whether the frequency of diet objects differed within each species. To compare between species, I converted the frequencies of diet objects into proportions (expressed as percent contribution of each diet object to the total gut content counts; Kurimoto and Tokeshi, 2010) and compared the species using a Fisher's Exact Test. To compare δ^{13} C and δ^{15} N values between the test species, I used Welch's t-tests because the data were normally distributed but violated the equal variances assumption of Student's t-tests. Variances for each isotope value were compared between species using an F-test.

RESULTS

FOOD CHOICE EXPERIMENTS IN THE FIELD

Both *C. digueti* ($\chi^2 = 115.45$, df = 5, *P* < 0.001) and *P. perrieri* ($\chi^2 = 20.57$, df = 5, *P* <

0.001) showed distinct preferences for the baits offered (Figure 3.1). The preferences of both species appeared to overlap, and there was no significant difference between species in the proportion of animals arriving at each bait (Fisher's Exact Test, P = 0.176; Figure 3.1). Squid

attracted the most animals of both species, indicating that it was the preferred bait type of those offered. Shrimp was the second most attractive bait source offered for both species, with oyster and fish being the next most visited baits. For both test species, the blank (control) attracted more animals than the algae bait, suggesting that algae is not an attractive food source for these animals in the context of this experiment.



FIGURE 3.1. Proportion of animals arriving at baits. Proportions were calculated for each species by dividing the total number of animals arriving at each bait by the total number of animals observed approaching the baits. N = 355 C. *digueti* and N = 37 P. *perrieri*.

GUT CONTENT ANALYSES

Comparisons of *C. digueti* and *P. perrieri* gut contents revealed a significant difference in the proportion of diet items in the guts of the species (Fisher's Exact Test, P < 0.001; Figure 3.2). Frequencies of the food types differed within species for both *C. digueti* ($\chi^2 = 73.66$, df = 2, P < 0.001) and *P. perrieri* ($\chi^2 = 27.93$, P < 0.001). *C. digueti* had a higher frequency of algal/plant tissue in its guts, with animal tissue the second most abundant food type. In contrast, *P. perrieri* had a higher frequency of animal tissue compared to algal/plant tissues. Cyanobacteria had the lowest frequency of the quantified food items in the guts of both species. Photosynthetic material was significantly more abundant than non-photosynthetic material in the guts of *C. digueti* ($\chi^2 = 58.80$, P < 0.001). No significant difference was observed between the frequencies of photosynthetic and non-photosynthetic materials in the guts of *P. perrieri* ($\chi^2 =$ 1.021, P = 0.312). Between species comparisons showed that *C. digueti* and *P. perrieri* differed significantly in the proportions of photosynthetic and non-photosynthetic materials in their guts (Fisher's Exact Test, P = 0.011).



FIGURE 3.2. Proportions of each dietary item in the guts of *C. digueti* and *P. perrieri*. A) Proportion of animal, algae/plant, and cyanobacteria in guts. B) Proportion of materials that were photosynthetic and non-photosynthetic.

STABLE ISOTOPE ANALYSES

Stable isotope analyses revealed a significant difference between the δ^{15} N (Welch t₄₉ = 17.20, *P* < 0.0001) and δ^{13} C (Welch t₄₈ = 10.49, *P* < 0.0001) values of *C. digueti* and *P. perrieri* (Figure 3.3). The difference between the mean δ^{15} N values of *C. digueti* (mean = 14.5‰) and *P. perrieri* (mean = 10.5‰) was equal to 4.0‰. The difference between the mean δ^{13} C values of *C. digueti* (mean = -9.4‰) and *P. perrieri* (mean = -12.1‰) was equal to 2.7‰. There was no significant difference in the variances of δ^{15} N values between *C. digueti* and *P. perrieri* (F_{27, 29} = 1.878, *P* > 0.05). There was, however, a significant difference between the variances of δ^{13} C values between the species (F_{28, 29} = 2.30, *P* = 0.0292).



FIGURE 3.3. Isotopic values of *C. digueti* and *P. perrieri* muscle tissue. Mean ± SD shown for each isotope value.

DISCUSSION

The fundamental food niches of *C. digueti* and *P. perrieri* appear to overlap based on the results of my bait preference experiments conducted in the field. *C. digueti* and *P. perrieri* showed similar preferences for the bait types offered, suggesting that they prefer to feed on similar food sources. Because the animals were collected as they approached the bait items, and thus did not aggregate around or feed directly on the bait items, competition for the food items was an unlikely factor influencing the baits that *C. digueti* and *P. perrieri* approached. I did not observe any instances of interspecific aggression during the trials that could have influenced the decision of either species to approach a bait source. Thus, bait preferences shown by *C. digueti* and *P. perrieri* in this experiment can be used to estimate the fundamental niches of the species.

Because of the similarity of food preferences between the species, I expected an overlap in the realized food niches of the species. However, differentiation in the realized food niches of *C. digueti* and *P. perrieri* was clearly demonstrated by the results of the gut content and stable isotope analyses. Counts of the frequencies of diet objects in the guts showed that *P. perrieri* fed equally on photosynthetic and non-photosynthetic materials, while *C. digueti* fed more on photosynthetic than non-photosynthetic tissues (Figure 3.2).

These gut content differences are consistent with the stable isotope results for muscle tissues. I found a significant difference in δ^{13} C values between *C. digueti* and *P. perrieri*, suggesting that the species may feed within food webs based on different primary producers (McCutchan Jr. et al., 2003). These different primary producers at the base of the food webs could include plant or algae species (1) using different photosynthetic pathways (i.e., C₃, C₄, or CAM), (2) originating in different habitats (i.e., aquatic vs. terrestrial; near shore vs. offshore), or (3) conducting photosynthesis under different levels of carbon dioxide limitation (O'Leary,

1988). Differences in δ^{15} N values between *C. digueti* and *P. perrieri* were also consistent with the results of my gut content analyses. I found the mean δ^{15} N value for *P. perrieri* to be 4.0‰ higher than that of *C. digueti*, consistent with the typical 3-4‰ increase in δ^{15} N values seen between trophic levels (Cabana and Rasmussen, 1994). Thus, my results suggest that *P. perrieri* feeds at a higher trophic level than *C. digueti*. The low δ^{15} N value of *C. digueti* relative to *P. perrieri* is consistent with the results of my gut content analyses, in which *C. digueti* guts had significantly more photosynthetic materials than *P. perrieri* guts.

Hermit crabs show a wide range of δ^{13} C and δ^{15} N values among species in the published literature. δ^{13} C values for previously studied hermit crab species range from -25 to -15‰, and δ^{15} N values range from 3 to 16‰ (Pinnegar and Pulunin, 2000; Moncreiff and Sullivan, 2001; Kieckbusch et al. 2004; Lugendo et al., 2006; McNatty et al., 2009). Thus, isotope data for hermit crabs shows a wide range of values which are likely influenced heavily by local environmental conditions. Because I was not permitted to collect and analyze local flora and fauna, it is difficult to draw conclusions on the absolute values of δ^{13} C and δ^{15} N shown by *C*. *digueti* and *P. perrieri*. However, in their analyses of habitats within the Gulf of California similar to those inhabited by *C. digueti* and *P. perrieri*, Anderson and Polis (1998) found that marine algae and plants had average δ^{13} C values of -11.00‰ and δ^{15} N values of 14.86‰. These values coincide with the range of δ^{13} C and δ^{15} N observed for *C. digueti* and *P. perrieri* (Figure 3.3). Generalist foragers have broad fundamental niches and are capable of consuming many different food resources. While the data suggest that the realized food niches of the two species are differentiated, it remains unknown whether this differentiation is the result of the species utilizing different resources altogether, or using the same resources in different proportions (Barnes, 1997; Behmer and Joern, 2008). Given the small size of the animals analyzed, and the nature of their feeding (tearing small pieces of food with the chelipeds before consuming), gut content analyses lacked the resolution needed to accurately identify diet objects to genus or species levels. Future studies could employ DNA testing of gut contents to achieve a finer scale of resolution.

There are a number of potential reasons why the food niches of *C. digueti* and *P. perrieri* are different. Interspecific competition has been shown to be an important determinant of the levels of food niche overlap between ecologically similar, sympatric species (Pianka, 1974; Behmer and Joern, 2008; McNatty et al., 2009). Differences in competitive abilities dictate which species will gain access to limited food resources, and thus be able to feed on their preferred food sources, when available. Previous studies on sympatric hermit crab species (Kaiser et al., 1996) have shown that food competition influences species access for shared food resources. Interspecific competitors (Hazlett, 1981; Kaiser et al., 1996; Laidre, 2007), with access to resources dictated by fighting ability. Indeed, Chapter 4 of this dissertation shows that *C. digueti* and *P. perrieri* differ in their rates of aggression and competitor than *P. perrieri*, with *C. digueti* relying heavily on aggression to gain increased access to shared food resources. At first glance, it seems counter-intuitive that the dominant competitor in the assemblage should feed at

a lower trophic level than the inferior competitor. However, there are a number of specific advantages to feeding at lower trophic levels, such as increased access to available food resources and higher efficiency of energetic transfer between lower trophic levels (Hairston and Hairston, 1993). In their analysis of Northern Gulf of California species, Morales-Zárate et al. (2004) found that cephalopods occupy an intermediate trophic level below fish and shrimp species. In my field food preference experiments, the largest proportion of animals of both species were attracted to squid tissue, suggesting that both species prefer to feed on carrion sources of intermediate trophic levels. If this is the preferred trophic level for both species to feed, and *C. digueti* can use aggression to dominate interspecific competition, it is plausible that *C. digueti* gains access to its preferred food items, thus forcing *P. perrieri* to feed on higher trophic-level foods, when available.

My results support the idea that the natural limitation of high-quality protein sources may influence hermit crabs decisions to consume lower-quality photosynthetic tissues (Wolcott and O'Connor, 1992). The natural diets of both *C. digueti* and *P. perrieri* consisted of a mix of photosynthetic and non-photosynthetic materials. However, when crabs were presented with an array of both animal tissues and algal tissues during our field experiments, algae were consistently the least visited bait behind all protein sources. This result suggests that hermit crabs supplement their diets with photosynthetic materials when animal carrion is not present, but when animal carrion is present, hermit crabs clearly show preferences for animal tissues over photosynthetic tissues. Photosynthetic material is generally harder for crabs to digest, and provides fewer essential nutrients (e.g., nitrogen and amino acids) than animal tissue (Wolcott and O'Connor, 1992). In this regard, it remains unclear why *C. digueti*, the presumed better competitor, was found to have a greater proportion of photosynthetic materials than *P. perrieri* in

its guts. Further research is needed to elucidate the links between interspecific competition and food resource use between *C. digueti* and *P. perrieri*.

While a plethora of knowledge exists on how hermit crabs use, and compete for, gastropod shells as ecological resources, very little is known about hermit crab feeding ecology. The results of this study confirm that the food niches of sympatric hermit crab species may be differentiated, despite the aura of extensive overlap created by their generalist detritivore feeding strategies (Hazlett, 1981). Specifically, greater attention needs to be directed to understanding the importance of food competition and food niche differentiation in hermit crab assemblages. By taking a more multidimensional approach to ecological studies of hermit crabs, we can better understand the factors influencing intertidal community dynamics and better explain the observed patterns of resource use exhibited by sympatric hermit crab species.

CHAPTER 4

AGGRESSION AND FOOD RESOURCE COMPETITION BETWEEN SYMPATRIC HERMIT CRAB SPECIES

Tran, M.V., O'Grady, M., Colborn, J., Van Ness, K., and Hill, R.W. Aggression and Food Resource Competition Between Sympatric Hermit Crab Species. Submitted to *PLOS ONE*.

INTRODUCTION

Hermit crabs have commonly been used as subjects in studies addressing resource use and competition. To date, research on hermit crabs has focused extensively on how they compete for shell resources by means of aggressive interactions. However, relatively little is known about how hermit crabs compete for other important ecological resources (i.e., food, shelter), and how the outcomes of these competitions influence the distributions and abundances of competing species. In particular, the potential importance of interspecific food competition on the structure of hermit crab assemblages has been largely overlooked.

Intertidal hermit crab assemblages offer an interesting study system to test hypotheses on the ecological effects of interspecific competition because (1) multiple ecologically similar species often live in close sympatry (Hazlett, 1981), and (2) sympatric species often show stereotypical patterns of vertical (depth) zonation (Ayón-Parente and Hendricx, 2010) with species segregating into distinct bands within the intertidal zone (Connell, 1961). The causes of these zonation patterns in hermit crabs are largely unknown, but are believed to be the result of a complex interplay between abiotic (e.g., desiccation risk, aerial exposure) and biotic (e.g., competition) pressures (Bertness, 1981; Stillman and Somero, 1997; Somero, 2002).

Hermit crabs are generalist detritivores (Hazlett, 1981), but many species show a strong affinity for consuming carrion, likely because of carrion's high nutritional value (Wilson and Wokovich, 2011) compared to other available food items (e.g., algae). Ecological studies have confirmed that carrion is a particularly scarce resource in the intertidal zone (Britton and Morton, 1994), and one that is believed to limit the sizes of scavenger populations (McKillup and McKillup, 1997; McNatty et al, 2009). Hermit crabs face intense competition for carrion resources because carrion is (1) distributed irregularly in space and time (Britton and Morton,

1994) and (2) often rapidly removed from a system because of being relied upon by numerous competing species (Britton and Morton, 1994; McKillup and McKillup, 1997; Kaiser et al., 1998; McNatty et al., 2009). Indeed, the scarcity of intertidal carrion resources has been shown to limit the population sizes of intertidal scavengers (McKillup and McKillup, 1997). Thus, it is likely that differences in the competitive abilities for food between sympatric species have important implications for the structure of hermit crab assemblages, including species distributions (Connell, 1961, Paine, 1974; Sousa, 1979, Lohrer et al., 2000) and abundances within the intertidal zone. Ecological research has shown that dominant competitors will inhabit the most profitable foraging grounds and geographically displace inferior competitors (Heller, 1971). Thus, the zonation patterns observed among sympatric hermit crab species may be the result of competitive displacement by the dominant food competitor from areas of highest food resource availability.

Hermit crabs rely on aggressive interactions to settle resource disputes. Differences in aggressive behaviors between species can result in unequal access to resources between competitors, with the outcomes usually favoring the more aggressive species (Kaiser et al., 1998; Pieman and Robinson, 2010). Thus, it can be predicted that species differing in competitive abilities will also differ in aggressiveness. In this study, we used behavioral bioassays to test the hypotheses that intertidal hermit crab species exhibiting vertical zonation differ in their (1) rates of aggression (both inter- and intraspecific) during the search phase of foraging and (2) competitive abilities for food. To test these hypotheses, we studied two ecologically similar, sympatric hermit crab species, *Clibanarius digueti* and *Paguristes perrieri*, from the Gulf of California. *C. digueti* is the most abundant hermit crab species in the Gulf of California (Snyder-Conn, 1980) and generally occupies areas ranging from the subtidal to the high intertidal zone

(Ayón-Parente and Hendrickx, 2010; Personal Observation; Figure 1.1.). *P. perrieri* is a common hermit crab that occupies a small band within the mid-intertidal zone (Ayón-Parente and Hendrickx, 2010; Personal Observation; Figure 1.1), but is not observed inhabiting the upper-intertidal zone (Personal Observation). Thus, the species overlap over all of *P. perrieri*'s distribution, with *C. digueti*'s distribution extending significantly higher in the intertidal zone than *P. perrieri*'s. A previous study (Harvey, 1988) has shown that abiotic factors (i.e., desiccation risk) are not strong determinants of *C. digueti* and *P. perrieri*'s distributional limits, suggesting that competitive exclusion may prevent *P. perrieri* from inhabiting the upper intertidal zone. The species are of similar body sizes (Harvey, 1988), and field observations (Chapter 3) have shown that they overlap in their carrion preferences. Thus, there is reason to believe that the species compete for food resources in nature.

MATERIALS AND METHODS

ANIMALS, HOUSING AND MAINTENANCE

Wild-caught *Clibanarius digueti* and *Paguristes perrieri* from the Gulf of California were obtained from a commercial distributor (A & M Aquatics, Lansing, MI) and acclimated to laboratory conditions for a minimum of two weeks prior to use in experiments. During acclimation, animals were held communally in mixed-species groups in 10-gallon glass aquaria containing aerated artificial saltwater (ASW; Instant Ocean) and kept under a 12h light: 12h dark cycle. This ASW, and all ASW mentioned in this report, was maintained at 24 - 28 °C, pH 8.2 - 8.4, and specific gravity of 1.022 - 1.025. Animals were fed 2-3 times per week with a krill meal-based pellet food (NewLife Spectrum).

Only males were used for aggression and competition experiments to control for any effects of sex on aggression or competitive ability. Animals were sexed by visually examining

the gonopores. Because of their dark body coloration, *C. digueti* could only be sexed after evacuation from its shell. The shells of *C. digueti* were removed by gently flaming the tip of the shell, causing the animal to evacuate without harm and preserving the integrity of the shell for re-entry. The animals appeared behaviorally normal following this manipulation, and readily reentered their shells. If any injury or behavioral abnormalities were noted, the animal was not used. *P. perrieri's* light body coloration allowed them to be sexed without removal of the shell by holding the animal inverted under water until the animal emerged from its shell and exposed the gonopores.

Following gender determination, animals were housed in small groups in plastic containers ($20 \times 13 \times 14$ cm or $26 \times 16 \times 17$ cm) containing ASW and a gravel substrate for a minimum of 4 days to allow sufficient time to recover from handling stress. Because we allowed a minimum of 4 days to recover from handling stress, it is highly unlikely that the different methods of sex determination used between species influenced the animals' behaviors in our experiments. The size of the containers used to house experimental animals had no effect on either species for any of the measured behaviors (Mann-Whitney U-tests, P > 0.05).

To test aggression and competitive abilities, we sized-matched pairs of animals. These pairs of animals were always drawn from separate plastic housing containers in order to alleviate the effects of any pre-existing dominance hierarchies that may have been formed among tankmates (Gherardi and Atema, 2005). For all experiments assessing interspecific differences, we housed animals in containers with conspecifics only, and for experiments assessing intraspecific differences, we housed animals in mixed-species containers. On the day the experiments were conducted, experimental pairs of animals were formed by selecting one animal from separate containers. All pairs of animals were size-matched within 3 mm shell length.

Size-matching controlled for the effect of body size on aggression and competitive abilities. Shell length was used as a proxy for body size because (1) the species show similar relationships between wet body weight (g) and length (cm) of shell inhabited (Figure 4.1), (2) during acclimation in housing tanks, animals were provided an abundance of empty shells of different shapes and sizes so they could choose optimally-fitting shells (Tricarico et al., 2008), and (3) using animals of equal shell size helped to ensure that aggressive interactions observed during the trials were the result of food competition, and not the result of motivation to switch shells, which could confound the results of these experiments. This method was effective since test crabs rarely showed shell investigation behavior, and only one case of shell switching was documented during the trials. Both species routinely occupied *Cerithium stercusmuscarum* shells.

For experiments assessing feeding times in the absence of a competitor, we used animals of both sexes (11 males: 4 non-gravid females of each species) that had previously been used in other, non-related behavioral experiments and were randomly drawn from mixed-species population tanks. We did this because we had a limited availability of *P. perrieri* males and had no *a priori* reason to believe that normal feeding behaviors (1) would be affected by the animals' previous use in other, non-related experiments, or (2) differed between sexes when no competitors were present. Animals were used only once in this experiment.



FIGURE 4.1. Relationship between wet weight (g) and shell length inhabited (cm). Data were drawn from lab populations of *C. digueti* (N = 75) and *P. perrieri* (N = 79) following acclimation in housing tanks. Wet weights were measured from animals after removal of their shells. Lines represent best fit lines from linear regression analyses. *C. digueti* best fit line: y = 0.2442x - 0.3584. *P. perrieri* best fit line: y = 0.2472x - 0.3764.

TESTING APPARATUS

The testing apparatus consisted of a 250 mL glass Erlenmeyer flask (8 cm bottom diameter) containing 250 mL of ASW and clean, white gravel substrate. This apparatus was large enough that the test animals could remain physically separated and were not forced to interact.

FOOD ODOR AND FOOD PELLET PREPARATION

A liquid food extract (FE) was prepared by macerating 2 g of the animals' normal pellet food diet (NewLife Spectrum) in 200 mL of ASW for 5 minutes. The liquid supernatant was frozen (-4°C) in ~5 mL aliquots in glass vials and thawed at room temperature immediately prior to use. Food pellets used in "Interspecific Competition in the Presence of Food" experiments were made by macerating 4 g of pellet food (NewLife Spectrum) in 10 mL dH₂O until blended. The contents were poured into a 5 cm diameter plastic petri dish and air dried at room temperature (~22°C) until solid. The solid food pellet was broken into ~0.5 cm³ pieces for use in the experiments. This pellet size was sufficient to allow both competitors to feed simultaneously, but small enough so that the animals would be forced to interact physically if feeding simultaneously.

INTERSPECIFIC AGGRESSION IN THE PRESENCE OF FOOD ODOR ONLY

Animals were given no food for 2 days prior to use to ensure motivation to forage during trials. On the day of the experiment a size-matched pair of heterospecific animals was formed,

placed into the testing apparatus, and given a minimum of 15 minutes to acclimate. Following acclimation, 2 mL FE were pipetted into the top of the apparatus using a glass pipette. The animals were allowed 30 seconds to initiate foraging behaviors, after which the numbers of aggressive and submissive behaviors (Table 4.1) exhibited by each animal were counted for 10 minutes. Only trials in which both animals showed obvious foraging behaviors (e.g., increased locomotion, substrate probing, feeding movements; Lee and Meyers, 1996) after the FE was introduced were included in analyses. Twenty trials of this experiment met these criteria and were used for analyses. We compared the number of (1) aggressive behaviors, and (2) submissive behaviors observed during the trials between the species using a two-sided Wilcoxon Sign-Ranks test for matched pairs. Paired analyses were required because the behaviors of one species could directly influence the behaviors of the other species during the trials. These, and all other statistical tests mentioned in this report, employed a significance cutoff of P = 0.05.

Behavior	Description
Aggressive	
Approach	Animal moves towards other animal
Display	Animal shows chelipeds and/or legs in threatening move
Attack	Animal strikes other animal with chelipeds/legs
Grasp	Animal grasps onto other animal's shell
Submissive	
Retraction	Animal pulls its body into shell
Retreat	Animal moves away from other animal

TABLE 4.1. Aggressive and Submissive Behaviors Observed During Foraging.

INTRASPECIFIC AGGRESSION IN THE PRESENCE OF FOOD ODOR ONLY

Size-matched pairs of conspecific animals were tested using the same procedure listed in the previous section. One *C. digueti* trial was excluded from analyses because the animals switched shells during acclimation, and we could not be sure that subsequent aggressive behaviors were not shell-related. Seventeen trials for each species were used for analyses (34 trials total). We compared the number of (1) aggressive, and (2) submissive behaviors shown during intraspecific trials between species using a two-sided Mann-Whitney U-test. Paired tests were not required for these statistical analyses because the species were tested independently of each other, and thus the behaviors of one species could not directly affect the behaviors of the other.

INTERSPECIFIC COMPETITION IN THE PRESENCE OF FOOD

Prior to use in these experiments, animals were withheld from food for 1 day prior to testing to ensure motivation to forage. Size-matched pairs of heterospecific animals were placed into the testing apparatus, and given a minimum of 15 minutes to acclimate. Following acclimation, a single food pellet ($\sim 0.5 \text{ cm}^3$) was placed an equal distance from both animals and 2 mL FE were immediately pipetted into the testing apparatus to initiate foraging behaviors. The animals were allowed 2 minutes to locate the food item. Observation time was started either when an animal contacted the food item or 2 minutes elapsed after food placement. The first animal to contact the food item and the total time spent feeding by each animal were recorded for a period of 10 minutes. Feeding was characterized by the animal controlling (grasping) the food item with its chelipeds or legs and picking off small pieces with the chelipeds. Only trials in

which (1) both animals showed foraging behaviors after food placement and (2) at least one animal fed were used for analyses. Twenty six trials of this experiment met these criteria and were used in the analyses. The proportion of trials in which each species was the first to contact the food item was compared using a chi-square goodness-of-fit test. Time spent feeding by each species was compared using a two-sided Wilcoxon Sign-Ranks test for matched pairs.

FEEDING TIMES WITHOUT COMPETITION

This experiment was done to determine if feeding times for both species were similar in the absence of a competitor. The same experimental procedure was used as in the previous section, except that only a single animal was placed in the apparatus during each trial. Feeding times were compared between species using a two-sided Mann-Whitney U-test.

RESULTS

INTERSPECIFIC AGGRESSION IN THE PRESENCE OF FOOD ODOR ONLY

During interspecific foraging bouts in the presence of food odor only, *C. digueti* showed significantly more aggressive behaviors than *P. perrieri* (W = 171.0, P = 0.0015; Table 4.2), while *P. perrieri* showed significantly more submissive behaviors than *C. digueti* (W = -92.0, P = 0.0172; Table 4.2). *C. digueti* frequently initiated the interactions by approaching *P. perrieri*, and *C. digueti* escalated the interactions by frequently attacking and grasping *P. perrieri* (Table 4.2). *P. perrieri* routinely responded to *C. digueti*'s aggressive behaviors by retracting into their shells (Table 4.2).

TABLE 4.2. Behaviors observed during interspecific aggression trials. Total cour	nts of
behaviors shown. Counts were summed among test animals of the same species. N	r = 20
trials.	

Behavior	Clibanarius digueti	Paguristes perrieri
Approach	53	10
Display	0	2
Attack	21	5
Grasp	46	15
Total Aggressive	120	32
Mean ± SEM Aggressive	6.00 ± 0.73	1.60 ± 0.50
Retreat	4	2
Retract	4	33
Total Submissive	8	35
Mean ± SEM Submissive	$\textbf{0.40} \pm \textbf{0.17}$	1.75 ± 0.45

TABLE 4.3. Behaviors observed during intraspecific aggression trials. Total counts of behaviors shown. Counts were summed among test animals of the same species. N = 17trials for each species.

Behavior	Clibanarius digueti	Paguristes perrieri
Approach	76	30
Display	12	2
Attack	43	23
Grasp	52	20
Total Aggressive	183	75
Mean ± SEM Aggressive	10.76 ± 1.90	4.41 ± 1.34
Retreat	31	9
Retract	19	12
Total Submissive	50	21
Mean ± SEM Submissive	2.94 ± 0.79	1.24 ± 0.64

INTRASPECIFIC AGGRESSION IN THE PRESENCE OF FOOD ODOR ONLY

The number of aggressive (U = 54.0, P = 0.0019) and submissive (U = 85.0, P = 0.0295) behaviors were significantly higher for *C. digueti* than *P. perrieri* during intraspecific foraging bouts (Table 4.3). The frequencies of all measured behaviors were higher for *C. digueti* than *P. perrieri* (Table 4.3).

INTERSPECIFIC COMPETITION IN THE PRESENCE OF FOOD

During interspecific food competition, *C. digueti* fed for significantly more time than *P. perrieri* (W = 179.0, P = 0.0238; Figure 4.2). Mean feeding times for *C. digueti* were substantially higher than for *P. perrieri* (means 360.6 s and 234.1 s, respectively). Differences in feeding times between the species can partly be explained by the fact that *C. digueti* was the first animal to contact and feed on the food item in 20 of the 26 trials analyzed ($\chi^2 = 7.54$, df = 1, P = 0.006; Figure 4.3).

FEEDING TIMES WITHOUT COMPETITION

In the absence of a competitor, the feeding times of *C. digueti* and *P. perrieri* were not significantly different (U = 102.0, P = 0.6738; Figure 4.4).


FIGURE 4.2. Outcome of food competition between *Clibanarius digueti* and *Paguristes perrieri*. Mean \pm SEM time spent feeding shown. N = 26 trials. * P < 0.05.



FIGURE 4.3. First animal to contact food item during interspecific food competition assays. The number of trials in which each species was first to contact the food item is shown. N = 26 trials. ** P < 0.01.



FIGURE 4.4. Feeding times when no competitor was present. Mean \pm SEM time spent feeding shown. N = 15 trials for each species. NS = not significantly different.

DISCUSSION

The results of this study suggest that the zonation patterns of intertidal hermit crab species may be influenced by differences in the species rates of aggression and competitive abilities for food resources. During interspecific aggression trials in the presence of food odor only, *C. digueti* showed significantly higher frequencies of aggressive behaviors than *P. perrieri*, whereas *P. perrieri* showed significantly higher frequencies of submissive behaviors. Intraspecific interactions between *C. digueti* individuals also resulted in more aggressive behaviors than were observed for *P. perrieri*, suggesting that *C. digueti* relies heavily on aggression to mediate intraspecific, as well as interspecific, resource disputes.

In the absence of interspecific competition, there was no difference in the time spent feeding by *C. digueti* and *P. perrieri*. This suggests the species have similar feeding rates when their feeding is not interfered with by a competitor. When the two species fed together, *C. digueti* fed for a significantly higher amount of time than *P. perrieri*, although both species did gain access to the food item and the species sometimes fed simultaneously. This suggests that *C. digueti* is dominant over *P. perrieri* in regards to food competition. Qualitative observations during interspecific feeding trials revealed that *C. digueti* would routinely aggress towards *P. perrieri* as *P. perrieri* either approached the food item or as the two species fed simultaneously. Interestingly, *P. perrieri* did not routinely abandon an already encountered food item upon *C. digueti*'s aggression. Instead, *P. perrieri* routinely withstood *C. digueti*'s aggressive acts and continued feeding. This is in contrast to qualitative observations made during aggression trials when no food was present, during which *C. digueti*'s aggressive actions sometimes resulted in *P. perrieri* decreasing the vigor with which it searched. These changes in foraging motivation by *P. perrieri* following *C. digueti*'s aggression were, however, not quantified as part of this study, but

do warrant further exploration as they could be important determinants of food competition outcomes between these species. Based on our findings, it is likely that *C. digueti* relies heavily on aggression during the search phase of foraging (i.e., between sensory detection and arrival at the food), when aggression towards *P. perrieri* is more likely to dissuade *P. perrieri* from continuing its search. When food is present and *P. perrieri* is feeding, it is harder for *C. digueti* to force *P. perrieri* off the food item.

The outcomes of food competition between *C. digueti* and *P. perrieri* could also be influenced strongly by the differential abilities of the species to locate food items. When food was present in the testing apparatus, *C. digueti* was the first species to contact the food item in 20 of the 26 trials conducted. Previous experiments have shown that the two species do not differ in their chemosensitivities to foraging cues (Tran, 2013; Chapter 2) and thus both species appear equally capable of locating food resources from a distance via their primary sensory input (olfaction). However, the locomotive abilities of the two species (i.e., speed of locomotion) could play an important role in determining which species arrives at a food item first. *C. digueti* appears to be more active and to cover more foraging space than *P. perrieri*, which likely increases *C. digueti* 's probability of encountering stationary food items. Alternatively, *P. perrieri* may require social information provided by conspecifics and heterospecifics to locate food resources (Laidre, 2010).

The results of this study highlight the need for further research on the feeding ecology of hermit crabs. Because so little is known about hermit crab food competition, it is difficult to compare its importance to that of shell competition in influencing species abundances and distributions. However, because hermit crabs also use aggression to contest for shells (Hazlett, 1981; Turra and Leite, 2004; Tricarico et al., 2008), it is likely that highly aggressive species

would be better at competing for both food and shells than less aggressive species. Thus, it seems unlikely that there would be an ecological trade-off between a species' ability to compete for food and shells.

The differences in competitive abilities for food between C. digueti and P. perrieri may have a number of ecological implications. First, the outcomes of food competition may influence species abundances in nature (McNatty et al., 2009). C. digueti was shown to be the dominant food competitor in this study, and is also the most numerically dominant species in the Gulf of California. Second, differences in competitive abilities may influence the distribution of C. digueti and P. perrieri within the intertidal zone. The zonation patterns observed in nature, with C. digueti being the only hermit crab species inhabiting the upper-intertidal zone, may reflect competitive (geographical) exclusion of *P. perrieri* from the upper-intertidal zone by *C*. *digueti*. Previous studies have shown that dominant competitors displace inferior competitors from the most profitable habitats (Connell, 1961; DeBach, 1966; Lohrer et al. 2000; Reitz and Trumble, 2002). In the context of our study, this competitive displacement explanation is dependent on food abundance being highest in the upper intertidal zone. While this has not been tested empirically within the natural ranges of these species, it is plausible given that carrion is often stranded at the land-water interface (i.e., the strand line). As the result of wave action, carrion may be lifted higher in the intertidal zone (closer to the shoreline) where it interacts with the land and becomes stranded (Polis and Hurd, 1996). Thus, proximity to the strand line would afford animals more frequent opportunities to encounter carrion resources. If competitive exclusion from the upper intertidal zone was not occurring, it is difficult to understand why P. *perrieri* would not be found higher in the intertidal zone, since they seem physiologically capable of tolerating the harsher environmental conditions of the upper-intertidal zone (Harvey,

1988). Finally, the differences in competitive abilities between the species may force *P. perrieri* to consume different food items than *C. digueti*. Indeed, evidence of food niche differentiation has been demonstrated through sensory differences between these species (Tran, 2013; Chapter 2) and through dietary analyses of the species (Chapter 3).

The zonation patterns of intertidal organisms can be influenced by a complex interplay among abiotic and biotic factors. Our data suggest that the outcomes of interspecific food competition may be an important determinant of the structure of intertidal hermit crab assemblages. While considerable attention has been focused on understanding how shell selection influences the structure of hermit crab assemblages and interspecific dynamics, further attention must be given to how these animals utilize other resources (e.g., food) to fully understand how species distributions are formed and maintained in nature.

CHAPTER 5

THE SCENT OF CANNIBALISM: THE OLFACTORY BASIS OF CANNIBALISM IN HERMIT CRABS

Tran, M.V. The Scent of Cannibalism: The Olfactory Basis of Cannibalism in Hermit Cabs. Submitted to the *Journal of Experimental Marine Biology and Ecology*.

INTRODUCTION

Cannibalism is widespread across the animal kingdom and is believed to be an adaptive foraging strategy in many ecological contexts (Fox, 1975). Although cannibalism offers nutritional benefits (Crossland et al., 2011; Mayntz and Toft, 2006; Meffe and Crump, 1987; Nagai et al., 1971), it also poses potential costs to the cannibal. One such cost is an increased risk of predation directed at the cannibal, especially for scavenging cannibals that feed on dead or injured conspecifics (hereafter referred to as CONs). Because the prey's cause of death is difficult to assess from a distance, scavenging cannibals that approach dead or injured CONs could also be approaching potential predators (Ferner et al., 2005). Thus, scavenging cannibals must either be able to accurately assess their risk of death by predation before approaching a potential food source, or prioritize feeding over the risk of predation. How foraging animals assess this risk and make the appropriate decisions is poorly understood. However, it has been predicted that animals should exhibit caution when approaching injured CONs and heterospecifics (HETs; animals of closely related, sympatric species) due to the strong selection pressures associated with risk of predation directed at the scavenger (Ferner et al., 2005; Moir and Weissburg, 2009).

Many aquatic animals rely heavily on olfaction to mediate vital processes in their daily lives, including foraging and predator avoidance (Hazlett, 2011; Rittschof, 1992). For aquatic crustaceans, risk of injury or death by predation is often assessed based on the presence or absence of "alarm" cues processed by the olfactory system (Hazlett, 2011). These alarm cues commonly consist of odors emitted from the tissues of injured CONs or HETs that are leached into the surrounding water following predation events (Hazlett, 2011). For scavenging cannibals foraging in aquatic environments, the cues leached from CON or HET tissues into the

environment following bodily damage offer conflicting signals to the cannibal because they could represent either the availability of a potential food resource or the proximity of a predator. Thus, scavenging crustacean cannibals face the dilemma of using the same olfactory cues as both foraging and alarm cues (Ferner et al., 2005; Moir and Weissburg, 2009). Recent studies on the cannibalistic blue crab (*Callinectes sapidus*) have shown that these animals respond to the odors of injured CONs in a manner more consistent with risk avoidance than foraging (Ferner et al., 2005; Moir and Weissburg, 2009). However, further research on other crustacean species is needed to determine if these responses are stereotypical of all crustaceans, or are species-specific.

Hermit crabs make excellent model systems for addressing hypotheses regarding the riskreward consequences of cannibalism because (1) they are generalist scavengers (Hazlett, 1981) that have been documented to exhibit cannibalistic behaviors in nature (Barnes, 1997), and (2) like most aquatic crustaceans, these animals rely heavily on olfaction to mediate their foraging behaviors (Rittschof, 1992). While recent studies (Laidre, 2010; Laidre 2013) indicate that terrestrial hermit crabs rely heavily on visual and social cues during foraging, olfaction is regarded as the primary sensory input for environmental information in aquatic hermit crabs. Additionally, ecologically similar hermit crab species often live in close sympatry (Hazlett, 1981; Mackie and Boyer, 1977; Snyder-Conn, 1980), presenting us with potentially excellent model systems for the exploration of behavioral reactions to both CON and HET odors (Hazlett and McLay, 2005).

Hermit crab CON and HET odors can be released into the surrounding water due to (1) nearby predation events or (2) injury during resource contests (e.g., limb loss during shell fights; Bertness, 1981; Neil, 1985; Scully, 1979; Scully, 1983). The intensity of resource contents, and

thus the amount of physical damage inflicted during the contests, is largely dependent on the motivation of the contestants and the value of the resource being contested for (Dowds and Elwood, 1983; Laidre, 2007). While resource contests may not escalate to physical damage every time, the frequency with which these contests occurs means that hermit crabs are routinely exposed to the odors of CONs and HETs outside of the context of predation directed at hermit crabs. Since hermit crabs are scavenging cannibals and rely heavily on shells previously occupied by CONs and HETs (Laidre, 2011), the frequent detection of CON and HET odors outside of the context of predation directed at hermit responses to CON and HET odors associated with foraging (cannibalism) or shell acquisition. While the behavioral reactions of hermit crabs to these odors have been examined in the context of shell-resource acquisition (Small and Thacker, 1994; Rittschof and Hazlett, 1997; Tricarico et al., 2011), they have not been examined in the context of cannibalism.

Examining the behavioral responses of hermit crabs to CON and HET odors in the context of cannibalism requires the use of procedures that are specifically designed to identify and quantify foraging behaviors. Previous studies suggest that when hermit crabs are stimulated to search for shells via olfactory inputs, they do not exhibit foraging behaviors (Rittschof, 1980; Rittschof et al., 1992). Thus, the behaviors of foraging and shell acquisition appear to be completely different, and the identification of foraging behaviors after presenting an animal with CON or HET odors should serve to indicate that the animals are foraging, rather than searching for new shells.

The objectives of this study were twofold. First, I sought to examine the behavioral responses of two sympatric hermit crab species to the odors of crushed CONs and HETs to determine whether these animals use these odors as foraging or alarm cues. Second, I sought to

determine if there were differences in the crabs' willingness to approach and consume dead CONs versus dead HETs. To accomplish the first objective of this study, I exposed hermit crabs to the odors of crushed CONs and HETs and measured the strengths of the crabs' foraging reactions using an established foraging behavioral assay (Tran, 2013). To accomplish the second objective, I quantified whether there were differences in the animals' (1) willingness to approach dead CONs compared to dead HETs (measured as latency to contact time) and (2) time spent feeding on dead CONs compared to dead HETs. Because both CONs and HETs represent potentially important food resources for hermit crabs, I predicted that the hermit crabs would (1) show foraging responses instead of anti-predation behaviors when exposed to the odors of both crushed CONs and HETs, (2) consume both CON and HET tissues equally, and (3) show no difference in willingness to approach dead CONs and HETs. Additionally, since an animal's risk of being eaten generally decreases with increasing body size (Moir and Weissburg, 2009), I predicted that cannibal body size would be positively correlated with (1) the strength of their reactions to CON and HET odors, (2) their willingness to approach dead CONs and HETs, and (3) their willingness to sustain feeding on dead CONs and HETs. It is important to note that the objective of this study was to quantify how hermit crabs respond to CON and HET odors, not to compare the strength of responses between test species. The two test species were used in order to determine if any trends observed were species-specific, or could be extrapolated to be considered more general behaviors of hermit crabs.

MATERIALS AND METHODS

STUDY SYSTEM

Clibanarius digueti (Bouvier, 1898) and *Paguristes perrieri* (Bouvier, 1895) are ecologically similar, sympatric hermit crab species that coexist in the intertidal region of the Gulf of California (Ayón-Parente and Hendrickx, 2010) and have been documented to form large, mixed-species clusters (Snyder-Conn, 1980; Personal Observation) which likely facilitate shell switching between individuals (Gherardi and Tricarico, 2011). Because of the aggressive nature of shell contests in hermit crabs, and the potential bodily damage that accompanies such aggression, these clusters likely also facilitate cannibalistic encounters. Like most hermit crab species (Hazlett, 1981), these species are detritus and carrion scavengers. Because the two species are sympatric and of similar body sizes (Harvey, 1988), they likely share common predators, although this has not been documented. Hermit crab predators generally include fish, octopi, and birds, all of which are present in the intertidal region of the Gulf of California.

ANIMAL HOUSING AND MAINTENANCE

Wild-caught *C. digueti* and *P. perrieri* were obtained from a commercial distributor (A & M Aquatics, Lansing, MI) and housed in mixed-species groups in 10-gallon glass aquaria containing sand substrate and aerated artificial salt water (ASW; Instant Ocean). This ASW and other ASW mentioned in this report was maintained at a specific gravity of 1.021 - 1.025, temperature 23 - 29 °C, and pH 8.2 - 8.4. Animals were acclimated to laboratory conditions for a minimum of 2 weeks prior to testing while being kept under a 12h light: 12h dark cycle and fed a diet of pellet food (NewLife Spectrum) three times per week. The quantity of food provided

(~2 pellets per animal) was adequate to keep the crabs in a healthy physiological state while avoiding the buildup of nitrogenous waste products emanating from uneaten food. During acclimation, aquaria were stocked with a number of empty shells of different sizes and shapes so the animals could selectively choose optimally-fitting shells.

TESTING APPARATUS

The testing apparatus used in all experiments consisted of a 250 mL glass Erlenmeyer flask filled with 250 mL ASW and clean gravel substrate.

BEHAVIORAL REACTIONS TO THE ODORS OF CRUSHED CONs AND HETS

The three test stimuli used in these experiments were crushed CONs, crushed HETs, and plain ASW (control). ASW was chosen as the control for these experiments because (1) it has been shown to elicit no behavioral response in these species (Tran, 2013), and (2) including a stimulus that elicits no behavioral response is necessary to ensure the unbiased scoring of behavioral responses across stimuli (i.e., it prevents the observer from biasing towards high behavioral scores). One medium sized animal (shell length 2.1-2.7cm) of each species was randomly selected from a population tank each day and used for stimulus preparation. Animals were removed from their shell, sexed, and placed separately into a glass beaker containing 60 mL ASW. The animals were physically euthanized via one swift, crushing blow from the blunt end of a glass pipette, macerated for two minutes and the liquid was filtered through medium filter paper. Stimuli were maintained at room temperature (~22 °C) in glass vials that were labeled so that the observer did not know the identities of the stimuli within them, ensuring blind scoring of behaviors. To ensure standardization of test stimuli across trials, stimuli were made

fresh on each test day, only healthy male animals were used to produce stimuli, and stimuliproducing animals were used only once.

Upon the detection of a chemical feeding effector (incitant or stimulant), hermit crabs show marked behavioral changes (see Lee and Meyers, 1996 for full description of crustacean foraging), including increasing the frequency of feeding movements (i.e., touching the mouthparts with the appendages). To quantify the animals' foraging responses to the test stimuli, I used the methods of Tran (2013), which are based on the quantification methods for crustacean foraging behaviors used by Field (1977) and Johnson and Atema (1986). This behavioral assay (Tran, 2013) measures stereotypical behaviors associated with hermit crab foraging. Thus, the behaviors measured in this procedure were those related only to foraging, and not shell acquisition. On the day before the trials began, 13 experimental animals of each species (mean \pm SD shell length inhabited: C. digueti = 2.03 ± 0.24 cm, P. perrieri = 2.08 ± 0.30 cm) were randomly selected from housing tanks and placed individually into 8.5 cm³ glass jars sunken into a 20-gallon glass aquarium containing ASW. Sample sizes were determined based on ethical considerations, results from previous experiments, and from sample sizes used in similar studies (Corotto et al., 2007; Hazlett et al., 2002; Johnson and Atema, 1986; Tierney and Atema, 1988; Tran, 2013). The two species were housed and tested separately during the experiment. Animals were denied food for 2 full days prior to the start of the experiments and were not fed for the duration of the experiment. A previous study (Tran, 2013; Chapter 2) has shown that the strength of behavioral responses to olfactory foraging cues is not correlated to hunger level in the test species used in this study. Each experimental animal was tested individually for responses to each of the three test stimuli. Animals received one stimulus per day over the course of three days. Both the order of stimulus introduction and the order of

animals tested on each day were randomized to control for the effects of prior stimulus exposure and time of day influences, respectively. A single animal was placed into the testing apparatus and allowed 15 minutes to acclimate. After acclimation, 2 mL of clean ASW were pipetted from a glass pipette onto the antennules of the animal from a distance of ~ 4 cm to control for the effects of disturbance (i.e., mechanosensory stimulation) on the animal. The number of feeding movements was counted for a period of 3 minutes following ASW introduction (pre-stimulus). After this 3 minute period, 2 mL of test stimulus were pipetted in the same manner as above, and the number of feeding movements was again counted for a period of 3 minutes (post-stimulus). Movement Scores were calculated to be the number of post-stimulus feeding movements minus the number of pre-stimulus feeding movements. During ASW (pre-stimulus) and test stimulus (post-stimulus) introduction, I documented whether or not the animals retracted into their shell. Retraction into the shell was used as a metric of anti-predation behaviors because it is the stereotypical anti-predation behavior in hermit crabs (Fink, 1941; Rosen et al., 2009). For a behavior to be scored as a retraction, the animal had to (1) pull all body parts completely inside the shell and (2) retract only during the test stimulus introduction and not during pre-stimulus ASW introduction. This second criterion controlled for the possibility of the animals retracting as a consequence of overhead movements. If retractions were in response to overhead movements, they would also be present during the ASW introduction, and thus would not be scored as retractions in the context of this experiment. Once all trials were complete, animals were removed from their shells and sexed. The sex ratios used in this experiment were 9 males: 4 females for C. digueti and 11 males: 1 female: 1 unidentifiable animal for P. perrieri.

All statistical tests reported in this study employed a significance level of $\alpha = 0.05$. Parametric statistical tests were used when data conformed to the test assumptions. Non-

parametric tests were used when the data did not satisfy the assumptions for parametric tests. Movement Scores were compared among test stimuli using a repeated-measures ANOVA with post hoc Bonferroni's multiple comparisons test for C. digueti and a Friedman's test with post hoc Dunn's multiple comparisons test for *P. perrieri*. Correlation analyses between body size (measured as shell length in cm) and Movement Score were conducted for each test species and each test stimulus using Pearson's correlation analyses for C. digueti data and Spearman's correlation analyses for P. perrieri. Since chemical alarm cues may degrade over time and therefore alter behavioral reactions to the cue, it is inherently possible that the chemical cues used in these experiments degraded between the time of production and the time of use in the trials. Since degradation of the cue could alter the resulting behaviors of the experimental animals, I tested the correlation between time of day that the trial was run (as a proxy of cue degradation potential) and the Movement Score exhibited by the experimental animals in response to the cue. I calculated time of day as total minutes into the test day (i.e., minutes since midnight) because the exact time that the cue was made was not recorded. If the cues degrade over time, it should be expected that Movement Scores would show either an increase or decrease in value over time. Correlation analyses were conducted for the CON and HET cues for C. digueti using a Pearson's correlation analysis, and for P. perrieri using a Spearman's correlation analysis.

CONSUMPTION ASSAYS

Bait animals were randomly selected from a 20-gallon housing tank containing a mixedspecies group of *C. digueti* and *P. perrieri*. To limit the environmental impact of using excess amounts of animals in these experiments, all animals used as bait sources had previously been

used in other behavioral experiments that did not entail animal tissue modification. Bait animals (mean \pm SD wet weight without shell = 0.18 \pm 0.06 g) were removed from their shells, sexed, and only male animals were used. A single bait animal was used for each trial (N = 80 individuals). After shell removal, bait animals were held individually in 8.5 cm³ glass containers containing a small amount of ASW until use. Just prior to use, a single animal was removed from its container and euthanized. To avoid excess leaching of chemical cues, euthanasia was done in a dry plastic weighing dish, bait animals were not macerated after euthanasia, and each bait animal was used for only one trial. Although these bait animals were not macerated in the exact same manner as the animals used to make CON and HET odors in the previous section, the cues released in both procedures should be "alarm" cues (Wisenden, 2000).

A single experimental animal (mean \pm SD shell length: *C. digueti* = 2.20 \pm 0.37 cm; *P. perrieri* = 2.10 \pm 0.28 cm) was randomly drawn from the housing tanks, placed in the testing apparatus, and allowed a minimum of 15 minutes to acclimate. Experimental animals were used without regard to sex, although no gravid females were used. A single bait animal was placed within 1 shell length of the anterior end of the experimental animal. For 10 minutes following bait placement, I recorded (1) whether the experimental animal accepted or rejected the food item, (2) the total time it took for the experimental animal. Feeding was defined as the experimental animal holding the bait animal with its walking legs and performing feeding movements (as defined previously). First contact with the bait animal was defined as any contact made with the bait animal by an appendage of the experimental animal. If the experimental animals did not contact the bait during the 10 minute test period, a latency time of 600 seconds and a feeding time of 0 seconds were assigned. This experiment was repeated so that there were

20 trials for each test species using both CONs and HETs (N = 80 trials total). Experimental animals were used only once, and all CON and HET trials for each test species were conducted within one week of each other.

For each species, counts of feeding acceptance or rejection for each bait source were compared using chi-squared goodness-of-fit tests. Latency and feeding times were compared between CON and HET baits for each test species using two-sided Mann-Whitney U-tests. The relationship between experimental animal body size (shell length inhabited) and feeding/latency times was analyzed using Spearman's rank correlations.

ETHICAL NOTE

Every attempt was made to limit pain and suffering of animals used in this study. Euthanasia methods were selected based on rapidity and effectiveness, as well as the need to maintain tissues in their natural state for consumption assays and stimulus extraction.

RESULTS

BEHAVIORAL REACTIONS TO THE ODORS OF CRUSHED CONs AND HETS

I found no evidence that the chemical cues used in these experiments degraded between when they were made and when they were used for testing. For both test species, correlation analyses showed no significant relationships between the time of day the trials were conducted and the Movement Scores elicited by either CON or HET odors (P > 0.05; Table 5.1). Movement Scores differed significantly among test stimuli for both *C. digueti* ($F_{2,12} = 12.42$, P< 0.001) and *P. perrieri* ($\chi^2_2 = 11.69$, P < 0.01). For *C. digueti*, the odors of both crushed CONs and HETs elicited significantly larger Movement Scores than did ASW (Figure 5.1), but no significant difference was found between responses to CON and HET odors. For *P. perrieri*, the odor of crushed HETs elicited a significantly larger Movement Score than ASW and CON odor, but there was no significant difference between Movement Scores for the CON odor and ASW (Figure 5.1). For *C. digueti* responding to the conspecific odor, there was a significant negative correlation between body size (shell length) and Movement Score (r = -0.65, N = 13, P = 0.02; Table 5.2). All other correlations between body size and Movement Scores were not significant (P > 0.05; Table 5.2) for either of the test species.

Only a single *C. digueti* animal retracted when presented with the CON odor. However, this retraction was likely in response to the mechanical stimulation caused by stimulus introduction rather than the odor itself, since two *C. digueti* animals retracted when presented with ASW. No retractions were documented for *P. perrieri*.

TABLE 5.1. Correlation analyses between the time of day trial was conducted and Movement Scores. N = 13 for all analyses. ^a Correlation coefficients are based on Pearson's correlation analyses (*C. digueti*) and Spearman's rank correlation analyses (*P. perrieri*).

Test Species	Stimulus	Correlation Coefficient ^a	<i>P</i> -value
C. digueti	Conspecific	r = -0.25	0.42
	Heterospecific	r = -0.04	0.90
P. perrieri	Conspecific	$r_s = -0.42$	0.15
	Heterospecific	$r_{s} = 0.07$	0.83

TABLE 5.2. Correlation analyses between body size (shell length) and Movement Scores. N = 13 for all analyses. ^a Correlation coefficients are based on Pearson's correlation analyses (*C. digueti*) and Spearman's rank correlation analyses (*P. perrieri*).

Test Species	Stimulus	Correlation Coefficient ^a	<i>P</i> -value
C. digueti	Conspecific	r = -0.65	0.02
	Heterospecific	r = 0.16	0.60
P. perrieri	Conspecific	$r_s = -0.25$	0.41
	Heterospecific	$r_s = -0.18$	0.56



FIGURE 5.1. Foraging responses to cannibalistic olfactory cues. Boxes depict interquartile ranges. Lines inside boxes represent medians. + denotes mean. Whiskers depict minimum and maximum values for each dataset. Different letters above whiskers denote that the values are significantly different in a multiple comparisons test (see methods section for details). ASW = artificial saltwater, HET = heterospecific odor, CON = conspecific odor.

CONSUMPTION ASSAYS

C. digueti fed on CONs in 15 of the 20 trials performed ($\chi^2_1 = 5$, P = 0.03) and HETs in all of the 20 trials performed ($\chi^2_1 = 20$, P < 0.001; Figure 5.2). Latency times for *C. digueti* were not significantly different between CONs and HETs (U = 195.0, $N_I = N_2 = 20$, P > 0.05; Table 5.3). The total time spent feeding by *C. digueti* was significantly greater for HETs compared to CONs (U = 78.50, $N_I = N_2 = 20$, P = 0.001), indicating that *C. digueti* was more willing to feed on HETs than CONs (Table 5.3).

P. perrieri fed on CONs in only 5 of 20 trials ($\chi^2_1 = 5$, P = 0.03), and HETs in 11 of 20 trials ($\chi^2_1 = 0.2$, P > 0.05; Figure 5.2). Latency times for *P. perrieri* were significantly higher for CONs than HETs (U = 114.5, $N_I = N_2 = 20$, P = 0.02; Table 5.4), indicating that they were more cautious about approaching CONs than HETs. The total time spent feeding by *P. perrieri* was not significantly different between CONs and HETs (U = 142.5, $N_I = N_2 = 20$, P > 0.05; Table 5.4). Although feeding times were higher, on average, for HETs, there was a large degree of variation in feeding times for *P. perrieri* animals, which likely resulted in the insignificant result.

The *a priori* prediction that experimental animal body size would be positively correlated with feeding times and negatively correlated with latency times was not supported (Spearman's rank correlations, P > 0.05). Neither latency times (Table 5.5) nor feeding times (Table 5.6) were associated with body size for either test species.



FIGURE 5.2. Acceptance and rejection rates for bait sources offered. N = 20 trials per bait. * denotes that acceptance and rejection rates for that bait source were significantly different in a chi-square goodness-of-fit test (P < 0.05).

	Latency Time (seconds)		Feeding Time (seconds)	
	Conspecifics	Heterospecifics	Conspecifics	Heterospecifics
Mean (SEM)	86.70 (36.25)	24.45 (7.54)	123.60 (49.62)	368.30 (53.36)
Median	7.00	7.00	11.50	467.50
25 th Percentile	0.25	3.00	0.50	62.25
75 th Percentile	70.75	48.00	92.00	592.50
Minimum	0.00	0.00	0.00	15.00
Maximum	600.00	112.00	600.00	600.00
Mann-Whitney U	195.00		78.50	
<i>P</i> – Value	0.90		0.001	

TABLE 5.3. Latency and feeding times of *C. digueti* on conspecific and heterospecific baits.

- 1	TABLE 5.4.	Latency and	l feeding times	s of P. <i>perrieri</i>	on conspecific and	l heterospecific baits.
------------	------------	-------------	-----------------	-------------------------	--------------------	-------------------------

	Latency Time (seconds)		Feeding Time (seconds)	
	Conspecifics	Heterospecifics	Conspecifics	Heterospecifics
Mean (SEM)	437.40 (49.10)	247.30 (54.68)	73.10 (36.50)	125.5 (45.90)
Median	600.00	136.50	0.00	15.00
25 th Percentile	169.50	47.75	0.00	0.00
75 th Percentile	600.00	600.00	6.00	183.30
Minimum	63.00	11.00	0.00	0.00
Maximum	600.00	600.00	510.0	575.00
Mann-Whitney U	114.50		142.5	
<i>P</i> – Value	0.02		0.08	

Test Species	Bait	Spearman r	<i>P</i> -value
C. digueti	Conspecific	$r_{s} = 0.04$	0.85
	Heterospecific	$r_{s} = 0.40$	0.09
P. perrieri	Conspecific	$r_{s} = -0.28$	0.23
	Heterospecific	$r_{s} = -0.25$	0.29

TABLE 5.5. Correlation analyses between body size (shell length) and latency times to contact bait item. N = 20 for all analyses.

TABLE 5.6. Correlation analyses between body size (shell length) and feeding times for baititems. N = 20 for all analyses.

Test Species	Bait	Spearman r	<i>P</i> -value
C. digueti	Conspecific	$r_{s} = 0.13$	0.58
	Heterospecific	$r_s = -0.22$	0.34
P. perrieri	Conspecific	$r_{s} = 0.34$	0.14
	Heterospecific	$r_s = 0.38$	0.10

DISCUSSION

The results of this study support the hypothesis that the odors of crushed CONs and HETs are used as foraging cues, rather than alarm cues, by scavenging hermit crab cannibals. The methods used in this study were specifically designed to identify and quantify foraging behaviors in response to odor exposure. While it is acknowledged that the crabs may show search behaviors associated with shell-availability when exposed to the odors of crushed CONs and HETs (Small and Thacker, 1994; Tricarico et al., 2011), the metric used in this study was specifically designed to measure foraging behaviors. Additionally, previous studies suggest that when hermit crabs are attracted to shell-related cues, they exhibit behaviors related only to shell resource acquisition, and not foraging (Rittschof, 1980; Rittschof et al., 1992). Thus, the behaviors of foraging and shell acquisition appear independent of each other, and the identification of stereotypical foraging behaviors. Therefore, it can be stated with confidence that the behaviors quantified in this study were those of foraging, and not shell investigation.

The results of this study contradict the findings of other studies in which animals exhibited predator-avoidance behaviors when presented with CON and HET odors (see Hazlett, 2011 for review). The similarities of the two test species in their responses to CON and HET odors suggest that animals' reactions to a conflicting environmental cue can be influenced by the ecological contexts in which the cue is detected, and may reflect a behavioral response that is stereotypical among hermit crab species. For hermit crabs, which routinely inflict bodily injury on each other during resource competition (e.g., shells, mates; Bertness, 1981; Neil, 1985; Scully, 1979; Scully, 1983), the odor of crushed CONs and HETs is likely a more accurate

indicator of food availability than predator proximity. Predation risk for hermit crabs is, of course, dependent on the local abundances of predators in the natural habitats of the species, as well as the availability of shells of suitable dimensions to protect against predator attacks. To what extent laboratory acclimation in the absence of predators influences the perception of predation risk in hermit crabs remains unknown, but could be elucidated by comparing the results of this study with those of future field studies using animals in their natural habitats. However, if the release of CON and HET cues due to aggression is more frequent than the release due to predation in nature, natural selection should favor the use of these cues as foraging cues rather than anti-predation cues. For these reasons, hermit crabs likely do not rely on the odors of damaged CONs /HETs alone to initiate predator-avoidance behaviors. Instead, hermit crabs likely rely on (1) the odor of predators themselves (Rosen et al., 2009), (2) the interaction between predator odors and CON/HET odors, or (3) the interaction between olfaction and other sensory inputs (e.g., visual, auditory), to assess predation risk. Future research on these topics is warranted because it could elucidate the importance of ecological contexts on animals' perceptions of olfactory cues.

My results suggests that some common aspect of the ecologies of *C. digueti* and *P. perrieri* influences how they perceive and respond to foraging cues mediating cannibalism. Although not always statistically significant, both *C. digueti* and *P. perrieri* showed (1) stronger foraging reactions to the odor of crushed HETs than CONs, (2) longer latency times for CON baits than HET baits, and (3) shorter feeding times on CON baits than HET baits. This suggests that both *C. digueti* and *P. perrieri* prefer to feed on HETs over CONs. One possible explanation for this finding is that feeding on CONs increases the likelihood of acquiring species-specific parasites. Parasite transmission resulting from cannibalism has been demonstrated in a number

of taxonomic groups, ranging from amphibians (Pfennig et al., 1998; Pizzatto and Shine, 2011) to amphipods (MacNeil et al., 2003). A tradeoff often exists between nutritional value and risk of parasite transmission for animals feeding on closely related prey (Pfennig et al., 1998; Pfennig, 2000). While closely related prey (i.e., low phylogenetic distance between species) generally provide higher nutritional value than distantly related prey, cannibalistic animals have been shown to prefer intermediately related prey, likely because it maximizes the net advantage of the tradeoff between nutritional value and risk of parasite transmission (Pfennig, 2000). Thus, for *C. digueti* and *P. perrieri*, feeding on HETs of a different genus could maximize nutritional benefits while minimizing the risk of parasite acquisition.

The results suggest that body size is not associated with cannibalistic tendencies in these animals. Experimental animal body size was not correlated to latency times or feeding times for either species, and only a single significant correlation was found between body size and reaction to cannibalistic odor cues (*C. digueti*'s response to CON odor). This suggests that cannibalism is a behavior exhibited by individuals of all size classes of these species.

In conclusion, the results of this study show that hermit crabs are capable of discriminating between the odors of dead CONs and HETs. While the odors of both CONs and HETs appear to be used as foraging cues, rather than cues mediating predator-avoidance, hermit crabs appear to be more willing to approach and consume dead HETs than dead CONs. Future research should focus on how animals discriminate the two cues, and how the specific ecologies of animals influence their risk-reward decisions to feed on CONs and HETs.

CHAPTER 6

BEHAVIORAL REACTIONS TO NOVEL FOOD ODORS BY INTERTIDAL HERMIT CRABS

INTRODUCTION

Generalist scavengers can utilize a wide array of food resources, and can gain nutritional benefits from using novel foods encountered during foraging. These nutritional benefits make it worthwhile for generalist scavengers to possess innate sensory mechanisms allowing them to discriminate between edible and inedible novel items upon first encounter. These mechanisms are likely innate sensory mechanisms (Schmidt and Mellon, Jr., 2010) that can aid in the detection and recognition of food resources that have not been previously encountered, and thus the animals require no associative learning of the food odors before finding the odors attractive.

Hermit crabs are generalist scavengers that rely heavily on olfactory cues to detect and locate food items (Rittschof, 1992). Thus, the first detection of novel food items by scavenging hermit crabs comes from the detection of novel food odors. Hermit crabs have been documented to consume novel foods (Barnes, 1997), and are known to be capable of rapid associative learning of the sensory cues provided by novel foods (Wight et al., 1990). The marine intertidal zone inhabited by hermit crabs provides foragers with opportunities to encounter novel food items because currents and wave action carry offshore carrion into the intertidal zone (Britton and Morton, 1994; Polis and Hurd, 1996). These offshore carrion sources can include migratory or seasonably abundant species which are not a staple of the local environment, and thus can serve as novel food items for intertidal hermit crabs. Additionally, human influences, such as agriculture, urban development, and improper waste disposal (e.g., beach litter) can introduce novel foods into hermit crab foraging environments. Indeed, studies have shown that hermit crabs will consume common human wastes, such as garbage and feces (Barnes, 1997).

The ability to detect, and use, novel food odors is likely innate and based on the animal's recognition of general blends of common foraging cues within the novel odor mixture (Schmidt

and Mellon, Jr., 2010). While research has shown that crustaceans possess neurophysiological mechanisms to detect and process information from novel odors (Schmidt and Mellon, Jr., 2010), little is known about the level of stimulus (odor) reinforcement that is needed to maintain behavioral reactions to these novel odors after the initial exposure. Indeed, no studies to date have addressed whether generalist scavengers maintain their baseline level of reaction to novel food odors over repeated, unreinforced exposures. In the context of novel food items and foraging, odor reinforcement would come from the animal detecting the novel food resource and then consuming the novel food. In contrast, sensory detection of the novel food item without subsequent feeding would constitute an unreinforced exposures. The objectives of this study were to (1) quantify the behavioral foraging reactions of intertidal hermit crabs to novel food odors, and (2) determine if reinforcement of the novel food odor is necessary for the animals to maintain their baseline reactions to the odor. I hypothesized that hermit crabs would (1) show foraging reactions to novel food odors upon first exposure, and (2) decrease the vigor of their foraging behaviors after repeated, unreinforced exposures.

MATERIALS AND METHODS

ANIMAL HOUSING AND MAINTENANCE

Clibanarius digueti, an abundant intertidal hermit crab from the Gulf of California, served as the study species. Animals were acquired from a commercial distributor (A & M Aquatics, Lansing, MI), housed communally in 10 gallon glass housing tanks filled with artificial saltwater (ASW; Instant Ocean) under a 12:12 light:dark cycle, and fed 2-3 times weekly with pellet food (NewLife Spectrum). All ASW used in this report was maintained at a specific gravity of 1.022 - 1.024, pH 8.2 - 8.4, and a temperature of 23 - 27.5 °C. Animals were acclimated to these housing conditions for a minimum of two weeks prior to use in experiments. On the day before testing began, animals were transferred to experimental housing units consisting of $26 \times 16 \times 17$ cm plastic pet containers containing ASW and gravel substrate. Animals were housed in groups of 10 individuals during the experiments.

STIMULUS PREPARATION

All stimuli, including ASW controls, were made fresh at the beginning of each experiment day and maintained on ice until use to preserve freshness. A known food odor (KO) was extracted from the animals normal pellet food and used as a control in these experiments. This odor represented a KO because (1) the animals were repeatedly exposed to it during their housing in the lab, and (2) it has been shown to elicit strong and consistent foraging responses in this species (Tran, 2013, Chapter 2). KO was made by macerating 1.0 g of food pellets in 100 mL ASW for 2 minutes and straining through medium filter paper. A novel food odor (NO) was made by repeating this same procedure, except substituting beef tissue for food pellets. Beef was purchased from a local vendor, cut into ~ 1.0 g pieces, frozen, and thawed until soft at room temperature on the day of use. Beef was used to make NO because beef (1) is not a component of the normal diets of hermit crabs (i.e., the animals are not regularly exposed to it in nature), and (2) was not an ingredient of the pellet food used to feed animals during acclimation in the lab. Thus, all animals were presumed naïve to the odor of beef prior to use in these experiments.

TESTING APPARATUS

The testing apparatus consisted of a 250 mL glass Erlenmeyer flask containing 250 mL ASW and clean gravel substrate.

QUANTIFYING FORAGING BEHAVIORS

I used the procedures of Tran (2013) to quantify foraging behaviors. A single animal was placed into the testing apparatus and given a minimum of 15 minutes to acclimate. Following acclimation, 2 mL ASW were pipetted into the top of the apparatus using a glass pipette. The number of feeding movements (cheliped- and dactyl-to-mouth movements) that the animal exhibited was counted for three minutes. This count represented the pre-stimulus count. Following the pre-stimulus count, 2 mL of test odor were pipetted into the apparatus and the number of feeding movements was again counted for 3 minutes. This count represented the post-stimulus count. Movement Scores were calculated to be the number of post-stimulus feeding movements minus the number of pre-stimulus feeding movements (Johnson and Atema, 1986; Tran, 2013).

TRACKING ANIMAL IDENTITIES

The experiments employed a repeated measures design, which required the identities of the test animals to be tracked throughout the experiment. Animal identities were tracked by observing the physical characteristics of the animal (wet weight, shell characteristics, leg and antennae length) before each trial.

REINFORCED FEEDING TREATMENT

Figure 6.1 shows the general schematic of the experimental design used in this study. Animals were randomly selected from group housing tanks and randomly assigned to either the reinforced or unreinforced feeding treatment. Animals within feeding treatments were randomly assigned to either the KO or NO odor group (N = 20 individuals per group). Within each odor group, animals were housed and tested in subgroups of 10 individuals. All animals were naïve to the NO at the start of the experiment. On the day before the experiments began, all animals were feed their normal pellet food diet (0.1 g per tank) to standardize hunger levels across individuals. On test day 1, animals were tested for behavioral reactions to their respective odors. Following testing, animals were held with their tankmates in covered buckets containing aerated ASW, and returned to their housing containers after the day's trials had ended. After test day 1, and between all subsequent test days, animals were fed a diet of beef tissue (0.1 g per tank). This beef was treated identically to that in the "Stimulus Preparation" section. Animals were observed during feeding to ensure they were showing foraging behaviors (Lee and Meyers, 1996) and consumed the food. The amount of food provided was generally in excess of what the animals would consume overnight. The housing containers were drained, rinsed of debris and food wastes, and refilled each morning before testing began to remove leftover chemical cues in the containers and reset the chemical environment.

UNREINFORCED FEEDING TREATMENT

Animals were treated in the same manner as stated in the previous section, except they were fed 0.1 g of their normal pellet food diet at all feeding events.



B.

Reinforced Feeding Treatment



FIGURE 6.1. Experimental Design. **A.** Schematic of how experimental groups were formed. **B.** Feeding and testing schedule of animals in this experiment. Words below arrows indicate the food item that was fed to the animals.

STATISTICAL ANALYSES

All statistical tests used employed a significance cutoff of P = 0.05. All data were rank transformed prior to statistical analyses using the RT-1 method (Conover and Iman, 1981) in order to achieve approximate normality and heterogeneity of variances. Before combining the data for subgroups within each odor group, I rank transformed the data between the two subgroups, and tested for a significant effect of subgrouping using a two-way repeated measures ANOVA (with subgroup and test day as factors). Subgroups within each odor group were combined after analyses revealed no significant effect of subgrouping (P > 0.05). Movement Scores within feeding treatments were analyzed using a two-way repeated measures ANOVA on the global ranks of the data (all data within each feeding treatment were ranked together). The two factors used in the two-way repeated measures ANOVA were odor group (KO or NO) and test day. Bonferroni multiple comparisons tests were used following significant ANOVA results to determine if Movement Scores differed among test days for each odor group.

RESULTS

REINFORCED FEEDING TREATMENT

Movement Scores did not differ among test days when the NO was reinforced through feeding (Table 6.1; Figure 6.2A). Two-way repeated measures ANOVA revealed a significant effect of odor group on the Movement Scores observed ($F_{1, 38} = 9.18$, P < 0.001; Table 6.1). KO consistently elicited stronger Movement Scores than NO. Test day did not significantly
influence Movement Scores (F_{3, 114} = 1.33, P > 0.05). The interaction between odor group and

test day was not significant (F_{3, 114} = 0.30, P > 0.05).

loi renijoreen leeung neunent.				
Source of Variation	df	F	P - value	
Odor Group x Test Day	3	0.30	0.8223	
Odor Group	1	9.18	0.0044	
Test Day	3	1.33	0.2682	
Subjects (matching)	38	2.59	< 0.0001	
Residual	114			

TABLE 6.1. Results of two-way repeated measures ANOVA for *reinforced* feeding treatment.

TABLE 6.2. Results of two-way repeated measures ANOVA for *unreinforced* feeding treatment.

Source of Variation	df	F	P - value
Odor Group x Test Day	3	7.61	0.0001
Odor Group	1	67.33	< 0.0001
Test Day	3	3.13	0.0284
Subjects (matching)	38	2.28	0.0004
Residual	114		

UNREINFORCED FEEDING TREATMENT

When the NO was left unreinforced, Movement Scores rapidly decreased after test day 1

(Figure 6.2B). Two-way repeated measures ANOVA revealed a highly significant effect of odor

group on the Movement Scores observed (F_{1, 38} = 67.33, P < 0.0001; Table 6.2), with KO

consistently eliciting higher Movement Scores than NO. A significant effect was also found for test day ($F_{3, 114} = 3.13$, P < 0.05). Interaction between odor group and test day was significant ($F_{3, 114} = 7.61$, P = 0.0001). Bonferroni multiple comparisons tests revealed no significant differences in Movement Scores among test days for KO. Significant differences were found for the NO between days 1 and 2, days 1 and 3, and days 1 and 4 (Figure 6.2B).



FIGURE 6.2. Behavioral reactions to novel and known food odors. A) Reinforced Treatment.
B) Unreinforced treatment. Mean ± SEM of untransformed data shown. Different letters below points denote significant differences in Movement Scores between test days in a Bonferroni multiple comparisons test.

DISCUSSION

The results of this study suggest that hermit crabs (1) possess innate sensory mechanisms that permit the recognition of novel food items upon first exposure, and (2) require the reinforcement of NOs to maintain their baseline levels of attraction during subsequent exposures. Naïve animals showed strong baseline responses to the novel odor at first exposure. However, test animals showed a significant decrease in foraging vigor after a single, unreinforced exposure to the NO in this study. When the NO was reinforced by allowing the animals to feed on the novel food, the animals showed no change in foraging vigor across exposures. In contrast, animals exposed to a KO exhibited consistent behavioral responses across exposures, and this trend was observed in both the reinforced and unreinforced feeding treatments.

The exact sensory mechanisms permitting hermit crabs to assess the edibility of potential food items upon first detection remains unknown. The main olfactory organs of decapod crustaceans, the antennules, are innervated by a diverse array of olfactory receptor neurons (ORNs) with different response profiles (Derby et al., 2002). This diversity of ORNs innervating the olfactory organs allows crustaceans to respond to a wide variety of odorants (Derby et al., 2002). Most food odors consist of a complex mixture of odorant molecules (e.g., amino acids, other muscle metabolites, etc.), with the concentrations of the specific odorant molecules differing subtly between food items (i.e., between prey species; Carr et al., 1996). Thus, the differentiation between odors of two distinct food items appears largely dependent on differences in the concentrations of shared odorants between the two odor mixtures, rather than one mixture being comprised of odorants that are not present in the second mixture. Since most food items consumed by hermit crabs possess similar types of odorants, but at different concentrations (Carr et al., 1996), hermit crabs likely assess the edibility of novel food items based on the similarity

100

between the odor profiles of the novel food item and known food items that the crabs have consumed in the past.

Learning to ignore environmental odors that do not provide reliable information appears beneficial to foraging scavengers. In the same way that prey animals habituate to predator cues under low predation threat (Kavaliers and Choleris, 2001), foragers should benefit from habituating to odors that do not provide accurate information regarding food availability. In this study, hermit crabs rapidly lost their baseline responses to the NO after a single, unreinforced exposure. However, the KO elicited consistent responses across test days even in animals that were repeatedly exposed to the odor while being fed beef. Thus, it appears that hermit crabs rapidly learn to ignore NOs that do not provide accurate information about food availability, but retain their attraction to KOs that have accurately predicted food availability in the past. An interesting follow up to this study would be to determine the number of repeated exposures without subsequent food reward it takes for hermit crabs to habituate to a previously known food.

The innate responses of hermit crabs to novel food odors is not surprising given that novel food items can provide valuable nutritional supplements to the normal diets of hermit crabs. Hermit crabs are generalist scavengers that show a particularly strong affinity for consuming carrion resources (Hazlett, 1981). Because intertidal carrion is distributed irregularly in space and time (Britton and Morton, 1994), it is generally contested for by numerous species and rapidly removed from the system. As the result of this rapid removal, hermit crabs may go long periods of time without consuming carrion, and this is likely a contributing factor to the evolution of broad dietary niches and their ability to consume plant and algal materials (Wolcott and O'Connor, 1992). In addition to the ability to consume plant and algal materials, the scarcity

101

of carrion encounter has likely contributed to the evolution of sensory mechanisms to assess the edibility of novel food items.

Hermit crabs appear to rely on post-ingestion physiological feedback mechanisms to assess the value or risks associated with consuming novel foods (Wight et al., 1990). In contrast to the neophobic behaviors exhibited by many scavengers, hermit crabs appear to readily consume novel foods, allow digestion to occur, and then assess the value of the novel food based on their resulting physiological state (Wight et al., 1990). Subsequent decisions to consume similar food items reflect the associative learning that occurred between the sensory detection of the food and the physiological state induced by consumption (Wight et al., 1990). When viewed in light of the scarcity of potential carrion sources, this strategy seems beneficial because it allows hermit crabs to exploit potentially beneficial novel foods when available, rather than risk losing the food to nearby competitors or to the tides. REFERENCES

REFERENCES

- Anderson, W.B. and Polis, G.A. 1998. Marine subsidies of island communities in the Gulf of California: Evidence from stable carbon and nitrogen isotopes. Oikos 81(1): 75-80.
- Ayón-Parente, M. and Hendrickx, M.E. 2010. Species richness and distribution of hermit crabs of the family Diogenidae (Crustacea: Decapoda: Anomura) in the Eastern Pacific. Nauplius 18(1): 1-12.
- Bach, C., Hazlett, B., and Rittschof, D. 1976. Effects of interspecific competition on fitness of the hermit crab *Clibanarius tricolor*. Ecology 57(3): 579-586.
- Barnes, D.K.A. 1997. Ecology of a tropical hermit crab at Quirimba Island, Mozambique: a novel locally important food source. Marine Ecology Progress Series 161: 299-302.
- Behmer, S.T. and Joern, A. 2008. Coexisting generalist herbivores occupy unique nutritional feeding niches. Proceedings of the National Academy of Sciences 105(6): 1977-1982.
- Bertness, M.D. 1981. Competitive dynamics of a tropical hermit crab assemblage. Ecology 62(3): 751-761.
- Britton, J.C. and Morton, B. 1994. Marine carrion and scavengers. Oceanography and Marine Biology 32: 369-434.
- Bouvier, E.-L. 1895. Sur une collection de crustacés décapodes recuellis en Basse-Californie par M. Diguet. Bulletin de Muséum d'Histoire Naturelle, Paris 1:6-9.
- Bouvier, E.-L. 1898. Sur quelques Crustacés anomures et brachyures recueillis par M. Diguet en Basse-Californie. Bulletin de Muséum d'Histoire Naturelle, Paris 4: 371-384.
- Cabana, G. and Rasmussen, J.B. 1994. Modelling food chain structure and contaminant bioaccumulation using stable nitrogen isotopes. Nature 372: 255-257.
- Carr, W.E.S., Netherton III, J.C., Gleeson, R.A. and Derby, C.D. 1996. Stimulants of feeding behavior in fish: analyses of tissues of diverse marine organisms. Biological Bulletin 1990: 149-160.
- Case, J. and Gwilliam, G.F. 1961. Amino acid sensitivity of the dactyl chemoreceptors of *Carcinides maenas*. Biological Bulletin 121: 449-455.
- Connell, J.H., 1961. The influence of interspecific competition and other factors on the distribution of the barnacle *Chthamalus stellatus*. Ecology 42(4), 710-723.

Conover, M.R. 2007. Predator-prey dynamics: the role of olfaction. CRC Press, Boca Raton.

- Conover, W.J. and Iman, R.L. 1981. Rank transformations as a bridge between parametric and nonparametric statistics. The American Statistician 35(3): 124-129.
- Corotto, F.S., McKelvey, M.J., Parvin, E.A., Rogers, J.L., and Williams, J.M. 2007. Behavioral responses of the crayfish *Procambarus Clarkii* to single chemosensory stimuli. Journal of Crustacean Biology 27(1): 24-29.
- Crossland, M.R., Heamden, M.N., Pizzatto, L., Alford, R.A. and Shine, R. 2011. Why be a cannibal? The benefits to cane toad, *Rhinella marine* [= *Bufo marinus*], tadpoles of consuming conspecific eggs. Animal Behaviour. 82: 775-782.
- DeBach, P., 1966. The competitive displacement and coexistence principles. Annual Review of Entomology 11: 183-212.
- Derby, C.D., Steullet, P., Cate, H.S., and Harrison, J.H. 2002. A compound nose: Functional organization and development of aesthetasc sensilla. In: Wiese, K. (Ed.), The Crustacean Nervous System. Springer-Verlag, Berlin: 346-358.
- Dowds, B.M., and Elwood, R.W. 1983. Shell wars: assessment strategies and the timing of decisions in hermit crab shell fights. Behaviour 85(1/2): 1-24.
- Ferner, M.C., Smee, D.L. and Chang, Y.P. 2005. Cannibalistic crabs respond to the scent of injured conspecifics: danger or dinner? Marine Ecology-Progress Series 300, 193-200.
- Field, L.H. 1974. Sensory and reflex physiology underlying cheliped flexion behavior in hermit crabs. Journal of Comparative Physiology 92: 397-414.
- Field, L.H. 1977. A description and experimental analysis of a stereotyped cheliped flexion behaviour in hermit crabs. Behavior 61(3/4): 147-179.
- Fink, H.K. 1941. Deconditioning of the "fright reflex" in the hermit crab, *Pagurus longicarpus*. Journal of Comparative Psychology 32(1): 33-39.
- Fox, L.R. 1975. Cannibalism in natural populations. Annual Review of Ecology and Systematics 6: 87-106.
- Fuzessery, Z.M., and Childress, J.J. 1975. Comparative chemosensitivity to amino acids and their role in the feeding activity of bathypelagic and littoral crustaceans. Biological Bulletin 149: 522-538.
- Gause, G.F. 1934. The struggle for existence. The Williams & Wilkins Company, Baltimore.
- Gherardi, F. and Atema, J. 2005. Memory of social partners in hermit crab dominance. Ethology 111: 271-285.

- Gherardi, F. and Tricarico, E. 2011. Chemical ecology and social behavior of Anomura. In: Breithaupt, T., Thiel, M. (Eds.), Chemical Communication in Crustaceans. Springer, New York: 297-312.
- Hairston, Jr., N.G. and Hairston, Sr., N.G. 1993. Cause-effect relationships in energy flow, trophic structure, and interspecific interactions. The American Naturalist 142(3): 379-411.
- Hardin, G. 1960. The competitive exclusion principle. Science 131(3409): 1292-1297.
- Harvey, A.W., 1988. Size and sex-related aspects of the ecology of the hermit crab *Clibanarius digueti* Bouvier (Decapoda: Anomura: Diogenidae). Ph.D dissertation, University of Arizona.
- Hazlett, B.A. 1981. The behavioral ecology of hermit crabs. Annual Review of Ecology and Systematics 12: 1-22.
- Hazlett, B.A. 1994. Crayfish feeding responses to zebra mussels depend on microorganisms and learning. Journal of Chemical Ecology 20(10): 2623-2630.
- Hazlett, B.A. 2011. Chemical cues and reducing the risk of predation. In: *Chemical Communication in Crustaceans* (Ed. by T. Breithaupt and M. Thiel), Springer, New York: 355-373.
- Hazlett, B.A., Acquistapace, P.. and Gherardi, F.. 2002. Differences in memory capabilities in native and invasive crayfish. Journal of Crustacean Biology 22(2): 439-448.
- Hazlett, B.A. and McLay, C. 2005. Responses of the crab *Heterozius rotundifrons* to heterospecific chemical alarm cues: phylogeny vs. ecological overlap. Journal of Chemical Ecology, 31(3): 671-677.
- Heller, C.H., 1971. Altitudinal zonation of chipmunks (Eutamias): Interspecific aggression. Ecology 52(2): 312-319.
- Hofknecht, G. 1978. Description and key to the intertidal sponges of the Puerto Peñasco area in the Gulf of California. Journal of the Arizona-Nevada Academy of Science 13(2): 51-56.
- Janecki, T. and Rakusa-Suszczewski, S. 2005. The influence of starvation and amino acids on metabolism of the antarctic amphipod *Waldeckia obesa*. Journal of Crustacean Biology 25(2): 196-202.
- Johnson, B.R. and Atema, J. 1986. Chemical stimulants for a component of feeding behavior in the common gulf-weed shrimp *Leander tenuicornis* (Say). Biological Bulletin 170: 1-10.

- Kaiser, M.J., Ramsay, K. and Hughes, R.N. 1998. Can fisheries influence interspecific competition in sympatric populations of hermit crabs? Journal of Natural History 32: 521-531.
- Kavaliers, M. and Choleris, E. 2001. Antipredator reponses and defensive behavior: ecological and ethological approaches for the neurosciences. Neuroscience and Biobehavioral Reviews 25 (7-8): 577-586.
- Kieckbusch, D.K., Koch, M.S., Serafy, J.E., and Anderson, W.T. 2004. Trophic linkages among primary producers and consumers in fringing mangroves of subtropical lagoons. Bulletin Of Marine Science 74(2): 271-285.
- Kurimoto, M. and Tokeshi, M. 2010. Variation on a theme of herbivory: *Corallina* hermit crab relationship on a temperate-subtropical rocky shore. Oikos 119: 1401-1408.
- Laidre, M.E. 2007. Vulnerability and reliable signaling in conflicts between hermit crabs. Behavioral Ecology 18(4): 736-741.
- Laidre, M.E. 2010. How rugged individualists enable one another to find food and shelter: field experiments with tropical hermit crabs. Proceedings of the Royal Society B 277(1686): 1361-1369.
- Laidre, M.E. 2011. Ecological relations between hermit crabs and their shell-supplying gastropods: Constrained consumers. Journal of Experimental Marine Biology and Ecology 397: 65-70.
- Laidre, M.E. 2013. Eavesdropping foragers use level of collective commotion as public information to target high quality patches. Oikos 122(10): 1505-1511.
- Lee, P.G. and Meyers, S.P., 1996. Chemoattraction and feeding stimulation in crustaceans. Aquaculture Nutrition 2: 157-164.
- Lohrer, A.W., Fukui., Y., Wada, K., and Whitlatch, R.B. 2000. Structural complexity and vertical zonation of intertidal crabs, with focus on habitat requirements of the invasive Asian shore crab, *Hemigrapsus sanguineus* (de Haan). Journal of Experimental Marine Biology and Ecology 244: 203-217.
- Lugendo, B.R., Nagelkerken, I., Van Der Velde, G., and Mgaya, Y.D. 2006. The importance of mangroves, mud and sand flats, and seagrass beds as feeding areas for juvenile fishes in Chwaka Bay, Zanzibar: Gut content and stable isotope analyses. Journal of Fish Biology 69: 1639-1661.
- Mackie, S.A. and Boyer, E.H. 1977. Intertidal zonation of macroscopic invertebrates on the Coquina Reef at Play de Estación, Puerto Peñasco, Mexico. Bios, 48(3), 120-128.

MacNeil, C., Dick, J.T.A., Hatcher, M.J., Fielding, N.J., Hume, K.D., and Dunn, A.M. 2003.

Parasite transmission and cannibalism in an amphipod (Crustacea). Int. J. Parasitol. 33, 795-798.

- Mayntz, D. and Toft, S. 2006. Nutritional value of cannibalism and the role of starvation and nutrient imbalance for cannibalistic tendencies in a generalist predator. Journal of Animal Ecology 75: 288-297.
- McCutchan Jr., J. H., Lewis Jr., W.M., Kendall, C., and McGrath, C. C. 2003. Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. Oikos 102: 378-390.
- McKillup, S.C., McKillup, R.V., 1997. The effect of supplemental feeding on the growth of an intertidal scavenger. Marine Ecology Progress Series 148: 109-114.
- McNatty, A., Abbot, K.L., and Lester, P.I. 2009. Invasive ants compete with and modify the trophic ecology of hermit crabs on tropical islands. Oecologia 160(1): 187-194.
- Meffe, G.K. and Crump, M.L. 1987. Growth and Reproductive benefits of cannibalism in the mosquitofish. American Naturalist 129(2): 203-212.
- Moir, F. and Weissburg, M.J. 2009. Cautious cannibals: Behavioral responses of juvenile and adult blue crabs to the odor of injured conspecifics. Journal of Experimental Marine Biology and Ecology 369: 87-92.
- Moncreiff, C.A. and Sullivan, M.J. 2001. Trophic importance of epiphytic algae in subtropical seagrass beds: evidence from multiple stable isotope analyses. Marine Ecology Progress Series 215: 93-106.
- Morales-Zárate, M.V., Arreguín-Sánchez, F., López-Martínez, J., and Lluch-Cota, S.E. 2004. Ecosystem trophic structure and energy flux in the Northern Gulf of California, México. Ecological Modelling 174: 331-345.
- Morton, B. and W.Y. Yuen. 2000. The feeding behaviour and competition for carrion between two sympatric scavengers on a sandy shore in Hong Kong: the grastropod *Nassarius festivus* (Powys) and the hermit crab, *Diogenes edwardsii* (De Haan). Journal of Experimental Marine Biology and Ecology 246(1): 1-29.
- Nagai, Y., Nagai, S., and Nishikawa, T. 1971. The nutritional efficiency of cannibalism and an artificial feed for the growth of tadpoles of Japanese toad (*Bufo vulgaris* sp.). Agricultural and Biological Chemistry (Tokyo) 35(5): 697-703.
- Neil, S.J. 1985. Size assessment and cues: Studies of hermit crab contests. Behavior 92(1/2): 22-37.
- O'Leary, M.H. 1988. Carbon isotopes in photosynthesis. BioScience 38(5): 328-336.

- Paine, R.T., 1974. Intertidal community structure, experimental studies on the relationship between a dominant competitor and its principal predator. Oecologia 15(2): 93-120.
- Pfennig, D.W. 2000. Effect of predator-prey phylogenetic similarity on the fitness consequences of predation: A trade-off between nutrition and disease? American Naturalist 155(3): 335-345.
- Pfennig, D.W., Ho, S.G., and Hoffman, E.A. 1998. Pathogen transmission as a selective force against cannibalism. Animal Behaviour 55: 1255-1261.
- Pianka, E.R. 1974. Niche overlap and diffuse competition. Proceedings of the National Academy of Sciences 71(5): 2141-2145.
- Pieman, K.S. and Robinson, B.W. 2010. Ecology and evolution of resource-related heterospecific aggression. The Quarterly Review of Biology 85(2): 133-158.
- Pinnegar, J.K. and Polunin, N.V.C. 2000. Contributions of stable-isotope data to elucidating food webs of Mediterranean rocky littoral fishes. Oecologia 122(3): 399-409.
- Pizzatto, L. and Shine, R. 2011. You are what you eat: Parasite transfer in cannibalistic cane toads. Herpetologica 67(2): 118-123.
- Polis, G.A. and Hurd, S.D. 1996. Linking marine and terrestrial food webs: Allochthonous input from the ocean supports high secondary productivity on small islands and coastal land communities. The American Naturalist 147(3): 396-423.
- Ramsay, K., Kaiser, M.J., and Hughes, R.N.. 1996. Changes in hermit crab feeding patterns in response to trawling disturbance. Marine Ecology Progress Series 144: 63-72.
- Reitz, S.R. and Trumble, J.T. 2002. Competitive displacement among insects and arachnids. Annual Review of Entomology 47: 435-465.
- Ristvey, A. and Rebach, S. 1999. Enhancement of the response of rock crabs, *Cancer irroratus*, to prey odors following feeding experience. Biological Bulletin 197(3): 361-367.
- Rittschof, D. 1980. Chemical attraction of hermit crabs and other attendants to simulated gastropod predation sites. Journal of Chemical Ecology 6(1): 103-118.
- Rittschof, D. 1992. Chemosensation in the daily lives of crabs. American Zoologist 32: 363-369.
- Rittschof, D. and Buswell, C.U. 1989. Stimulation of feeding-behavior in 3 species of fiddler crabs by hexose sugars. Chemical Senses 14(1): 121-130.

Rittschof, D. and Hazlett, B.A. 1997. Behavioral repsonses of hermit crabs to shell cues,

predator haemolymph and body odour. Journal of the Marine Biological Association of the United Kingdom 77(3): 737-751.

- Rittschof, D., Tsai, D.W., Massey, P.G., Blanco, L., Kueber, Jr., G.L., and Haas, Jr., R.A. 1992. Chemical mediation of behavior in hermit crabs: Alarm and aggregation cues. Journal of Chemical Ecology 18(7): 959-984.
- Rosen, E., Schwartz, B., and Palmer, A.R. 2009. Smelling the difference: Hermit crab responses to predatory and nonpredatory crabs. Animal Behaviour 78: 691-695.
- Safi, K. and Siemers, B.M. 2010. Implications of sensory ecology for species coexistence: biased perception links predator diversity to prey size distribution. Evolutionary Ecology 24: 703-713.
- Schmidt, M. and Mellon, Jr., D. 2010. Neuronal processing of chemical information in crustaceans. In: Chemical Communication in Crustaceans (Ed. by T. Breithaupt and M. Thiel), Springer, New York: 355-373.
- Scully, E.P. 1979. The effects of gastropod shell availability and habitat characteristics on shell utilization by the intertidal hermit crab *Pagurus longicarpus* Say. Journal of Experimental Marine Biology and Ecology 37: 139-152.
- Scully, E.P. 1983. The effects of shell availability on intraspecific competition in experimental populations of the hermit crab, *Pagurus longicarpus* Say. Journal of Experimental Marine Biology and Ecology 71: 221-236.
- Siemers, B.M. and Schnitzler, H-U. 2004. Echolocation signals reflect niche differentiation in five sympatric congeneric bat species. Nature 429: 657-661.
- Siemers, B.M. and Swift, S.M. 2006. Differences in sensory ecology contribute to resource partitioning in the bats *Myotis bechsteinii* and *Myotis natteri* (Chiroptera: Vespertilionidae). Behavioral Ecology and Sociobiology 59: 373-380.
- Small, M.P. and Thacker, R.W. 1994. Land hermit crabs use odors of dead conspecifics to locate shells. Journal of Experimental Marine Biology and Ecology 182(2): 169-182.
- Snyder-Conn, E. 1980. Tidal clustering and dispersal of the hermit crab *Clibanarius digueti*. Marine Behaviour and Physiology 7: 135-154.
- Snyder-Conn, E.K. 1981. The adaptive significance of clustering in the hermit crab *Clibanarius digueti*. Marine Behaviour and Physiology 8: 43-53.
- Somero, G. N. 2002. Thermal physiology and vertical zonation of intertidal animals: Optima, limits, and cost of living. Integrative and Comparative Biology 42: 780-789.
- Sousa, W.P., 1979. Disturbance in marine intertidal boulder fields: The nonequilibrium

maintenance of species diversity. Ecology 60(6): 1225-1239.

- Stillman, J.H. and Somero, G.N, 1996. Adaptation to temperature stress and aerial exposure in congeneric species of intertidal porcelain crabs (Genus *Petrolisthes*): Correlation of physiology, biochemistry and morphology with vertical distribution. Journal of Experimental Biology 199: 1845-1855.
- Thacker, R.W. 1996. Food choices of land hermit crabs (*Coenobita compressus* H. Milne Edwards) depend on past experience. Journal of Experimental Marine Biology and Ecology 199(2): 179-191.
- Thacker, R.W. 1998. Avoidance of recently eaten foods by land hermit crabs, *Coenobita compressus*. Animal Behaviour 55: 485-496.
- Tierney, A.J. and Atema, J. 1988. Behavioral responses of crayfish (*Orconectes virilis* and *Orconectes rusticus*) to chemical feeding stimulants. Journal of Chemical Ecology 14(1): 123-133.
- Tran, M.V. 2013. Divergent reactions to olfactory foraging cues between two ecologically similar, sympatric hermit crab species. Journal of Crustacean Biology 33(4): 512-518.
- Tricarico, E., Benvenuto, C., Buccianti, A., Gherardi, F., 2008. Morphological traits determine the winner of "symmetric" fights in hermit crabs. Journal of Experimental Marine Biology and Ecology. 354, 150-159.
- Tricarico, E., Breithaupt, T., and Gherardi, F. 2011. Interpreting odors in hermit crabs: A comparative study. Estuarine, Coastal and Shelf Science 91: 211-215.
- Trott, T.J. and Robertson, J.R. 1984. Chemical stimulants of cheliped flexion behavior by the Western Atlantic ghost crab *Ocypode quadrata* (Fabricius). Journal of Experimental Marine Biology and Ecology 78(3): 237-252.
- Turra, A. and Leite, F.P.P. 2004. Shell-size selection by intertidal sympatric hermit crabs. Marine Biology 145: 251-257.
- Vance, R.R. 1972. Competition and mechanism of coexistence in three sympatric species of intertidal hermit crabs. Ecology 53(6): 1062-1074.
- Weissburg, M.J., and R.K. Zimmer-Faust. 1991. Ontongeny versus phylogeny in determining patterns of chemoreception: Initial studies with fiddler crabs. Biological Bulletin 181: 205-215.
- Wight, K., Frances, L., and Eldridge, D. 1990. Food aversion learning by the hermit crab *Pagurus granosimanus*. Biological Bulletin 178: 205-209.

Wilson, E.E. and Wolkovich, E.M. 2011. Scavenging: How carnivores and carrion structure

communities. Trends in Ecology and Evolution 26(3): 129-135.

- Wisenden, B.D. 2000. Olfactory assessment of predation risk in the aquatic environment. Philosophical Transactions of the Royal Society of London B: Biological Sciences 355(1401): 1205-1208.
- Wolcott, D.L. and O'Connor, N.J. 1992. Herbivory in crabs: Adaptations and ecological considerations. American Zoologist 32: 370-381.
- Zimmer, R.K., Commins, J.E., and Browne, K.A. 1999. Regulatory effects of environmental chemical signals on search behavior and foraging success. Ecology 80(4): 1432-1446.
- Zimmer-Faust, R.K., Tyre, J.E., Michel, W.C., and Case, J.F. 1984. Chemical mediation of appetitive feeding in a marine decapod crustacean: The importance of suppression and synergism. Biological Bulletin 167: 339-353.