HABITAT SELECTION OF CORAL REEF INVERTEBRATES
MEDIATED BY ENVIRONMENTAL STIMULI

by

Molly M. Ashur

A dissertation submitted to the Faculty of the University of Delaware in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Marine Studies

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First, habitat selection is not a singular event, but rather, an evolving process throughout development. I investigated the developmental variations in coral larvae preference and associated behaviors in response to general reef cues using three species of Caribbean corals. Larvae of Acropora palmata, Orbicella annularis, and Pseudodiplora strigosa were exposed to general reef or offshore cues each day of their larval duration. A. palmata were tested in both flowing water, using a two-channel choice flume, and in a novel still-water behavioral assay; due to weaker swimming capabilities, the other species were only tested in the still-water assay. A. palmata larvae prefer reef cues compared to offshore cues throughout development, but all three species displayed minute, yet distinct, behaviors that varied based on cue and developmental stage. These behaviors may be indicative of emerging sensitivities to environmental stimuli and should be investigated further.

ABSTRACT

Habitat selection is a critical process for most organisms that are mobile for at least a portion of their lives and can influence survival and lifelong fitness. Selection of optimal habitat is accomplished through the detection and assessment of environmental cues such as waterborne chemicals or structural morphology. Substrate availability on coral reefs is limited, giving rise to high competition between benthic species. Here, I assessed different aspects of sensory-mediated habitat selection of reef invertebrates through a variety of research methods, invertebrate species, and geographic locations to develop an integrated and diverse understanding of the process and implications of habitat selection.

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Sensory-mediated habitat selection is not solely dependent on general reef cues but may also rely on specific cues of the benthic reef environment. *A. palmata* were tested in the still-water behavioral assay to determine attraction or repulsion to algal and cyanobacterial chemical cues. Regardless of benthic species, coral larvae increased the percent of time spent moving after the cues were presented. Notably, coral larvae significantly increased the proportion of time spent in directed motion or swimming in small circles only when presented with crustose coralline algae (CCA), potentially revealing these minute behaviors as indicators of positive habitat cues.

Habitat selection is not only relevant early in organismal life history, but is also vital for roving, migrating, or homing adults. Sensory-mediated adult habitat selection was investigated in the specialized reef shrimp, *Lysmata pederseni*, which resides within Caribbean tube sponges, but nocturnally vacates to feed or mate. Using mark and recapture techniques, reef transects, cue isolation experiments, and patch reefs, I discovered that while shrimp use both chemical and morphological cues to assess potential habitats, the optimal sponge habitat is defined more by shape than by chemistry.

Morphological characteristics of specialized habitat are further investigated in my final chapter, where I attempt to relate habitat to fitness of a coral-dwelling Indo-Pacific shrimp, *Coralliocaris graminea*. Transects reveal non-random associations of shrimp within 7 species of *Acropora* corals. Shrimp abundance is closely related to coral morphology, especially coral head diameter and branch height. Further, *C. graminea* individuals were collected from host corals to reveal that many aspects of body and claw size, as well as fecundity, can be positively correlated with coral size.
The potential exists for this habitat-specialized shrimp to survive within structurally similar, non-host corals if preferred hosts are stressed or degrading.

In all, this body of work contributes to our understanding of habitat selection, from ontogenesis to adults. Knowledge of both the environmental stimuli that influence habitat selection and the implications of that choice can guide research and management practices. An integrated approach was taken to each of these projects, where multiple methods were incorporated and compared to determine varying behaviors, motivations, and repercussions of correctly interpreting stimuli to select the optimal habitat. However, during the current period of unprecedented environmental change, the optimal habitats are changing and so are the various cues by which organisms can detect and select this habitat. This research has made strides towards not only understanding habitat selection of cryptic, specialized, and/or foundational species, but also predicting how these sensory-mediated behavioral responses might shift as environmental conditions decline.
Habitat selection in the marine environment is a function of physical processes and animal behavior (Meadows and Campbell 1972; Cowan and Sponaugle 2009). The relative influence of each of these factors depends on size, swimming capabilities, responsiveness to cues, and necessity of specific habitats for individual species (Meadows and Campbell 1972; Pawlik 1992; Raimondi and Morse 2000). Habitat selection occurs at several points throughout organismal life history (Gillanders et al. 2003), from initial habitat selection early in development (Buttman 1987; Lecchini et al. 2005) to homing or migration of adults (Thorrold et al. 2001; White and Brown 2013). In highly complex coral reefs, available substrate is limited; while appropriate habitat is important for all organisms, it is especially critical for animals that require specialized habitat (Wilson et al. 2008) or are permanently sessile once habitat has been selected (Hadfield 1986). In both situations, the selection of inappropriate habitat can lead to reduced fitness or, in some cases, death (Raimondi and Morse 2000).

Organisms orient to and choose between habitats using available environmental cues, including waterborne chemicals, soundscapes, and structural morphology, assessed through visual and tangible cues. Chemosensory reception is one of the primary senses used by most marine organisms to detect habitat (Atema et al. 2002; Hay 2009) and chemical cues can be detected up to 2km from the source (Lecchini et al. 2014). The chemical signature of a coral reef relays information
regarding the health of the ecosystem (Lecchini et al. 2013; Dixson et al. 2014). Additionally, soundscapes made of snapping shrimp and fish vocalizations allow potential recruits to successfully orient to coral reefs (Simpson et al. 2005). Visual and tactile cues can inform organisms about the morphological characteristics of potential habitats to ensure the optimal location is chosen (Ambrosio and Brooks 2011).

Coral reefs around the world are experiencing unprecedented deterioration (Hughes 1994; Hoegh-Guldberg et al. 2007). Environmental stressors from myriad sources contribute to phase shifts from coral to algal dominated reefs (Done 1992; Knowlton 1992), with associated reductions in biodiversity and species abundance. Consequently, the environmental cues that organisms use to select habitat reflect these changes. For example, chemical signatures of reefs shift as species compositions change; dead and living reefs not only produced different chemicals, but these chemical differences were detectable to reef fish, with fish preferring the cues associated with a living reef (Lecchini et al. 2014).

This compilation of studies investigates habitat selection of reef invertebrates at different life stages. First, the development of chemical sensitivity to the reef environment is assessed in three species of Caribbean coral larvae (chapter 1). Subsequently, coral larval response to specific components of the reef were observed to understand the influence of benthic cues on habitat selection of reef-building corals (chapter 2). From there, I investigated which environmental cues - chemical or morphological - were more relevant for habitat selection of an adult Caribbean reef shrimp (chapter 3). Finally, habitat associations of an Indo-Pacific reef shrimp were
analyzed in an attempt to correlate habitat with organismal fitness, a potential long-term consequence of habitat selection (chapter 4).
REFERENCES


Chapter 1

CHEMICAL PREFERENCE AND ASSOCIATED BEHAVIORS OF FREE-SWIMMING SCLERACTINIAN CORAL LARVAE THROUGH EARLY DEVELOPMENT

1.1 Abstract

Most coral species have a split life cycle, where a relatively sessile adult produces free-swimming larvae or planula. Larvae of broadcast-spawning coral species are subject to physical ocean processes and may develop offshore before returning to the reef to settle. Orienting back to the reef environment requires the use of numerous environmental cues, including water-soluble chemicals. Sensitivity and attraction to these cues may change as larvae develop and seek settlement sites. Temporal variations in coral larvae behavior when exposed to general reef cues was investigated using three species of Caribbean coral. Larvae of *Acropora palmata*, *Orbicella annularis*, and *Pseudodiploria strigosa* were collected in situ and reared until they were capable of swimming before being exposed to reef and offshore cues for two separate assays: a two-channel choice flume and a novel still-water behavioral assay. The choice flume experiment revealed that *A. palmata* invariably preferred the chemical cues associated with a reef compared to offshore cues. However, in the still-water assay larvae displayed a reversal in some behaviors based on cue depending on developmental stage; the proportion of time coral larvae spent traveling through the dish was greater in offshore water early in development, but greater in reef water later in development. For all three coral species, four distinct behaviors were characterized and recorded, with reef cue initiating significant changes in time spent stationary or in
non-directional movement. These behaviors may be analogous to benthic probing or testing behaviors and potentially indicate recognition of preferred habitat cues. Investigations of chemical preferences and the associated minute behaviors of coral larvae throughout development can augment our understanding of the process of habitat selection in foundational coral species.

1.2 Introduction

Coral reefs have been described as the rainforests of the ocean, accounting for more than a quarter of all marine species (Knowlton et al. 2010). Like the trees in rainforests, coral colonies create the architecture of the ecosystem and provide dozens of ecological services to the local denizens (Moberg and Folke 1999). Unlike trees, however, corals can actively select their habitat during a self-locomotive larval stage (Raimondi and Morse 2000). In the marine environment, most organisms that are permanently sessile at the adult stage have a motile larval stage that allows for behavioral choices before and during the settlement process (Chia 1989; Pawlik 1992; Rodríguez et al. 1993; Katz et al. 1994; Tamburri et al. 1996; Raimondi and Morse 2000; Jenkins 2005). These choices are mediated by environmental cues perceived by the sensory systems of the individual (Hadfield and Paul 2001; Vermeij et al. 2010). For example, barnacle larvae use the chemical cues of conspecifics to navigate to appropriate sites for gregarious settlement (Knight-Jones 1953; Burke 1986). An appropriate settlement site among barnacle conspecifics increases fitness because proximity facilitates breeding (Buschbaum 2001).

Corals reproduce by either brooding or broadcast spawning (Szmant 1986). Brooders produce fully-functional planulae that settle immediately, often in the vicinity of their parental colony. Broadcast spawners, however, release gametes into
the water column for fertilization, a process that typically occurs annually. The fertilized cells develop in the water column and are often transported by currents from their native reef (Wood et al. 2014). Coral larvae have a limited free-swimming period before settlement, and choosing an appropriate settlement site is critical, as nascent corals are permanently sessile after metamorphosis (Burke 1983). Motile coral larvae must first select habitat at the ecosystem level using appropriate whole-reef cues and then must find suitable settlement sites within the reef matrix using specific benthic cues (Ritson-Williams et al. 2010, 2016). This process is mediated by environmental cues such as light (Maida et al. 1994; Mundy and Babcock 1998), sound (Vermeij et al. 2010), pressure (Stake and Sammarco 2003), and soluble chemicals of both the general reef and specific benthic species (Dixson et al. 2014).

Research into chemically-mediated habitat selection in corals has primarily focused on the near-settlement stage larvae that are actively probing the bottom (Hadfield and Paul 2001; Gleason et al. 2009; Price 2010; Tebben et al. 2015). Laboratory and field-based settlement assays help to determine the chemical cues involved in both settlement and metamorphosis. Using a two-channel choice flume, Dixson et al (2014) found that pre-settlement stage, free-swimming coral larvae are capable of distinguishing chemical signatures of both the general reef and specific benthic components. Indo-Pacific acroporid species swam towards the chemical cues of healthy, diverse reefs yet actively avoided the chemical cues of degraded, algae-dominated reefs.

The next step in this field is to determine if this behavior is universal to all coral species and across ocean ecosystems. Further, the timing of these behaviors will augment our understanding of the life history of these vital reef builders. For example,
during pelagic development anemonefish display alternating chemical preferences for their host sea anemones compared to untreated water; larval anemonefish avoid water containing habitat-rich cues until mid-way through their developmental pelagic period when returning to the reef for settlement is required (Dixson et al. 2011). It is unknown if coral larvae share this fluctuating preference. During the planktonic development of scleractinian coral larvae, lipid energy reserves are depleted (Arai et al. 1993; Harii et al. 2007), locomotive cilia form, and epidermal cells specialize into sensory and secretory cells (Heyward 1987). As settlement competency approaches, larvae become increasingly mobile and more sensitive to external cues. Sensory cells are concentrated at the aboral end, from which they typically probe the substrate and eventually attach (Tran and Hadfield 2013). Sensory capabilities and associated behaviors during this critical planktonic period between fertilization and settlement can impact individual survival and population replenishment.

Due to species differences in larval size and swimming capabilities, the two-channel choice flume used in previous experiments may not be an appropriate behavioral assay for all coral species, as it requires the larvae to continually swim against a 70 mL min\(^{-1}\) (0.72 cm sec\(^{-1}\)) flow rate. Additionally, the flume protocol can only determine chemical preferences but cannot assess minute changes in behavior associated with environmental cues, such as changes in velocity. To address these issues, I created a novel assay to test behavioral changes to environmental cues for coral species that are not compatible in the flume.

This chapter investigates both the chemical preferences and the associated behaviors of coral larvae throughout their free-swimming development. By video recording still-water assays after reef cues have been introduced to three species of
Caribbean coral larvae, I investigated the influence of early development on cue sensitivity and associated behaviors. Behavioral data are viewed in light of data collected using the traditional flume method to determine preference or avoidance of general reef and offshore cues. I hypothesized that coral larvae would exhibit differential behaviors depending on the cue that was presented and that these behaviors would shift as the larvae approached settlement. This research allows us to compare species-specific developmental shifts in response to reef chemistry in order to gain a more thorough understanding of how coral larvae select habitat. This is especially critical for coral species that are endangered, such as two of the focal species, as knowledge of habitat preferences can inform conservation practices.

1.3 Methods

1.3.1 Study site and organisms

Research was conducted at the Smithsonian Carrie Bow Cay field station off the coast of Belize (16°48'9.26"N, 88° 4'54.87"W). The larvae of three Caribbean scleractinian, corals were used in the behavioral assays. *Acropora palmata* is a foundational branching coral among Caribbean reefs but is critically endangered and has faced as much as 80% reduction in coverage (Bruckner 2002; National Marine Fisheries Service 2006). *Orbicella annularis* (previously *Montastraea annularis*) and *Pseudodiploria strigosa* (previously *Diploria strigosa*) are both massive boulder corals dominant in the Caribbean, however, *O. annularis* has been classified as endangered due to excessive population declines (Edmunds and Elahi 2007; National Marine Fisheries Service 2014). All three species are broadcast spawners and usually experience a peak spawn in August. Typically, *A. palmata* synchronously spawns 3-5
days after the full moon and up to 4.5 hours after sunset (Jordan 2018). *O. annularis* and *P. strigosa* spawn later in both regards – usually 6-8 days after the full moon and up to 5.5 hours after sunset (Jordan 2018). Larvae of all three species are lecithotrophic and depend on lipid-rich energy reserves during their planktonic period (Baird et al. 2009).

### 1.3.2 Larval collection and rearing

*Acropora palmata* larvae were collected on 17 August 2014 and 5-6 August 2015, while *Orbicella annularis* and *Pseudodiploria strigosa* larvae were collected on 8 August 2015. Coral colonies (<4m depth) were observed by divers and tented similar to Sharp et al. (2010), once pre-released gamete bundles were observed at the tip of the polyps. Upon spawning, the positively buoyant gamete bundles accumulated in a falcon tube at the top of the tent structure. After spawning, bundles were transported to the Carrie Bow Cay field station for fertilization. Eggs and sperm from separate colonies were cross-fertilized for one hour before the excess sperm was rinsed off with seawater. Coral larvae were reared in 4L larval containers with continual seawater flow (Ritson-Williams et al. 2010). Environmental conditions of the rearing tanks, i.e. temperature and light cycle, mirrored conditions found in the natural reef environment. Throughout development, deceased larvae and debris were removed from the containers with pipettes.

### 1.3.3 Two-channel choice flume

A two-channel choice flume (Gerlach et al. 2007) was used to assess chemical preference of coral larvae to either offshore or general reef cues throughout early development. The choice flume (23.5 x 4 cm) allows an individual organism to
experience the chemical cues of two different water sources simultaneously by presenting them side-by-side. Water from the two sources were gravity-fed from buckets through tubing and into the choice chamber. Laminar flow was maintained at 70 mL min\(^{-1}\) (0.72 cm sec\(^{-1}\)) using flow meters (Dwyer MMA-40) and checked periodically using dye tests. A coral larva was pipetted into the center of the flume and given a 30s habituation period, during which time the larva was free to explore either cue. During the habituation period, individuals were carefully observed to ensure that they swam against the current. Individuals that could not maintain their position were removed from the trial because they likely indicated a lack of swimming capability rather than a lack of preference. After the habituation period, larva position in each stimulus was recorded at 5s intervals for 2 min. The larva was then removed from the flume for 1 minute while the water sources were switched to eliminate a potential side bias. The same individual was then placed back into the center of the flume for another 30s habituation and 2 min test. Experimenters were unaware of which side held which cue and thus remained blind during the entirety of the trials to avoid bias.

1.3.4 Still-water behavioral assay

Plastic petri dishes (90 mm) were marked with a 1 cm\(^2\) grid and filled with 30 mL filtered seawater. Microscope lights (Omano, MikeLite) with duel gooseneck arms illuminated the dish from opposite sides such that the photosynthetically active radiation (PAR) at the surface of the dish was 95-111 µmol m\(^{-2}\) s\(^{-1}\) (Hansatech light meter). Cameras (Apple iPod Touch) attached to adjustable tripods were positioned above the petri dishes for aerial footage of each behavioral assay. An individual coral larva was pipetted into the center of the petri dish and allowed a 1 min habituation period, after which video recording commenced. After 2 min, the larva was relocated
back to the center of the dish using a glass pipette. Immediately, 2 mL from each water source was carefully inserted into opposite sides of the dish using 5 mL syringes. Video recording continued for 2 min before the conclusion of the trial. The cue was inserted into alternating sides of the dish for each trial to avoid any potential side bias. Dye tests revealed the chemical plume distance to be 3-4 cm from the insertion point.

1.3.5 Chemical cues

During both the choice flume and the still-water assay, larvae were presented with reef water and offshore water each day (n=20 for each assay). Individual larvae were only used for one trial. An additional 20 larvae per assay per day acted as a control and were tested when exposed to offshore water on both sides. Reef water was collected by boat from surface water flowing over a reef <3 m below the surface. Offshore water was collected by boat >1 km from the nearest land mass. Water cues were used in behavioral assays within 6 hours after collection.

1.3.6 Video analysis

Behavioral videos were analyzed using Tracker 5.0 (Brown 2019) to assess velocity. Autotracker was initiated when conditions permitted (i.e. the larva was distinguishable from the background), and larval position was tracked manually when autotracking failed. Video frame rate was 29.97 frames/s and larval position was recorded every 5 frames. Frames involving larval relocation or cue insertion were removed from the analysis. Behavioral videos were also analyzed manually to characterize behavior type. Four general behaviors were identified: (1) Stationary Rotations, in which the larva either remained motionless or rotated in place, (2) Small
Circles, in which the larva swam in confined circles <1 cm, yet did not experience any overall change in position within the dish, (3) Directed Movement, in which the larva swam in one direction with limited variations in speed; and (4) Non-directed Movement, in which the larva traveled in an unpredictable fashion involving spiraling, drifting, bouncing, or serpentine motion. For both Tracker and manual analyses, the first 10 seconds of footage after the cue was introduced was removed to account for any water movement due to the induction of the cue rather than larval self-propulsion.

1.3.7 Statistical analysis

Chemical choice fluming experiments were analyzed using the Wilcoxon signed rank test (JMP Pro 13). For the still-water analysis, average velocity and percent time spent in each behavior were analyzed in R (R Core Team 2017) using a between subjects analysis of covariance (ANCOVA) to assess the effect of cue type, after controlling for pre-cue insertion data. Post-cue data (average velocity or percent time in specific behaviors after the cue was presented) acted as the dependent variable, cue type (reef vs offshore) acted as the independent variable, and pre-cue data (average velocity or percent time in specific behaviors before the cue was presented) acted as the covariate. Data from each day post fertilization were analyzed separately because the covariate, pre-cue data, can be influenced by developmental age. In each test, the data was screened for assumptions and tested for outliers using Mahalanobis distance. When an outlier was detected (n=2), it was removed from the analysis. Linearity, homogeneity (Levene’s tests), homoscedasticity, and normality (Shapiro-Wilks test) were verified for all tests. When data were not normally distributed, a square-root transformation was applied.
1.4 Results

Larvae of *A. palmata*, *O. annularis*, and *P. strigosa* had varied developmental times and swimming capabilities. For the fluming trials, larvae were required to swim against a 70mL min\(^{-1}\) flow rate. This swimming speed was attainable for *A. palmata* larvae on developmental days 4-6 (Table 1.1). Larvae of *O. annularis* and *P. strigosa* were never capable of swimming against this current and were thus incapable of being tested in the two-channel choice flume. In contrast, the still-water behavioral assays were an appropriate method of observing larval behavior throughout development, regardless of swimming capabilities. Experiments were conducted on each species during their early development only while swimming was detectable, i.e. beginning when larvae first showed signs of movement and ending when larvae immediately settled when placed in the assay and did not react to any cue (Table 1.1). *O. annularis* larvae were actively swimming on day 7 post-fertilization; however, a population decline in the reared larvae forced an early termination of the experiment for that species, as well as a reduced number of individuals per test on days 6 (n=16) and 7 (n=10). A fraction of videos could not be analyzed and were removed from the analysis. Final n-values of each trial are provided in Table 1.1.
Table 1.1: Number of days post fertilization during which behavioral assays could be conducted either in the two-channel choice flume or the still-water assay. The included n-values signify the final n-values used in the analyses after faulty videos were removed. N-values of *O. annularis* were low on day 6 and 7 due to limited number of larvae. Red X’s indicate days in which larvae of that species were not viable to be tested, either because they were not yet swimming (*O. annularis*, Day 3) or because they had already reached settlement-stage (all others).

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<td><em>P. strigosa</em></td>
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<td>Still-water assay: Reef: n=17 Offshore: n=19</td>
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1.4.1 *Acropora palmata*

*A. palmata* larvae discriminated reef and offshore chemical cues within the two-channel choice flume between days 4-6 post-spawn. Larvae consistently spent more time in the flume side containing cues associated with reef water compared to offshore water (p<0.001 for all trials) (Figure 1.1).

![Chemical preferences of *Acropora palmata* larvae throughout early development in the two-channel choice flume. Mean percent time (±SE) spent in general reef water (dark blue bars) or offshore water (light blue bars) is depicted for each day that the larvae were free-swimming. Wilcoxon signed rank test, α=0.05, * indicates significance.](image)

Figure 1.1: Chemical preferences of *Acropora palmata* larvae throughout early development in the two-channel choice flume. Mean percent time (±SE) spent in general reef water (dark blue bars) or offshore water (light blue bars) is depicted for each day that the larvae were free-swimming. Wilcoxon signed rank test, α=0.05, * indicates significance.
Even though larvae could not swim against the flume current earlier than day 4, behavioral changes could be observed in the still-channel assay on days 3-6. The percentage of time spent traveling was divergent between cue types for *A. palmata* larvae (Figure 1.2A). On day 3, larvae spent significantly more time traveling only when exposed to the offshore cues (*p*=0.022), whereas on day 5 and 6, larvae spent significantly more time traveling when the reef cue was added (*p*=0.022, *p*=0.028, respectively). Average velocity was higher on day 3 and 4 when larvae were presented with only the offshore cue (*p*<0.001, *p*=0.026, respectively) (Figure 1.3A). However, on day 5 and 6, no difference in velocity was detected.
Figure 1.2: Variations in mean percent time (±SE) spent traveling during early development of (A) Acropora palmata, (B) Orbicella annularis, and (C) Pseudodiploria strigosa larvae after they were presented with either general reef water (dark blue bars) or offshore water (light blue bars) ANCOVA, α=0.05, * indicates significance.
Figure 1.3: Variations in mean velocity (±SE) during early development of (A) *Acropora palmata*, (B) *Orbicella annularis*, and (C) *Pseudodiploria strigosa* larvae when presented with either general reef water (dark blue bars) or offshore water (light blue bars). ANCOVA, $\alpha=0.05$, * indicates significance.
Four broad behavioral classifications were identified during the video analysis and each were analyzed separately (Figure 1.4). On day 3, *A. palmata* larvae spent a greater amount of time moving in a directed manner when presented with the offshore cue (p=0.021). On day 4, larvae spent more time stationary or performing rotations in place when presented with the reef cue (p=0.025). On day 5 and 6, the percent time spent on each of the four behaviors did not significantly vary between the cues. Small circles were a distinct, but relatively rare behavior regardless of the day or cue and could not be analyzed with this method as the distributions were not normal, despite transformations.
Figure 1.4: Variations in behavior of *Acropora palmata* larvae during early development. The mean percent time (±SE) spent displaying each of the four behavioral classifications is depicted for each day (listed at the top) and each cue (listed at the bottom). ANCOVA, $\alpha=0.05$, * indicates significance.
1.4.2 *Orbicella annularis*

*O. annularis* larvae did not significantly vary in the amount of time spent travelling on day 4, 5 or 7. On day 6, however, larvae traveled a greater percentage of time when exposed to offshore cues (*p*=0.049) (Figure 1.2B). Despite variations in travel time, *O. annularis* larvae did not experience any changes in velocity in response to cue type, regardless of developmental day (Figure 1.3B).

Behavior type varied significantly on day 4 and 5 (Figure 1.5). On day 4, larvae spent more time stationary while in offshore water compared to reef water (*p*=0.031). This behavior shifted on day 5, with larvae spending more time stationary when presented with reef water (*p*=0.038). This increase in time spent stationary in reef water was paired with a significant reduction in time spent in non-directed movement (*p*=0.021).
Figure 1.5: Variations in behavior of *Orbicella annularis* larvae during early development. The mean percent time (±SE) spent displaying each of the four behavioral classifications is depicted for each day (listed at the top) and each cue (listed at the bottom). ANCOVA, α=0.05, * indicates significance.
1.4.3  *Pseudodiploria strigosa*

During the two days that *P. strigosa* could be tested, larvae did not exhibit any significant differences in travel time (Figure 1.2C) or mean velocity (Figure 1.3C) depending on cue. Regarding the four behavior classifications, *P. strigosa* larvae did not show any significant variation between cues on day 3. However, on day 4, larvae spent more time in non-directed motion when presented with the reef cue (p=0.044) (Figure 1.6).
Figure 1.6: Variations in behavior of *Pseudodiploria strigosa* larvae during early development. The mean percent time (±SE) spent displaying each of the four behavioral classifications is depicted for each day (listed at the top) and each cue (listed at the bottom). ANCOVA, $\alpha=0.05$, * indicates significance.
1.5 Discussion

The successful settlement of planktonic reef larvae is determined by both water currents and animal behavior (Meadows and Campbell 1972; Raimondi and Morse 2000), but the relative role of each of these factors has been debated. While coral larval dispersal can be highly dependent on hydrodynamic forces (Wood et al. 2014), larvae can indeed alter their behavior in response to hydrostatic pressure (Stake and Sammarco 2003), light availability (Gleason et al. 2006), and waterborne chemical cues (Dixson et al. 2014) and this behavior can play a vital role in successful settlement (Raimondi and Morse 2000). During development of planktonic larvae, the relative role of behavior increases, as larvae become more functional and their preferences change (Dixson et al. 2011). The period of time between coral larvae fertilization and metamorphosis involves critical biochemical and physiological changes, including development of sensory cells and adhesion mechanisms (Chia and Bickell 1978; Gleason and Hofmann 2011). Metamorphic competence is the threshold at which environmental cues can trigger permanent attachment and metamorphosis and requires both the sensory capabilities of detecting external cues as well as the presence of appropriate chemical or physical cues (Gleason and Hofman 2011; Hadfield et al. 2011). Some coral larvae can remain planktonic for extended periods of time when suitable environmental cues are not present, but strategies for length of pelagic duration are variable between species (Bishop et al. 2006). This study investigated behavior of free-swimming coral larvae during the critical days leading up to metamorphic competence when the larvae were exposed to reef and offshore cues.

As soon as A. palmata larvae were capable of swimming against the 70 mL min⁻¹ flow in the flume, they displayed an unfailing preference for reef water over
offshore water throughout the remainder of their free-swimming period. Unlike reef fish (Dixson et al. 2011), no alteration in chemical choice was detected during their limited developmental time period. It is unknown if this behavior is universal among planktonic coral larvae, as A. palmata was the only species tested that was capable of swimming in the flume. A palmata larvae can reach settlement competency within 5 days but can remain planktonic up to 20 days post-fertilization (Baums 2005). With a relatively short free-swimming period, it may be advantageous for the larvae to remain invariably attracted to the reef. The majority of lecithotrophic coral larvae, which depend solely upon parental provisions during planktonic development, settle within a few days. However, some coral larvae can survive in the planktonic zone for upwards of 100 days (Graham et al. 2013). Coral species with extensive larval durations may display ontogenetic shifts in preference for reef water.

The planktonic larval period of P. strigosa and O. annularis are unusual in opposite ways. P. strigosa can reach settlement competency in as little as 2.5 days post fertilization, and their larval duration usually lasts no longer than 8 days (Davies et al. 2017). In contrast, when the appropriate cue is present, Orbicella spp. usually settle within 5-14 days, however larvae of O. franksi, can survive up to 120 days without metamorphosing (Davies et al. 2017; Rippe et al. 2017). P. strigosa might be expected to behave similarly to A. palmata and remain attracted to reef water throughout their free-swimming period. It is possible that Orbicella spp. may avoid reef water during their early development, as they can remain in the pelagic zone longer (Davies et al. 2017; Rippe et al. 2017). However, it is equally as likely that extensive larval durations are caused by lack of desirable cues, so preference for reef water may occur as soon as it is detected.
The still-water behavioral analysis displayed variable results depending on cue, day post fertilization, and specific behavior. The clearest trend was observed in *A. palmata*; larvae spent more time traveling when only presented with offshore cues on day 3, but on day 5 and 6, the addition of reef water was related to an increase in movement. Ontogenetic increases in movement have been observed in other coral species, although cue was not taken into effect. For example, the percentage of slowly-moving *Orbicella faveolata* larvae increased through development, before dropping off again as the larvae neared settlement age (Vermeij et al. 2006).

In this study, percent time spent traveling could be split into two distinct behaviors, directed and non-directed movement. Directed movement in coral larvae has been observed in other species, such as *Dendrogyra cylindrus* (Marharver et al. 2015), and is often related to vertical movement and positive geotaxis, or swimming towards the substrate. Non-directional movement was an all-encompassing term, which included such movements best described as spiraling, bouncing, and undulating. These same behaviors were noted in brooding coral larvae as well (Goffredo and Zaccanti 2004). Some of the behaviors associated with non-directional movement could potentially be examples of benthic probing (Gleason et al. 2009)—especially “bouncing” behaviors, typified by larvae moving along the dish by hopping forward and touching down again. This behavior was observed across both the bottom of the petri dish and along the rim. Benthic probing in other studies is accompanied by a vertical shift in position from swimming in the water column to probing the substrate. An expected developmental shift in geotaxis was observed in the scleractinian coral, *Oculina varicosa*, as larvae transitioned to the substrate in search of settlement locations (Brooke and Young 2005). This study focused on the horizontal movement...
of coral larvae and the water depth in the still-water assay was not deep enough to permit the examination of vertical movement.

Although not evident in this study, other researchers have observed an increase in coral larvae velocity as development progressed and the larvae became more developed (Brooke et al. 2005; Stromberg and Larsson 2017). In this study, mean velocity per individual was analyzed, regardless of the percent time the larva spent moving. Larvae that spent more time stationary displayed disproportionately lower mean velocities, even if their maximum speed was high. Therefore, velocity was highly related to travel time and should be teased apart in future studies. Regardless, it is unknown whether or not interaction with a positive habitat cue would influence velocity of coral larvae although it is likely. Aquatic chemical cues propagate through the environment in a filamentous manner, whereby a larva passes through a series of individual streams of the cue, rather than a continuous wall of cue (Koehl et al. 2007). The larvae of the sea slug, *Phestilla sibogae*, ceased swimming and subsequently sank whenever the cue was encountered, and then resumed swimming in the absence of the cue (Koehl et al. 2007). This shift in velocity allowed for greater settlement success and may be a potential mechanism for coral larvae as they approach the turbulent boundary layer just above the substrate.

The still-water behavioral assay also analyzed the proportion of time the larvae spent stationary or revolving in place, and variations related to cue were detected. Two days after the initiation of swimming behaviors, larvae of *A. palmata* and *O. annularis* both experienced an increase in time spent stationary or revolving in place when exposed to the reef cue. This trend is a reversal of the previous day for *O. annularis* larvae, at which point they spent more time stationary in offshore water compared to
reef water. This definition of Stationary/Rotations aligns with what Miller and Mundy (2003) identifies as “testing” habitat, where larvae were loosely attached and spun in place. This behavior occurs before settlement, which involves both permanent attachment and subsequent metamorphosis (Miller and Mundy 2003). We did not statistically compare the variation in time spent stationary between developmental days, and no trend was clearly visible during the free-swimming period. However, on day 7 for A. palmata and day 5 for P. strigosa, larvae immediately attached themselves to the bottom upon entering the dish and did not move during the habitation period, marking the end of the free-swimming larval phase and end of the testing period.

For the still-water analysis, proportion of time in each behavior and average velocity, regardless of behavior type, were compared between cues. It is possible that other parameters not addressed here may reveal variations in behavior across time. Common parameters of interest include the proportion of larvae that perform each behavior, rather than the proportion of time an individual spent preforming a specific behavior (Vermeij et al. 2006; Gleason et al. 2009). In this study, inter-individual variation was an influential factor in larval behavior post cue insertion and may have masked relevant behavioral trends. Future studies investigating chemical cue-mediated behaviors at the population level rather than the individual level, e.g. by assessing percent of stationary larvae rather than percent of time an individual larva remained stationary, may reveal temporal trends.

Previous studies have assessed free-swimming coral larvae behavior when exposed to general reef cues, with variable results, which could be a factor of methodology or species-specific differences. Larvae of three Pacific acroporids
successfully discriminated between the chemical cues associated with a healthy, coral-dominated reef versus a degraded, algae-dominated reef (Dixson et al. 2014). In comparison, chemical cues from both a degraded and a healthy reef initiated benthic-probing and positive geotaxis in the planula of two Caribbean brooders (Gleason et al. 2009). General reef cue used in this study comes from a healthy, coral-dominated reef, although the specific chemical components of the general cue that play a role in larval behavior are unknown. Investigations into whether Caribbean larvae can differentiate between a healthy and degraded reef and what chemical components of these general reef cues initiate certain behaviors are areas of future study.

In all, this study has revealed interesting temporal trends in the behavior of planktonic coral larvae when exposed to reef and offshore cues during their critical pre-settlement period. Further development of the still-water behavioral assay and associated analysis will aid in the understanding of the ecological significance of these behaviors. The still-water assay, while far removed from the turbulent, complex environment of the reef ecosystem for settling larvae, can facilitate observations of coral larvae strictly in response to a chemical cue, rather than physical movement of the water. Successful coral settlement is vital for long-term, reef revitalization efforts throughout the Caribbean. Investigations into the chemically-mediated behavior of coral larvae during habitat selection can inform research and management practices.

1.6 Acknowledgements

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Chapter 2

CORAL LARVAE BEHAVIORAL RESPONSE TO BENTHIC CUES DURING HABITAT SELECTION

2.1 Abstract

Environmental chemical cues relay valuable information about the health of an ecosystem to potential larval recruits. Coral larvae recognize not only general reef cues but also those of specific benthic components of the reef ecosystem. Here, the behavioral response of *Acropora palmata* larvae to the chemical cues released from benthic algae and cyanobacteria was investigated using a still-water assay. Video recordings of larval behavior before and after the cue was inserted allowed for an analysis of minute behavioral changes in response to algal and cyanobacterial cues. Overall, larvae increased the time they spent traveling, regardless of the cue that was presented, but most significantly when introduced to *Dictyota* sp, *Halimeda tuna*, *Galaxaura rugosa*, *Paragoniolithon solubile*, and *Hydrolithon boergesenii*. Four broad behavioral classifications were identified during the assay: Stationary/Rotations, Small Circles, Non-directed Movement, and Directed Movement. Notably, the percent of time spent in directed motion or swimming in small circles significantly increased when the larvae were presented with positive cues of crustose coralline algae. These behaviors may indicate recognition of a positive settlement cue, highlighting an area for future study. Identifying positive and negative chemical cues for settling coral larvae can provide valuable insights into conservation strategies in a rapidly changing world.
2.2 Introduction

Coral reefs are intricately complex ecosystems, replete with mobile fish and invertebrates as well as abundant benthic species of corals, algae, and sponges. All of these organisms release chemicals into their environment, either passively or actively (Hay 1996). These species-specific chemicals create an amalgam of cues that can be perceived as a general reef cue by many marine animals (Atema et al. 2002). This general reef cue reflects the species composition of the ecosystem and relays important information to potential incoming settlers regarding its quality (Dixson et al. 2014). For example, pelagic reef fish larvae can distinguish between the chemical signatures of reefs that are algal versus coral-dominated and significantly prefer healthy, coral-dominated reefs (Lecchini et al. 2013). Pelagic larvae, as well as migrating adults, can use these broad reef cues to not only orient back to a reef, but also to choose the healthiest reef in the region.

The overall chemical signature of many coral reefs is changing as species distributions and abundances shift in response to environmental stressors (Nyström et al. 2000; Hughes et al. 2003). Overfishing, eutrophication, bleaching, and structural damage can result in phase shifts characterized by an alteration in the dominant benthic species on a reef (Hughes 1994; Bruno et al. 2009). For example, in the 1980s, the Caribbean experienced massive mortality rates of the long-spined sea urchin, *Diadema antillarum*, a grazer vital in controlling marine macroalgae (Lessios 1988). The functional extinction of this species, along with other local stressors, led to a rapid shift from coral-dominated reef systems to algal-dominated systems (Liddell and Ohlhorst 1986). Progress towards recovery has occurred, but it has been extremely slow (Lessios 2016).
As Caribbean reefs continue to experience phase shifts towards algal-dominance, with limited capacity for recovery (Mumby et al. 2007), research has focused on the impact of benthic algae on reef-building corals throughout their life history, from fertilization to sexual maturity (Birrell et al. 2008a). Within the reef matrix, upright macroalgae of many different taxonomic and functional groups directly compete with settled corals for the limited available substrate on which to grow (McCook et al. 2001). Generally, both corals and benthic algae require solid substrate to anchor themselves as well as unobstructed sunlight for photosynthesis. Mechanisms for competition between algae and corals include overgrowth, shading, abrasion, epithelial sloughing, recruitment barriers, and chemical defense (McCook et al. 2001). In this race for both settlement sites and light, many benthic species produce defensive compounds that are repulsive or toxic to potential consumers (Hay and Fenical 1988). Further, chemicals are also used for direct competition with neighbors through allelopathic warfare (Littler and Littler 1997; Gross 2003; Rasher and Hay 2010, 2014; Rasher et al. 2011).

Settlement in an area replete with upright benthic algae can be detrimental to corals of all sizes, therefore pre-settlement larvae should aim to avoid these areas. Mobile coral larvae are capable of recognizing environmental cues and can modify their behavior in response to these cues (Dixson et al. 2014). Detection of external cues is a requirement of metamorphic competency and is thus a critical aspect of early development (Gleason and Hofman 2011; Hadfield et al. 2011). Research has shown that the presence of cues from a host of upright benthic macroalgae and cyanobacteria can deter settlement in coral larvae (Kuffner et al. 2006; Birrell et al. 2008a), yet research into specific behavioral responses before settlement is limited.
While the presence of some macroalgal cues can deter settlement, other cues can induce settlement. Crustose coralline algae (CCA) are a group of calcareous red algae that act as cement in a reef ecosystem (Steneck 1986; Adey 1998) and contain chemical cues, potentially in their associated biofilm, that are attractive to coral larvae (Morse et al. 1988; Heyward and Negri 1999; Hadfield and Paul 2001; Negri et al. 2001; Harrington et al. 2004; Golbuu and Richmond 2007; Kitamura et al. 2007, 2009; Sneed et al. 2014; Tebben et al. 2015). Larval preference for CCA is species specific as CCA growth forms can vary, with some species providing solid substrate ideal for settling larvae and other species being unsuitable for permanent settlement due to epithelial sloughing (Harrington et al. 2004). It would be beneficial for free-swimming coral larvae within the reef matrix to distinguish between chemicals produced by different reef algae, as an inappropriate settlement choice can lead to decreased fitness or death.

The species-specific chemical preferences of free-swimming coral larvae has been investigated in the Indo-Pacific using a two-channel choice flume (Dixson et al. 2014). Coral larvae are not only capable of using general reef cues to distinguish between healthy and degraded reefs, but they also respond to species-specific benthic cues of corals and algae. Coral larvae are chemically attracted to CCA and adult corals but are repulsed by several species of macroalgae (Dixson et al. 2014).

Research into pre-settlement behavioral response of coral larvae to algal cues is ongoing in the Caribbean. The current study attempts to determine the minute changes in behavior of Caribbean coral larvae after exposure to common algal and cyanobacteria cues. I developed a novel still-water behavioral assay to investigate the response of *Acropora palmata* larvae to common Caribbean macroalgae and
cyanobacteria of various taxonomic and functional groups. As settlement is permanent after metamorphosis and the consequences of inappropriate settlement sites are high, I hypothesized that the introduction of benthic cues, either positive or negative, would incite a behavioral reaction in free-swimming coral larvae. Specifically, due to the detrimental effects of upright macroalgae on corals at all life stages and the benefits of CCA for inducing metamorphosis, I hypothesized that coral larvae would display distinctive behaviors when presented with traditionally negative versus positive cues.

2.3 Methods

2.3.1 Larval collection and rearing
This study was conducted at the Carrie Bow Cay field station, off the coast of Belize (16°48'9.26"N, 88° 4'54.87"W). Acropora palmata is a critically-endangered, yet vital reef-building coral throughout the Caribbean (see Chapter 1 methods for species information). Acropora palmata coral larvae were collected from the reefs surrounding Carrie Bow Cay, Belize on 5-6 August 2015 and 23-24 August 2016 according to methods described in Chapter 1. Fertilized eggs were reared in 4 L larval containers with continual seawater flow (Ritson-Williams et al. 2010). Throughout development, deceased larvae were removed from the container using pipettes. Larvae were reared for at least 4 days before initiating behavioral assays. Assays were run on larvae 4-6 days post spawn, after which the larvae ceased swimming in behavior trials and remained attached to the substrate.

2.3.2 Cue generation
Common Caribbean algal and cyanobacterial species of various taxonomic and functional groups (Table 2.1) were used as environmental cues in the still-water assays
and were collected from the barrier reef around Carrie Bow Cay in waters ranging from 1-15 m. Macroalgae (Galaxaura rugosa, Halimeda tuna, H. opuntia, Stylopodium zonale, Dictyota sp., and Lobophora sp.) and a cyanobacterium (Hormothamnion enteromorphoides) were semi-dried in a salad spinner for 25 revolutions to reduce excess water before being weighed to 20 g (Dixson 2011).

Crustose coralline algae (Paragoniolithon solubile and Hydrolithon boergesenii) were collected from reef rubble (1 m depth) using rock hammers and trimmed with wire clippers to eliminate additional cues before being weighed to 50 g. After being weighed to the right amount, cues were individually soaked in 10 L seawater for 1 hour (Dixson 2011) to generate the cues for the experiments.
Table 2.1: Functional and taxonomic groups of benthic algae and cyanobacteria used in this study (from ¹Steneck and Dethier 1994, ²Littler et al. 1983, ³Diaz-Pulido 2010).

<table>
<thead>
<tr>
<th>Species</th>
<th>Functional group</th>
<th>Taxonomic group</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Stypopodium zonale</em></td>
<td>¹Corticated foliose, ²Thick leathery group, ³Upright, fleshy</td>
<td>Phaeophyceae (Brown algae)</td>
</tr>
<tr>
<td><em>Lobophora sp.</em></td>
<td>¹Corticated foliose, ²Sheet group, ³Upright, fleshy</td>
<td></td>
</tr>
<tr>
<td><em>Dictyota sp.</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Halimeda tuna</em></td>
<td>¹Articulated calcareous, ²Jointed calcareous group, ³Upright, calcareous</td>
<td>Chlorophyta (Green algae)</td>
</tr>
<tr>
<td><em>Halimeda opuntia</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Galaxaura rugosa</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Hydrolithon boergesenii</em></td>
<td>¹, ², ³Crustose calcareous</td>
<td>Rhodophyta (Red algae)</td>
</tr>
<tr>
<td><em>Paragoniolithon solubile</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Hormothamnion enteromorphoides</em></td>
<td>¹Microalgae</td>
<td>Cyanophyta (Cyanobacteria)</td>
</tr>
</tbody>
</table>

2.3.3 Still-water behavioral assay

A still-water assay was used to assess minute changes in coral larvae behavior when exposed to algal cues. As described in more detail in chapter 1, a larva was pipetted into a petri dish filled with 30mL filtered seawater. After a 1 min habituation period, video recording above the dish commenced. After 2 min, the larva was pipetted back to the center of the dish and 2 mL of cue water and 2 mL of control water were carefully inserted into the far sides of the dish. Video recording continued for 2 min before the conclusion of the trial.
2.3.4 Video analysis

Average velocity was analyzed using Tracker 5.0 (Brown 2019), as described in chapter 1. Additionally, videos were analyzed manually to characterize behavior type. Four general behaviors were identified: (1) Stationary Rotations, in which the larva either remained motionless or rotated in place, (2) Small Circles, in which the larva swam in circles <1cm, yet did not experience any overall change in position within the dish, (3) Directed Movement, in which the larva swam in one direction with limited variations in speed; and (4) Non-directed Movement, in which the larva traveled in an unpredictable fashion involving spiraling, drifting, bouncing, and serpentine motions. For both Tracker and manual analyses, the first 10 seconds of footage after the cue was introduced was removed to account for any water movement due to the induction of the cue rather than larval self-propulsion.

2.3.5 Statistics

This study investigated behavioral changes of coral larvae after a benthic cue was presented; therefore, analyses focused on the differences in pre-cue and post cue data. Mean velocity and percent time spent displaying each behavior were analyzed using the Wilcoxon signed rank test (JMP Pro 13), a non-parametric alternative to the paired t-test as the data was not normally distributed despite transformations.

2.4 Results

2.4.1 Brown algae

Three species of brown algae were tested in the still-water assays, *Stypopodium zonale*, *Dictyota* sp., and *Lobophora* sp. Neither the introduction of *S. zonale* nor *Lobophora* sp. chemical cues resulted in significant variations in larval
velocity or percentage of time spent in any particular behavior. Only the introduction of chemical cues from *Dictyota* sp. resulted in a significant increase in the time the larvae spent traveling (p=0.025, Figure 2.1) as well as average larval velocity (p<0.001, Figure 2.2). Additionally, a significant decrease in stationary/rotation behavior (p=0.005, Figure 2.3A) was paired with an increase in non-directed movement (p=0.023, Figure 2.3C) after *Dictyota* sp. cue was presented to the coral larvae.

**Figure 2.1:** Comparison of mean percent time (±SE) spent traveling before (lighter colors) versus after (darker colors) the chemical cue of each benthic species was inserted into the petri dish. Brown bars=brown algae, green bars=green algae, red bars=upright red algae, blue bars=cyanobacteria, purple bars=CCA. Wilcoxon signed rank test, α=0.05, * indicates significance.
Figure 2.2: Comparison of mean velocity (±SE) before (lighter colors) versus after (darker colors) the chemical cue of each benthic species was inserted into the petri dish. Brown bars=brown algae, green bars=green algae, red bars=upright red algae, blue bars= cyanobacteria, purple bars=CCA. Wilcoxon signed rank test, α=0.05, * indicates significance.
Figure 2.3: Comparison of pre-cue and post-cue mean percent time (±SE) spent in each of the four behavioral classifications, (A) Stationary/Rotations, (B) Small Circles, (C) Non-Directed Movement, and (D) Directed Movement. Lighter colors refer to pre-cue data and darker colors refer to post-cue data. Brown bars=brown algae, green bars=green algae, red bars=upright red algae, blue bars= cyanobacteria, purple bars=CCA. Wilcoxon signed rank test, α=0.05, * indicates significance.

2.4.2 Green algae

Analysis of the still-water behavioral videos revealed no change in coral larvae behavior when presented with chemical cues of *H. opuntia*. However, when the larvae were presented with *H. tuna* cues, they exhibited an increase in percent time traveling (p=0.008, Figure 2.1) and an associated increase in velocity (p=0.035, Figure 2.2). Specifically, an increase in the percent time in non-directed motion (p=0.004, Figure
2.3C) was reflected in a decrease in the percent time stationary or revolving in place (p=0.005, Figure 2.3A) after the cue was presented.

### 2.4.3 Red algae

*Galaxaura rugosa* was the only species of upright red algae used in the behavioral assays. *A. palmata* larvae significantly increased their percent time spent traveling when presented with the chemical cues of *G. rugosa* (p=0.018, Figure 2.1), but an associated increase in average velocity was not detected (Figure 2.2). Larvae significantly increased the percent time spent in non-directed motion (p=0.005, Figure 2.3C) and reduced the percent time spent stationary or revolving in place (p=0.026, Figure 2.3A) after exposure to *G. rugosa* cues.

### 2.4.4 Cyanobacteria

*Hormothamnion enteromorphoides* acted as a representative cyanobacterium during the still-water trials. No significant changes in mean velocity or behavior were detected when *A. palmata* larvae were presented with cues from *H. enteromorphoides*.

### 2.4.5 Crustose coralline algae (CCA)

The addition of chemical cues from the two CCA species, *P. solubile* and *H. boergesenii*, into the petri dishes of the still-water assays resulted in a significant increase in coral larvae travel time (p<0.001, Figure 2.1) as well as mean velocity (*H. boergesenii*: p<0.001, *P. solubile*: p=0.048, Figure 2.2). Larvae consistently displayed a decrease in time spent stationary or revolving in place after the cues were inserted (*H. boergesenii*: p<0.001, *P. solubile*: p=0.002, Figure 2.3A). The addition of cues from *P. solubile* was the only alga to cause an increase in amount of time spent swimming in small circles (p=0.012, Figure 2.3B). When waterborne cues of *H.
boergesenii were presented, coral larvae displayed a significant increase in both
directed and non-directed movement (p=0.012, Figure 2.4D; p=0.007, Figure 2.4C,
respectively)

2.5 Discussion

Upright macroalgae and cyanobacteria can directly compete with coral larvae
for space on the substrata to settle, therefore avoidance of chemical cues associated
with these species would be beneficial for coral fitness. Additionally, benthic algae
and cyanobacteria from a variety of taxonomic and functional groups produce
compounds that can be detrimental for survival and recruitment of invertebrate larvae
(Walters et al. 1996), including coral larvae (Diaz-Pulido et al. 2010). Numerous
studies have detected a reduction in survival and/or settlement of coral larvae after
exposure to chemical cues of brown algae (Morse 1996; Baird and Morse 2004;
Kuffner 2006; Birrell 2008b; Vermeij et al. 2009; Diaz-Pulido et al. 2010; Paul et al.
2011; Fong et al. 2019), green algae (Birrell et al. 2008b; Vermeij et al. 2009; Diaz-
Pulido et al. 2010; Lee et al. 2012), upright red algae (Vermeij et al. 2009; Diaz-Pulido
et al. 2010), and cyanobacteria (Kuffner and Paul 2004; Kuffner 2006). However,
interactions are not consistent and various species of coral larvae respond positively or
indifferently to cues of some upright benthic algae and cyanobacteria (Maypa and
Raymundo 2004; Nugues and Szmant 2006; Birrell 2008b). Encrusting red algae, or
the associated biofilm, is a known attractant to coral larvae and can induce settlement
and metamorphosis (Morse et al. 1988; Heyward and Negri 1999; Hadfield and Paul
2001; Negri et al. 2001; Harrington et al. 2004; Webster et al. 2004; Golbuu and
Richmond 2007; Kitamura et al. 2007, 2009; Ritson-Williams et al. 2010; Sneed et al.
2014; Tebben et al. 2015); however, compounds have been derived from some CCA that are toxic to coral larvae (Kitamura et al. 2005).

Most of the above studies assessed settlement success, but pre-settlement coral larvae are also capable of altering their swimming direction to reflect their preference (Dixson et al. 2014). In the Indo-Pacific, *Acropora tenuis* larvae swam towards cues associated with CCA and away from chemical cues of upright brown and red algae (Dixson et al. 2014). Movement towards or away from a cue may not be the only behavioral response initiated by a coral larva after exposure to a repulsive or attractive cue. Coral larvae have been observed performing a diversity of behaviors including, bouncing, spiraling, rotating in place, and swimming in small circles (Miller and Mundy 2003; Goffredo and Zaccanti 2004; Gleason et al. 2009; Marharver et al. 2015). This study assessed behavioral changes of coral larvae when presented with common Caribbean algal cues, and these deviations were variable depending on the cue, even within algal groups.

Overall, the introduction of algal cues resulted in decreased stationary/rotations and increased non-directional movement. This trend, most apparent when the larvae were exposed to *Dictyota* sp., *H. tuna*, *G. rugosa*, and the CCA species, *P. solubile* and *H. boergesenii*, and was unrelated to algal taxonomic or functional relatedness. While CCA typically induce settlement, upright brown, red, and green algae typically deter settlement (Birrell et al. 2008a). An increase in movement regardless of cue may indicate that the sensory capabilities of coral larvae are developed enough to perceive environmental chemicals. Additionally, coral larvae may utilize the same behavioral mechanisms to both avoid or swim towards a cue.
Notably, the increase in the proportion of time spent in small circles or directed movement is unique to the introduction of potentially positive cues. Coral larvae spent more time performing small circles after the CCA, *P. solubile*, was introduced. Swimming in uniform small circles, without changing overall position within the dish was a very distinct, but relatively rare behavior. Bryozoan larvae display this behavior when an appropriate settlement site has been located (Ryland 1974; Chia et al. 1983), suggesting that swimming in tight circles might be indicative of a positive cue for coral larvæ settlement as well. Additionally, zooplankton utilize a similar pattern of cyclical loops when they encounter a high-density food patch (Williamson 1981; Hamner et al. 1983; Buskey 1984). Swimming in small circles allows zooplankton such as copepods to remain longer within the area of abundant food resources. Coral larvæ may engage in cyclical swimming in an attempt to remain near the source of a positive cue so that settlement can occur. Swimming in small circles was also distinct but rare throughout the development of *A. palmata*, *Orbicella annularis*, and *Pseudodiploria strigosa* larvæ (see Chapter 1). Further investigations into the nature and prevalence of this behavior would likely prove insightful in the identification of positive settlement cues.

*A. palmata* larvæ increased the amount of time traveling in a directed motion only when the CCA, *H. boergesenii*, was detected, although the directionality of that movement was not assessed. *H. boergesenii* has been shown to be a settlement inducer for several species of coral larvæ (Ritson-Williams et al. 2010, 2016) and in a paired choice settlement assay, *A. palmata* preferred to settle onto *H. boergesenii* over *P. solubile* (Ritson-Williams et al. 2010). An increase in directed motion after the addition of *H. boergesenii* cues indicates that directed motion might be indicative of a
positive benthic settlement cue. Directed motion was observed in larvae of the Pillar coral, *Dendrogyra cylindrus*, while the larvae were positively geotactic and swimming near the bottom of the container (Marharver et al. 2015). The shallow nature of our testing arena revealed directed motion along a horizontal plane, but vertical directed motion may be evident in deeper containers, especially as larvae reach settlement age.

Of the brown algae, only *Dictyota* sp. cues triggered significant variations in larval behavior in the still-water analyses, despite all three Phaeophyceae species containing significant quantities of secondary metabolites, some known to inhibit coral settlement (Gerwick et al. 1985; Kuffner et al. 2006; Diaz-Pulido et al. 2010; Paul et al. 2011; Fong et al. 2019). Similarly, introduction of the chemical cues of the cyanobacterium, *H. enteromorphoides*, did not incite a significant reaction in coral larvae during the behavioral assays, despite similar cyanobacteria species deterring settlement of other scleractinian coral species (Kuffner and Paul 2004; Kuffner 2006). These upright macroalgae and cyanobacteria are abundant on degraded reefs, leading us to incorrectly hypothesize that cues associated with these species would cause a reaction in coral larval behavior in the still-water assay. Perceived indifference to waterborne cues of *Lobophora* sp., *S. zonale*, and *H. enteromorphoides* in the still-water trials could potentially be due to high inter-individual variation or lack of cue detection but could also indicate that these benthic species do not emit compounds that are relevant to coral larvae.

Results of this study corroborate previous studies suggesting that response to benthic cues depends on the focal larval species and remains highly variable regardless of algal taxonomic or functional relatedness. For example, *H. opuntia* inhibits settlement of the brooder, *Pocillopora damicornis* (Lee et al. 2012), yet larvae
of *Favia fragrum* surprisingly preferred to settle directly on *H. opuntia* individuals (Nugues and Szmant 2006). In this study, *A. palmata* larvae were indifferent to *H. opuntia* cues, but increased movement and velocity after exposure to chemical cues of the congeneric, *H. tuna*. Similarly, *A. palmata* larvae were indifferent to cues of *H. enteromorphyoides*, despite this species producing secondary metabolites deterrent to herbivores (Pennings et al. 1997). However, the presence of this cyanobacterium was correlated to an increase in successful recruitment of coral larvae in Hawaii (Cover 2011). This correlation, however, may be an indirect result of a reduction in grazers that scrape off coral larvae due to the presence of the cyanobacterium. Therefore, even though the specific chemical cues of these species deter grazers, they might reflect potentially beneficial macrohabitat, resulting in conflicting responses.

Definitive avoidance of macroalgal chemical cues may offer an evolutionary advantage to coral larvae for two reasons. First, secondary metabolites produced by benthic algae may directly inhibit coral growth (Tanner 1995; Lirman 2001; Rasher and Hay 2010, 2014; Rasher et al. 2011). Second, even if some algal cues are not acutely toxic, they may still relay important information about potential settlement locations at both the macro ecosystem scale and the microhabitat scale. Within the reef matrix, it would be disadvantageous for a coral larva to settle adjacent to a fast-growing, chemically-rich alga with the potential to shade sunlight. Additionally, as Caribbean reefs rapidly shift from coral-dominated to algae-dominated, an abundance of algal cues may warn free-swimming coral larvae of a degraded reef (Lecchini et al. 2013).

In conclusion, observing and quantifying the behavioral patterns of coral larvae is an important step in coral larval ecology and requires the use of diverse and novel
assays that can be adapted based on coral species, relevant environmental cues, and behavioral traits of interest. As behavioral patterns can influence recruitment success (Raimondi and Morse 2000), determining how coral larvae will respond to environmental cues can be vital for conservation efforts. Identification of positive and negative chemical cues that impact recruitment and settlement of coral larvae can inform reef management. For example, herbivores that consume repulsive algae can be granted high protection status (Bellwood et al. 2004), and settlement-inducing chemicals can be incorporated into artificial reefs. An interdisciplinary approach to reef management, incorporating aspects of animal behavior and environmental cues, can allow for more efficient, ecosystem-level conservation.

2.6 Acknowledgements

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Chapter 3

MULTIPLE ENVIRONMENTAL CUES IMPACT HABITAT CHOICE DURING NOCTURNAL HOMING OF SPECIALIZED REEF SHRIMP

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3.1 Abstract

Habitat selection is a critical process for animals throughout their life, and adult organisms that travel to forage or mate must re-select habitat frequently. On coral reefs, competition for space has led to a high proportion of habitat specialists. Habitat selection is especially vital for organisms that require specialized habitat; however, research has primarily focused on the initial habitat choice made during the larval/juvenile stage. Here, we analyze habitat selection in the adult sponge-dwelling reef shrimp, *Lysmata pederseni*. Using a mark-and-recapture technique, belt transects, patch reefs, and cue isolation experiments, this study reveals that adult *L. pederseni* diurnally re-select habitat and a natural preference exists for specific sponge species and shapes. This natural preference is a function of chemical and morphological cues as well as sponge distribution. As habitat specialists can drive biodiversity, understanding the mechanisms behind habitat selection can inform research and management practices.
3.2 Introduction

Animals typically occupy distinct habitats ranging in specificity from the entire ecosystem to specialized single-host microhabitats. Habitat selection has been researched in a variety of taxa in an attempt to understand the balance between environmental variables (Cowen and Sponaugle 2009) and animal behavior (Meadows and Campbell 1972). While some organisms choose appropriate habitat once during their life history – typically at the juvenile or larval stage – others must re-select habitat frequently if they migrate, disperse, or temporally home. Roaming animals exist in both the terrestrial environment (e.g. cougars [Dickson and Beier 2002], golden eagles [Domenech et al. 2015], and foxes [Chamberlain and Leopold 2000]) and the aquatic realm (e.g. catfish [Kadye and Booth 2012], octopuses [Regueira et al. 2013 and cardinalfishes [Marnane 2000]). Though these animals migrate frequently, they often have home bases and exhibit high site-fidelity. Insight into adult habitat selection at all scales is critical as the ecological baselines of numerous systems are rapidly shifting. This shift is projected to most devastatingly influence species that are tightly coupled with specific habitats (Munday 2004).

Specialized habitat selection is particularly important in the intensely complex and diverse coral reef ecosystem (Huston 1985). The selection of favorable habitat can increase overall fitness through predator avoidance (Caley and St John 1996), food availability (Pereira et al. 2012), and access to mating opportunities (Baeza et al. 2016). Due to competition for space and the potential consequences of selecting inappropriate habitat (e.g., death), natural selection should favor individuals that choose suitable sites. On coral reefs, this selection has resulted in a high proportion of species exhibiting obligate habitat use (Connell 1978).
Obligate habitat associations often begin at the conclusion of the larval stage. Thereafter, the inhabitant rarely leaves the host, as seen in the anemonefish – sea anemone relationship (Dixson et al. 2014). However, some reef animals occupy specific habitats during rest periods, yet vacate to forage or mate. Cardinalfishes (Apogonidae), for example, reside in caves or dendritic corals during the day (Greenfield and Johnson 1990) but leave at night to forage throughout the reef (Chave 1978). Host specificity in cardinalfishes is pronounced, with individuals showing high site fidelity by homing back to specific locations within a coral matrix after being displaced 1-2 km (Marnane 2000). Such temporal homing has been observed in multiple species of reef teleosts (Ogden & Buckman 1973; Quinn and Brodeur 1991), as well as several invertebrate groups including crustaceans (Herrnkind and McLean 1971; Hahn and Itzkowitz 1986; Vannini and Cannicci 1995), cephalopods (Mather 1991), and limpets (Cook et al. 1969), yet the mechanisms behind the selection of habitat are relatively unknown.

Shrimp associations within tube sponges are rare among the family, Hippolytidae, which consists primarily of cleaner and peppermint shrimp (Rhyne and Lin 2006). The peppermint shrimp, Lysmata pederseni, exhibit obligatory associations with tube sponges, particularly the sponge genera Callyspongia, Niphates, and Aplysina throughout Caribbean reefs (Rhyne and Lin 2006; Baeza 2010; Baeza et al. 2016). Although L. pederseni is not a true cleaner shrimp, it may passively clean its surroundings (Rhyne and Lin 2006), potentially contributing to sponge health. Further, these cathemeral shrimp are ecologically distinct from other peppermint shrimp due to their propensity for living alone or in small groups, rather than gregariously (Rhyne
and Lin 2006). Little is known about the external factors that influence the unique habitat association of *L. pederseni* within tube sponges.

The evaluation of potential hosts can be accomplished through chemosensory perception or an assessment of morphological characteristics (Enright 1978). The relative use of these stimuli depends on species, spatial proximity, and environmental variables (Kingsford et al. 2002). *Lysmata pederseni* assess chemical cues using olfactory receptors in aesthetascs on their antennules (Hallberg et al. 1992). These shrimp are capable of detecting and responding to the chemical cues from the sponge *Callyspongia vaginalis* (Baeza et al. 2016); however, the role chemical cues play in species-specific habitat selection has yet to be understood. Additionally, host morphology can influence habitat choice, as size and shape can be critical not only for protection from predators, but also for space required to perform basic functions such as mating (Vytopil and Willis 2001). Morphological assessment can be accomplished using visual and tactile cues, making it a reliable source of information even for visually-limited shrimp (Caves et al. 2016).

In this study, we investigated the tube sponge habitat selection of adult *L. pederseni*. First, the movement patterns of adult peppermint shrimp were identified using a mark-and-recapture experiment. Then, belt transects were conducted to investigate natural variations in shrimp-associated sponge species and morphologies. Subsequently, to determine which sensory cues are pertinent for habitat selection, both chemical and morphological preferences were tested in isolation. Finally, as the natural distribution of sponge species varies spatially, patch reefs were constructed to test sponge preference when all cues were present, and sponges were equidistant. Habitat specialists are more susceptible to the shifting species composition on coral
reefs, especially when associated with living hosts. A better understanding of vital processes, such as specialized habitat selection, can lead to more thorough predictions of how ecosystems and communities will respond to future conditions. We use *L. pederseni* as a model organism in this study, but the results have the potential to inform habitat selection across diverse taxa.

### 3.3 Methods

#### 3.3.1 Temporal movements

*Lysmata pederseni* habitat fidelity was monitored during the day and night to gain insight into the extent and temporal scale of adult movement and habitat selection. Short-term habitat fidelity was assessed using an overnight mark-and-recapture technique at two reefs near Carrie Bow Cay, Belize (16°48'9.26"N, 88°4'54.87"W; fore reef: 17 m max depth, surveyed August 23-25, 2016; lagoon reef: 9 m max depth, surveyed June 17-18 and 24-27, 2017). Sponges containing *L. pederseni* were marked with flagging tape in the afternoon (~16:00). If multiple sponge tubes hosted shrimp, each tube was individually flagged. Each shrimp within the sponge was carefully extracted by squeezing the sponge so that the shrimp rose through the column into a net (Baeza et al. 2016) and tagged using an elastomer tag (Northwest Marine Technology, Inc., USA) on the ventral side of the sixth abdominal segment (Baeza 2010). After tagging, shrimp were returned to their original sponge tube. All shrimp were alive after release (n=26).

Shrimp locations were assessed three times following tagging. First, presence of shrimp was recorded the morning after the tagging procedure (~10:00) to appraise site fidelity. The subsequent night, shrimp-sponge associations were evaluated using a
1000 lumen flashlight at ~21:00 to determine whether shrimp nocturnally vacate their host sponge. Shrimp could be detected in a few seconds, which minimized disturbance by dive lights. Finally, flagged sponges were checked for shrimp presence the morning after the nocturnal observations (~10:00) to confirm site fidelity despite disturbance.

3.3.2 Natural habitat associations

Natural variability in shrimp-sponge associations was determined using 15x1 m belt transects on reefs around Carrie Bow Cay, Belize between March 31-April 2, 2016 (depth range: 3-14 m, n=22). Along each transect, all tube sponges were inspected to quantify abundance of resident *L. pederseni* and four sponge parameters were recorded: (1) sponge species, (2) number of tubes, (3) tube height from base to tip, and (4) osculum diameter, measured at the widest point.

3.3.3 Morphological preference

Shrimp used in the cue isolation and patch reef experiments were collected using methods described above and transported to the Smithsonian Research Station on Carrie Bow Cay, where they were held in ~15 L flow-through aquaria with coral rubble for shelter until testing. Cafeteria-style choice experiments were used to assess shrimp morphological preference to host sponges. Four morphological types of artificial tube sponges were constructed from black foam pool noodles (Fig. 3.1): tall wide (20 cm high, 7 cm osculum diameter), short wide (10 cm high, 7 cm osculum diameter), tall narrow (20 cm high, 3 cm osculum diameter), and short narrow (10 cm high, 3 cm osculum diameter). One of each sponge morphotype was fixed in a random configuration to an acrylic plate such that each artificial sponge was 30 cm from the center. The acrylic plates with attached artificial sponges were placed into circular
aquaria (40L) and weighed down with dive weights. Each aquarium was filled with seawater and sand was added to eliminate all crevices. A 10 cm diameter habituation chamber, constructed from plastic mesh (1.27 x 1.27 cm) and fly screen (1x1 mm), was positioned upright in the sand such that the top of the chamber was above the waterline. Each aquarium contained an airstone. The sand and water were replaced after each trial.

After sunset (~21:00), one shrimp was carefully placed inside the habituation chamber for 10 min. At the end of the habituation period, the chamber was slowly removed, and the aquarium was covered with a mesh screen. This allowed natural light to penetrate but prevented the shrimp from escaping. After 12 hours, the shrimp location was recorded (n=36).
3.3.4 Chemical preference

To assess the use of chemical cues in habitat selection, shrimp preference was tested using an Atema two-channel choice flume (Gerlach et al. 2007). The choice flume (23.5 x 4 cm) allows an individual organism to experience the chemical cues of two different water sources simultaneously by presenting them side-by-side. The two water sources were gravity-fed from buckets through tubes into the choice chamber. Laminar flow was maintained at 100 mL min\(^{-1}\) using flow meters (Dwyer MMA-40) and checked periodically using dye tests. A shrimp was placed in the center of the flume and given a 2 min habituation period, during which time the shrimp was free to move throughout the chamber and explore either cue. After the habituation period, the

Figure 3.1: Infogram of the experimental methods to test sponge morphology preferences in isolation. Grey cylinders represent artificial sponges made of foam pool noodles. Experimental results are shown in text boxes with a significant preference for tall and narrow tube morphology.
shrimp’s position in each stimulus was recorded at 5-second intervals for 2 min. The water sources were then switched to eliminate a potential side bias with a 2-min flushing period and the entire test was repeated. Each shrimp was tested only once per trial (n=10) and all trials were run blind.

Sponge species used as cues in fluming trials were chosen based on habitat associations observed during the transects (Table 3.1). *Lysmata pederseni* associated with the tube sponges, *Callyspongia vaginalis*, *C. plicifera* and *Niphates digitalis*. In contrast, another common tube sponge, *Aplysinia fistularis*, never hosted peppermint shrimp and was used as a negative control cue. Sponges used for cue generation were cut at the base of their structures and transported to the lab. Only sponges lacking resident shrimp were collected and any additional epibionts were removed. To generate chemical cues, each species of sponge was spun 20 times in a salad spinner to eliminate excess water before being weighed to 20 g and added to 2 L of reef water for 1 hour in a closed system. This solution was diluted to 10 L using reef water. Water collected directly from the reef acted as a general reef water cue. Chemical preferences were analyzed using the Kolmogorov-Smirnov (K-S) nonparametric test. The proportion of time spent in each cue was compared to the proportion of time spent on one side of the choice flume when no cues were present (blank control).
Table 3.1: Chemical choice comparisons. * indicates non-associated habitat based on transect data.

<table>
<thead>
<tr>
<th>Choice trials</th>
<th>Preference hierarchy trials</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. fistularis</em> vs Reef water</td>
<td><em>C. vaginalis</em> vs <em>A. fistularis</em></td>
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<tr>
<td><em>C. vaginalis</em> vs Reef water</td>
<td><em>C. vaginalis</em> vs <em>C. plicifera</em></td>
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<tr>
<td><em>C. plicifera</em> vs Reef water</td>
<td><em>C. vaginalis</em> vs <em>N. digitalis</em></td>
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<tr>
<td><em>N. digitalis</em> vs Reef water</td>
<td><em>C. plicifera</em> vs <em>N. digitalis</em></td>
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<td>Reef water vs Reef water</td>
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3.3.5 Patch reefs

The patch reef experiment was conducted in a large sand patch (8m depth, 16°48'45.15"N, 88° 5'8.29"W) to test the preference for different sponge species when all cues were present and sponge distribution was equal (Fig. 3.2). Eight patch reefs were constructed, and each contained one sponge of each of the four test species, *C. vaginalis*, *C. plicifera*, *N. digitalis* and *A. fistularis*. Sponges were arranged randomly and size matched based on height and overall mass, as mass can influence the quantity of chemical cues released. Number of tubes and tube width were distinct morphological characteristics typical of specific sponge species and were therefore not size matched. Two rubble pieces were added to the center of the patch and each sponge was 0.5 m from this point and equidistance from each other.

Experimental shrimp were tagged with an elastomer tag to indicate the sponge species from which it was collected. Shrimp were placed on patch reefs at ~21:00 (n=36). Dive lights never directly illuminated the patch reefs. At each patch, a 10 cm habituation chamber was wedged into the sand between the rubble pieces and one shrimp was carefully placed within this chamber. The chamber was open at the top,
allowing the shrimp to leave and explore. After 30 min, the habituation chambers were removed. If the shrimp was still inside, the sides of the chamber were gently squeezed until the shrimp crawled onto the rubble pieces. After a 12-hour overnight period, shrimp location was recorded, and shrimp were removed from the patch reef. The sponges were shifted one position clockwise each morning to eliminate potential biases due to current direction or light availability. The experiment was conducted over six nights using different shrimp each time.

Figure 3.2: Infogram of the experimental methods to test sponge preference when all cues were present, and sponges were equidistant. Experimental results are shown in text boxes with a significant preference for *C. vaginalis*. 
3.4 Results

3.4.1 Temporal movements

Morning observations following afternoon tagging revealed that 96.2% ± 7.3 of tagged shrimp were found in the same sponge individual, and only 8.0% ± 10.6 of those found were within a different tube in the same sponge. Nocturnal observations conducted the subsequent night demonstrated definite movement from host sponges; 80.8% ± 15.1 of tagged shrimp vacated their host sponge entirely, with an additional 11.5% ± 12.3 migrating between different tubes of the same sponge. The majority of the tagged shrimp were absent from within or around the sponge individual; however, five tagged shrimp were observed outside of their host sponge tubes over the course of the experiment. Multiple untagged shrimp that were not present during the day were observed in flagged sponges at night. The morning following the nocturnal observations, 80.8% ± 15.1 of the tagged shrimp were found in the same sponge that they were originally tagged in, and only 23.8% ± 18.2 of those were found in a different tube within the same sponge. The proportion of shrimp present in their host sponges during the day compared to the night was statistically different (z-test for proportions in R [R Core Team 2017]; p<0.0001).

3.4.2 Natural habitat associations

Transects confirmed the natural association of *L. pederseni* to specific tube sponge species (See Supplementary Data). The number of tubes per sponge significantly influenced shrimp occupancy rates (ANOVA [JMP Pro 13]; F$_{7,148}=56.956$, p<0.0001) and among naturally shrimp-associated sponges, both sponge species (ANOVA; F$_{2,102}=4.8863$, p=0.0094) and number of tubes (ANOVA; F$_{6,102}=99.64$, p<0.0001) affected shrimp presence. The number of tubes, however, was
highly correlated to the sponge species (ANOVA; $F_{9,148}=7.056; p<0.0001$). When sponge tube morphology was analyzed independent of sponge species, *L. pederseni* were more abundant on sponges with high height to diameter ratios (Wilcoxon signed-rank test [JMP Pro 13]; $p=0.0477, n=234$), thereby favoring tall, narrow sponges opposed to short, wide sponges (Fig. 3.3). An analysis of individual species morphologies reveals that *L. pederseni* tend to occupy *N. digitalis* tubes with high height to diameter ratios (Wilcoxon signed-rank test; $p=0.0012, n=89$). No morphological inclinations were observed for *C. vaginalis* (Wilcoxon signed-rank test; $p=0.1605, n=16$) or *C. plicifera* (Wilcoxon signed-rank test; $p=0.4768, n=42$). The natural abundance of sponge species varies spatially; when the relative population abundance of each sponge species was considered, a greater percentage of *C. vaginalis* tubes hosted *L. pederseni* (18.8%, $n=16$), followed by *N. digitalis* (8.9%, $n=89$) and lastly *C. plicifera* (4.8%, $n=42$). Additionally, tube sponges hosted other epibionts including fish, crabs, brittle stars, and non-conspecific shrimp, but the presence of other inhabitants did not preclude the focal shrimp from association with sponge individuals.
3.4.3 Morphological preference

When morphological preferences were tested in isolation using artificial sponges, no shrimp were found in association with short sponges, regardless of osculum width (Fig. 3.1). Shrimp significantly preferred taller ($\chi^2$ goodness-of-fit test in [R Core Team 2017]; $\chi^2 = 28.033$, $p<0.0001$) and narrower ($\chi^2$ goodness-of-fit test; $\chi^2 = 5.633$, $p=0.0176$) tubes. Of the 36 shrimp tested, 6 did not choose an artificial sponge, 22 selected tall, narrow sponges and 8 selected tall, wide sponges.

3.4.4 Chemical preference

Shrimp preferred the chemical cues of *C. vaginalis*, *C. plicifera* and *N. digitalis* to the general reef cue, by spending >80% of their time in the side of the
flume containing the sponge cue (K-S test [JMP Pro 13]; p<0.0001). However, *L. pederseni* avoided the chemical cue from the negative control, *A. fistularis*, only spending 21% ± 2.66 SE of the time in this cue (K-S test, p<0.0001) (Fig. 3.4a). When presented with two different sponge cues simultaneously, peppermint shrimp preferred *C. vaginalis* to all other species tested (K-S test; p<0.0001 for either comparison). When chemical cues from *C. plicifera* were tested against the chemical cues of *N. digitalis*, shrimp preferred *C. plicifera* (K-S test; p<0.0001) (Fig. 3.4b).
Figure 3.4: Chemical choice trials. (A) Shrimp response to individual sponge cues compared to general reef cues. (B) Shrimp response when presented with two sponge cues simultaneously to determine a hierarchical preference. All chemical data are mean percent time ± standard error. * indicates significance.
3.4.5 Patch reefs

The sponge species that shrimp were collected from did not impact sponge choice in the patch reef experiment (Fisher’s exact test in R [34]; p=0.3512), allowing data from all of the shrimp to be pooled. Sponge selection by *L. pederseni* was not random ($\chi^2$ goodness-of-fit test in [R Core Team 2017]; $\chi^2=14.032$, p=0.002862); rather, *L. pederseni* significantly preferred *C. vaginalis*, with 51% (n=16) of shrimp associating with this species the following morning. *C. plificera* was selected by 16% (n=5) of the shrimp and 6% (n=2) of the shrimp selected *N. digitalis* (Fig. 3.2). Surprisingly, 26% of the shrimp (n=8) selected *A. fistularis*, an association never observed naturally. Five shrimp either abandoned the patch reef or succumbed to predation.

3.5 Discussion

As the shifting climate causes changes in local species abundances (Hughes et al. 2003), habitat specialists will be some of the most vulnerable due to their association with living hosts (Munday 2004). Understanding specialized habitat associations and the mechanisms used in assessing host individuals is vital at both the larval and adult stages. Here, we show that adult *L. pederseni* vacate and re-select habitat daily, with strong site fidelity towards the same host individual. These shrimp demonstrate a preference for the chemical cues of specific sponge species as well as the morphological characteristics of tall narrow sponge tubes, a pattern that is reflected in both natural benthic transects and the *in situ* patch reef experiment. The natural distribution of sponges, however, can also influence sponge-shrimp associations across the reef, thereby creating high variation in chosen host sponges.
Most obligate habitat associations begin at the larval stage and thereafter the inhabitant remains solely within the host. In contrast, *L. pederseni* show high site fidelity to individual sponges, yet vacate at night, suggesting that adult habitat re-selection is frequent. Previous studies have indicated that *L. pederseni* host fidelity can be constant for up to two months (Baeza 2010), depending on the sexual phase or mating system (Baeza et al. 2016). *Lysmata pederseni* are protandric simultaneous hermaphrodites, where all juveniles are male and develop into functional hermaphrodites (Baeza 2009). Populations can tend towards monogamy or polygynandry depending on location. Populations at Carrie Bow Cay, Belize are primarily monogamous (Baeza et al. 2016), whereas populations in the Florida Keys exist in polygynandrous relationships characterized by promiscuity and frequent host switching (Baeza 2010). Previous studies, however, have only assessed site fidelity during the day. Our study confirms that regular nocturnal vacancies occur despite apparent daytime fidelity to specific sponges, suggesting that habitat selection is an essential process at the adult stage even among monogamous populations.

Morphological characteristics tested in isolation revealed a preference for tall, narrow sponges. This morphological affiliation is confirmed in field transects, but whether these natural associations are due to shrimp selection or differential survival remains to be determined. Specifically, morphological characteristics of *C. vaginalis* and *C. plicifera* do not influence the likelihood of shrimp association, possibly due to their ubiquitous tube-like shapes. Conversely, shrimp are more abundant on tall, narrow morphotypes of *N. digitalis*. Tube shape of *N. digitalis* varies with some having an irregularly shaped osculum that is long and narrow. Measurements were taken at the widest diameter of the osculum, causing irregular morphotypes to have
low height to diameter ratios. Wider openings may allow easier predator access to the epifaunal community within and may promote shrimp to avoid this morphotype. Height preference in this species is further confirmed in the Florida Keys where _L. pederseni_ were never found in sponges smaller than 10 cm high (Baeza et al. 2016). While our isolation experiment validates this, one shrimp was found naturally within a 6 cm _C. plicifera_ tube.

_Lysmata pederseni_ easily discriminated between different sponge odours. Chemical preferences (_C. vaginalis, C. plicifera_ and _N. digitalis_) and chemical deterrents (_A. fistularis_) matched natural presence-absence field observations. Previous work on the chemical detection of _L. pederseni_ found that these shrimp positively respond to the chemical cues of _C. vaginalis_ (Baeza et al. 2016). Our study corroborates and expands on this research by testing the response to additional host species and identifying hierarchical preferences. _Lysmata pederseni_ have the strongest preference for the chemical cues of _C. vaginalis_, followed by _C. plicifera_ and _N. digitalis_. This ranking is supported by previous field studies stating that _L. pederseni_ are more commonly or only found in _C. vaginalis_ (Rhyne and Lin 2006; Baeza 2010; Baeza et al. 2016).

Reef sponges can be classified into 3 categories in relation to chemical defenses and predation rates: (1) chemically-defended species, (2) chemically-undefended species that persist because of high rates of growth, reproduction or healing, and (3) chemically-undefended species that persist in secluded refuges (Pawlik 2011). _Callyspongia_ and _Niphates_ are fast-growing and chemically undefended (Pawlik _et al_. 1995), whereas _Aplysina_ sponges produce secondary metabolites that defend against predation (Pawlik _et al_. 1995), fouling (Willemsen
(Kelly et al. 2005), and allelopathic attacks (Pawlik et al. 2007). *Aplysina fistularis*, in particular, naturally extrudes secondary metabolites, but the rate of extrusion can increase when damaged (Walker et al. 1985). Transect and fluming data indicate that *L. pederseni* prefer the fast-growing, chemically undefended species, suggesting that sponge morphology may play a larger role in shrimp preference than the chemical defense of the habitat itself.

The differential distribution of sponges across the reef could be a major factor contributing to sponge-shrimp associations. Transects revealed more *L. pederseni* residing in *N. digitalis* than other species; however, after factoring in sponge population sizes, peppermint shrimp inhabited a higher proportion of *C. vaginalis* sponges, matching the chemical preference data. When the influence of distance and scarcity was eliminated in the patch reef experiment, *L. pederseni* preferred *C. vaginalis*, regardless of origin sponge. Although natural sponge associations and chemical cue preferences corroborate one another, the patch reef data demonstrated the willingness of shrimp to associate with *A. fistularis*. This sponge never hosted a shrimp naturally and was avoided using chemical cues alone. The association of eight *L. pederseni* individuals with *A. fistularis* in the patch reefs indicates that the secondary metabolites produced by this sponge are not acutely toxic to the shrimp; however, as seen with other sponge dwelling species, these secondary metabolites could have chronic effects (Henkel and Pawlik 2011). The growth rate of the brittle star, *Ophiothrix lineata*, was significantly reduced when experimentally forced to reside in a sponge species that produces secondary metabolites rather than in a preferred non-defended sponge (Henkel & Pawlik 2011). The selection of *A. fistularis* by peppermint shrimp in the patch reef also supports the idea that sponge morphology
has a greater influence than sponge chemistry on short-term habitat selection. *Aplysina fistularis* had the highest height to diameter ratio of all tube sponges on the transects, potentially making this species a beneficial choice for a shrimp looking to quickly escape from predators. The absence of *L. pedersenii* in *A. fistularis* sponges in nature suggests that despite beneficial morphology, the secondary metabolites produced by this sponge may have long-term consequences.

In conclusion, *L. pedersenii* utilize morphology as an effective habitat selection tool and also show distinct chemical preferences for specific hosts. Habitat selection in adults is understudied compared to habitat selection in larvae but is no less important as movements between distinct habitats can influence material transport, nutrient fluxes and community dynamics (Marnane 2000). Understanding how and why adult habitat specialists choose their particular habitat is important in today’s rapidly changing world. Habitat degradation is occurring at an unprecedented rate, especially in reef environments (De’ath et al. 2012). Habitat specialists may be more threatened by environmental change when they are associated with a living host because the host can also be impacted by changing conditions (Munday 2004). Since habitat specialists can drive biodiversity (Sale 1977), an understanding of the mechanisms behind habitat selection can inform research and management practices.

### 3.6 Acknowledgements

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REFERENCES


Chapter 4

CORAL MORPHOLOGY IS LINKED TO INCREASED ABUNDANCE AND FITNESS OF OBLIGATE CORAL-DWELLING SHRIMP, CORALLIOCARIS GRAMINEA.

4.1 Abstract

Branching corals act as hosts to a wide variety of epibionts, many of which are obligate associates that depend on corals for survival. Appropriate hosts can increase the fitness of inhabitants through greater access to resources. However, the suitability of a host can vary based on species and morphological characteristics. This study investigates the influence of coral species and morphology on the fitness and abundance of the obligate coral-dwelling shrimp, Coralliocaris graminea. Transects were conducted at two locations in the Fijian Islands and morphological parameters were recorded from each corymbose or digitate acroporid coral intersected. Resident C. graminea were removed from the corals to assess body size, claw size, and fecundity. Shrimp associated with 7 species of Acropora corals, and the distribution of shrimp within these corals was not random. Coral morphology was a strong indicator of shrimp association; C. graminea tended to inhabit corals with larger diameters and higher branch heights. Fitness of C. graminea, quantified by body size, claw size, and fecundity, was higher for those inhabiting larger, deeper corals and corals that hosted conspecifics. Movement of C. graminea between corals has not been documented but must be occurring, evidenced by the presence of gravid females residing in corals without males present. This study suggests that coral preference of C. graminea is not species specific and coral morphology may have a greater influence on shrimp fitness.
and abundance; therefore, these obligate coral inhabitants may survive on structurally similar, non-host corals. As shrimp abundance and fitness increased with coral head diameter, conservation efforts should aim to increase individual colony size along with overall coral coverage.

4.2 Introduction

Coral reefs have declined rapidly in the last few decades in both the Caribbean (Hughes and Tanner 2000) and the Indo-Pacific (Bruno and Selig 2007; Hughes et al. 2018). This deterioration is characterized by reduced coral coverage (Gardner et al. 2003; Bruno and Selig 2007) and phase shifts (Hughes et al. 2007), which lead to dwindling fish abundance and diversity (Jones et al. 2004), local extinctions (Munday et al. 2004), and decreased resilience (Anthony et al. 2011). Differences in life history, evolutionary experiences, and genetic diversity have resulted in variable tolerance to stress among corals species (Hughes et al. 2003; Pandolfi et al. 2011). This tolerance variation may allow coral reefs as an ecosystem to withstand environmental change, but sensitive species, as well as coral obligate species, will face the threat of extinction. For example, the vital reef-building genus, Acropora, is among the most sensitive to environmental change (Loya et al. 2001; Comeau et al. 2014) and has already experienced massive declines worldwide (Williams and Miller 2012). The loss of high-complexity corals, such as Acropora spp., is especially devastating as they provide structure and substrate for numerous organisms (Jones et al. 2004; Wild et al. 2011).

Large, structurally complex corals host numerous obligate associates that depend on the coral for survival and are invariably found in association with their hosts (Stella et al. 2010). These obligate inhabitants acquire food resources while in
their habitat and these resources are allocated to various functions including tissue maintenance, reproduction, defense, and storage (Cronin 2001). In a resource-limited environment, acquired energy must be distributed between these functions in a way that provides the best chance of survival. However, when resources abound, more energy can be allocated to more functions. The availability of resources can be highly correlated to habitat (MacArthur and Levins 1964), especially for obligate inhabitants (Munday 2004). A suitable habitat will allow residents to acquire more resources, thereby increasing individual growth, fecundity, and overall fitness.

The relationship between coral morphology and epibiont fitness is investigated using the obligate coral-dwelling shrimp, *Coralliocaris graminea*. This cryptic snapping shrimp is found within numerous species of corymbose (bushy) or digitate corals throughout the Indo-Pacific (Bruce 1998). While these shrimp typically associate exclusively with acroporid corals, rare individuals have been found within *Stylophora*, *Seriatopora* and *Pocillopora* (Miyake 1968; Bruce 1972, 1977). Shrimp within these alternative genera were characterized as small juveniles, indicating that these corals may not be ideal habitat but rather a transitional host (Bruce 1977). Among the acroporids, *C. graminea* has been observed associating with at least 18 species (Miyaki 1968; Bruce 1972, 1976, 1977; Patton 1994; Stella et al. 2010; Vytopil and Willis 2011).

This study seeks to understand the influence of coral species and morphology on the abundance and condition of *C. graminea*. Using extensive transects at two Fijian locations, the percent occupancy of the focal shrimp within *Acropora* corals was quantified. Body size, claw size and fecundity were used as proxies for general fitness of the individuals. Studies involving *C. graminea* are rare in established literature and
none to date focus explicitly on the habitat preferences and associated fitness of this cryptic species. Declining reefs impact habitat specialized organisms disproportionately; therefore, investigating the full impact of coral coverage and morphology on the habitat associations of selective organisms can inform our understanding of future reef ecosystems.

4.3 Methods

Forty-five 25 m transects assessing shrimp-coral associations were conducted along the reefs of Tavewa and Kadavu Islands, Fiji. Twenty transects were run on the southern coast of Kadavu Island (19° 2'46.53"S, 178°23'57.75"E) from 17-24 October 2016; an additional twenty-five transects were conducted on the eastern shore of Tavewa Island (16°55'23.32"S, 177°22'1.40"E) from 13-17 July 2017 and 24 September 2018. In addition to transects, in order to increase the power of the analysis, corals throughout the uninspected reef were haphazardly sampled and searched for presence of *C. graminea* and only corals that housed the focal shrimp were recorded.

At each corymbose or digitate *Acropora* coral intersected by the transect (n=462), coral parameters were recorded, including: 1) coral species, 2) diameter, measured at the widest point, 3) height of branches, measured from the deepest point, and 4) the number of resident *C. graminea*. In addition, each coral was photographed from above to measure distance between branch tips (inter-brachial width). Mean inter-brachial spacing was analyzed using ImageJ software by averaging the distance (mm) between two adjacent axial tips from five haphazardly selected points per coral head.
*C. graminea* observed within *Acropora* corals at Tavewa Island were extracted from their host corals using a clove oil, ethanol mixture (Javahery et al. 2012). Shrimp (n=69) were euthanized and preserved in 4% formaldehyde before being transferred to a 60% ethanol solution. Eggs were extracted from fecund shrimp (n=35) by carefully massaging the pleopod area below the abdomen with blunt forceps and counted under a dissection microscope (Stella et al. 2011). To assess impact of coral morphology on shrimp size, *C. graminea* total length (TL), standard length (SL), claw length and width, and carapace width were measured from images analyzed in ImageJ (Schindelin et al. 2012) (Figure 4.1). Additionally, observations of community ecology were recorded including sex of collected shrimp, sex ratios of populated corals, and rate of limb loss. Claw dimensions were only recorded for the largest fully-grown claw because numerous shrimp were experiencing various stages of claw regeneration in at least one claw.
Figure 4.1: Focal shrimp, Coralliocaris graminea, depicting body (Standard length (SL), Total length (TL), Carapace width) and claw (length, width) measurements. Inlay: Posterior ventral view of gravid female C. graminea.
4.4 Results

Fifteen species of corymbose or digitate Acropora corals were identified during transects; C. graminea were associated with seven of these species: Acropora divaricata, A. gemmifera, A. humilis, A. millepora, A. nasuta, A. tenuis and A. valida, henceforth known as preferred corals. Of the preferred corals, the majority of shrimp were associated with A. nasuta (57.5%), A. tenuis (15%), and A. millepora (14.2%); however, these species were among the most common along the transects. Therefore, coral species abundance was incorporated into the expected frequencies of a \( \chi^2 \) goodness-of-fit test (R, R Core Team 2017) to assess the role of coral species on shrimp abundance (Table 4.1). Observed shrimp-coral associations deviated significantly from what would be expected from a random distribution of shrimp \( (\chi^2=32.442, \ p<0.001) \). An assessment of standardized residuals indicated that the abundance of shrimp within A. humilis, A. nasuta, A. tenuis, and A. valida is higher than expected and the abundance within A. divericata, A. gemmifera, and A. millepora is lower than expected (Table 4.1).
Table 4.1: Observed and Expected shrimp abundance by coral species used in the \( \chi^2 \) goodness-of-fit test (\( \chi^2=32.442, p<0.001 \)). The abundance of each coral species along the transect (column 2) was incorporated into the expected frequency of shrimp associations. Standardized Residuals (Std. Res.) indicate the extent and direction of deviation from expected values.

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</tr>
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<td>A. nasuta</td>
<td>216</td>
<td>69</td>
<td>61.86</td>
<td>0.91</td>
</tr>
<tr>
<td>A. tenuis</td>
<td>35</td>
<td>18</td>
<td>10.02</td>
<td>2.52</td>
</tr>
<tr>
<td>A. valida</td>
<td>5</td>
<td>4</td>
<td>1.43</td>
<td>2.15</td>
</tr>
</tbody>
</table>

Shrimp associations were not only impacted by coral species, but also by coral morphologies. A comparison of preferred corals with and without shrimp occupants indicated that shrimp resided in above average (\( \bar{x} = 24.5 \text{ cm} \)) coral head diameters (Wilcoxon signed rank test for non-normal data, \( p<0.001 \), JMP Pro 13) and above average (\( \bar{x} = 6.4 \text{ cm} \)) branch heights (\( p=0.021 \)), but inter-brachial spacing was not a relevant factor in likelihood of shrimp association (Figure 4.2, \( n=420 \)). A negative binomial generalized linear model (GLM) was created in R to assess how coral morphology influenced quantity of shrimp within preferred corals. This model was chosen because the data consisted of counts and had an abundance of zeros. Within this model, coral head diameter was the only morphological characteristic that significantly influenced quantity of shrimp within preferred corals (Table 4.2), with shrimp quantity increasing with coral diameter.
Figure 4.2: Habitation of *C. graminea* within *Acropora* corals is associated with (A) coral head diameter and (B) coral branch height, but not with (C) interbranchial spacing. Dark grey bars represent corals without resident *C. graminea* while light grey bars represent corals with shrimp inhabitants. Wilcoxon signed rank test, $\alpha=0.05$, * indicates significance.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Estimate</th>
<th>SE</th>
<th>z value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diameter</td>
<td>0.058</td>
<td>0.015</td>
<td>3.883</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Height</td>
<td>0.002</td>
<td>0.098</td>
<td>0.023</td>
<td>0.981</td>
</tr>
<tr>
<td>Inter-brachial spacing</td>
<td>-0.046</td>
<td>0.486</td>
<td>-0.950</td>
<td>0.342</td>
</tr>
</tbody>
</table>

In addition to shrimp abundance within corals of varying species and morphologies, *C. graminea* were collected for analyses of fitness and community structure. In all, 44 females, 18 males and 7 juveniles were collected from corymbose or digitate corals. The sex ratio of shrimp within corals was diverse (Figure 4.3), with solitary females (n=6) and solitary males (n=7) occurring frequently. Mixed sexes of one male and one or multiple females and juveniles were found on 10 corals. Multiple
females often occupied a single coral colony (n=4); however, multiple males were never found on the same coral. Juveniles whose sex could not be accurately determined were found in isolation or with other males or females. Gravid females were found in corals hosting mixed sexes, solitary females, and multiple females.

Figure 4.3: Sex ratios of *C. graminea* within *Acropora* corals. 69 shrimp were collected from 31 coral individuals: 44 females, 18 males, and 7 juveniles.
Male *C. graminea* exhibited smaller SL (t-test in JMP, *p*=0.015), TL (*p*=0.019), and carapace width (*p*=0.047) but comparable claw sizes to females (Figure 4.4). In contrast, the ratio of claw length to width was significantly smaller in males (t-test, *p*<0.001). Four shrimp (3 females, 1 juvenile) were missing both claws; 5.5% of males, 22.7% of females, and 57.1% of juveniles were missing one claw. Among shrimp that possessed both claws, the size difference between the two claws was much more pronounced in females than in males (t-test, *p*<0.001). Juveniles were not included in this analysis as only three individuals possessed both claws.

![Figure 4.4](image.png)

**Figure 4.4:** Sexual dimorphism between male and female *C. graminea*. Males are represented by the dark grey bars while females are represented by the light grey bars. t-test, \(\alpha=0.05\), * indicates significance.
Shrimp fitness was assessed through body size (TL, SL, carapace width), defensive strategies (claw length, width, L/W ratio), and fecundity (egg count). Only adult shrimp that could be sexed were used in this analysis to control for life stage (n=62). Additionally, two shrimp hosted the isopod parasite, *Neophryxus globicaudatus* (Bruce 1973, Bruce 2007), beneath their anterior abdominal segment and were removed from the fecundity analysis, as this parasite may influence egg retention. Body length (SL, TL) was log transformed to normalize the data.

A multiple linear regression model was constructed to assess the influence of coral head diameter and branch height on shrimp body characteristics. Since male *C. graminea* were smaller than females, sex was added as a categorical predictor into the model. Only significant interactive effects were left in the model. Graphical linear regressions are depicted in the supplementary figures listed in Appendix A. Coral diameter, branch height, and sex were all significant predictors of body size (SL, TL and carapace width) (Table 4.3, Figure A.1). The interaction between sex and coral diameter significantly influenced SL and TL, but not carapace width. Inter-brachial spacing was not recorded for 36 corals, so these data were analyzed in a separate regression model, revealing that both inter-brachial spacing and sex were influential factors of shrimp body size (Table 4.4). Additionally, the interaction between inter-brachial spacing and sex was a significant predictor of all three characteristics of body size.
Table 4.3: Multiple linear regression results to predict body size characteristics (TL, SL, carapace width). Diameter, height and sex were used as predictors in the model. The only significant interaction effect was between coral diameter and shrimp sex. Regression graphs are depicted in Appendix A. α=0.05, * indicates significance.

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Body Size Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total Length</td>
</tr>
<tr>
<td>Diameter</td>
<td>( F_{1,62}=13.92, p&lt;0.001^* )</td>
</tr>
<tr>
<td>Height</td>
<td>( F_{1,62}=5.94, p=0.018^* )</td>
</tr>
<tr>
<td>Sex</td>
<td>( F_{2,62}=24.74, p&lt;0.001^* )</td>
</tr>
<tr>
<td>Diameter:Sex</td>
<td>( F_{2,62}=4.08, p=0.022^* )</td>
</tr>
</tbody>
</table>

Table 4.4: Multiple regression analysis to determine body size characteristics (TL, SL, carapace width), using inter-brachial spacing and sex as predictors. The interaction between sex and inter-brachial spacing was significant and remained in the model. α=0.05, * indicates significance.

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Body Size Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total Length</td>
</tr>
<tr>
<td>Inter-brachial spacing</td>
<td>( F_{1,31}=7.45, p=0.010^* )</td>
</tr>
<tr>
<td>Sex</td>
<td>( F_{1,31}=27.51, p&lt;0.001^* )</td>
</tr>
<tr>
<td>Inter-brachial spacing:Sex</td>
<td>( F_{2,31}=4.61, p&lt;0.018^* )</td>
</tr>
</tbody>
</table>

Influence of coral morphology on claw size (length and width) was also assessed through a multiple linear regression. Coral diameter was an influential factor for mature claw length and width, but coral branch height only impacted claw length (Table 4.5, Figure A2). Sex was a significant factor for both measurements of claw size.
size, and the interaction between branch height and sex was relevant for claw width measurements. Claw L/W ratio was not influenced by coral diameter or height; however, sex and the interaction term were significant predictors. Inter-brachial spacing was not a significant factor in any claw characteristics (Table 4.6).

Table 4.5: Multiple linear regression results to predict shrimp claw dimensions (length, width, L/W ratio). Diameter, height and sex were used as predictors in the model. The only significant interaction effect was between coral diameter and shrimp sex. Regression graphs are depicted in Appendix A. α=0.05, * indicates significance.

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Claw Size Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Claw Length</td>
</tr>
<tr>
<td>Diameter</td>
<td>F₁,58=18.93, p&lt;0.001*</td>
</tr>
<tr>
<td>Height</td>
<td>F₁,58=5.70, p=0.020*</td>
</tr>
<tr>
<td>Sex</td>
<td>F₂,58=20.56, p&lt;0.001*</td>
</tr>
<tr>
<td>Diameter:Sex</td>
<td>F₂,58=2.19, p=0.121</td>
</tr>
</tbody>
</table>

Table 4.6: Multiple regression analysis to determine shrimp claw dimensions (length, width, L/W ratio), using inter-brachial spacing and sex as predictors. The interaction term was not significant and was thus removed from the model. α=0.05, * indicates significance.

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Claw Size Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Claw Length</td>
</tr>
<tr>
<td>Inter-brachial spacing</td>
<td>F₁,32=1.77, p=0.193</td>
</tr>
<tr>
<td>Sex</td>
<td>F₁,32=13.97, p&lt;0.001*</td>
</tr>
</tbody>
</table>
Fecundity, measured through egg counts of gravid shrimp, was analyzed using a multiple linear regression with height and diameter as predictor variables. The interaction term was not significant and was therefore removed. Coral diameter significantly influenced fecundity ($F_{1,31}=5.08$, $p=0.031$), but branch height did not ($F_{1,31}=1.42$, $p=0.243$) (Appendix A). Additionally, inter-brachial spacing was a significant predictor of fecundity; however, the n-value of observations of fecund shrimp within corals with recordings of inter-brachial spacing was low ($n=13$), thus the power of the test was minimal. Egg quantity was positively correlated with shrimp SL, carapace width, and claw length, but not influenced by TL, claw width or claw L/W ratio (Table 4.7). Finally, shrimp morphometric measurements were correlated with the community of conspecifics within the coral hosts. Carapace width, and claw length and width were positively correlated with the number of resident *C. graminea* within the coral (Table 4.8) After Bonferroni adjustment, fecundity, SL, TL and claw L/W ratio were not significantly impacted by quantity of shrimp ($\alpha=0.007$).
Table 4.7: Multiple regression analysis to determine influence of body (TL, SL, carapace width) and claw (length, width, ratio) dimensions on shrimp fecundity measured by egg count. Body size predictors and claw size predictors were analyzed in two separate models. No interaction terms were significant were thus removed from the model. $\alpha=0.05$, * indicates significance.

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Fecundity Parameter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Egg count</td>
</tr>
<tr>
<td>Body size</td>
<td></td>
</tr>
<tr>
<td>Total Length</td>
<td>$F_{1,30}=2.66$, $p=0.113$</td>
</tr>
<tr>
<td>Standard Length</td>
<td>$F_{1,30}=75.91$, $p&lt;0.001^*$</td>
</tr>
<tr>
<td>Carapace Width</td>
<td>$F_{1,30}=11.94$, $p=0.002^*$</td>
</tr>
<tr>
<td>Claw size</td>
<td></td>
</tr>
<tr>
<td>Claw Length</td>
<td>$F_{1,28}=43.58$, $p&lt;0.001^*$</td>
</tr>
<tr>
<td>Claw Width</td>
<td>$F_{1,28}=1.78$, $p=0.193$</td>
</tr>
<tr>
<td>Claw L/W Ratio</td>
<td>$F_{1,28}=0.60$, $p=0.444$</td>
</tr>
</tbody>
</table>

Table 4.8: Simple linear regressions to determine correlation between *C. graminea* fitness (body size, claw size, fecundity) and the number of resident shrimp within each coral colony. $\alpha=0.007$ after Bonferroni correction, * indicates significance.

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Body size parameters</th>
<th>Claw size parameters</th>
<th>Fecundity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Standard Length</td>
<td>Total Length</td>
<td>Carapace Width</td>
</tr>
<tr>
<td># of shrimp inhabitants</td>
<td>$F_{1,50}=6.8$, $p=0.012$</td>
<td>$F_{1,60}=7.3$, $p=0.009$</td>
<td>$F_{1,60}=16.3$, $p&lt;0.001^*$</td>
</tr>
</tbody>
</table>
4.5 Discussion

Obligate epibionts have evolved to associate within specific habitats that maximize survival. This specificity is not limited only to the coral species but also to intraspecific variations in coral morphological characteristics. The association between shrimp fitness and host morphology can reveal the ecological significance of these associations, especially during changing conditions. *Coralliocaris graminea* distributions among corymbose or digitate corals was not random and was impacted by both coral species and morphologies. Additionally, shrimp fitness was mainly impacted by coral head size, branch height and number of resident conspecifics.

Although the majority of *C. graminea* were found within *A. nasuta*, *A. millepora*, and *A. tenuis* corals, these associations were not random once coral abundance was incorporated into the expected values. Shrimp abundances were higher than expected within *A. humilis*, *A. nasuta*, *A. tenuis*, and *A. valida* corals. While *C. graminea* were found within 7 acroporids in this study, and at least 18 acroporids in other studies, (Miyaki 1968; Bruce 1972, 1976, 1977; Patton 1994; Stella et al. 2010; Vytopil and Willis 2011), they may preferentially select for *A. humilis*, *A. nasuta*, *A. tenuis*, and *A. valida*. This preference may be morphologically driven as each of these corals, except *A. tenuis*, exhibited above average coral diameters, branch heights, and inter-brachial spacing.

Coral colony size was positively correlated with abundance of *C. graminea*, a pattern that is mirrored in several other invertebrate coral inhabitants (Abele 1976, Abele and Patton 1976, Coles 1980, Enochs 2012). Larger corals host bigger and fitter populations of *C. graminea* – potentially due to greater access to food. While the precise diet of *C. graminea* is unknown, they have the appendages required for suspension feeding (Bruce 1976) and have been observed ingesting zooplankton.
A larger coral would increase food availability by allowing more channels through which water, laden with consumable particles and plankton, can flow. Alternatively, the larger coral area would increase the amount of coral tissue and mucus, as well as provide additional habitat for small organisms such as amphipods. Both coral tissue and small invertebrates may provide additional food resources to *Coralliocaris graminea*, if suspension feeding is not its sole source of nutrition.

The increase in complexity associated with branch height and inter-brachial spacing may provide better protection from predators. *Coralliocaris graminea* that have been removed from their coral habitat are quickly consumed by reef fish such as *Thalassoma* sp., and Bruce (1972) postulated that this may be due to their bright coloration. Even within the coral matrix, these shrimp face predation threat and can be targeted by crabs, stomatopods, and small fish (Bruce 1972). However, *C. graminea* are able to coexist with the other commensal and symbiotic organisms that reside within the branches and incidence of cohabitation is high (pers. obs.). Moreover, an analysis of the gut content of *Trapezia* crabs, one of the few epibionts large enough to consume *C. graminea*, reveals that these crabs subsist on a diet of coral mucus, not shrimp (Castro 1976).

Despite the protection that the coral matrix offers, *C. graminea* still face predation. The frequency of missing or partially regenerated claws is high, which is likely due to predation attempts (Bruce 1972) but could also be a result of competition or mating. Unlike most true snapping shrimp, which have one large claw (Johnson 1947), both claws of *C. graminea* are modified to produce high-velocity “punches” for protection. These shrimp also use both claws to grasp the coral branches such that they are oriented facedown inside the coral (Patton 1966). Large claw size increased with
coral diameter and branch height, suggesting that high rates of resource acquisition allowed for the investment in robust defensive structures. *Coralliocaris gramminea* have evolved to have two large claws, therefore size discrepancies between the large and small claws likely reflect recent limb loss and subsequent regeneration. This study determined that females not only displayed a larger size discrepancy between claw sizes, but also showed higher rates of single or double limb loss, potentially indicative of either slower rates of recovery or more frequent limb loss in females. Whether limb loss is a function of predation or competition remains to be determined.

Females were more common than males, and the sex ratio within individual coral colonies varied from solitary males and females to mixed sexes of one male and multiple females. Gravid shrimp were not only located in corals with both males and females, but also in corals hosting solitary or multiple females only. Movement of males between colonies may be occurring, although the life history and reproductive ecology of these cryptic shrimp remain unknown.

As corals continue to decline and die, these obligate shrimp may be forced to relocate to healthy hosts or even non-host species, although movement of *C. graminea* between corals has not yet been documented. In an investigation of the frequency of obligate coral hosts associating with dead corals, Head et al. (2015) observed only one *C. graminea* residing within a dead *Acropora*. The current study indicates that these specialized shrimp can inhabit several species of structurally-similar *Acropora* corals, yet specific coral morphologies can significantly influence shrimp abundance and fitness. Therefore, in a system barren of acroporids, these obligate coral associates may be able to survive on non-host corals, provided that the alternate hosts possess similar corymbose or digitate morphologies. Most significantly, shrimp abundance and
fitness increased with coral head diameter, highlighting the need for conservation efforts aimed at increasing individual colony size along with overall coral coverage.

4.6 Acknowledgements

I would like to thank P. Leingang and A. Good for their assistance with data collection and D Miller for statistical support. I would also like to thank the members of the Dixson Lab for editorial comments as well as the staff of Coralview Island Resort and Matava Resort for logistical support.
REFERENCES


Chapter 5

CONCLUDING REMARKS

Through an analysis of coral larvae and habitat-specialized shrimp, I have investigated critical aspects of habitat selection in coral reef invertebrates. Habitat selection is vital at many points throughout an organism’s life and can be mediated by numerous environmental cues, depending on the sensory capabilities and ecological requirements of the species. Regardless of timing or scope, selection of habitat can have pervasive impacts on organismal fitness.

Caribbean scleractinian coral larvae are invariably attracted to general reef cues throughout their ontogeny but reveal diverse behaviors regardless of cue. Observations of minute behaviors revealed that directed movement and small circles might be indicative of positive benthic cues. Appropriate habitat selection is essential for coral larvae because corals only have a single opportunity to make the optimal decision before becoming permanently sessile after metamorphosis (Hadfield 1986). The significance of this decision is accentuated by the unmitigated importance of corals as a foundational species in the reef ecosystem (Graham and Nash 2013). Successful recruitment of coral larvae is vital to reef recovery (Dixson et al. 2014).

Habitat selection is not limited to the larval stage of reef invertebrates but can also be relevant for homing adults. Peppermint shrimp, Lysmata pederseni, rely on both chemical and morphological cues to select appropriate tube sponges after nightly forays on the reef. Invertebrates that rely on other living organisms for habitat are at a heightened vulnerability, especially during periods of environmental change. Not only
can specialists be directly threatened by change, but they can also be indirectly affected if their host cannot tolerate the stress (Wilson et al. 2008). The appropriateness and health of a host can impact the fitness of inhabitants, as investigated in the shrimp, Coralliocaris graminea. As morphology is potentially more relevant than habitat species in maintaining fitness, the potential exists for these habitat specialists to adapt to environmental change by transferring to hardier, yet morphological similar, non-host corals.

Coral reefs are declining at an unprecedented rate due to local and global stressors (Knowlton and Jackson 2008). While the potential remains for local stressors to be mitigated by community efforts (Cinner et al. 2005; Hamilton et al. 2011), global stressors, such as climate change and ocean acidification, will continue to exacerbate the deterioration (Hoegh-Guldberg et al. 2007). Projected reductions in ocean pH can have devastating impacts on the sensory biology of marine organisms, sometimes completely reversing ecological behaviors critical for survival (Ashur et al. 2017; Appendix B). All of the behaviors discussed in the previous chapters have the potential to be influenced by this change in ocean chemistry. Continuing to explore the influences of environmental stimuli on habitat selection in reef invertebrates is an important foundational step in coral reef ecology. From there, we can advance our knowledge of how these behaviors might be affected by changing conditions so that we can aim to effectively protect the vital coral reef ecosystem.
REFERENCES


Figure A.1: Linear regression graphs displaying relationship between body size characteristics (TL, SL, carapace width; y-axis) and coral morphology (diameter, height; x-axis). Female shrimp are depicted with red markers while males are depicted with blue markers. Full statistical outputs are described in Figure 4.3.
Figure A.2: Linear regression graphs displaying relationship between claw size characteristics (length, width, y-axis) and coral morphology (diameter, height; x-axis). Female shrimp are depicted with red markers while males are depicted with blue markers. Full statistical outputs are described in Figure 4.5.
Figure A.3: Linear regression graphs displaying relationship between fecundity (number of eggs) and coral morphology (diameter, height; x-axis). Full statistical outputs are described in Figure 4.5.
Appendix B

IMPACTS OF OCEAN ACIDIFICATION ON SENSORY PERCEPTION: UNDERLYING MECHANISMS AND TAXA COMPARISONS

This appendix has been published in the journal Integrative and Comparative Biology.
Co-Authors: Nicole K. Johnston\textsuperscript{1}, Danielle L. Dixson\textsuperscript{1,2}

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B.1 Introduction

The global decline of oceanic ecosystems has been attributed to various natural and anthropogenic stressors, with ocean acidification identified as a major threat to marine life. Ocean acidification is caused by the influx of atmospheric CO\textsubscript{2} into the oceans, resulting in an increase in pCO\textsubscript{2} and reduction of surface water pH (Doney et al. 2009). This change in acidity has significant impacts on the physiology (Fabry et al. 2008), ontogeny (Byrne 2011), behavior (Clements and Hunt 2015; Nagelkerken and Munday 2016) and survivorship (Kroeker et al. 2010) of marine organisms, which can escalate to the ecosystem level. Recently, numerous studies covering a myriad of taxa have observed an impairment of organismal sensory perception when exposed to ocean acidification conditions (Munday et al. 2009; Dixson et al. 2010).
Sensory stimuli allow organisms to perceive their external environment. Perception of chemical, auditory, and visual cues plays an integral role in the daily life and survival of marine organisms by influencing homing (Simpson et al. 2005; Dixson et al. 2008), settlement (Pawlik et al. 1992), predator detection and evasion (Lima and Dill 1990), foraging (Tomba et al. 2001), conspecific social interactions (Cole and Shapiro 1995) and mating (Thomas 2011). Additional senses - including the lateral line system, electrosense in elasmobranchs and the ability to detect magnetic fields - offer unique conduits of perceiving the world to specialized marine species.

A critical distinction exists when describing sensory ecology that becomes a challenge when designing experiments – sensory input versus central processing. *Sensory input* is the process of transmitting impulses from sensory organs to nerve centers at which point *central processing* converts the stimuli into behavior (The American Heritage Medical Dictionary 2007). Studies that strictly examine sensory input accurately reveal the proximate causes of the affected behaviors. However, the majority of studies assessing sensory perception under ocean acidification use observable behaviors as a proxy for sensory perception, yet often fail to distinguish whether the behavioral differences are a result of distorted sensory input or altered central processing, or both. In this study, we attempt to characterize relevant studies based on the true message each experiment reveals.

At the organismal scale, ocean acidification directly affects the perception of a suite of sensory systems, including chemosensory (Munday et al 2009), vision (Ferrari et al. 2012a) and sound (Simpson et al. 2011) and additional daily cognitive functions including activity level and boldness (Munday et al. 2010), lateralization (Domenici et al. 2011) and learning (Ferrari et al. 2012b). These disturbances have the potential to
The ability to detect chemical cues is the primary sense used by most organisms across different environments and taxa (Ache and Young 2005). Recognition of chemical cues is initiated by the binding of a chemical molecule to a receptor protein on a primary chemoreceptor neuron. In all animals, this binding initiates a G protein-based signaling cascade that transmits a signal to the central nervous system (Buck and Axel 1991). While the physiology of chemosensory perception is relatively conserved across species, the morphology of chemoreception organs can vary greatly; crustaceans use aesthetascs on their antennules to “sniff” their environment (Schmitt et al. 1979) and fish and cephalopods use specialized olfactory organs exposed to chemical cues through nasal cavities or olfactory pits (Kasumyan
It is still debated whether the reception of chemical signals is through gustation or olfaction (Hara 1994). Therefore in the context of this review, the term chemosensory perception is used when referring to either sense.

Chemosensory perception and communication are critical in the marine environment and are relevant at all spatial scales (Hay 2009). The ability to accurately detect and respond to environmental chemical cues is critical for homing (Atema et al. 2002; Dixson et al. 2014), settlement (Lecchini et al. 2005), foraging (Tomba et al. 2001) and predator evasion (Lima and Dill 1990). The highly conserved nature of chemosensory perception has led to numerous studies investigating the effect of ocean acidification on this essential sense across many taxa. Chemosensory perception and associated behavioral responses are influenced by elevated CO$_2$ (Table A.1), although the physiological or biochemical origin of these changes varies among taxa.
Table B.1: Synopsis of research studies evaluating the influence of ocean acidification on chemosensory perception. The table highlights focal species, experimental CO$_2$ levels, control CO$_2$ levels, sensitivity at which an effect was observed and methodology. NH = Newly Hatched, * indicates species was treated with high CO$_2$ starting at birth/hatching.

<table>
<thead>
<tr>
<th>Study</th>
<th>Species</th>
<th>CO$_2$ (pCO$_2$)</th>
<th>Control (pCO$_2$)</th>
<th>Sensitivity (pCO$_2$)</th>
<th>CO$_2$ Exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Munday et al. 2009</td>
<td><em>Amphiprion percula</em></td>
<td>1000, 1700 ppm</td>
<td>380 ppm</td>
<td>1000 ppm</td>
<td>11d; settlement*</td>
</tr>
<tr>
<td>Albright et al. 2010</td>
<td><em>Acropora palmata</em></td>
<td>560,800 μatm</td>
<td>400 μatm</td>
<td>560 μatm</td>
<td>6d; settlement</td>
</tr>
<tr>
<td>Dixson et al. 2010</td>
<td><em>Amphiprion percula</em></td>
<td>1000 ppm</td>
<td>380 ppm</td>
<td>1000 ppm</td>
<td>24h, NH, 11d, settlement*</td>
</tr>
<tr>
<td>Munday et al. 2010</td>
<td><em>Amphiprion percula, Pomacentrus wardi</em></td>
<td>550, 700, 850 ppm</td>
<td>390 ppm</td>
<td>700 ppm</td>
<td>1-10d*; 1-4d; settlement</td>
</tr>
<tr>
<td>Albright and Langdon 2011</td>
<td><em>Porites astreoides</em></td>
<td>560,800 μatm</td>
<td>380 μatm</td>
<td>560 μatm</td>
<td>2d; settlement*</td>
</tr>
<tr>
<td>Cripps et al. 2011</td>
<td><em>Pseudocromis fuscus</em></td>
<td>600,950 μatm</td>
<td>400 μatm</td>
<td>600 μatm</td>
<td>4-7d; adult</td>
</tr>
<tr>
<td>Ferrari et al. 2011</td>
<td><em>Pomacentrus chrysurus, P. moluccensis, P. amboiensis, P. nagasakiiensis</em></td>
<td>700, 850 ppm</td>
<td>390 ppm</td>
<td>700 ppm</td>
<td>4d; settlement</td>
</tr>
<tr>
<td>de la Haye et al. 2012</td>
<td><em>Pagurus bernhardus</em></td>
<td>pH 6.8</td>
<td>pH 8.20</td>
<td>pH 6.8</td>
<td>5d; adult</td>
</tr>
<tr>
<td>Devine et al. 2012a</td>
<td><em>Pomacentrus amboiensis, P. chrysurus, P. moluccensis</em></td>
<td>700, 850 ppm</td>
<td>440 ppm</td>
<td>700 ppm</td>
<td>4-5d; settlement</td>
</tr>
<tr>
<td>Devine et al. 2012b</td>
<td><em>Cheilodipterus quinquelineatus</em></td>
<td>550, 700, 950 ppm</td>
<td>390 ppm</td>
<td>550 ppm</td>
<td>4d; adult</td>
</tr>
<tr>
<td>Doropoulos et al. 2012</td>
<td><em>Acropora millepora</em></td>
<td>800, 1300 μatm</td>
<td>401 μatm</td>
<td>800 μatm</td>
<td>6d; settlement</td>
</tr>
<tr>
<td>Nilsson et al. 2012</td>
<td><em>Amphiprion percula, Neopomacentrus azysron</em></td>
<td>900 μatm</td>
<td>450 μatm</td>
<td>900 μatm</td>
<td>11d; 18d; settlement</td>
</tr>
<tr>
<td>Chivers et al. 2013</td>
<td><em>Pomacentrus amboiensis</em></td>
<td>987 μatm</td>
<td>440 μatm</td>
<td>987 μatm</td>
<td>4d; juvenile</td>
</tr>
<tr>
<td>Devine and Munday 2013</td>
<td><em>Paragobiodon xanthosomus</em></td>
<td>880 μatm</td>
<td>440 μatm</td>
<td>880 μatm</td>
<td>4d; adult</td>
</tr>
<tr>
<td>Study</td>
<td>Species/Species Group</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>-------------------------------</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Jutfelt and Hedgarde 2013</td>
<td><em>Gadus morhua</em></td>
<td>1170 μatm</td>
<td>550 μatm</td>
<td>No effect</td>
<td>35-41d; juvenile</td>
</tr>
<tr>
<td>Lönnstedt et al. 2013</td>
<td><em>Pomacentrus amboiensis</em></td>
<td>880 μatm</td>
<td>440 μatm</td>
<td>880 μatm</td>
<td>4d; settlement</td>
</tr>
<tr>
<td>Munday et al. 2013</td>
<td><em>Plectropomus leopardus</em></td>
<td>570, 700, 960 μatm</td>
<td>490 μatm</td>
<td>700 μatm</td>
<td>4d, 28 d; juvenile</td>
</tr>
<tr>
<td>Webster et al. 2013</td>
<td><em>Coral larvae/CCA</em></td>
<td>822, 1187, 1638 μatm</td>
<td>464 μatm</td>
<td>822 μatm</td>
<td>CCA: 6 weeks; settlement</td>
</tr>
<tr>
<td>Barry et al. 2014</td>
<td><em>Strongylocentrotus fragilis</em></td>
<td>3255 ppm</td>
<td>1028 ppm</td>
<td>3255 ppm</td>
<td>30d; adult</td>
</tr>
<tr>
<td>Dixon et al. 2014</td>
<td><em>Mustelus canis</em></td>
<td>741, 1064 μatm</td>
<td>405 μatm</td>
<td>1064 μatm</td>
<td>5d; adult</td>
</tr>
<tr>
<td>Manriquez et al. 2014</td>
<td><em>Concholepas concholepas</em></td>
<td>700, 1000 μatm</td>
<td>390 μatm</td>
<td>1000 μatm</td>
<td>5mo; juvenile</td>
</tr>
<tr>
<td>Munday et al. 2014</td>
<td><em>Dascyllus aruanus, Pomacentrus moluccensis, Apogon cyanosoma, Cheilodipterus quinquelineatus</em></td>
<td>441-998 μatm</td>
<td>346-413 μatm</td>
<td>No effect</td>
<td>Entire life; juvenile*</td>
</tr>
<tr>
<td>Welch et al. 2014</td>
<td><em>Acanthochromis polyacanthus</em></td>
<td>656, 912 μatm</td>
<td>446 μatm</td>
<td>656 μatm</td>
<td>40-45d; juvenile*</td>
</tr>
<tr>
<td>Queiros et al. 2015</td>
<td><em>Nucella lapillus</em></td>
<td>750, 1000 μatm</td>
<td>380 μatm</td>
<td>1000 μatm</td>
<td>14mo; adult</td>
</tr>
</tbody>
</table>
Many marine organisms have a dispersive larval stage followed by a relatively sessile adult stage (Caley et al. 1996). Most development occurs in the pelagic environment and the successful return and recruitment of juveniles is vital for species persistence in any marine ecosystem. When in the pelagic environment, larvae primarily utilize chemoreception (Atema et al. 2002) and sound (Simpson et al. 2005) to navigate towards a suitable habitat. For example larval anemonefish, *Amphiprion percula*, use the chemical signatures of island leaf litter to navigate to reef systems supporting their symbiotic host anemones (Dixson et al. 2008). After exposure to future ocean acidification conditions, however, *A. percula* displayed a weaker positive reaction to native trees, indicative of reef habitat, and significant positive response to non-native plants, a cue not correlated to beneficial habitat (Munday et al. 2009). Additionally, larval damselfish (family Pomacentridae) in ambient conditions have specific habitat cue preferences, depending on the species. However, once exposed to lower pH, each species was unable to distinguish between odor cues of different habitat when the chemical cues alone were tested, however, settlement was successful when all cues were present, suggesting sensory compensation (Devine et al. 2012a).

Accurate settlement decisions are especially critical for sessile invertebrates, as habitat choice is permanent once settlement and metamorphosis have occurred. Invertebrate larvae use chemoreception to choose specific habitat locations and settlement can be induced by an extensive array of cues allowing gregarious (i.e. conspecific cues) and associative (i.e. parasitic, herbivorous, microbial cues) settlement (Pawlick 1992). Ocean acidification reduces the percentage of successful settlement of several species of coral, including *Acropora millepora* (Webster et al. 2013; Doropoulos et al. 2012), *A. tenuis* (Webster et al. 2013), *A. palmata* (Albright et
al. 2010), and *Porites astreoides* (Albright and Langdon 2011). Additionally, under elevated CO\(_2\) conditions, *A. millepora* switches preferred substrate preference (Doropoulos et al. 2012). Thus far, no study has tested the effects of ocean acidification on chemoreception in isolation, i.e. within a chemical choice flume so that only chemical cues are assessed; it is therefore unknown if the reduction in settlement success is a function of chemosensory impairment. The reduction in settlement may be related to a change in microbial composition on preferred settlement substrates. The microbial shift may change the chemical signature, resulting in reduced settlement success (Webster et al. 2013).

Habitat selection is also important for other life stages beyond recruits. Chemosensory mediated impairment of habitat selection in adult fish is evidenced by (1) adult gobies, *Paragobiodon xanthosomus*, that after high CO\(_2\) exposure no longer show strong preference for their sole coral host and neglect to relocate to preferred habitat if placed on a dead coral (Devine and Munday 2013) and (2) CO\(_2\)-exposed adult cardinalfish, *Cheilodipterus quinquelineatus*, demonstrate impairment in the ability to navigate back to their resting sites after nocturnal feeding (Devine et al. 2012b). The effect of ocean acidification on the navigational capabilities of adult invertebrates has not been assessed.

Beyond homing and settlement, chemosensory cues are vital for predator detection and evasion. Innate predator recognition is imperative for larvae that recruit to predator-rich habitats such as reefs. Studies on several species of juvenile reef fish (Families: Pomacentridae, Apogoninae, Serranidae) show that under ocean acidification conditions, recognition of predator odor is reversed or the antipredator response behavior is reduced (Dixson et al. 2010; Munday et al. 2010; Munday et al.
Larval fishes preferentially swim towards a predator or no longer show preference away from the threat. In contrast, anemonefish, *A. percula*, when exposed to elevated CO$_2$ conditions only during the egg stage maintained predator avoidance behavior post hatching, suggesting adaptation potential relating to natural variations of pH and CO$_2$ within the reef matrix or the higher levels of CO$_2$ within the egg itself due to embryonic respiration (Dixson et al. 2010). Altered chemosensory perception of predators is not observed in all species of fishes, with juvenile Atlantic cod (*Gadus morhua*) avoiding predator cues even after month-long high CO$_2$ exposure (Jutfelt and Hedgarde 2013).

As for invertebrates, predator avoidance behaviors in two species of Mollusca, including the fast-escape jump response of *Gibbosus gibbosus* (Watson et al. 2014) and the ability of *Concholepas concholepas* to distinguish a predator odor from a neutral cue (Manriquez et al. 2014), were impaired by ocean acidification. The physical escape responses of these gastropods were not affected, i.e. *G. gibbosus* could still jump, however the chemosensory or decision-making capabilities of the organisms were impaired.

Predators are also affected by ocean acidification. Chemosensory tracking or attraction to prey cues is diminished after exposure to elevated CO$_2$ in an adult elasmobranch (smooth dogfish, *Mustelus canis*; Dixson et al. 2015) and a teleost (brown dottyback, *Pseudochromis fuscus*; Cripps et al. 2011). Foraging ability in adult invertebrates appears to be weakened, by elevated CO$_2$ in species of Mollusca (Queirós et al. 2015), Echinodermata (Barry et al. 2014) and Crustacea (de la Haye et al. 2012), although the specific parameters tested are variable among research groups.
In the deep ocean, Barry et al. (2014) documented that deep-sea urchins, *Strongulocentrotus fragilis*, exposed to elevated CO$_2$ experienced significantly longer foraging time, but not a change in speed, indicating difficulty locating the cue rather than locomotor impairment. Conversely, the gastropod, *Nucella lapillus*, did not experience increased foraging time, but the distance covered was significantly greater (Queirós et al. 2015). The hermit crab, *Pagurus bernhardus*, spent significantly less time in contact with the prey cue and experienced reduced antennular flicking (“sniffing”) rates (de la Haye et al. 2012). In contrast, juvenile muricid snails, *Concholepas concholepas*, show no impaired ability to locate prey (Manriquez et al. 2014). Each of these experiments incorporated chemoreception and implied impairment, but chemosensory cues in isolation have yet to be tested.

Some studies have implied that in future predator-prey interactions, both players will be affected by reduced pH, but ocean acidification will have a more deleterious impact on prey than predators. Manriquez et al. (2014) tested *C. concholepas* as both a predator and a prey and found that predator avoidance behavior was reduced by ocean acidification, but prey detection was unaffected. Cripps et al. (2011) found that a mesopredator spent 20% less time in prey cue, which is disadvantageous, but minimal compared to the 80% (check) switch observed in prey species (Dixson et al. 2010). Other factors outside of sensory impairment and behavioral alterations can influence future predator-prey interactions, including growth, shell thickness and strength, strength of offensive weapons and reproduction (Kroeker et al. 2014). After taking into account the effects on both predators and prey, Kroeker et al. (2014) predicts that per capita predation rates of coastal molluscs will increase with ocean acidification. Evolutionarily, it is far more detrimental to have
reduced predator avoidance than prey detection because the worst result is death rather than undernourishment. Therefore, we can hypothesize that selection pressure will be higher for prey species, possibly suggesting evolutionary adaptation. Studies comparing sensory impairment from both the predator and prey perspective are necessary to scale-up the effects of ocean acidification to the ecosystem level.

### B.3 Audition

Audition in the marine environment depends on the definition of sound detection (Budelman 1992). If broadly defined as the ability to detect non-contact vibrational stimuli (Pumphrey 1950), then response to auditory cues has been observed in fish (Popper and Fay 1973), arthropods (Lovell 2005; Derby 1982), cephalopods (Kaifu 2008) and cnidaria (Vermeij 2010). Auditory morphology in fish is similar to that observed in other vertebrates, with the majority of sound detection dependent on the inner ear, which consists of three chambers containing sensitive hair cells (Popper and Fay 1973). When sound waves pass through a fish, the otoliths move within their fluid chambers and press against the hairs, sending impulses to the brain. Marine invertebrates lack inner ears, but can sense vibrational stimuli through other structures, including external sensory hairs in lobsters (Derby 1982), statocysts (gravistatic organs) in shrimp (Lovell et al. 2005) and squid (Kaifu 2008) or external cilia in coral larvae (Vermeij et al. 2010).

Sound production and detection is ecologically important for conspecific communication, foraging, predator detection, and navigation (Myrberg 1997). Ecosystem-specific sounds, both biologic, i.e. fish vocalization or crackle of snapping shrimp (Simpson et al. 2010), and physical, i.e. wave action (Urick 1984), can be
detected from thousands of meters away and can be a reliable source of information for homing fish as sound transmits through water regardless of water movement (Simpson et al. 2005). Auditory cues from the marine environment influence the settlement of crab zoea and megalopae (Jeffs et al. 2003), temperate blennies (Tolimieri et al. 2000), coral larvae (Vermeij et al. 2010) and coral reef fish (Leis et al. 2002, Simpson et al. 2005).

Ocean acidification alters this ability of fish larvae to navigate using acoustic cues, affecting both the timing of settlement (Simpson et al. 2011) and the attraction to preferred habitat cues (Rossi et al. 2015, 2016a) (Table A2). In ambient conditions, settlement-stage fish are attracted to nocturnal reef sounds but repelled by daytime, predator-rich reef sounds (Heenan 2012, Leis 1996). However, when exposed to elevated CO$_2$, larval anemonefish, *A. percula*, experience an attraction to daytime reef noises, which could have implications for survival (Simpson et al. 2011). Similar tests were conducted on temperate mulloway (*Argyrosomus japonicas*) (Rossi et al. 2016a) and the catadromous fish, barramundi (*Lates calcarifer*) (Rossi et al. 2015). In present-day conditions, these fishes are attracted to acoustic habitat cues, but avoid them once treated with lower pH. Not only are auditory capabilities altered in mulloway exposed to ocean acidification, but also the soundscape of the preferred habitat is degraded as a result of a reduced intensity and frequency of snapping shrimp sounds (Rossi et al. 2016b). Therefore, ocean acidification has the potential to directly, through sensory impairment, and indirectly, through soundscape depreciation, affect navigation of settlement-stage fish.
Table B.2: Synopsis of research studies evaluating the influence of ocean acidification on auditory perception and otolith development. The table highlights focal species, experimental CO$_2$ levels, control CO$_2$ levels, sensitivity at which an effect was observed and methodology. NH = Newly Hatched, * indicates species was treated with high CO$_2$ starting at birth/hatching.

<table>
<thead>
<tr>
<th>Study</th>
<th>Species</th>
<th>CO$_2$ (pCO$_2$)</th>
<th>Control (pCO$_2$)</th>
<th>Sensitivity (pCO$_2$)</th>
<th>CO$_2$ Exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Auditory perception</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Simpson et al. 2011</td>
<td><em>Amphiprion percula</em></td>
<td>600, 700, 900 μatm</td>
<td>390 μatm</td>
<td>600 μatm</td>
<td>17-20d; post-settlement*</td>
</tr>
<tr>
<td>Checkley et al. 2009</td>
<td><em>Atractoscion nobilis</em></td>
<td>993, 2558 μatm</td>
<td>380 μatm</td>
<td>933 μatm</td>
<td>7-8d; larvae*</td>
</tr>
<tr>
<td>Franke and Clemmensen 2011</td>
<td><em>Clupea harengus L.</em></td>
<td>1260, 1859, 2626, 2903, 4635μatm</td>
<td>480 μatm</td>
<td>No effect</td>
<td>NH*</td>
</tr>
<tr>
<td>Munday et al. 2011a</td>
<td><em>Amphiprion percula</em></td>
<td>1050, 1712 μatm</td>
<td>404 μatm</td>
<td>1721 μatm</td>
<td>11d; settlement*</td>
</tr>
<tr>
<td>Munday et al. 2011b</td>
<td><em>Acanthochromis polyacanthus</em></td>
<td>600, 725, 850 μatm</td>
<td>450 μatm</td>
<td>No effect</td>
<td>21d; juvenile*</td>
</tr>
<tr>
<td>Bignami et al. 2013a</td>
<td><em>Rachycentron canadum</em></td>
<td>800, 2100, 3500, 5400 μatm</td>
<td>300 μatm</td>
<td>800 μatm</td>
<td>20d; larvae</td>
</tr>
<tr>
<td>Bignami et al. 2013b</td>
<td><em>Rachycentron canadum</em></td>
<td>800, 2100, 3500, 5400 μatm</td>
<td>300, 500 μatm</td>
<td>800 μatm</td>
<td>14,22d; larvae</td>
</tr>
<tr>
<td>Maneja et al. 2013</td>
<td><em>Gadus morhua</em></td>
<td>1800, 4200 μatm</td>
<td>370 μatm</td>
<td>1800 μatm</td>
<td>7-53d; larvae*</td>
</tr>
<tr>
<td>Schade et al. 2014</td>
<td><em>Gasterosteus aculeatus</em></td>
<td>1000 μatm</td>
<td>400 μatm</td>
<td>1000 μatm</td>
<td>30,60,90d; larvae*</td>
</tr>
<tr>
<td>Perry et al. 2015</td>
<td><em>Stenotomus chrysops</em></td>
<td>1726; 1861 μatm</td>
<td>1205 μatm</td>
<td>No effect</td>
<td>8w; adult</td>
</tr>
<tr>
<td>Reveillac et al. 2015</td>
<td><em>Sparus aurata</em></td>
<td>700, 1200, 2000 μatm</td>
<td>475 μatm</td>
<td>700 μatm</td>
<td>40d; juvenile</td>
</tr>
</tbody>
</table>
Impairments to the auditory system in fish suggest that sensory systems with internal receptors are affected by changing ocean chemistry. Whereas olfactory receptors in most organisms are located externally, fish auditory capabilities are dependent on internal structures such as the inner ear and otoliths. A change in shape, size or symmetry of otoliths can be detrimental to organisms (Gaglio et al. 2008). While elevated-CO\textsubscript{2} induced alterations in otolith symmetry have not been observed (Munday et al. 2011a,b; Maneja et al. 2013; Reveillac et al. 2015), an increase in otolith size in fish exposed to elevated CO\textsubscript{2} conditions has been recorded in numerous species (Checkley et al. 2009, Schade et al. 2014, Bignami et al. 2013a,b, Munday et al. 2011a, Hurst et al. 2012, Maneja et al. 2013, Frommel et al. 2013, Reveillac et al. 2015, Rossi et al. 2016) (Table A.2), suggesting a possible mechanism for auditory alteration. In contrast, the otoliths of some species remain unaffected by even high levels of CO\textsubscript{2} (Munday et al. 2011b, Franke and Clemmesen 2011, Simpson et al. 2011, Perry et al. 2015), indicating species-specific sensitivity. An increase in otolith size corresponds to more sensitive hearing and models predict up to a 50% increase in hearing range for CO\textsubscript{2}-treated fish larvae due to enlarged otoliths (Bignami et al. 2013a). An improvement in audio detection could be beneficial for perceiving ecologically important cues, however, could be disadvantageous through increased sensitivity to background noise, thereby obscuring relevant audio information (Bignami et al. 2013a). Further, the benefits or detriments of increased sensitivity could be species dependent. Evolution of benthic organisms has already selected for comparatively large otoliths, thus these species would likely benefit from increased sensitivity (Lychakov and Rebane 2000). However, pelagic fish developed small otoliths and an increase in sound detection may distract or confuse these less sensitive
organisms (Lychakov and Rebane 2000). Typically, the level of CO$_2$ exposure needed to increase otolith size (avg levels) is higher than levels needed to induce altered auditory perception (avg levels). Therefore, behavioral effects will most likely occur sooner than otolith enlargement, lessening the likelihood that otolith enlargement is responsible for altered perception.

B.4 Vision

Vision in marine organisms is remarkably diverse, with visual structures ranging from pigmented photoreceptors in some invertebrates and early chordates to the complex and highly functional camera eyes found in fish and cephalopods (Arendt 2003). Morphologically, there are dozens of different eye types, with almost every form existing in the aquatic environment (Land and Nilsson 2002). In aquatic systems, the ability to focus an image depends on the formation of either pinhole eyes or lenses (Land and Fernald 1992). The physiological basis of vision relies on the photon-activated G-protein phototransduction cascade that is present in nearly every light-sensing organism (Arshavsky et al. 2002). Despite variations among taxa, this cascade is overall conserved.

While auditory and olfactory cues are integral for navigation across large spatial scales, vision becomes critical over a smaller geographic area once an organism has located a specific habitat. Depending on conditions, visual cues can provide the most accurate information about the environment, particularly predation risk (Ferrari et al. 2010). Several studies have indicated deficient response to visual cues and altered retinal functioning under ocean acidification conditions (Table A.3).
Table B.3: Synopsis of research studies evaluating the influence of ocean acidification on visual perception. The table highlights focal species, experimental CO$_2$ levels, control CO$_2$ levels, sensitivity at which an effect was observed, methodology and the specific component of vision that was tested. NH = Newly Hatched, * indicates species was treated with high CO$_2$ starting at birth/hatching.

<table>
<thead>
<tr>
<th>Study</th>
<th>Species</th>
<th>CO$_2$ (pCO$_2$)</th>
<th>Control (pCO$_2$)</th>
<th>Sensitivity (pCO$_2$)</th>
<th>CO$_2$ Exposure</th>
<th>Visual components</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ferrari et al. 2012a</td>
<td><em>Pomacentrus amboiensis</em></td>
<td>550, 700, 850 μatm</td>
<td>440 μatm</td>
<td>850 μatm</td>
<td>4d; settlement</td>
<td>Visual perception</td>
</tr>
<tr>
<td>Ferrari et al. 2012b</td>
<td><em>Pomacentrus amboiensis</em></td>
<td>700, 850 μatm</td>
<td>440 μatm</td>
<td>850 μatm</td>
<td>4d: settlement</td>
<td>Visual social Learning</td>
</tr>
<tr>
<td>Forsgren et al. 2013</td>
<td><em>Gobiusculus flavescens</em></td>
<td>1400 μatm</td>
<td>370 μatm</td>
<td>1400 μatm</td>
<td>9-12d; NH*</td>
<td>Phototaxis</td>
</tr>
<tr>
<td>Lönnstedt et al. 2013</td>
<td><em>Pomacentrus amboiensis</em></td>
<td>880 μatm</td>
<td>440 μatm</td>
<td>880 μatm</td>
<td>4 d; settlement</td>
<td>Vision/Chemosensory</td>
</tr>
<tr>
<td>Chung et al. 2014</td>
<td><em>Acanthochromis polyacanthus</em></td>
<td>944 μatm</td>
<td>466 μatm</td>
<td>944 μatm</td>
<td>6-7d; small</td>
<td>Retinal function</td>
</tr>
<tr>
<td>Spady et al. 2014</td>
<td><em>Idiosepius pygmaeus</em></td>
<td>626, 956 μatm</td>
<td>447 μatm</td>
<td>626 μatm</td>
<td>5d: adult</td>
<td>Vision/Predation threat</td>
</tr>
</tbody>
</table>
The preference of larval fish to swim towards a light source (phototaxis) is essential for feeding and survival (Karlsen and Mangor-Jensen 2001). Under elevated CO₂ conditions, newly-hatched larvae of a temperate goby (*Gobiusculus flavescens*) experienced a 65-70% increase in positive phototaxis, both in terms of proportion of larvae to reach the light source in 2 minutes and the speed of the fastest larvae per trial (Forsgren et al. 2013). Although positive phototaxis is beneficial to larvae, an increase this drastic suggests visual hypersensitivity due to an overload of brain activity, which could be maladaptive (Forsgren et al. 2013).

Anti-predator behavior is crucial to the survival of marine organisms and can include reduced foraging, increased shelter use and “bobbing” signaling behavior when threatened. Appropriate anti-predator behavior can be innate or learned through visual social cues (Kelley and Magurran 2003). When exposed to visual cues from a predator, CO₂-treated juvenile damselfish, *Pomacentrus amboinensis*, displayed weaken anti-predator behavior by continued foraging, lack of bobbing behavior, and normal activity levels (Ferrari et al. 2012b). Moreover, *P. amboinensis* exposed to ocean acidification conditions were unable to socially learn appropriate anti-predator behavior from visual cues from conspecifics (Ferrari et al. 2012a).

Anti-predatory behavior has also been tested in the invertebrate tropical squid, *Idiosepius pygmaeus*. When presented with a visual threat stimulus, squid exposed to elevated CO₂ retained their predator escape ability (no difference in reaction time or distance moved), but the choice of escape behavior shifted to jet propulsion over defensive arm positioning (Spady et al. 2014). While this study shows critical behavioral changes to a visual threat, it suggests a neural alteration rather than sensory impairment.
From the few studies so far conducted, it appears that anti-predatory responses to visual cues are affected but not entirely lost (Ferrari et al. 2012a,b; Spady et al. 2014). In contrast, the response to olfactory cues can be completely reversed (Dixson et al. 2010). Visual perception may provide sensory compensation for the loss or alteration of chemosensory perception (Lonnstedt et al. 2013; Cripps et al. 2011; Devine et al. 2012a).

A possible mechanism for altered behaviors in response to visual cues may be slowed retinal function, characterized by critical clicker fusion (CFF) (Chung et al. 2014). CFF is the threshold at which an animal perceives a flickering light as continuous and is variable depending on lifestyle and environmental illumination (Horodysky et al. 2008; Smolka et al. 2013). The CFF of damselfish, *Acanthochromis polyacanthus*, after exposure to ocean acidification conditions was significantly reduced compared to controls. A decrease in CFF could weaken the ability to react to fast events during foraging and predator avoidance. This decrease in CFF was negated after exposure to the GABA$_A$ antagonist, gabazine, suggesting an underlying influence of neurotransmitter overactivation (Nilsson et al. 2012; see mechanisms below).

**B.5 Other senses**

Migrating marine animals are thought to rely on a magnetic sense for navigation using the Earth’s magnetic field or anomalies within the Earth’s crust at mid-ocean ridges (Walker et al. 2002). The ability to discriminate between magnetic fields has been shown in teleosts (Quinn 1980), elasmobranchs (Kalmijn 1978), spiny lobsters (Lohmann et al. 1995) and sea turtles (Lohmann and Lohmann 1996). The precise mechanism by which marine organisms detect magnetic fields is unknown,
although multiple hypotheses have been proposed (Lohman and Johnsen 2000). While no definitive studies have been conducted, it is plausible to hypothesize that the magnetic sense in marine organisms would be influenced by ocean acidification due to neural impairment. The reception of the magnetic field cues may not be affected, but the interpretation of these cues may be influenced, leading to complications with navigation.

The lateral line sense is highly conserved among aquatic vertebrates including fishes and amphibians and allows for the detection of water currents and pressure gradients (Bleckmann and Zelick 2009). The lateral line system is composed of a network of mechanoreceptors, or neuromasts, that reside in canals over the head and along the side of the organisms (Flock and Wersall 1962) and plays a critical role in rheotaxis, or the orientation into an oncoming current (Montgomery et al. 1997). Currently, no studies have been conducted on the effects of elevated CO$_2$ on the effectiveness of the lateral line sensory system. We hypothesize that the perception of external cues through the lateral line system (pressure, current direction) will be influenced due to the neural component of sensory perception and advocate for research to test this hypothesis.

The lateral line system has evolved specialized functions in sharks, skates and rays, which have the unique ability to detect bioelectric stimuli using ampullary electrosense (Kalmijn 1972). Elasmobranchs use this sense during foraging, social interactions, reproduction and anti-predatory behaviors (Wilkens and Hofmann 2005). Structurally, elasmobranchs are equipped with electroreception organs called ampullae of Lorenzini, clusters of which can detect not only small local electric fields emanating from prey, but also uniform electric fields from background structure
Similar to chemoreceptors, electroreceptors are located on or directly connected to the surface of the organism, possibly making them more vulnerable to changing ocean chemistry. No studies to date have assessed the effect of ocean acidification on electrosense. However it is reasonable to assume changes will occur for two reasons: (1) changes in salt concentration, temperature and pH are known to influence the conductivity of bioelectric fields and (2) similar to other senses, the endpoint of electrorception is in neural processing and therefore elasmobranchs may experience alterations in how they perceive electrical pulses.

B.6 Mechanisms

Several physiologic mechanisms have been proposed to explain the disruption of sensory perception due to elevated CO$_2$. Briffa et al. (2012) highlight possible mechanisms for info-disruption in aquatic organisms. Chemoreception relies on the connection between sensory receptor molecules and odor molecules. A change in seawater chemistry may shift the ionic charge of the receptor sites (Tierney and Atema 1987) or the chemical molecules (Hara 1976; Brown et al. 2002; Leduc et al. 2004). While this has mainly been tested in freshwater systems, which experience different chemical environments, it may be a potential mechanism of sensory impairment in the marine system. In fact, Roggatz et al. (2016) discovered that an acidified ocean could modify the charge, shape and hydrophobicity on peptide signaling cues, thereby affecting receptor-cue interactions and requiring a higher cue concentration to induce a response. On the other hand, sensory organs as a whole may be affected by elevated CO$_2$, however a connection between structural alterations and sensory impairment has
not been observed thus far for chemoreception (Munday et al. 2009; de la Haye et al. 2012) or sound (Simpson et al. 2011).

Finally, evidence including multiple sensory system impairment, cognitive alterations involving lateralization (Domenici et al. 2011) and learning (Ferrari et al. 2012b), and the observation that high CO₂ treated fish received chemical cues but responded inappropriately, suggests a disruption in processing at the brain or neural level.

The GABA<sub>A</sub> receptor is the primary neurotransmitter receptor in the vertebrate brain (Bormann et al. 1987), and many invertebrates have GABA<sub>A</sub>-like receptors (Hutton et al. 1993; Lunt 1991). These receptors are vital to multiple sensory systems across diverse taxa (ex: Pierobon et al. 2004; Igarashi et al. 2009; Yang et al. 2004). Nilsson et al. (2012) revealed that the inhibitory neurotransmitter receptor, GABA<sub>A</sub>, plays a role in the chemosensory and lateralization changes observed in reef fish.

Acid-base balance allows fish to maintain functional extracellular pH through the adjustment of blood plasma HCO₃⁻ and Cl⁻. The ability to maintain appropriate intercellular pH is critical to cell function (Putnam and Roos 1997) and can be linked to metabolism and iono-regulation (Widdicombe and Spicer 2008). In ambient seawater conditions, the ion gradients allow an influx of Cl⁻ and HCO₃⁻ through the neuronal membrane, which leads to hyperpolarization and inhibition of the neuron (Heuer and Grosell 2014). When held in elevated pCO₂, fish control their acid-base balance by accumulating HCO₃⁻ and ejecting Cl⁻, which stabilizes the intracellular pH but increases the concentration of HCO₃⁻ and pCO₂ in the extracellular fluid (Nilsson et al. 2012). The expulsion of Cl⁻ changes the ion gradients, resulting in the extrusion rather than the incursion of these ions when GABA binds to GABA<sub>A</sub> receptors.
Therefore, the membrane switches from a hyperpolarizing, inhibitory current to a depolarizing, excitatory current. Fish treated with gabazine, a GABA_A antagonist, returned to normal olfactory and lateralization behaviors (Nilsson et al. 2012). This mechanism has been confirmed in retinal function and learning in damselfish (Chung et al. 2014; Chivers et al. 2014), predator evasion in a gastropod (Watson et al. 2014), lateralization in three-spined stickleback (Lai et al. 2015) and anxiety in juvenile salmon (Ou et al. 2015) and rockfish (Hamilton et al. 2014). In a novel approach, Regan et al. (2016) observed the effect of low CO_2 exposure to a fish native to hypercapnic waters (Pangasianodon hypophthalmus) and found behavioral alterations that were repaired by the addition of gabazine.

**B.7 Comparison between taxa**

An understanding of the sensory systems affected by ocean acidification and the mechanisms behind this impairment can guide our predictions of which marine taxa are at greater risk of sensory impairment. As mentioned above, the excitation of GABA_A receptors as a side effect of acid-base regulation is thought to be a primary mechanism for sensory impairment. Therefore we might expect organisms with partial or complete acid-base regulation to be more susceptible to altered sensory processing (Nilsson et al. 2012). The ability to regulate acid-base compensation can be a function of metabolic rate, metabolic rate fluctuations, current environmental hypercapnia, and ontogenetic hypercapnia (Melzner et al. 2009). High-power organisms or those that experience long developmental periods within their egg, such as fish, cephalopods and some crustaceans, as well as organisms that experience elevated pCO_2 levels (intertidal zones, estuaries, upwelling areas, etc) typically exhibit complete or near
complete extracellular compensation. Conversely, organisms characterized by hypometabolism and primarily pelagic development such as bivalves, echinoderms and sipunculid worms, as well as those located in stable environmental conditions such as the deep sea typically show incomplete or inferior compensation (Meltzner et al. 2009; Widdicombe and Spicer 2008). However, the current review shows modified sensory perception in fish (elasmobranchs and teleosts), gastropods, crustaceans, echinoderms and cnidarians, a pattern that does not match the extent of acid-base regulation.

Beyond relative completion of acid-base regulation, extent of sensory impairment may reflect adaptation or acclimation potential. Numerous studies have shown transgenerational acclimation of physiological or metabolic alterations under high CO$_2$ conditions (Miller et al. 2012; Murray et al. 2014; Ho and Berggren 2012). However, transgenerational plasticity is absent or partial in studies assessing behavior or sensory function (Allan et al. 2014; Welch et al. 2014). In general, adaptational differences may be evident when life span and reproductive strategies are compared, with shorter-lived r-selected species able to more readily pass on epigenetic plasticities than longer-lived k-selected organisms.

Finally, taxa differences in sensory impairment in a high CO$_2$ ocean may reflect the ecology of the organism in regards to relative use of sensory systems. While antipredatory responses to visual cues appear to be weakened by ocean acidification (Ferrari et al. 2012b; Lonnstedt et al. 2013), they are not completely reversed or lost as in chemosensory cues (Dixson et al. 2010; Lonnstedt et al. 2013). This suggests that more visually-dependent organisms may be better equipped to handle ocean acidification conditions. Further CO$_2$-exposed damselfish have altered responses to
predatory (Lonnstedt et al. 2013) and habitat (Devine et al. 2012a) chemosensory cues, however, these effects are nonexistent when multiple cues were presented, suggesting sensory compensation. Therefore, organisms with multiple different sensory systems may be more equipped to perceive their environment with the loss or impairment of one, compared with simpler organisms that lack multiple senses, therefore the loss of critical senses are felt more soundly.

**B.8 Future directions**

This review highlights the current knowledge on the effects of ocean acidification (Fig.1). Synthesizing this field has revealed trends, biases, and a lack of standardized methodology that, if rectified, would significantly improve our understanding of the full extent of elevated CO₂ on sensory systems.
Notable variations in the CO$_2$ level tested, length of exposure and habituation period has made species or systems comparisons difficult if not impossible (Table A1-A3). Variations in CO$_2$ levels tested are expected and acceptable as long as these levels reflect the projected levels expected in that particular region. However, using global averages for ocean acidification may not be the most accurate prediction for specific regions, thus local and regional future pH estimates should be determined before experimentation. Habituation period is critical at the start of experiments as acid-base regulation can begin within 15 minutes, with full compensation within a few hours in at least one species (Espaugh et al. 2012). There is no established
standardized methodology for habituation length or exposure time, both of which can have substantial influence on results and should be normalized.

Just as the synthesis of current studies revealed high variability, it also reveals the biases in organisms and species studied. The majority of studies focus on fishes, larvae or juveniles, reef ecosystems, and chemosensory perception. Expansion of this field involves (1) researching the diversity of responses exhibited by marine invertebrates and non-fish vertebrates, (2) tracking individual responses through ontogeny and into adulthood to understand the influence of life stage, (3) focusing on other ecosystems that may be even more threatened by ocean acidification than reefs, including estuaries (Meltzner et al. 2013) and upwelling zones (Steckbauer et al. 2015) because of the additive effect of hypoxia on hypercapnia response (Meltzer et al. 2013), and (4) designing behavioral or physiological assays to test neglected sensory systems such as electrosense, magnetic perception and the lateral line system.

As the field advances, focus should shift towards creating more realistic conditions while testing sensory perception. Realistically, marine ecosystems will face multiple stressors in concordance with ocean acidification, including temperature, pollution, hypoxia and habitat degradation. A plethora of studies have investigated the combined effects of temperature and lowered pH on calcification, survival and growth (Harvey et al. 2013; Lefevre 2016), but few have addressed behavior or sensory impairment. Similarly, physiological studies often address both acidification and hypoxia (Pörtner 2008; Meltzer et al. 2013), but this has yet to be assessed for behavior. Additionally, an effort to produce more realistic studies will incorporate natural pH variation into laboratory design. Often, the nightly decrease in pH from respiration is lower than the oceanic pH expected in the next century (Hofmann et al.
yet few researchers have included these fluctuations in their experimental design. Natural variations in environmental pH are predicted to expedite the onset of elevated CO₂ thresholds (Shaw et al. 2013a) and the magnitude of variation may increase non-linearly due to a weakening of the environmental buffering system (Shaw et al. 2013b). However, exposure to variable environmental pH conditions can reduce sensitivity to manipulated pH conditions at least in terms of survival (Lewis et al. 2013), but this has yet to be tested in terms of sensory impairment. Further research focusing on acclimation potential would also be critical since ocean acidification is expected to occur gradually throughout the next century (see taxa comparison section).

Finally, more realistic studies must scale-up to determine community-level effects. This can begin by testing multiple species in an interaction; since both partner in predator-prey interactions have the potential to experience sensory impairment in future conditions, studies investigating both sides simultaneously could provide valuable information beyond individual species. Mesocosm experiments are an obvious next step and have already been conducted when testing for survival, bleaching and community structure changes (ref). Some work has shown increase mortality due to high CO₂ behavioral or sensory alterations after transporting treated fish back to the natural environment (Munday et al. 2010). CO₂ seeps in Papua New Guinea can act as an ecologically relevant natural laboratory, where community-scale effects of sensory impairment can be observed without typical experimental caveats including habituation, shock potential and handling stress. Previous work has shown that fish from the natural seeps exhibited the same altered behaviors as seen in the laboratory, although there was no difference in community structure (Munday et al. 2014). Natural CO₂ seeps are close predictors of future conditions, yet still retain the
ability to act as sinks to allochthonous larvae, therefore results should be considered with caution. Ultimately as the field advances, effective and meaningful studies must strive for realistic studies incorporating whole-community dynamics, whether through multiple species interactions in the laboratory setting, mesocosm experiments, or the use of natural CO₂ environments.

B.9 Conclusions

Ocean acidification will have a profound effect on the sensory perception of marine organisms, with highly variable responses based on species, life-stage, ecosystem, and parental influence. The field has overwhelmingly been dominated by studies of coral reef vertebrate larvae, and this significant bias needs to be addressed and corrected if we want to develop a more accurate picture of future ocean ecosystems. Further studies should focus on (1) expanding the range of species studied, (2) including marine ecosystems outside of coral reefs, especially estuaries and mangroves, (3) assessing the potential of transgenerational acclimation or genetic adaptation to elevated CO₂ levels, (4) emphasizing more realistic laboratory experiments to include multiple stressors and environmental variability, and (5) scaling up to reveal community-wide effects of sensory and behavioral adjustments to increased CO₂. This diversification of ecosystem perspectives will expand our understanding of sensory impairment under ocean acidification conditions.

B.10 References


Appendix C

PERMISSIONS

C.1 Multiple environmental cues impact habitat choice during nocturnal homing of specialized reef shrimp

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C.2 Impacts of Ocean Acidification on Sensory Function in Marine Organisms

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