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CHEMICALLY STIMULATED BEHAVIOR OF THE HERMIT CRAB *CALCINUS LATENS* (RANDALL 1840) AND THE ROLE OF CHEMICAL SIGNALING AS A MODE OF SENSORY PERCEPTION WITHIN THE CORAL RUBBLE HABITAT OF MOOREA, FRENCH POLYNESIA

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Abstract Aquatic invertebrates utilize multiple forms of sensory perception including chemical signaling, to evaluate their surrounding environment. The hermit crab *Calcinus latens* is able to detect external chemical cues within the complex coral rubble habitat. These discrete chemicals whether emanated from a potential predator, competitor or conspecific are received through chemosensory structures and elicit a specific behavioral response. This study examines the effect of four chemical treatments (control-ambient sea water, predator-Octopus bocki, potential competitor-Saron marmoratus and conspecific-Calcinus latens) on the number of times an individual *Calcinus latens* is observed in active, exploratory behavior verses stationary, defensive behavior. The results demonstrate a significant difference in the amount of time observed in defensive behaviors by the hermit crabs exposed to the treatment containing octopus chemical cues when compared to the other treatments. Across the four chemical treatments, there was a significant difference in the observed use of six specific behaviors, indicating a patterned behavioral response, unique for each treatment. Additionally, an experiment testing the response of *Calcinus latens* individuals to artificially introduced treatment species, (octopus Octopus bocki, shrimp Saron marmoratus as well as conspecifics) in which tested individuals could utilize all modes of sensory perception, was compared to the chemically stimulated behaviors. Analysis of the response behavior to chemical cues verses multimodal sensory assessment of actual treatment species demonstrated a statistically significant similarity in elicited behavior which underlines the importance of chemical signaling in modulating the behavior of *Calcinus latens* within the coral rubble microhabitat.

Keywords: chemical cues, *Calcinus latens*, hermit crab behavior, Moorea, French Polynesia, coral rubble microhabitat

INTRODUCTION Multimodal sensory perception is critically important for organisms in assessing their surrounding environmental conditions. The ability of organisms to garner and synthesize information from any combination of visual cues, tactile senses, sound perception and chemoreception, near-range to considerable distances away (Mellon 2007) allows for informed behavioral decisions. This is especially true for organisms living within topographically complex habitats such as coral rubble where complex distributions of microhabitats have lead to high invertebrate species diversity and distribution (Kohn 1983, Kohn and Leviten 1976, Abele 1974, Gischler 1997, Turra and Denadai 2002, Choi and Ginsburg 1983).

The coral rubble habitat is a common hard substrate on most coral reefs (Gishler 1997, Choi and Ginsburg 1983) and the biotopes created within its cavities offer refuge from predators and physical disturbances (Choi and Ginsber 1883), as well as create feeding sites of settled detritus (Turra and Denadai 2002), and prey abundance. These factors allow for sympatric species from discrete trophic levels to coexist in close proximity (Monteforte 1987, Poupin 1998). The community structure and spatial distribution observed within these microhabitats is influenced by complex species behavior and interactions dependent upon sensory perception; namely the influence of chemical cues (Reese 1999, Brooks 1991, Chiussi et al 2001, Hazlett 1981, Gilchrist 2003).

Within the coral rubble habitat, the dynamic fluid movements of the surrounding aquatic environment, combined with local low light conditions and turbidity has lead to the ubiquity of chemical signaling (Marcotte 1999) both at close range and over great distances (Briffa and Williams 2006, Mellon 2007). Aquatic crustaceans, one of the most represented groups in this environment, have developed diverse chemoreceptive structures to process chemical signals for surrounding environment. The interpretation of chemosensory inputs occur by means of cuticular sensilla on the body and appendages (Mellon, 2007), specifically through

the antennules and chemoreceptive units on walking legs in decapod crustacea (Mesce 1993). Receptors receive and process chemical input from surrounding currents, which elicit responses of feeding, predator avoidance, settlement, and intraspecific communication in the individual (Herring 1979). The ability of individual prey organisms to chemically detect the presence of potential predators, surrounding competitors and neighboring conspecifics influences behavior and life history strategies.

One common chemoreceptive organism of the coral rubble environment of Moorea, French Polynesia is a hermit crab species *Calcinus latens* (Randall 1840). In addition to structures on antennules and walking legs, hermit crabs have developed chelar simple setae and ambulatory dactyls (Hazlett 1971 and Mesce 1993) to receive chemical signals. Hermit crabs are known to modulate their behavior in response to surrounding chemical signals as a strategy of predator avoidance (Brooks 1991, stone crab Rittschof and Hazlett 1997, green crab Rotjan et al 2004, fish juice Chiussi et al 2001, predatory crab Mima et al 2003, lobster model Scarrat and Godin 1992 and fish juice Hazlett 1971), identifying conspecifics (Rittschof et al 1992, Gherardi and Tiedemann 2004, Gherardi and Atema 2005, Gherardi et al 2005 and Briffa and Williams 2006), to mediate exploratory shell seeking behavior (Hazlett and Rittschof 2005, Rittschof et al 1992, Pezzuti et al 2002, Gherardi and Atema 2005 and Orihuela et al 1992) and orientation of visual cues (Briffa and Williams 2006, Chiussi et al 2001, Diaz et al 1994, and Orihuela et al 1992). However, little is known about the comparative effects of chemical cues emanating from a predator, competitor and conspecific as they relate to the stimulated behavior of *Calcinus latens*.

To quantify and contrast stimulated behavior of tested *Calcinus latens* individuals in response to four chemical treatments, an ethogram of defensive (stationary) verses exploratory (active) behavior will be used to determine the impact of the following four chemical treatments: 1. Ambient seawater 2. Chemical cues from the marbled shrimp *Saron marmoratus* (Olivier, 1811), an invertebrate whose distribution in the coral rubble habitat overlaps with that of *Calcinus latens*, and is thus assumed to interact in some capacity 3. A pygmy octopus *Octopus bocki* (Adams, 1941), a predator of *Calcinus latens* within the coral rubble habitat. 4. Chemical cues from

conspecifics of the hermit crab species *Calcinus latens.*

This study aims to quantify the differential behavioral response (namely amount of time observed in specific behaviors) of *Calcinus latens* to treatments of water containing various chemical cues with the hypothesis that the predator odors (of *Octopus bocki*) will elicit a more severe response in exploratory verses locomotory behavior than those treatments of a potential competitor odor (*Saron mamoratus*) and conspecific (*Calcinus latens*).

The second facet of this study aims to investigate the associations between the chemically stimulated behavioral responses of C. *latens* individuals and the elicited behavior in response to the artificial introduction of actual treatment species individuals. The actual interactions of hermit crab *Calcinus latens* individuals with *Saron marmoratus*, *Octopus bocki* and conspecifics will allow tested individuals to utilize all of their modes of sensory perception (mainly tactile, auditory, visual and chemical cues) to assess their environment and respond accordingly. Using the same behavioral criterion as in the chemosensing experiment, I plan to compare and analyze the response behavior of *Calcinus latens* between chemical cue and actual interaction experiments in order to compare the relative importance of chemical signaling (between individuals of the same species, competitors and predators) to other modes of environmental perception in modulating the activity and behavior of *Calcinus latens* to sympatric species of the coral rubble habitat.

METHODS
Study site
Fieldwork took place on the French Polynesian island of Mo'orea. A volcanic island that is a part of the society island chain, Mo'orea has extensive marine habitats and immense biodiversity. Lab work was conducted at the Richard Gump field station in Cook's bay.
*Focal Species Biology*
Calcinus latens (the white tip hermit crab) is a common species within the lagoons of Moorea and has the spatial distribution ranging from the infralittoral fringe to the sublittoral (Gherardi and Nardone 1997, Reese 1969) and predominantly aggregates under dead coral rubble (Reese 1969, Personal Observation). *Calcinus latens* has been observed in the field to demonstrate complex shell selection behavior which often permeates many aspects of observed

behavior and biology (Shih and Mok 2000, Kosuge and Imafuku 1997). As with most hermit crab species, *Calcinus latens* is an omnivorous detritvore, opportunistically foraging for a variety of energy sources within its range (Hazlett 1981). Although an indepth description of interspecific species interactions of *Calcinus latens* is currently not available in the literature, personal observations indicate a predator-prey relation ship between *Octopus bocki* and *Calcinus latens*.

Collection

Species of interest from the coral rubble habitat (*Octopus bocki, Saron mamoratus* and *Calcinus latens*) were collected from inside the reef crest regions in the coral lagoons at the following sites around the island of Moorea, French Polynesia: Temae bay, outside Opunohu public beach (near the Sheraton), and the lagoon outside Motu Tiahura (See Figure1). I fastened a number of buckets to the top of a large kayak and filled the large bins with porous coral rubble ranging in size from 5 to 50 centimeters in diameter. The rubble was then left to drain for approximately 30 minutes at which point I would search through the contents on the bottom of the bucket for desired organisms. Animals were transported back to the Richard Gump field station via aerated plastic containers.
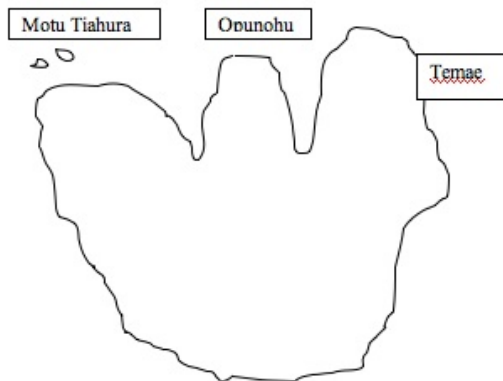
Care at the Station

Calcinus latens and *Saron mamoratus* were stored in individual plastic containers so as to aid in identification and prevent interactions and fighting between individuals. Both species were fed every other day on fish food pellets. Water was cleaned on a daily basis from the running seawater system in the wet lab. *Octopus bocki* were stored in 2 Liter plastic containers with ample holes in the lids and fed every other night with various crustaceans gathered from the coral rubble (predominantly xanthid crabs, stomatopods and various shrimp sp.). Water was changed daily.

Chemosensing Experiments

Two series of 40 *Calcinus latens* individuals (for a total of 80 crabs) were collected from the field and numbered. Twenty individuals were used per treatment and selected using Excel random number generator. There were a total of four chemically stimulated behavior tests, each one varying in their chemical treatment:

1. *Control Chemical Treatment* Three 2 Liter containers were each filled with 750 mL of ambient seawater taken from the source in the wetlab. The three containers were then left to sit for a total of 14 hours (overnight). This procedure was repeated until all twenty individuals were tested.

2. *Predator (Octopus bocki) Chemical treatment* The same three 2 Liter plastic containers were each filled with 750 mL of plain sea water and then an adult *Octopus bocki* was added to the container and allowed to sit for 14 hours in the container without feeding or other known chemical inputs.

3. *Potential Competitor treatment (Saron mamoratus)* Same procedure as for *Octopus bocki* but instead one *Saron marmoratus* was added per container.

4. *Conspecific treatment (Calcinus latens)* Same procedure as above but one hermit crab of the species *Calcinus latens* was added per container.

Experimental Procedure: Chemically stimulated behavior

All of the experiments were conducted between the hours of 8am and 12pm and held within a small, indoor room to ensure similar activity levels of test species and minimum disturbance from outside factors. An individual hermit crab (selected randomly) was placed in a 14-cm diameter clear Petri dish placed above a paper grid and allowed to acclimate for a total of 60 seconds. Following the 60 seconds, 150mL of a chemical treatment (one of the four listed above) was then added to the Petri dish. The subsequent behavior of the hermit crab was noted every 10 seconds for a total of 300 seconds (Although only the first 60s were used in behavioral comparisons and data analysis). An ethogram of exploratory behavior (adapted from Briffa and Williams 2006 and combined with personal observations) was used to describe behavior with the following six behaviors recorded, the first three being grouped as stationary positions, the latter three considered locomotory positions:

Withdrawn all arms withdrawn into the shell *Stationary* no observed movement but appendages visible *Stationary-appendage movement* while the whole body remained in the same position, appendages were still waved around *locomotion* moving around in any direction *climbing* attempts to climb the side of the Petri dish and *shell raising* the behavior of physically lifting the shell up against the side of the Petri dish (similar to climbing but appendages remain on the bottom of the pi-tri dish). Following the 300 seconds, the *Calcinus latens* individual was returned to its cup and stored for the second experiment involving interactions.

Experimental Procedure: Artificial Introduction of actual treatment species

This set of experiments was designed to observe the behavior of hermit crabs *Calcinus latens* stimulated in response to an introduced treatment species individual. Tested individuals could utilize all modes of perception (namely visual, tactile, auditory and chemical cues) in gaining information about their experimental environment and the species artificially placed within it. First, each 2 Liter container (same ones used in above experiment) was filled with 750 mL of ambient seawater collected from the wet lab. Of 80 individuals collected, 20 were used per trial for a total of four trials and 80 individuals and were selected randomly using Excel random number generator. The test crab was placed into the container with the plain seawater and left to acclimate for one minute. After 60 seconds, either one adult *Octopus bocki* one *Saron marmoratus*, plain seawater or one *Calcinus latens* was physically added to the container already holding the focal hermit crab. Using the same ethogram as the chemical sensing tests, behavior was recorded every 10 seconds for a total of 60 seconds (for a total of 7 data points). This procedure was then repeated 20 times for every four treatments until the behavioral response of all 80 individuals was documented.

RESULTS

Preliminary Observations

In preliminary experiments, an observation of the feeding behavior of *Octopus bocki* upon *Calcinus latens* was repeatedly attempted. Although actual feeding was never observed (most likely as a result of the intrusive procedure for feeding the octopus in lab conditions), when left overnight the hermit crab shell in the morning was either empty, indicative of its consumption by the octopus, or the hermit crab was observed maintaining a withdrawn position, in which it stayed until returned to an individual container with ambient sea water.

Another preliminary experiment designed to test the relationship of individual *Calcinus latens* within the coral rubble habitat involved placing multiple individuals in a container containing one piece of coral rubble and observing latency to approach and behavior upon arrival to the substrate. In all cases the *Calcinus latens* individuals approached the hunk of coral rubble immediately upon introduction into the container and selected cavities on all surfaces of the coral rubble large enough to allow shell fit. In some cases individuals would actually climb on top of each other, vying for the desired position within the rubble, while those that could not fit on the small piece of dead coral aggregated around the substrate.

A final preliminary observation involved the simulation of natural conditions for the three treatment species in order to gain insight of their interactions in the field. A large container was filled with coral rubble (collected from above mentioned collection sites) and all three treatment species, *Calcinus latens, Saron mamoratus and Octopus bocki* were added simultaneously to the arena. Observations were made over a thirty-minute period. During the whole 30 minutes of observation, none of the individuals were seen to physically interact as each species immediate sought refuge in the abundant coral rubble interstices and remained for the duration of the time. While experiment was not reproduced or continued over a greater amount of time, it demonstrated the importance of coral rubble cavities as refuges to interactions in field conditions.

*Chemical cue experiments: Behavioral responses to chemical stimuli*

A behavioral activity bioassay was conducted to compare the response of hermit crabs exposed to ambient sea-water, predator odor (*Octopus bocki*), potential competitor chemical cues (*Saron mamoratus)* as well as conspecific cues (other *Calcinus latens*). Using the behavioral ethogram outlined in the materials and methods section, average activity levels (measured in number of times observed in a locomotory position for the first 60 seconds of observation) were compared across treatments (figure 2) and analyzed using one-way analysis of variance (ANOVA) statistical test on JMP 5.1.2 software (see table 1). The results (df=3, F ratio= 22.7

and Prob>F of <0.0001) demonstrate a significant difference in the average activity levels across chemical treatments. Further examination of the data using a Tukey-Kramer HSD comparison test revealed that the chemically stimulated behavior of focal hermit crabs, measured in number of times observed in a stationary vs active position responded to the chemical cue of a predator (*Octopus bocki*) by spending significantly greater time in a stationary behavior than the hermit crabs in the other chemical treatments that were comparatively more active.

Table 1 Analysis of Variance (ANOVA) results:

Experiment	DF	F ratio	Prob> F
Average activity across chemical treatments	3	22.7104	<0.001
Withdrawn behavior compared across chemical treatments	3	14.5891	<0.001
Stationary behavior compared across chemical treatments	3	10.0918	<0.001
Stationary with Apendage movement behavior compared across chemical treatments	3	0.04424	0.7233
Locomotory behavior compared across chemical treatments	3	21.3370	<0.001

Figure 2 This graph describes the average activity of hermit crab individuals in response to four chemical treatments
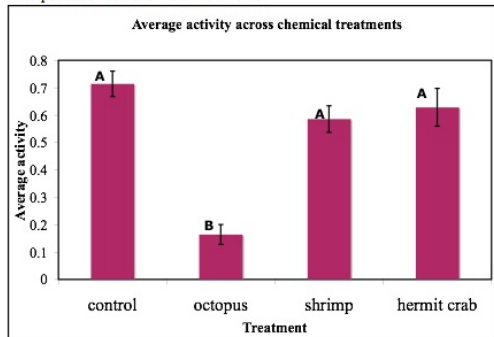
Comparison of specific behaviors across chemical treatments

Although the above test demonstrated a significant difference in the amount of time focal hermit crabs were observed in stationary vs locomotory behaviors in response to varying chemical cues, specifically predator odors, it does not describe the specific behaviors demonstrated. A comparison of the percentage of times individuals were observed in particular behaviors gives detailed information about hermit crab behavior in response to these specific cues (see figure 3). A comparison of the number of times the specified six behaviors (withdrawn, stationary, stationary with arm movements, locomotion, climbing and shell raising) were observed was analyzed using a contingency chi-squared test on JMP 5.1.2 software (the climbing and shell raising categories were combined because of such low frequency). The Pearson test value (chisquared= 181.535, df=12, P<0.0001) (see table 2) revealed that the types of behaviors observed were significantly different across the four treatments. Next I analyzed the average number of times specific response behaviors were observed across chemical treatments:

Figure 3 This graph describes the frequency of six specific observed behavior across four chemical treatments
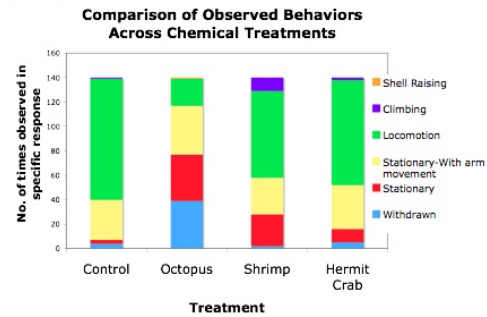

Table 2 Chi squared analysis : the comparison of six specific observed behaviors across chemical treatments

Source	DF	RSquare (U)
Model	12	0.1169

| Test	Chisquare	Prob> Chisq
Pearson	182.042	<0.0001

Withdrawn Behavior A one-way ANOVA run on JMP 5.1.2 software demonstrated a significant difference in the amount of time the withdrawn behavior was stimulated across the four treatments (df=3, F ratio 14.5891, Prob>F <0.0001) (table 1). Further analysis using a Tukey-Kramer HSD analysis on JMP 5.1.2 software showed that the hermit crabs exposed to treatments containing octopus chemical cues spent significantly more time in the withdrawn position that the hermit crabs from the other three treatments (control, shrimp and conspecific)(see table 1 and figure 4).

Withdrawn Behavior Across Chem Treatments
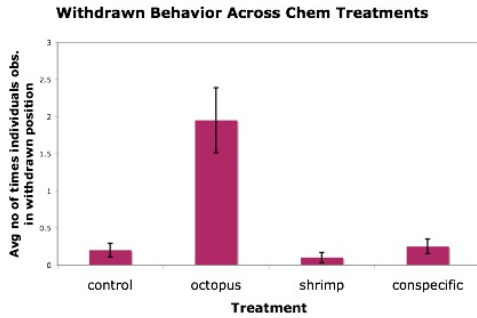
Stationary Behavior A comparison of the average number of times tested hermit crabs were observed in a stationary position across treatments was analyzed using a one-way ANOVA on JMP 5.1.2 software (table5 and figure 5). The results (df=3, F ratio 10.0918 and Prob>F <0.001) indicate that the variance in observed stationary behavior was significant across treatments. Further analysis using the Tukey-Kramer HSD analysis on JMP 5.1.2 software demonstrated there was no significant difference in the observed stationary behavior between the hermit crabs exposed to octopus and shrimp treated water, the shrimp and conspecific treatments and between the control and conspecific chemical treatments. There was a significant difference however between the observed stationary behavior of hermit crabs exposed to control and conspecific verses the octopus and shrimp treatments.

Figure 5 Graph comparing the number of times treatment *Calcinus latens* elicited **stationary** behavior in response to four varying chemical treatments

Stationary Behavior Across Chem Treatments
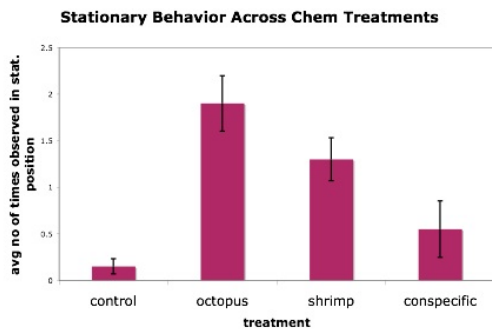
Stationary-Appendage movement behavior The graph depicts the average number of times hermit crabs from each chemical treatment were observed in a stationary-with appendage movement. A oneway ANOVA carried out on JMP 5.1.2 software gave a p value of 0.7233 (df=3, F ratio 0.4424, Prob>F, 0.7233, see figure

6), which indicates there is no significant difference in the observed stationary with appendage movements across the four chemical treatments.

Figure 6 Graph comparing the number of times treatment *Calcinus latens* elicited **stationary-appendage movement** behavior in response to four varying chemical treatments

**Stationary-arm movement Behavior Across Chem Treatments**
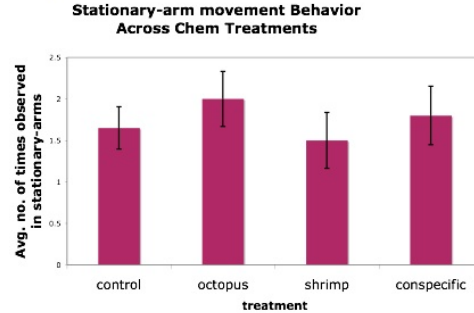
Locomotry Behavior The average number of times hermit crabs were observed in a locomotry position across four chemical treatments (control, octopus, shrimp and conspecific) was graphed (figure 7) and analyzed for statistical significance using a oneway ANOVA on JMP 5.1.2 and Tukey test. There was a significant difference (Prob>F, <0.0001) in the amount of time hermit crabs exposed to the octopus chemical cues compared to the other three treatments. There was no significant difference between the locomotory behavior of hermit crabs exposed to the control and conspecific treatment and the shrimp and conspecific chemical cues.

Figure 7 Graph comparing the number of times treatment *Calcinus latens* elicited **locomotory** behavior in response to four varying chemical treatments

Locomotory Behavior Across Chem Treatments
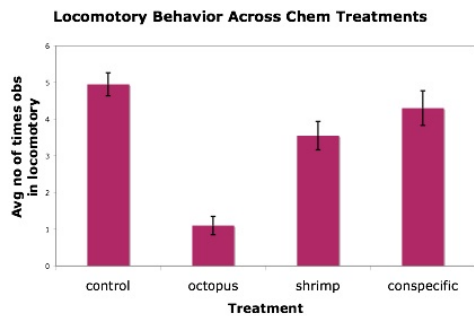
The remaining two behaviors, shell raising and climbing were observed with such low frequency that no statistical analysis could be run (see figure 8 for a graph of number of times observed). However, it is important to note that shell-raising behavior, although only observed 13 times across all individuals and treatments, was observed 10 times in the shrimp chemical cue treatments. Shell raising behavior was only observed once in all trials and was in response to treatment containing octopus chemical cues.

Climbing and Shell Raising Behavior Across Chemical Treatments
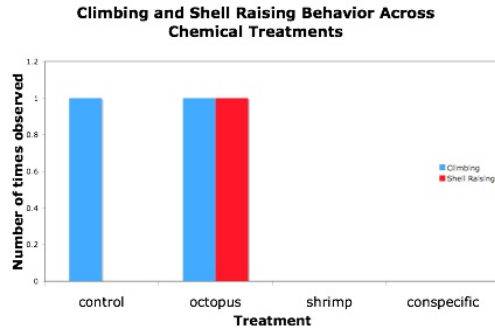
Comparison of elicited behavioral response between chemical treatments and actual interactions

      In order to compare the behavioral response of *Calcinus latens* to chemical treatments with their reaction to actual treatment species, intended to simulate natural conditions (see figure 9), I ran a two-way ANOVA on JMP 5.1.2 software in order to determine the similarities and differences in elicited behavioral response between chemical treatments and actual interactions with the species. As the graph of observed activity levels across chemical treatments and actual interactions illustrate, there was no significant difference in the average activity levels across experiments (chemical and actual) (df =3, Prob 0.973) (table 3). Whereas there were significant differences between treatments, specifically between the average activity of individual hermit crabs in response to octopus and octopus chemically treated water when compared to the other preparations.

Figure 9 Graph comparing the response behaviors of tested *Calcinus latens* individuals from the chemosensing experiment and "actual interaction" experiments, across chemical treatments

Comparison of Observed Activity Levels Across Treatments and Experiments (Chemical cues vs Actual Interactions)
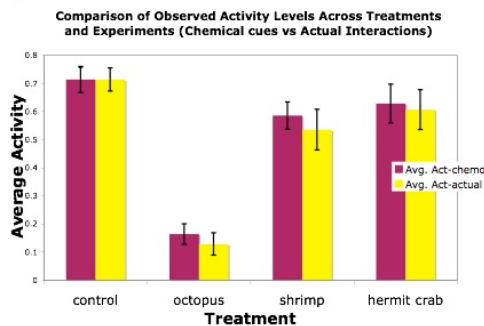

Table 3 Analysis between two experiments: Actual interactions experiment compared to Chemosensing experiment: and compared across four chemical treatments (control, predator, shrimp and conspecific) MANOVA statistical test:

Source	DF	F ratio	Prob> F
Treatment	3	41.4735	<0.0001
Actual Interactions	1	0.4745	0.4920
Actual Interactions*Treatment	3	0.0752	0.9732

DISCUSSION

      The chemically stimulated behaviors of the hermit crab *Calcinus latens* to treatments containing the chemical cues of sympatric species within the coral rubble habitat (predator *Octopus bocki*, a potential competitor, *Saron marmoratus* and conspecifics) agree with previous findings on other species of hermit crabs that individuals are capable of detecting and responding to chemical signals in the surrounding aquatic environment (Brooks 1991, Gherardi and Atema 2005, Pezzuti et al 2002, Rittschof et al 1992, Mima et al 200, Orihuela et al 1992, Diaz et al 1994, Chiussi et al 2001 and Hazlett 1970). The first experiment observed behaviors of hermit crabs in response to different treatments of chemical cues, and demonstrated a difference across the four treatments in the amount of time individuals were observed in stationary, defensive positions compared to active, exploratory behaviors. The predator chemical cues (*Octopus bocki)* had the single greatest effect on the amount of time individuals were observed in defensive behaviors, as predicted in the hypothesis. A more detailed comparison of six specific observed behaviors indicates that the grouping of behavior into stationary or locomotory showed a difference in frequency across treatments for three of the six behaviors. The withdrawn and stationary behaviors appear to be associated with defensive positions, and are displayed with the greatest frequency by hermit crabs exposed to octopus or shrimp chemical treatments. The stationary with appendage behavior conversely is equally observed across all treatments, which suggests its common use in facilitation of chemical reception. Further, the high frequency of locomotory behavior shown in the control and conspecific chemical treatments accentuates the frequent foraging and shell seeking behavior of individuals who do not perceive a threat in their surrounding environment. Finally, the remaining two behaviors, although not displayed with great frequency give details on the plasticity of behavior of the *Calcinus latens* hermit crab.

      The first major finding of this study is the significant difference in the average activity level of tested hermit crabs across chemical treatments. Those individuals exposed to predator (*Octopus bocki)* chemical cues are more likely to be observed in stationary positions then active ones. The literature on hermit crab behavior describes stationary behavior as a defensive position, most often demonstrated in

response to predators or predator cues (Rotjan et al 2004, Scarratt and Godin 1992, Mima et al 2003). Active, exploratory behavior on the other hand, is typically observed in hermit crabs displaying foraging behavior or shell seeking behavior. In a natural setting, hermit crabs must change shells as they increase in size or suppress their body size growth to compensate (Sato and Seno 2006). Additionally, predators select for hermit crabs inhabiting shells of inadequate size, in one study preferentially eating hermit crabs in shells that were too small (Vance 1972). These pressures to find an adequate shell, combined with a limited supply of gastropod shells in any environment is responsible for the frequency of observed locomotory shell seeking behavior of hermit crabs, in the absence of a perceived predation threat. Some hermit crab species aggregate around gastropod kill sites to facilitate exchange of shells (Pezzuti et al 2002 Rittschof et al 1992, Gherardi and Benvenuto 2001), others simply spend more time in locomotion, fast and meandering, in order to increase their chances of encountering empty shells or conspecifics (Tricarico and Gherardi 2006). This explains the active, exploratory behavior observed in the three treatments where the tested individual did not receive chemical cues indicating any threat in their surroundings and thus wandered around the Petri dish either exhibiting foraging or shell seeking behaviors.

The second comparison of observed behaviors across chemical treatments considers six elicited behaviors outlined in the methods (withdrawn, stationary, stationary-arm movement, locomotion, climbing and shell raising.) The first specific behavior examined across the four chemical treatments is withdrawn behavior. The number of times individual hermit crabs are observed in a withdrawn position when exposed to chemical cues of the predatory octopus *O. bocki* varied from the other treatments, which rarely display this behavior. When hermit crabs are faced with the chemical cue of a predator, they have two typical antipredator behaviors: fleeing from the area or seeking refuge in their acquired gastropod shells (Scarratt and Godin 1992, Mima et al 2003). The frequency of hermit crabs withdrawing into their shells for protection against predators is influenced by the shell fit of the hermit crab (crabs with a large shell fit tend to withdraw more often than flee Scarrat and Godin 1992), and the quality of the shell it occupies (some shells are better adapted to defend against predation). Hermit crabs have been documented

actively selecting gastropod shells better adapted for physical protection against potential predators when there is selective pressure from predators on a population (Bertness 1981) or when exposed to predator chemical cues and other shells are available for occupation (Rojan et al 2004, Mima et al 2003). Although both shell fit and shell type could describe the observed frequency of withdrawn behavioral response to octopus chemical treatments, I believe it is elicited as a behavioral adaptation to their environment. The complex substrate of the coral rubble habitat affords *Calcinus latens* refuge within its cavities and attempting to flee from potential predators incidentally may compromise this refuge and make the individual more exposed and vulnerable to attack. A comparison of antipredator success comparing fleeing verses withdrawing behavior in the coral rubble habitat would be an interesting topic for future research and might explain the preference of this antipredator tactic in *Calcinus latens.*

Another specific response behavior of tested *Calcinus latens* individuals compared across the four chemical treatments is stationary behavior (where an individual was out of its shell but not observed moving). The two treatment groups that display this behavior with the greatest frequency are the individuals tested from the octopus and shrimp treatments. Although stationary behavior is not a direct antipredator response, the refrain from locomotion, which could put the individuals in a position more vulnerable to predation, demonstrates that tested hermit crabs can detect and respond to chemical signal differentially. The groups displaying stationary behavior the least are the hermit crabs exposed to treatments of control water and conspecific chemical cues. A study by Briffa and Williams (2006) with the hermit crab *Pagurus bernhardus* demonstrated that focal crabs spent less time in a stationary position and a greater proportion of time on locomotion when cues from conspecifics were present (as long as they were previously non-fighting crabs) (Briffa and Williams, 2006). If *Calcinus latens* individuals exposed to the conspecific chemical signals are stimulated into locomotory shell seeking behavior, then they would not be expected to demonstrate high instances of stationary behavior. An examination of the specific locomotory behavior (movement of focal crabs in any direction) of tested *Calcinus latens* individuals across treatments illustrates this, see figure 8. Another reason for the observed locomotory behavior of hermit crabs

exposed to conspecific odors could be due in part to their ability to recognize the odor of other *Calcinus latens* individuals (Gherardi and Tiedemann 2004, Gherardi et al 2005 and Briffa and Williams 2006). A study by Gherardi et al in 2005 found that individual hermit crabs of the species *Pagurus longicarpus* could chemically distinguish between larger crabs inhabiting higher-quality shells and smaller crabs inhabiting lower-quality shells if they had some past association. Not knowing the previous interactions of tested individuals (due to random collection methods), it is possible that the potential previous encounters could influence the observed behavior within these treatments. However, because the hermit crabs exposed to the control treatment of ambient seawater had no statistical difference to the elicited behavior of hermit crabs in the conspecific treatment, we can assume that the potential influence of previous encounters does not have a severe impact on the recorded behaviors.

Another specific response behavior examined across chemical treatments is "stationary with appendage movement. These experiments demonstrate that this behavior is elicited equal across all four chemical treatments. Although little literature reports on this specific response behavior in hermit crabs, considering the location of chemoreceptive structures on hermit crabs, the movements of appendages (typically the front chelar structures as well as walking legs) could be a means for the individual hermit crab to generate mini currents around its sensors in order to detect the presence of chemicals within its environment. Because I only examined the behavioral responses of *Calcinus latens* to the four treatments within the first 60 seconds of exposure to the treatment, the ubiquitous use of this behavior across treatments supports the postulation that arm moving behavior is a means to distinguish chemical cues when placed in a novel environment as the tested individuals of this study are. It would be interesting in the future to compare the frequency of this behavior over a longer amount of observation.

The chemosensing experiment demonstrates that hermit crabs *Calcinus latens* is capable of detecting chemical cues and can modulate its behavioral based on whether it is sensing chemical signals from conspecifics, predator or potential competitors. *Calcinus latens* responds strongest to the chemical cues of its predator *Octopus bocki* by spending a significant amount of time in a withdrawn

behavior to avoid predation. These predator-induced changes in behavior may have implications for population dynamics and interspecific interaction within the coral rubble habitat.

The results of the second experiment conducted in this study demonstrate that the behavioral responses of individual *Calcinus latens* to actual treatment species artificially introduced to the same containers as individual hermit crabs, had no impact on elicited behavior when compared to the chemically stimulated behaviors in the first set of experiments. The similarity of these "actual interactions", which allow for multimodal sensory perception (namely auditory, visual, chemical and tactile cues) to the chemically stimulated behavior experiment, in which tested hermit crab individuals were only able to discern perceived threat through chemoreception, underlines the importance of chemical signaling to *Calcinus latens* in perceiving their environmental surroundings.

The observed response behavior of tested *Calcinus latens* individuals to the forced introduction of a treatment species (namely *Octopus bocki, Saron marmoratus* and conspecific) mirrors the cross treatment results of the chemosensing experiment: hermit crabs exposed to the treatment containing chemical cues from the predatory octopus were observed in stationary, defensive behaviors with greater frequency compared other chemical treatments. The response behavior of *Calcinus latens* individuals to these "actual interactions" compared to chemical sensing experiments demonstrate that they are not distinguishable from one another. The experimental design of the chemosensing experiments were intended to eliminate the ability of hermit crab individuals to utilize multiple modes of sensory perception. The clear Petri dish without contained no visual or auditory information, forcing the individuals being tested to rely on their ability to detect chemicals in the surrounding environment. To contrast, the second set of experiments, which physically placed a treatment species individual into a container of ambient sea water with the hermit crab being tested allows the use of visual cues to aid in directional orientation, auditory cues between species and tactile stimulation all in addition to chemical sensing information. The fact that tested *Calcinus latens* individuals respond the same whether they have all sensory faculties or only chemical perception underlines the importance of chemical signaling in

evaluating the proximate environment and responding with appropriate behavior.

Although hermit crabs have a range of sensory tools for interpreting their ambient environment, often one cue is not enough to establish directional orientation. In lab experiments, shape discrimination, background pattern, and other visual orientation cues have been tested either in the presence or absence of chemical signals representative of background cue, for example, the availability of a shell combined with calcium cues, and gastropod haemolymph extract or environment sea grass scent corresponding to a stripped background, and found that the response behavior of the hermit crab being tested are activated by chemical cues (Orihuela et al 1992, Diaz et al 1994, Chiussi 2001). Further, one study which additionally observed orientation of a tested hermit crab individual to a predator (fish odor) chemical cue discovered that the orientation away from a target (indicating an avoidance response) was only observed following the presence of predator odor (Chiussi et al 2001).

The requirement of chemical cues in synthesizing other sensory inputs to process information about surrounding conditions indicates the importance of chemical cues in determining directional orientation within a complex environment. Within the complex interstices of the coral rubble environment, there is a need to know the location of predators, food, mates and availability of shells without compromising a position in a refuge, or risking predation by sympatric predatory species. However, due to low light conditions, physical disturbances and complex microhabitats, many of the potential sensory cues can be obstructed by environmental conditions. Chemical sensing however, appears to be a relatively important mode of perception in these complex environments because of the observed specificity in signaling and the ability to garner information from proximate to distant ranges.

Hermit crabs are able to distinguish between the four chemical treatments of this study and modulate behavior according to the perceived threat. When a threat is received in the form of chemical cues emanating from a predator, appropriate antipredator tactics are induced in the individual, and other potential behaviors are not observed. This may have implications for the shell selection behavior of Calcinus, latens in the coral rubble habitat, as well as affect their locomotory behavior within their range. Future studies should consider the a

priori shell fit and quality shells occupied by individuals when comparing behaviors. It would also be interesting to compare the results of this study to an experimental design mimicking natural conditions to get a better understanding of hermit crab behavior in their natural, dynamic environment. This study also outlined the importance of chemical cues, in combination with other sensory modes, as well as the only means of evaluating environment conditions with the demonstration of chemical cues in determining directional orientation to sympatric species presence. This observation would benefit from future research on the comparative roles of other sensory modes of perception within this complex habitat to better understand the plasticity of behavior and sensory perception modes of *Calcinus latens* in the coral rubble microhabitat.

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