COMPETITION AND RESOURCE PARTITIONING BETWEEN CONGENERIC CORAL-DWELLING SCORPIONFISHES (*SEBASTAPISTES* SPP.) IN HAWAI‘I

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ABSTRACT

Exposed coral reefs in Hawaiʻi are largely dominated by the small branching cauliflower coral (*Pocillopora meandrina*), which supports diverse communities of fishes and invertebrates. Two of the most common fishes that reside in these corals are the Speckled Scorpionfish (*Sebastapistes coniorta*) and the Galactic Scorpionfish (*S. galactacma*). These small nocturnal reef mesopredators use the complex branching morphology of cauliflower coral as protection during the day and feed in and around their host corals at night. Despite their apparent ecological similarities, these species coexist locally, at times even co-occupying the same host coral. Surveys of cauliflower coral communities around Oʻahu revealed inverse depth distributions, with Galactic Scorpionfish found across all depths surveyed, though more commonly in deeper reef areas (> 11 m), while Speckled Scorpionfish almost exclusively occurred in shallow reef areas (5 – 10 m deep). These species also used microhabitat in subtly different ways, with the larger species (Speckled Scorpionfish) being more associated with coral colonies with wider spacing between branches. Reciprocal removal experiments across depths, among clusters of corals, and within individual colonies suggest that competition between these species is not currently occurring, and recolonization experiments showed that colony characteristics played a large role in determining which species recolonized corals. Diet analyses using both visual examination and DNA metabarcoding of stomach contents revealed that Speckled Scorpionfish and Galactic Scorpionfish have minimally overlapping diets with significantly different compositions, though both species typically consume small crustaceans that co-occur with them in cauliflower corals. Further, the diets of these species did not significantly differ whether they co-occurred or not, suggesting that these species have evolved different diets, possibly due to past competition. Thus, these species partition food and microhabitat resources to a degree that presently allows for their coexistence on reefs in Hawaiʻi.

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I also thank Patrick Nichols, who is co-author on my third dissertation chapter. I knew that I wanted to incorporate DNA molecular methods into my diet study but was cautious about trying to tackle it by myself. Patrick was willing and eager to collaborate on this project, adding his molecular expertise to my field science. He was always available to talk things over and was incredibly quick at getting me figures or statistics that I needed when trying to finish up my final data chapter just weeks before defending. I also would like to thank Peter Marko, Patrick's advisor, for allowing us to use his space and resources to work on my project.

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CONTRIBUTION OF AUTHORS

The third chapter of my dissertation was a collaboration between myself and Patrick Nichols. I conceived the project design, collected the field data and specimens, dissected and prepped stomach contents, and was responsible for writing most of the manuscript. Mr. Nichols conducted all molecular lab work, bioinformatics, and data analysis, and contributed to the editing of the manuscript.

For my fourth chapter, my co-authors were Mark Hixon and Erik Brush. This project was born out of the many conversations and plans developed between me and Dr. Hixon, and we worked together to design this field study. Mr. Brush helped during the planning stages to solidify the field procedures and was integral helping conduct these experiments underwater. I was responsible for execution of the experiments, data analysis, and preparation of the manuscript. Both Dr. Hixon and Mr. Brush provided substantial contributions and feedback to the manuscript.

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CHAPTER 1

General Introduction

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Coral reefs are one of the most diverse ecosystems on the planet and are home to nearly a third of all marine species despite comprising less than 1% of the world's oceans (Fisher et al. 2015). This amazing diversity is largely possible because of the habitat complexity created by reef-building coral species (Holbrook et al. 2002; Gratwicke and Speight 2005; Messmer et al. 2011; Agudo-Adriani et al. 2016). Corals generate vertical relief and complex interstitial spaces that serve as refugia for a many species of reef fishes (Coker et al. 2014) and invertebrates (Johnson et al. 2011), and the diversity and physical attributes of corals can directly affect the health and fitness of the species that reside in and around these colonies (Pereira and Munday 2016). How coral reef fishes use coral habitat is highly species specific (Depczynski and Bellwood 2004; Messmer et al. 2011), and competition for habitat resources can be intense between species that highly overlap in their use of coral habitat (Bonin et al. 2015).

Interspecific competition is an important ecological process that occurs between species that share resources in short supply (Birch 1957; Hixon 2006) and is capable of structuring communities (Schoener 1983). Competition can take the form of direct negative interactions between organisms (interference competition), such as territoriality or harassment. Alternatively, competition can occur via indirect negative interactions between organisms (exploitative competition), when a limited resource is consumed by one organism, leaving less of that resource available for others. The effects of competition are strongest among organisms that are comparably sized and that occupy similar ecological roles (Schoener 1974). Consequently, competition within species is thought to be more intense than that between species, the latter being more intense among species occupying the same ecological guild (Simberloff and Dayan 1991).

For coral-reef fishes, competition has been shown capable of limiting abundance via demographic density dependence (review by Hixon and Webster 2002), which arises from an increase in mortality or a reduction in fitness (e.g. growth rates, health, fecundity) from a decrease in access to resources. Even in cases where predation appears to be the primary agent of mortality, competition may be the ultimate cause by increasing vulnerability to predation due to a reduction in individual growth or health or access to refugia (Hixon and Jones 2005).

The coexistence of ecologically similar species has long intrigued ecologists (Macarthur 1958). Understanding how species use resources, generally food and habitat, can help explain how high levels of biodiversity are maintained. One mechanism that can lessen the strain of competition between species is if they partition their use of shared resources (Schoener 1974; Diamond 1978). Resource partitioning can happen on ecological timescales if species shift their resource use away from overlapping fundamental niches when cooccurring with a competitor species. Alternatively, resource partitioning can arise over evolutionary timescales, where species have evolved to use different resources, or the same resources in different ways, as a result of past competition, such that their fundamental niches no longer overlap substantially (Connell 1980). Partitioning of food and habitat has been documented as a mechanism allowing for coexistence within guilds of coral-reef fishes (Munday et al. 2001; Goatley et al. 2016; Whitney et al. 2018).

The main Hawaiian Islands (MHI) are densely populated, with urban areas concentrated near coral reefs. Anthropogenic stressors, such overfishing, nutrient runoff, and pollution, have resulted in relatively degraded reef ecosystems. Additionally, coral communities in Hawaiʻi are less diverse than other reefs throughout the Indo-Pacific and Caribbean, likely due to the isolation of the Hawaiian archipelago. Consequently, Hawaiian reefs are generally less structurally complex than many other coral reef systems (Grigg 1983; Friedlander et al. 2008).

One coral species that helps create complex habitat on Hawaiian reefs is the small branching cauliflower coral (*Pocillopora meandrina*), which is common across the MHI (Friedlander et al. 2008). This species is roughly spherical in shape, around 30 cm in diameter, and is one of the few coral species prevalent along coastlines with high wave energy (Smith 2019; Fukunaga et al. 2022). Cauliflower coral is one of only three branching coral species that commonly occur in the MHI. Despite their relatively small size, their abundance and complex branching morphology make cauliflower corals ideal habitat for a variety of fishes (Coker et al. 2014) and invertebrates (Counsell et al. 2018).

Two fish species that commonly inhabit cauliflower coral are the congeneric Speckled Scorpionfish (*Sebastapistes coniorta*) and Galactic Scorpionfish (*S. galactacma*), which occupy nearly a quarter of all cauliflower coral colonies around Oʻahu (Counsell et al. 2018). These species are nocturnal cryptobenthic reef fishes that shelter within the branches of cauliflower coral during the day. At night, they feed in and around their host corals on various invertebrates and small fishes (Hobson 1974). Despite their apparent ecological similarities (size, body shape, host coral species), the Speckled Scorpionfish and Galactic Scorpionfish are able to coexist on reefs in Hawaiʻi, occasionally co-occupying the same host colony. The focus of this dissertation is to investigate whether these two species compete for resources and explore the mechanisms that allow for their coexistence.

The second chapter of this dissertation, “Habitat partitioning by two coral-dwelling scorpionfishes in Hawaiʻi,” uses observational methods to document the habitat characteristics associated with Speckled Scorpionfish and Galactic Scorpionfish around Oʻahu, Hawaiʻi. This study first looked at reef scale differences in the distribution of these two species across depths. Patterns of bathymetric segregation between congeneric marine fishes have previously been documented in temperate systems (Hixon 1980; Larson 1980). This study also documented the physical attributes of host cauliflower coral colonies associated with Speckled Scorpionfish and Galactic Scorpionfish to determine the amount of overlap in microhabitat use between the two species. I hypothesized that these species partition within-reef habitats in response to present-day or past competition (Connell 1980) and predicted that they would be associated with cauliflower coral colonies whose interstitial branch spaces best matched each species average body size (Hixon and Beets 1993).

Speckled Scorpionfish and Galactic Scorpionfish may also share and may compete for food resources, the existence and extent of which is explored in the third chapter, “Dietary partitioning in congeneric Hawaiian scorpionfishes revealed by DNA metabarcoding.” Prior to this study, little was known about the diets of these two species. Hobson (1974) described the diet of Speckled Scorpionfish as generally consisting of various invertebrates, mostly xanthid crabs and caridean shrimps, and small unspecified fishes. The diet of Galactic Scorpionfish has not been previously documented.

Diet analyses using traditional visual methods can be challenging when working with small predatory fishes whom consume even smaller prey infrequently (Longenecker 2007).

Visual analyses of stomach contents require prey items to be at least partially intact, possess identifiable characteristics, and can overrepresent prey with body parts that are slower to digest, such as chitinous crustacean exoskeletons (Beukers-Stewart and Jones 2004). An alternative approach that is becoming cheaper and easier for ecologists is using DNA metabarcoding, the simultaneous identification of multiple species from a pooled sample of DNA, to identify prey from stomach contents (Valentini et al. 2009). DNA analysis is effective at identifying even heavily digested prey items from stomachs and intestines (Symondson 2002), and has been widely used to describe the diets of reef fishes (Leray et al. 2015; Harms-Tuohy et al. 2016; Dahl et al. 2017; Brandl et al. 2020; Nalley et al. 2022; Coker et al. 2023). This study used both traditional visual methods and modern DNA metabarcoding techniques to describe the diets of Speckled Scorpionfish and Galactic Scorpionfish.

We collected specimens from areas where only one species or the other were present and from areas where both species occupied the same host coral. This “natural experiment” design (Diamond 1978) allowed us to compare the diets of these two species when occurring alone and together. We hypothesized that these species overlap in their diets and predicted that if they are competing for food resources, then their diets will be constrained by competition when cooccurring. Alternatively, these species may have evolved to consume different prey whether or not they cooccur.

Understanding how the Speckled Scorpionfish and Galactic Scorpionfish use and share food and habitat resources may uncover how these species are able to coexist on reefs in Hawaiʻi. These species may exhibit niche complementarity and overlap largely in their use of one resource axis while partitioning resources along a second axis (Werner 1977). Alternatively, these species may overlap only moderately in their use of both food and habitat resources.

While documenting resource overlap can suggest that competition is potentially occurring between species, observational studies alone are insufficient for demonstrating that competition is actually occurring (Connell 1975). Chapter four, “Absence of present-day competition in two congeneric species of coral-dwelling scorpionfish,” reports manipulative field experiments that directly test whether Speckled Scorpionfish and Galactic Scorpionfish compete with one another on reefs in Hawaiʻi. This experiment employed a reciprocal press removal of each species from reef areas where they both naturally occur at two different spatial scales: 1) clusters of cauliflower coral colonies and 2) individual cauliflower colonies. We hypothesized that these species are competing for resources and predicted that the removal of one species from an area would result in an increase in the local abundance of the other species. Additionally, we conducted pulse removals of all *Sebastapistes* scorpionfishes from clusters of cauliflower corals in both deep and shallow reef areas to explore the effects of habitat preference and priority effects on the presence and abundance of each species.

This dissertation employs a three-pronged approach using an observation study, a natural experiment, and a set of manipulative experiments to describe how Speckled Scorpionfish and Galactic Scorpionfish use habitat and food resources and whether or not competition is presently occurring between these species in Hawaiʻi.

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CHAPTER 2

Habitat Partitioning by Two Coral-Dwelling Scorpionfishes in Hawai‘i

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Submitted

Abstract

Coral reefs are among the most biodiverse ecosystems on Earth, largely due to the structural complexity created by corals. Coral habitat is crucial refuge for numerous small animals, and competition for habitat can structure populations if in short supply. Reefs in Hawaiʻi are largely dominated by the small branching coral *Pocillopora meandrina*, which supports diverse communities of fishes and invertebrates. Two species of nocturnal scorpionfishes, *Sebastapistes coniorta* and *S. galactacma*, are particularly common in *P. meandrina*, inhabiting the complex branching morphology of their host corals during the day. Surveys of scorpionfishes in *P. meandrina* around the island of Oʻahu revealed inverse depth distributions over which these species occurred. *Sebastapistes coniorta* almost exclusively occurred in coral colonies between 5 and 10 m deep, while *S. galactacma* were found across all depths surveyed, though were far more common and abundant in deeper coral colonies below the range of *S. coniorta*. Further, these species appeared to use microhabitat in subtly different ways. The larger-bodied species, *S. coniorta*, was more commonly found in *P. meandrina* with wider spaces between branches, while the smaller-bodied species was negatively associated with wider branch spacing. These patterns indicate habitat partitioning between *S. coniorta* and *S. galactacma*, though some overlap does occur, particularly in shallow reef areas.

Key Words

Coral reefs, habitat use, Hawaiʻi, resource partitioning, Scorpaenidae, *Sebastapistes*,

2.1 Introduction

Coral reefs are among the most complex ecosystems on Earth, sustaining remarkably high diversity and abundance of marine life (Connell 1978; Reaka-Kudla 2005). Despite occupying less than 1% of the world's oceans, coral reefs are home to nearly one-third of all marine species (Fisher et al. 2015; Strain et al. 2019). Such high diversity is largely possible because of the structural complexity created by reef-building scleractinian corals (Holbrook et al. 2002; Gratwicke and Speight 2005; Messmer et al. 2011; Agudo-Adriani et al. 2016).

The diversity of coral species and growth forms provides heterogeneous habitat for a variety of fishes and invertebrates that use the resulting structural complexity for shelter, either temporarily or as permanent residents (Gratwicke and Speight 2005; Johnson et al. 2011). Coral habitat use is highly species-specific, ranging from habitat generalists using temporary refugia to highly specialized species that associate with only a single species of coral or an individual coral colony (Depczynski and Bellwood 2004; Messmer et al. 2011). Many nocturnal and diurnal reef fishes are heavily dependent on the refuge provided by coral colonies during quiescent periods (Hobson 1972; Michael and David 2002). The physical attributes of host coral colonies are also important for the health and fitness of associated reef fishes (Pereira and Munday 2016). For example, both colony size and interbranch spacing have been identified as important drivers of habitat preference in coral-dwelling gobies (Pereira and Munday 2016).

Reefs around the heavily populated Main Hawaiian Islands (MHI) are relatively degraded, have low relief, and lack the species diversity and structural complexity characteristic of many other coral reefs throughout the Pacific (Grigg 1983; Friedlander et al. 2008). In Hawaiʻi, coral reefs are dominated by just a handful of coral species, including the small, branching coral *Pocillopora meandrina* (Friedlander et al. 2008). Roughly spherical in shape and typically about 30 cm in diameter, this coral is prevalent and abundant along coastlines with high wave energy, is among the earliest coral colonizers of newly exposed substrate (Smith 2019; Fukunaga et al. 2022), and is one of only three species of branching coral that commonly occur in the MHI. Despite their small size, the high abundance and complex branching morphology of *P. meandrina* make them ideal habitat for a variety of fishes (Coker et al. 2014) and invertebrates (Counsell et al. 2018).

Around O‘ahu, the most common fishes inhabiting *P. meandrina* are small scorpionfishes of the genus *Sebastapistes* (Greenfield 2003), which occupy nearly a quarter of all *P. meandrina* colonies around the island (Counsell et al. 2018). The two most common *Sebastapistes* species habiting live *P. meandrina* on spur-and-groove reefs in Hawai‘i are the Speckled Scorpionfish (*Sebastapistes coniorta*) and the Galactic Scorpionfish (*S. galactacma*; Greenfield 2003; Counsell et al. 2018). These nocturnal, coral-associated cryptobenthic reef fishes use *P. meandrina* as refuge during the day (Hobson 1974). At night, larger individuals will leave the confines of their host coral and feed on small invertebrates, especially xanthid crabs and caridean shrimps (Randall 2007), as well as small fish. Smaller individuals, however, tend to remain at the base of their host coral (Hobson 1974). Before dawn, these fish return to protection among the branches of *P. meandrina* (Hobson 1972, 1974). Despite being common, little else is known about their ecology and habits, including how these species can coexist given their apparent ecological similarities.

Since *S. galactacma* and *S. coniorta* are closely-related, demersal microcarnivores found in the same general habitat (Counsell et al. 2018), interspecific competition could be an important interaction structuring their populations if any of their shared resources are in short supply (Hixon 2006). Competition could be direct via interference (e.g., territoriality) and/or indirect via exploitation of either food or habitat resources. One possible mechanism explaining the coexistence of *S. galactacma* and *S. coniorta* could be resource partitioning via the occupation of different microhabitats, thereby lessening the intensity of competition for space (Schoener 1974; Diamond 1978). Partitioning of microhabitats has been previously documented in other guilds of coral-associated fishes (Munday et al. 2001; Goatley et al. 2016; Whitney et al. 2018) and could be an important factor explaining the coexistence of coral-dwelling *Sebastapistes* in Hawaiʻi.

Here I document the habitat characteristics associated with *S. coniorta* and *S. galactacma* distributions and abundance around the island of Oʻahu, Hawaiʻi. I hypothesized that these species partition within-reef habitats in response to present-day or past competition (Connell 1980). Specifically, I predicted that the larger-bodied species, *S. coniorta*, would occur more frequently and in greater abundance in larger *P. meandrina* colonies and colonies with wider spaces between branches that provide refuge space more closely matching their body size (Hixon and Beets 1993). Conversely, I predicted that *S. galactacma* would be more commonly found in smaller *P. meandrina* colonies and colonies with more narrow spaces between branches.

2.2 Materials and Methods

Study Sites

Coral colonies for this study were surveyed across five sites on the south and west shores of the island of Oʻahu, Hawaiʻi (Fig. 1). The main site was an exposed spur-and-groove reef approximately 800 m off Waikīkī Beach (21°16'11.00"N, 157°50'18.53"W) that ranged from 5 to 20 meters in depth. This site was characterized by relatively degraded reef consisting of large tracks of hardbottom with roughly 8% coral cover (Franklin et al. 2013). Like most exposed reefs around O‘ahu, the reefs here are numerically dominated by *Pocillopora meandrina* and *Porites lobata*. Large fishes are scarce on these reefs, likely due to high fishing intensity (Williams et al. 2006).

The second site on the south shore, Kewalo Basin (21°17’21.15”N, 157°51’49.10”W), is approximately 3.5 km west of Waikīkī Beach. The composition of coral species here was similar to that at Waikīkī, though coral cover was much higher and *Montipora* corals were more common.

The two study sites on the west side of the island were off of Kahe Point (21°21'13.37"N, 158° 7'54.83"W) and Makaha Beach (21°28'28.90"N, 158°13'34.37"W). These reefs were similar to those on the south shore, though were generally shallower (5-15m).

The final site, Hanauma Bay (21°16'7.35"N, 157°41'34.47"W), is located on the southeastern end of O‘ahu. This site, an ancient volcanic crater that opened to the sea, is unique in that it is designated as a Marine Life Conservation District, and all fishing and boating activities are strictly prohibited. The reefs here are more continuous than at any other site surveyed and are largely dominated by *Porites lobata*, *P. compressa*, and *Montipora* spp. with some *Pocillopora meandrina* scattered throughout. Because this area is protected, large herbivorous and piscivorous fishes are common throughout the bay.

Pocillopora meandrina surveys

Pocillopora meandrina colonies were sampled haphazardly to encompass the range of sizes representative of adult colonies (>10 cm diameter) throughout each study area. An initial series of surveys of 133 *P. meandrina* colonies were conducted between 2018 and 2019, primarily at Waikīkī. These surveys consisted of a full census of resident fishes, including an estimation of total body length of each individual. Coral colonies were characterized by four parameters: depth, size, colony branch spacing, and isolation. Coral colony size was determined by taking measurements of maximum diameter length, maximum orthogonal width, and height. This allowed calculation of an estimated volume of the colony ($volume = \pi\left(\frac{length}{2}\right)\left(\frac{width}{2}\right)height$), assuming an elliptic cylinder. To quantify the amount of spacing between branch tips, I averaged five measures of the distance between the middle and center of two adjacent branch tips representative of each colony. An index of colony isolation was calculated as the average distance to the nearest three colonies of *P. meandrina*.

In 2021, I conducted additional surveys of 325 *P. meandrina* colonies across five field sites (Waikīkī: n = 44, Kewalo Basin: n =78, Kahe Point: n = 77, Makaha: n = 50, and Hanauma Bay: n = 76). Depth and colony size were recorded in the same way as in previous surveys, but colony isolation was not recorded. For these surveys, I changed how branch spacing was measured to better estimate the amount of available habitat space between the branches of the host colony. Instead of measuring from the center of the branch tips, I measured the distance between the nearest points of two adjacent branch tips at five random places around each colony and used these measurements to calculate a colony average.

Scorpionfish Colony Fidelity

Tagging allowed me to determine the level of fidelity of scorpionfish to particular coral colonies. In the fall of 2018, I selected five clusters of three *P. meandrina* colonies (<1 m apart) between 7 and 10 m deep and tagged all *Sebastapistes* spp. in each colony with Visible Implant Elastomer (VIE; Northwest Marine Technology, Inc.) tags (n = 28 *S. galactacma* and n = 2 *S. coniorta*). Fish were collected using a non-lethal, low-concentration mixture of clove oil (<7%)

and seawater, chopsticks to guide the fish out of the coral, and hand nets (DAR SAP No. 2019-45). Each individual received two tags, one on each side of the dorsal portion of the body. For each fish, a tag was placed on the left side of the body with a color corresponding to the coral colony where the individual was originally captured. A variety of tag colors were used on the right side of the body to identify individuals within each colony. After tagging, individuals were measured to the nearest millimeter total length and returned to the colony from which they were collected. Coral colony clusters were then monitored every one to two weeks over the next month, specifically looking for movement of tagged fish between *P. meandrina* colonies within a cluster. If a tagged individual was not observed during a survey, surrounding colonies outside of the cluster were searched for evidence of broader movements. Fish that could not be found anywhere near the study colonies were assumed to have died or left.

Statistical Analysis

Data were processed in the statistical analysis program R (R Core Team 2020). To determine the effects of habitat characteristics on the presence or absence of *S. coniorta* and *S. galactacma*, generalized linear mixed-effects models with binomial error distributions were constructed using the *glmer* function in the R package *lme4* (Bates 2015). Models testing the effects of habitat characteristics on within colony abundance used Poisson error distributions. Both sets of models included host coral colony volume, mean branch spacing, and depth as fixed effects and study site, reef area, and month surveyed as random effects. Conditional R^2 values, the amount of variance explained by the models (fixed and random effects), were calculated using the *r.squaredGLMM* function, also in *MuMIn* (Bartoń 2020).

I compared the habitat characteristics of colonies occupied by either species of scorpionfishes, both species, and neither species using a non-metric multidimensional scaling analysis using a Bray-Curtis distance matrix. This analysis was completed using the *metaMDS* function in the "vegan" package (Oksanen et al. 2019). A Permutational Multivariate Analysis of Variance (PERMANOVA) using distance matrices was completed with the function *adonis*, also in the "vegan" package. Pairwise comparisons between groups were adjusted with a Bonferroni correction to account for multiple comparisons.

2.3 Results

Pocillopora meandrina Characteristics

Surveyed *Pocillopora meandrina* colonies ranged in estimated volume from 0.002 m^3 to 0.061 m^3 ($\bar{x} \pm SE = 0.012 \pm 0.00034\ m^3$; Fig. 2a). Colony volume was significantly and positively correlated with depth (LM: $p << 0.001$, $R^2 = 0.19$; Fig. 3a), with larger colonies being more common in deeper reef areas. Colonies also varied in the amount of occupiable space between their branches (Fig. 2b), the colony average ranging from of 9.4 mm to 24.2 mm between branches ($\bar{x} = 15.15 \pm 0.12$ mm). The spacing between branches did not vary significantly across the range of depths surveyed (Fig. 3b). Finally, *P. meandrina* colonies were significantly more isolated in deeper reef areas than in shallower reef areas (LM: $p << 0.001$, $R^2 = 0.14$; Fig. 3c).

Scorpionfish Colony Fidelity

Thirteen of 28 tagged *S. galactacma* were recaptured one month after they were tagged. Of these 13 individuals, 11 were recaptured in the same colony as they were originally tagged in

(84.62%). Only two individuals were captured in colonies other than their original host colony, both of which were colonies less than half a meter away from their original colony. No tagged fish were found in coral colonies surrounding the tagged clusters. Periodic surveys of the clusters also showed that one other individual moved between colonies prior to recapture, though this individual was not found at the end of the survey. The two tagged *S. coniorta* remained in their original host colony throughout the study. Based on these data, I concluded that coral colony surveys accurately reflected the true patterns of within-habitat distribution and abundance of these fishes.

Sebastapistes Habitat Use

Among the 458 *Pocillopora meandrina* colonies surveyed, I observed 538 individuals of *S. galactacma* and 76 individuals of *S. coniorta*. The body length (mean ± SE) of *S. galactacma* was 3.41 ± 0.03 cmTL, significantly smaller than that of *S. coniorta* (5.5 ± 0.12 cmTL; t-Test: $p << 0.001$).

Sebastapistes was the most commonly occurring genus of fish found in *Pocillopora meandrina* during this study and was present at all five study sites around Oʻahu. *Sebastapistes galactacma* was by far the most frequently occurring species, present in 42.14% of all colonies surveyed, as well as numerically dominant. *Sebastapistes coniorta* occurred in only 8.95% of *P. meandrina* colonies. The two species occurred together in only 1.97% of colonies surveyed, significantly less often than would be expected by chance ($p = 0.01$; $\chi^2 = 6.65$; $df = 1$).

Sebastapistes coniorta typically occurred in a coral colony as either a single individual or in pairs (Fig. 4a). There were only five colonies out of 41 where *S. coniorta* occurred in higher abundance, including one colony occupied by 10 individuals. While the majority of *S.*

galactacma also occurred singly or in pairs, 38.86% of occupied colonies contained more than two individuals, with a maximum of 15 individuals within a single colony (Fig. 4b). Within colony abundance was greater in larger coral colonies for *S. galactacma* (GLMM: p = 0.001; χ^2 = 10.21; df = 1) but did not significantly vary across colony sizes for *S. coniorta* (GLMM: p = 0.65 ; χ^2 = 0.21; df = 1).

For *S. galactacma*, the fixed effects (depth, branch spacing, and colony volume) in the GLMM explained 20.8% of the variance in presence or absence. Including random effects for site, survey cluster, and month surveyed, the model explained 48.5% of variance. The model for *S. coniorta* explained 36.4% of variance with fixed effects and 53.0% using the full model.

The depth distributions of these species were inversely correlated across sites. *Sebastapistes galactacma* was significantly more common in the deeper reef areas (GLMM: p << 0.001 ; χ^2 = 19.6; df = 1), present in 71.74% of *P. meandrina* between 15 and 20 m deep, and only 35.50% of colonies between 5 and 10 m deep (Fig. 5). In contrast, *S. coniorta* presence was significantly negatively correlated with depth (GLMM: p = 0.004; χ^2 = 8.3; df = 1), and this species was almost exclusively found in shallow colonies between 5 and 10 m deep, occupying 10.69% of colonies in that range. Only two of the 151 colonies surveyed deeper than 10 m (1.32%) contained *S. coniorta*, both of which were at Kewalo Basin. Within the depth range where both species were commonly found (5 – 10 m), the rate of cooccurrence within colonies was not less frequent than would be expected by chance (p = 0.31; χ^2 = 1.0389; df = 1). Finally, depth did not have a significant effect on within colony abundance for either *S. galactacma* (GLMM: p = 0.99; χ^2 = 0.00; df = 1) or *S. coniorta* (GLMM: p = 0.56; χ^2 = 0.34; df = 1).

There was also an inverse relationship in the space between coral branch tips occupied by these species. *Sebastapistes galactacma* were less frequently found in coral colonies with more space between coral branch tips (GLMM: $p = 0.01$; $\chi^2 = 6.5$; df = 1) while *S. coniorta* more commonly occurred in colonies with wider branch spacing, though marginally significant (GLMM: $p = 0.05$; $\chi^2 = 3.8$; df = 1). *Sebastapistes coniorta* presence had a marginally significant positive correlation with increasing coral colony volume (GLMM: $p = 0.06$; $\chi^2 = 3.4$; df = 1). In contrast, *S. galactacma* presence did not significantly correlate with the size of their host coral (GLMM: $p = 0.86$; $\chi^2 = 0.03$; df = 1).

Colonies occupied by *S. galactacma* tended to be in deeper reef areas, while colonies occupied by *S. coniorta* tended to have more space between branches, as seen in an NMDS plot based on habitat characteristics (depth, colony volume, and colony interstitial space; Fig. 6). Colony volume did not correlate with either species. Colonies unoccupied by either species spanned the entire plot, overlapping with colonies occupied by either or both species of scorpionfish. A PERMANOVA indicated a significant difference among the four categories of coral colonies ($p = 0.008$). Pairwise comparisons showed that the only categories which were significantly different were colonies occupied by only *S. galactacma* and colonies unoccupied by both species of *Sebastapistes* ($p = 0.02$).

Sebastapistes galactacma at a site where no *Sebastapistes coniorta* occur

Both *S. coniorta* and *S. galactacma* were found at four of the five sites surveyed (Waikīkī, Kewalo Basin, Kahe Point, and Makaha). At Hanauma Bay, however, *S. coniorta* was absent in all *P. meandrina* colonies surveyed. Nonetheless, coral colonies at Hanauma Bay did not significantly differ from those at any of the other four sites for either colony volume (t-Test: $p = 0.15$) or branch spacing (t-Test: $p = 0.64$). The depth distribution of *S. galactacma* at

Hanauma Bay showed similar trends to the depth distributions at Waikīkī, Kewalo Basin, Kahe Point, and Makaha, even in the absence of *S. coniorta* (Fig. 7).

2.4 Discussion

The most striking pattern observed during this study was the inverse depth distributions of these species, with *S. galactacma* being more common in deeper reef areas and *S. coniorta* almost exclusively restricted to shallow areas. Similar trends of bathymetric segregation have been observed in other species of the Family Scorpaenidae (Larson 1980) and could be due to gradients of food or habitat availability and/or competitive interactions (Hixon 2006). The similarity of *S. galactacma*'s depth distribution across sites, including at Hanauma Bay where *S. coniorta* were absent, acts as a "natural experiment" (Diamond 1978) that provides some evidence against competitive exclusion of *S. galactacma* from shallow areas by *S. coniorta,* but further experiments to directly test for competition would need to be conducted to fully evaluate this possibility (Connell 1975). Despite this bathymetric segregation, there was an overlapping range of depths, roughly between 5 and 10 m deep, where both species were found in similar frequencies. Within this range, I observed both species in adjacent coral colonies, and occasionally co-occupying the same coral colony. Over all colonies surveyed, *S. coniorta* and *S. galactacma* inhabited the same colony less often than would be expected by chance. However, considering only colonies within the zone of overlap (5 – 10 m deep), the rate of cooccurrence was no longer different than would be expected by chance. Therefore, taking species distributions into account is important when inferring relationships between species even when working within a single, relatively small study site.

Coral colony characteristics were also correlated with *Sebastapistes* distribution and abundance. *Sebastapistes galactacma* occurred less frequently in colonies with more spacing between branches. This pattern could indicate that, at some point, the space between branches no longer matches their body size and is wider than would be required to provide refuge from external predators (Hixon and Beets 1993). Conversely, *S. coniorta* had a positive correlation with increasing branch size, albeit of marginal significance ($p = 0.05$). Given that *S. coniorta* are, on average, larger-bodied than *S. galactacma*, they may be restricted from coral colonies with too narrow branch spacing and may selectively settle to or move to colonies with more space between branches, better matching their adult size (Buchheim and Hixon 1992; Hixon and Beets 1993). Coral colony volume was not significantly correlated with the presence of *S. galactacma*, but there were significantly more individuals in larger colonies than in smaller colonies. Larger colonies were marginally correlated with *S. coniorta* presence though were not correlated with abundance. *Sebastapistes coniorta* most frequently occurred only singly or in pairs. However, one exceptionally large colony was inhabited by 10 individuals of *S. coniorta*. Other colonies with high numbers of *S. coniorta* were observed at other sites outside of this study, but it is unclear whether these high densities are maintained over time or are temporary aggregations.

A multivariate analysis of colony characteristics indicated that *S. coniorta* and *S. galactacma* occur in habitats with subtly different yet largely overlapping characteristics. Unoccupied corals, however, spanned the entire colony trait space. This pattern leads to two possible alternative explanations: 1) that coral habitat is not in short supply for these species, or 2) that there is some unmeasured colony characteristic that precludes or discourages occupation by *Sebastapistes* spp. Habitat could be available in excess if populations of *Sebastapistes* are maintained at low levels due to factors such as recruitment limitation (Doherty 2002). This is especially likely in Hawaiʻi given the historically low levels of juvenile fish recruitment (Walsh 1985, 1987) possibly resulting from the geographic isolation of the archipelago from other Indo-Pacific populations (Bowen et al. 2016).

This study focused on only one general resource axis, habitat, at both the reef scale (depth) and colony scale (colony size and interstitial branch space). Even though these species seemingly use microhabitat in subtly different ways, the ability for them to coexist within close proximity to one another, even in the same colony, is perplexing. While this study documented conditions where competition is likely to occur between these species, namely in the depth zone of overlap between 5 and 10 m deep, carefully designed manipulative experiments would be required to demonstrate whether competition is occurring (Schoener 1983). If these species do compete, then the presence of one species within an area would limit the abundance of the other species, and vice versa (Robertson 1996). Conducting a reciprocal removal experiment (e.g. Larson 1980) could help elucidate whether or not these species presently compete with one another and limit each other's local abundances.

It is also possible that these species exhibit niche complementarity (Werner 1977) and partition food resources rather than habitat. Given that these species are nocturnal, there are very few recorded observations of their foraging behavior, so it is unknown whether they differ in temporal feeding patterns or foraging ranges. Additionally, the diets of these two species are also poorly described. Hobson (1974) generally categorized the diet of *S. coniorta* as consisting mainly of xanthid crabs, caridean shrimps, and occasionally small fishes. There is less information about the diet of *S. galactacma*, though it is likely similar with some possibility of gape limitation due to their smaller body size. A more thorough description of the diets of *S.*

coniorta and *S. galactacma* could reveal if food resource partitioning is occurring and could explain how these species are able to coexist.

Cryptobenthic reef fishes like *S. coniorta* and *S. galactacma* act as an important link in the trophic chain of coral reefs, serving as a bridge between macroinvertebrates and higher trophic species that are often economically and culturally important (Depczynski and Bellwood 2003; Brandl et al. 2018). Coral-associated species also often act as mutualists with their coral hosts (Liberman et al. 1995; Pratchett 2001; Holbrook et al. 2008; Stier et al. 2012). For example, the feces of reef fishes provide nutrients to their host colony (Meyer and Schultz 1985), and the movements of resident fish increase the circulation of water in the interstitial spaces within coral colonies (Goldshmid et al. 2004). Resident invertebrates, which are potential prey of coral-dwelling scorpionfishes, have also been documented protecting their host colonies from coralivorous species such as the Crown-of-Thorns Starfish, *Acanthaster planci* (Pratchett 2001; McKeon and Moore 2014). Understanding the ecology of these species is therefore important for any management efforts aimed at conserving and protecting coral reef ecosystems.

Hawaiʻi is both geographically isolated and densely populated by humans, resulting in a community of corals that face a multitude of acute local stressors in addition to chronic global stressors, such as ocean warming and acidification. Hawaiʻi, once thought to be immune from coral bleaching (Jokiel and Brown 2004), has experienced three major bleaching events in the main Hawaiian Islands within the past 8 years (Bahr et al. 2015; Cunning et al. 2016; Rodgers et al. 2017; Jones et al. 2021), some of which led to widespread coral mortality (Kramer et al. 2016). Pocilloporid corals are particularly susceptible to heat stress, frequently bleaching earlier and more severely than other genera (McClanahan et al. 2020; Burgess et al. 2021). Further, coral-associated fishes, such as *Sebastapistes*, are acutely vulnerable to habitat loss following such disturbances (Feary et al. 2007; Lefèvre and Bellwood 2015). Climate change and other human-caused disturbances threaten coral reef ecosystems worldwide (Hoegh-Guldberg et al. 2007). It is therefore imperative that we understand the ecology of the species most likely to be affected by future disturbance as we seek to manage and conserve this vulnerable and valuable ecosystem.

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2.6 Figures

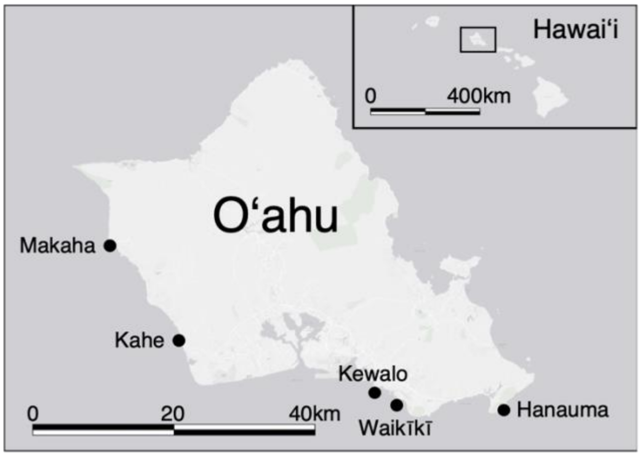

Figure 1. Map of five field sites used to assess resource partitioning of scorpionfishes (genus *Sebastapistes*) on the south and west shores of O‘ahu, Hawai‘i.

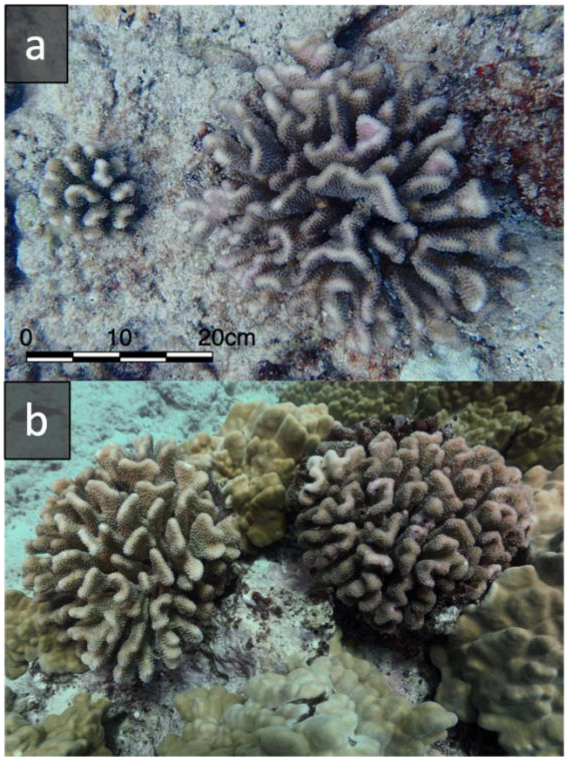

Figure 2. Phenotypic plasticity of *Pocillopora meandrina* in **a** adult size and **b** spacing between branches.

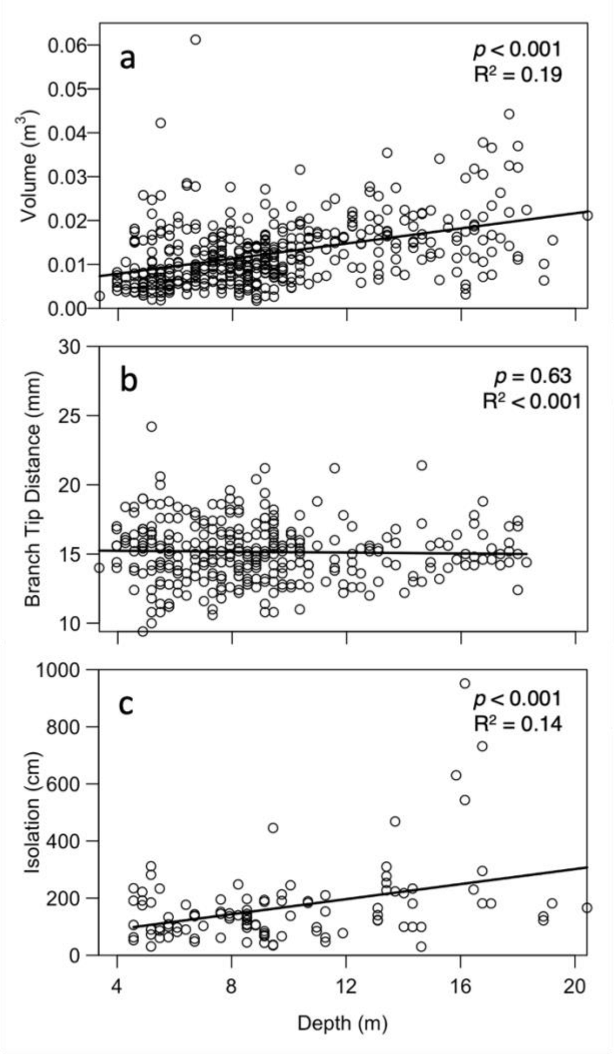

Figure 3. *Pocillopora meandrina* **a** volume, **b** branch spacing, and **c** isolation across the range of depths surveyed on O‘ahu, Hawai‘i.

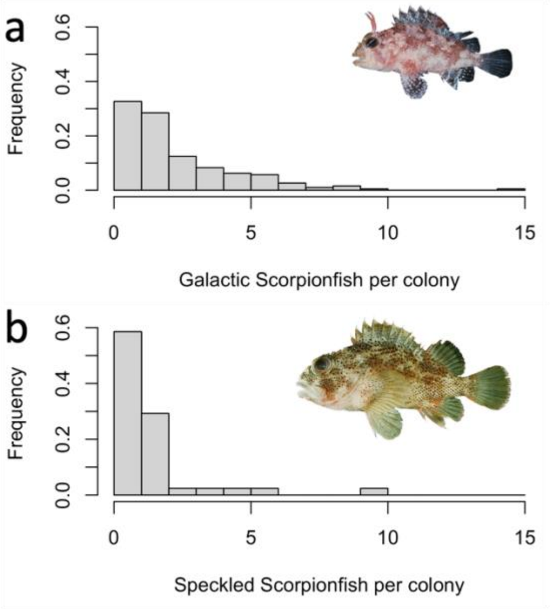

Figure 4. Number of **a** *Sebastapistes galactacma* and **b** *S. coniorta* individuals per coral colony on O‘ahu, Hawai‘i.

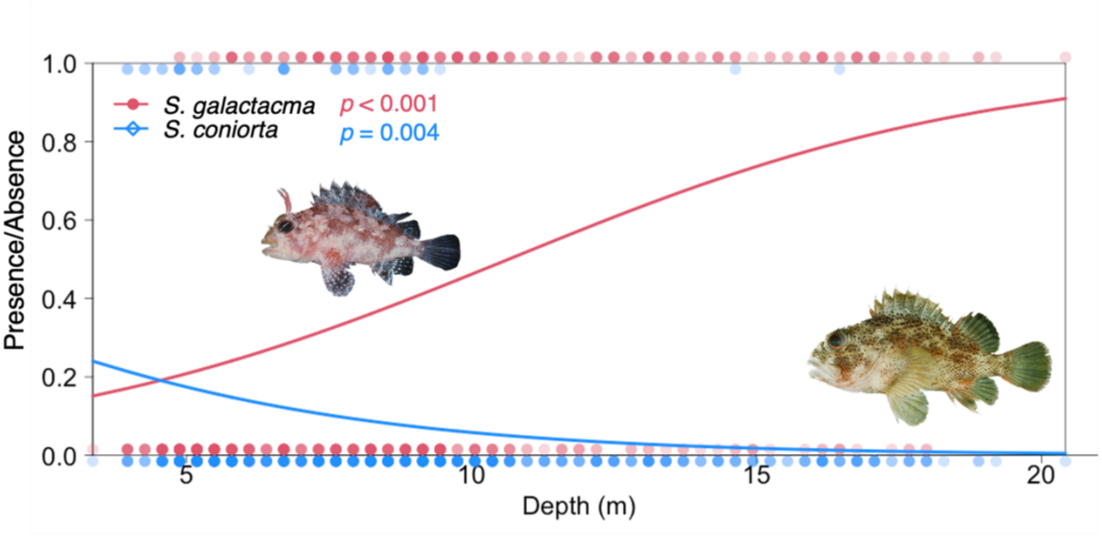

Figure 5. *Sebastapistes coniorta* (blue) and *S. galactacma* (red) presence (1.0) and absence (0.0) in *Pocillopora meandrina* colonies across the range of depths surveyed on O‘ahu, Hawai‘i.

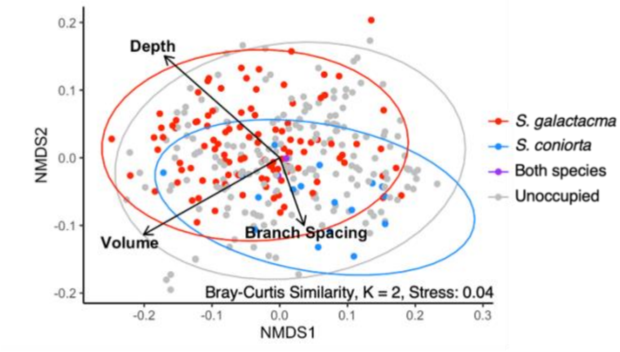

Figure 6. Non-metric multidimensional scaling ordination plot comparing the habitat characteristics of 319 *Pocillopora meandrina* colonies on O‘ahu, Hawai‘i inhabited by *Sebastapistes coniorta* alone, *S. galactacma* alone, both species, or neither species using a Bray-Curtis distance matrix. Ellipses represent 95% confidence intervals around the centroids of each group. Only two colonies were occupied by both species, both of which are located near the origin of the three variable vectors. An analysis of variance showed a significant difference among all groups (p = 0.008). Pairwise comparisons between each group found a significant difference only between colonies inhabited by *S. galactacma* alone and colonies unoccupied by both species (p = 0.02).

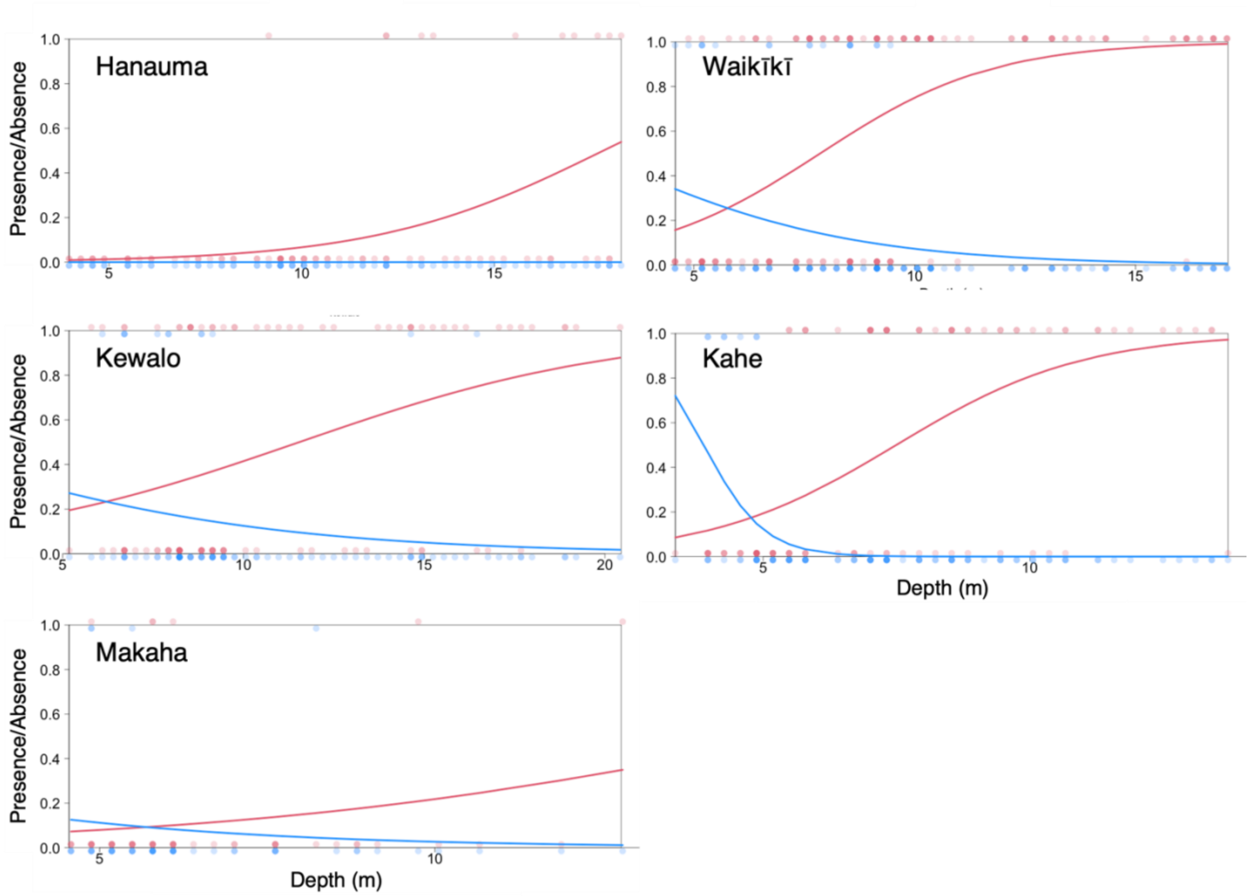

Figure 7. Presence (1.0) or absence (0.0) of *Sebastapistes galactacma* (red) and *S. coniorta* (blue) in *Pocillopora meandrina* colonies at Hanauma Bay, Waikīkī, Kewalo Basin, Kahe Point, and Makaha across depths surveyed at each site on O‘ahu, Hawai‘i. Both species were present at all sites except Hanauma Bay, where no *S. coniorta* were observed.

CHAPTER 3

Dietary Partitioning in Congeneric Hawaiian Scorpionfishes Revealed by DNA Metabarcoding

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Abstract

The Speckled Scorpionfish (*Sebastapistes coniorta*) and Galactic Scorpionfish (*S. galactacma*) are cryptobenthic reef mesopredators common throughout the Hawaiian Islands. Both species reside within the branches of cauliflower coral (*Pocillopora meandrina*) during the day and feed in and around their host corals at night. Despite their apparent ecological similarities, these species coexist in shallow reef areas, occasionally co-occupying the same host coral, yet Galactic Scorpionfish also occur in deeper areas in the absence of its congener. To determine the extent of dietary overlap, we documented the diets of each of these species in both the presence and absence of the other using a combination of visual diet analyses and DNA metabarcoding. Visually, we were able to identify prey in only 2% of specimens while metabarcoding identified prey to at least the family level in 81% of individuals. Based on molecular data, these species primarily consumed crabs (Domeciidae, Portunidae, and Xanthidae) and shrimps (Palaemonidae and Alpheidae). We found that the diets of these scorpionfishes had significantly different prey compositions and that they did not shift their diets when they occurred together vs. alone. Thus, coexistence of these species appears to be mediated by resource partitioning involving moderate overlap in both food and space. Alternatively, they simply may not presently compete. Further, we found some evidence that Galactic Scorpionfish have different diets in shallow vs. deep reef areas. This latter pattern suggests that prey availability may vary across depths, which could explain why these species occupy different though overlapping depth ranges.

Key Words

Diet, Hawaiʻi, interspecific competition, reef fishes, resource partitioning, *Sebastapistes*

3.1 Introduction

Coral reef fishes are the most diverse assemblages of vertebrates on Earth (Hixon and Randall 2019). Mechanisms allowing the coexistence of similar species inhabiting the same reef have focused on a variety of hypotheses (Jones 1991), and the effects of competition between species have received particular attention (Bonin et al. 2015).

Studies of freshwater fishes have demonstrated that partitioning of food resources can allow for ecologically similar species to coexist (Werner 1977). Dietary studies of reef fishes are more problematic. First, observing predation events directly can be challenging. Second, stomach content analyses of sufficient resolution can be difficult, especially in studies of small predatory fishes that consume even smaller prey (Longenecker 2007). Additionally, prey items are not always digested at the same rate. Prey species with hard body parts, like chitinous exoskeletons of crustaceans, are digested slower than fish prey (Beukers-Stewart and Jones 2004). Therefore, visual diet studies of small coral reef fishes that feed on both fishes and crustaceans are likely to overrepresent crustacean prey and underrepresent soft-bodied fishes.

DNA metabarcoding, the simultaneous identification of multiple species from a pooled sample of DNA, has become easier and more affordable through time (Valentini et al. 2009), can be used to identify prey from the stomachs of predators (Sheppard and Harwood 2005; de Sousa et al. 2019), and has been widely applied to describe the diets of reef fishes (Leray et al. 2015; Harms-Tuohy et al. 2016; Dahl et al. 2017; Brandl et al. 2020; Nalley et al. 2022; Coker et al. 2023). Unlike visual methods, which require prey items to be at least partially intact to detect identifiable characteristics, DNA can be recovered from heavily digested material in both the stomach and intestines and does not require extensive taxonomic expertise (Symondson 2002).

Stomachs that would be visually categorized as empty can still yield trace amounts of useable DNA, thus reducing the number of specimens required to attain a suitable number of samples.

Cauliflower coral (*Pocillopora meandrina*) is a common branching species on Hawaiian reefs that supports diverse communities of fishes and invertebrates (Counsell et al. 2018). Two of the most common species that inhabit this coral are the Speckled Scorpionfish (*Sebastapistes coniorta*) and Galactic Scorpionfish (*S. galactacma*). These nocturnal, cryptobenthic reef fishes shelter within the branches of cauliflower coral during the day and feed in and around their host colonies at night (Hobson 1974; Randall 2007). These species commonly cooccur within the same areas of reefs at shallow depths (5 – 10 m) and sometimes inhabit the same coral colonies, though Galactic Scorpionfish are also common deeper than 10 m, where Speckled Scorpionfish do not typically occur (Jones *submitted*). Despite their apparent ecological similarities, comparable habitat use, and overlapping within-reef distributions (Jones *submitted*), manipulative experiments were unable to detect present-day competition between these species (Jones et al. *in prep*). Three possible explanations for their coexistence are that these species (1) exhibit niche complementarity and partition food resources more than habitat (Werner 1977), (2) overlap only moderately in both space and diet (Schoener 1974), or (3) simply do not presently compete with each other.

Hobson (1974) described the diet of Speckled Scorpionfish as consisting mainly of xanthid crabs and caridean shrimps, many of which co-occur with this fish within the branches of cauliflower coral. Of the 34 individuals collected in that study, only one contained fish (species unspecified). While the diet of Galactic Scorpionfish has not been formally described, *Sebastapistes* spp. generally feed on various small crustaceans (crabs, shrimps, amphipods, and isopods) and fishes (Harmelin-Vivien and Bouchon 1976).

Here we describe the diets of both Speckled Scorpionfish and Galactic Scorpionfish using a combination of visual diet analyses and DNA metabarcoding of stomach and intestine contents. We used two molecular markers, one designed generally for marine metazoans (COI) and the other targeting crustaceans (16S), which were the dominant type of prey seen in the COI analysis. Specimens were collected from areas where only one species or the other occurred as well as from coral colonies inhabited by both species. This "natural experiment" design (Diamond 1978) allowed us to compare the diets of both species in the absence of the putative competitor to their diets where they co-occur. If these species are competing for food resources, then we predict that their diets will be constrained by competition when co-occurring to subsets of their possible diets and/or they will shift their diet to less preferred prey, in either case exhibiting diet partitioning. Alternatively, these species may have evolved to consume different food resources, regardless of whether or not they overlap spatially, potentially as a result of past competition (Connell 1980) or even because they never competed. Adult Speckled Scorpionfish are, on average, larger-bodied than adult Galactic Scorpionfish, and thus may have a wider range of prey due to gape limitations. Consequently, these species may have greater dietary overlap as juveniles when similarly sized. Finally, because differences in prey distributions may help explain the bathymetric segregation of reef fishes (Hixon 1980; Larson 1980), we compared the diets of Galactic Scorpionfish from different depths.

3.2 Materials and Methods

Specimen Collections

We collected 40 Speckled Scorpionfish and 60 Galactic Scorpionfish from reefs off Waikīkī Beach on the south shore of Oʻahu, Hawaiʻi, between September 2021 and July 2022.

Specimens were collected from cauliflower coral colonies occurring in clusters with at least three neighboring colonies within three meters. We identified coral clusters across our study site between 5 and 10 m deep that contained 1) only Speckled Scorpionfish, 2) only Galactic Scorpionfish, or 3) both species (20 clusters each). We sampled only clusters where the central colony contained at least one individual of whichever species were represented in that cluster. Additionally, we collected specimens from 20 coral clusters between 11 – 15 m deep containing only Galactic Scorpionfish.

Prior to collections, we surveyed each of the four coral colonies within each cluster for all fishes and macroinvertebrates. For each central colony, we documented physical characteristics (depth, volume, and interbranch spacing; see Jones *submitted* for methods). Finally, we measured the distance from the central colony to each of the three closest neighboring colonies.

For each coral cluster, we collected one individual of either Speckled Scorpionfish or Galactic Scorpionfish where each species occurred alone and one individual of each species where they inhabited the same colony. We collected fish using chopsticks to coerce individuals into small hand nets and then placed them in pre-labeled zip-top baggies. At the end of the dive, all fish were immediately placed on ice for approximately one hour and were then transferred to vials containing 70% ethanol and remained on ice until they were dissected in lab the same day. We weighed and measured all fish and removed the entire digestive tracts, which were stored in vials containing 100% ethanol in a freezer at -20 °C. Frozen stomachs were later thawed and carefully dissected to separate any contents from the stomach lining. Stomach contents that were potentially visually identifiable were weighed and photographed. All stomach and intestine contents were then transferred to vials containing 95% ethanol and stored in a freezer at -20 °C.

DNA Extraction and Metabarcoding

Total nucleic acids were extracted using the DNeasy Blood and Tissue Kit (Qiagen) on dissected stomach and intestine content samples (following the manufacturer's protocols) and eluted in 100 µL AE buffer. Eluted DNA was quantified using a Qubit fluorometer and dsDNA HS detection kit (Thermo Fisher Scientific). PCR was first performed using a universal primer set targeting marine metazoans: mlCOIintF/jgHCO2198 (Leray et al. 2013) which amplifies a 304-313 bp region of the mitochondrial cytochrome c oxidase subunit I (COI). Samples were also amplified using a second marker, Crust16S_F/Crust16S_R, targeting a 90-213 bp region of crustacean mitochondrial 16S rDNA (Berry et al. 2017). Reactions were amplified in 13 µL volumes consisting of 6.3 µL MyTaq 2x (Bioline), 0.65 µL BSA (Thermo Fisher Scientific, 20 mg/mL), 4.45 µL nuclease-free water (Growcells), 1.0 µL template DNA (9.9 ± 0.8 ng/µL SE), and 0.3 µL of each forward and reverse primers (10 µM). Thermal cycling parameters were as follows: denaturation for 2 min. at 95 °C, followed by a touchdown profile with 16 cycles of 95 °C for 30 s, annealing at 62 °C (decreasing 1 °C per cycle) for 30 s, and 72 °C for 30 s, followed by 25 cycles with an annealing temperature of 46 °C (COI) or 51 °C (16S), and ending with a 5 min. extension at 72 °C. The quality of all amplifications was assessed using gel electrophoresis, running samples and PCR negative controls through a 1.5% agarose gel stained with GelRed (Biotium), and visualized on an ultraviolet imaging platform. A clear single band at the expected length indicated amplification success, whereas multiple bands, or absence of a band indicated PCR failure. Failed amplifications were repeated and libraries were prepared for sequencing at the University of Hawaiʻi Microbial Genomics and Analytical Laboratory by purification and normalization of PCR products, followed by indexing using Nextera XT v2 (Illumina) adapters and an additional purification step. Amplicons were then quantified using a Qubit fluorometer and pooled equimolar amplicons (including no template controls) were then pair-end sequenced on an Illumina MiSeq platform using the V3 600-cycle reagent kit at the Advanced Studies in Genomics and Proteomics facility at the University of Hawaiʻi at Mānoa.

All laboratory surfaces and equipment were sterilized before and after sample processing using 10% bleach, followed by a 70% ethanol rinse. Amplification products (as well as negative controls) were prepared for sequencing in a post-PCR work area physically separated from pre-PCR areas (using dedicated post-PCR pipettors, plastics, and reagents) to prevent contamination.

Bioinformatics

After sequencing, a total of 10,241,506 demultiplexed COI reads (50,700 ± 3880 SE sequences from gut content extractions) and 7,067,006 demultiplexed 16S reads (45,814 ± 2773 SE) were processed by paired-end merging, followed by primer, adapter, and quality trimming in Geneious Prime v2023.0.1 (Biomatters, Ltd.). Next, 3,478,275 COI and 3,737,551 16S paired sequences were then processed using the R modular package for metabarcoding bioinformatics: Just Another Metabarcoding Pipeline (JAMP—https://github.com/Vasco Elbrecht/JAMP), which integrates USEARCH v11 (Edgar 2010), VSEARCH v2.23 (Rognes et al. 2016), and CutAdapt v4.4 (Martin 2011). More specifically, sequence lengths (CutAdapt) and any remaining low-quality sequences were filtered (UPARSE; fastq_filter with maxee = 0.25 and qmax = 60) and discarded (Edgar and Flyvbjerg 2015). A total of 968,450 COI and 292,722 16S sequences were then dereplicated (min. unique size = 2) and clustered with simultaneous de novo removal of chimeras using USEARCH (cluster_otus 97% identity). Singletons were discarded, leaving 178,798 COI and 82,134 16S dereplicated sequences to be matched to their respective clustered molecular operational taxonomic units (MOTUs) with a minimum match of 97% (USEARCH; usearch_global and strand plus).

We then used the R package, Informatic Sequence Classification Trees (INSECT), which implements a hidden Markov model approach to assign taxonomies against a classification tree built from reference COI and 16S sequences (Wilkinson et al. 2018). Finally, we cross-checked MOTU sequence classifications by querying against the National Centre for Biotechnology Information's (NCBI) GenBank nucleotide database (accessed October 21, 2022) using BLASTn with the following parameters: percent identity=99, query coverage=100, and E-value=1e-10. Only metazoan MOTUs were kept for downstream analysis and MOTUs assigned to the genus *Sebastapistes* (5 COI MOTUs) or class Mammalia (1 COI MOTU) were removed from the dataset (Appendix A : SM1). One metazoan MOTU was present in negative control sample (although not visible on the gel). The microDecon package (McKnight et al. 2019) was used to identify and remove contaminants from the final COI and 16S MOTU tables used in our analysis.

Statistical Analyses

Data were analyzed using R v4.2.3 (R Development Core Team, 2022). Diet compositions using all prey identified to at least phylum were plotted using non-metric multidimensional scaling (nMDS) with square-root transformed Bray-Curtis similarity matrices. Scorpionfish dietary overlap from shallow habitats where they potentially co-occur was tested with a PERMANOVA in the vegan package (Oksanen et al. 2017) using species, co-occurrence (together or alone), and body size (mm) as model predictors (~ Species * Cooccurrence * Size, permutations = 9999). For Galactic Scorpionfish occurring in both shallow and deep habitat, a PERMANOVA was implemented using depth (shallow vs. deep) and body size (mm) as predictors of dietary dissimilarities (~ Depth * Size, permutations = 9999).

Relative DNA reads resolved to taxonomic family were used to calculate indices of diet (richness, evenness, Shannon composite diversity H’) for each species of scorpionfish using the otuSummary package (Yang 2018). Schoener’s index (SI) of dietary overlap (Schoener 1971) between species was calculated with the dietOverlap function in the package FSAmisc (Ogle 2015) using the number of DNA reads from each family of prey for each scorpionfish species. Generalized linear mixed models (GLMs) were used to compare diversity metrics between species. Bipartite plots were constructed for scorpionfish species' dietary overlap using taxa that were matched to the family level (Dormann et al. 2008).

3.3 Results

The 40 collected Speckled Scorpionfish ranged in total length from 47 to 68 mm (μ ± SE = 56.2 ± 0.9 mm), and the 60 Galactic Scorpionfish ranged from 31 to 66 mm (μ = 42.7 ± 1.0 mm). Of the 100 stomachs dissected, 63 were visually categorized as empty (i.e., containing no solid or identifiable objects). Thirty-four stomachs contained unidentifiable crustacean body parts, mostly appendages, heads, or sections of exoskeleton. One stomach contained a small section of a fin ray but was otherwise empty. Only two stomachs, both from Speckled Scorpionfish, had contents visually identifiable to the species level. In both cases, the identifiable characteristic was a distinct banding pattern on the end of a palaemonid shrimp pereopod unique to the two-claw shrimp (*Brachycarpus biunguiculatus*).

DNA analysis of the stomach and intestine contents using the marker for marine metazoans (COI) produced a total of 1,743,482 reads assigned to 518 molecular operational taxonomic units (MOTUs) for both species, with 203 MOTUs being identified to at least the phylum level in 86 of the 100 specimens. We were able to assign MOTUs to the family level in 77 of the 100 specimens, and to the genus and species in 62 and 32 individuals, respectively (Table 1). After filtering out mammals (likely human contamination), *Sebastapistes* spp., and unidentifiable sequences, 535,966 reads from 203 MOTUs were used in our analysis. There was an average (± SE) of 2498 ± 1296 reads for Speckled Scorpionfish and 9240 ± 2992 reads for Galactic Scorpionfish. While Speckled Scorpionfish had a relatively low proportion of unidentified reads (4.2% at the family level), the majority of Galactic Scorpionfish reads could not be classified at the family level (59.9%; Fig. 1), most of which were identified to the phylum Arthropoda (95.6%).

A second analysis targeting only crustaceans (16S) generated a total of 3,474,993 reads that were assigned to 2,094 MOTUs, 1688 of which were assigned to at least the phylum level in 96 of 100 specimens. We were able to assign MOTUs to the family level in 81 individuals (562 MOTUs), to the genus in 73 individuals (55 MOTUs), and to the species in 64 individuals (20 MOTUs; Table 1). After filtering (see above), we had 3,365,142 reads from 1688 MOTUs, with an average (± SE) of 30,051 ± 7647 reads for Speckled Scorpionfish and 38,626 ± 8303 reads for Galactic Scorpionfish. Unlike the COI assay, using the 16S marker gave us similar proportions of DNA that was unidentified to the family level for both species, 88.9% of which was identified to the phylum Arthropoda.

The diets of both scorpionfishes largely comprised taxa that commonly co-occur with them in cauliflower coral (Table 2). The diet of Speckled Scorpionfish was primarily domeciid crabs (COI: 55.4%; 16S: 22.6%), alpheid shrimps (16S: 18.4%), and palaemonid shrimps (COI: 21.6%; 16S: 16.9%), while Galactic Scorpionfish mainly consumed alpheid shrimps (COI: 44.3%; 16S: 17.5%), palaemonid shrimps (COI: 26.3%; 16S: 14.4%), portunid crabs (16S: 24.3%), and xanthid crabs (COI: 22.1%; 16S: 9.6%; Fig. 2). The dietary overlap between these species calculated using the metazoan assay (SI = 27%) was less than what is generally considered ecologically relevant (SI > 60%, Wallace and Ramsey 1983), however, there was greater dietary overlap using the crustacean specific assay (SI = 82%). For marine metazoan prey, Speckled Scorpionfish and Galactic Scorpionfish individuals had similar Chao diet richness (4.92 ± 0.80 SE and 4.88 ± 0.71 SE, respectively), evenness (0.10 ± 0.02 SE and 0.09 ± 0.02 SE, respectively), and composite diversity (Shannon's H': 0.48 ± 0.07 SE and 0.50 ± 0.08 SE, respectively), and none of these metrics were statistically different between species (GLM: Richness/Evenness/Diversity ~ Species, p > 0.05; Appendix B: SM1a-c). Similarly, for crustacean prey, Speckled Scorpionfish and Galactic Scorpionfish individuals had similar Chao diet richness (30.2 ± 4.02 SE and 27.3 ± 5.68 SE, respectively), evenness (0.05 ± 0.007 SE and 0.06 ± 0.01 SE, respectively), and composite diversity (Shannon's H': 1.09 ± 0.13 SE and 0.83 ± 0.097 SE, respectively), none of which were statistically different between species (GLM: Richness/Evenness/Diversity ~ Species, p > 0.05; Appendix B: SM1d-f)

A PERMANOVA analysis of the composition of stomach contents in Speckled Scorpionfish and Galactic Scorpionfish showed that these species had significantly different diets generally using both the marine metazoan (Species: $F = 1.65$; df = 1; $P = 0.008$) and crustacean metabarcoding assay (Species: $F = 1.2$; df = 1; $P = 0.048$; Fig. 3). Diet composition was not significantly correlated with body size across species (Size: COI: $F = 1.04$; df = 1; $P = 0.37$; 16S: $F = 1.05$; df = 1; $P = 0.31$) and the effect of size did not differ between species

(Species × Size: COI: $F = 0.92$; df = 1; $P = 0.63$; 16S: $F = 0.90$; df = 1; $P = 0.84$). This analysis also showed that the difference between species in diet did not depend on whether the species occurred in the absence of its congener or co-occurred (Species × Cooccurrence: COI: $F = 0.78$; df = 1; $P = 0.90$; 16S: $F = 0.86$; df = 1; $P = 0.92$).

Separately, we examined the diets of Galactic Scorpionfish collected from deep reef areas (> 11 m) and shallow reef areas (5 – 10 m). Galactic Scorpionfish caught in shallow reef areas primarily consumed palaemonid shrimps (COI: 32.8%; 16S: 16.2%), alpheid shrimps (COI: 32.4%; 16S: 14.7%), xanthid crabs (COI: 28.1%; 16S: 4.1%), and portunid crabs (16S: 26.0%; Fig. 4). For the marine metazoan assay, the diet of deep specimens overwhelmingly consisted of alpheid shrimps (86.0%), but the crustacean assay revealed a more diverse diet consisting of xanthid crabs (40.6%), alpheid shrimps (33.4%), and portunid crabs (14.8%). A PERMANOVA analysis of diet composition using metazoan DNA found a significant difference between specimens collected from the two depth zones (Depth: $F = 1.30$; df = 1; $P = 0.042$; Fig. 5) and showed a significant effect of body size on diet composition (Size: $F = 1.28$; df = 1; $P = 0.036$). Smaller individuals (31 – 41 mm; n = 36) consumed mainly labrid fish recruits and rhynchocinetid shrimps, whereas larger specimens (54 – 66 mm; n = 5) tended to eat xanthid crabs and alpheid shrimps. In contrast, the crustacean specific assay did not find a significant difference in diet composition between depth zones (Depth: $F = 1.03$; df = 1; $P = 0.37$) or across body sizes (Size: $F = 1.14$; df = 1; $P = 0.10$).

For marine metazoan prey, Galactic Scorpionfish from deep and shallow habitats had diet compositions with similar richness (4.53 ± 1.3 and 4.87 ± 0.71, respectively), evenness (0.10 ± 0.03 and 0.09 ± 0.02, respectively), and composite diversity (Shannon's H': 0.38 ± 0.08 and 0.50 ± 0.08, respectively), and none of these metrics were significantly different between depth zones

(GLM; Richness/Evenness/Diversity ~ Depth, $P > 0.05$; Appendix B: SM2a-c). Similarly, for crustacean prey, the two depth zones had similar prey richness (deep: 16.42 ± 4.0; shallow: 27.3 ± 5.7), evenness (deep: 0.055 ± 0.016; shallow: 0.056 ± 0.01), and composite diversity (Shannon's H': deep: 0.60 ± 0.1; shallow: 0.83 ± 0.10), and there were no significant differences between depth zones for any of these metrics (GLM; Richness/Evenness/Diversity ~ Depth, $P > 0.05$; Appendix B: SM2d-f).

3.4 Discussion

This study documented significant differences between the diets of Speckled Scorpionfish and Galactic Scorpionfish, two congeneric coral-dwelling fishes that co-occur across the Hawaiian Islands. Previous studies have shown that these species also exhibit moderate overlap in habitat use, co-occurring in 33.3% of reef areas (n = 27) and 10.8% of cauliflower coral colonies (n = 65) surveyed between five and 10 m deep at this same study site (Jones *submitted*). Despite overlap in their use of both food and space, these species presently do not compete to a level that limits local abundance over the course of a year (Jones et al. *in prep*). Therefore, moderate differences in how these species use both resources (Schoener 1974) apparently provides sufficient resource partitioning for these species to coexist in the same reef habitats. Alternatively, these species simply may not presently compete with each other.

By using a combination of DNA barcoding assays, one generally targeting marine metazoans (COI) and one specifically targeting crustaceans (16S), we generated a more robust description of the diets of these two species. Universal COI primers (Leray et al. 2013) captured both invertebrate and fish DNA, while the crustacean specific 16S primers (Berry et al. 2017) gave a greater number of prey detections of invertebrates, which appeared to be the primary prey recovered in the COI assay. The large discrepancy in the proportion of unknown DNA between species observed in the COI analysis was not present in the 16S analysis. It is unclear, however, whether the 16S analysis provided more resolution for the unknown Galactic Scorpionfish DNA or whether the unknown DNA was from non-crustacean prey. If the latter, and the unknown DNA was from prey that was not consumed by Speckled Scorpionfish, then the actual overlap in these species' diets may be even less than what was detected in this study.

Both analyses of metazoan and crustacean prey revealed significant differences in the diet compositions between these species, however, the metazoan analysis indicated there was only moderate dietary overlap (SI = 27%) while the crustacean specific diet analysis showed much greater overlap (SI = 82%). Traditional measures of overlap, such as Schoener's Index, may be less informative than multivariate analyses that account for more than just predator species groups and use all prey identified from phylum to species level, not just those identified to family. Additionally, the threshold for ecological significance for Schoener's Index may be somewhat arbitrary (Mabragaña and Giberto 2007).

The diets of both Speckled Scorpionfish and Galactic Scorpionfish were primarily composed of taxa that also inhabited cauliflower coral colonies (e.g., Alpheidae, Domeciidae, Palaemonidae, and Xanthidae). Both species consumed similar proportions of palaemonid shrimps, which are very common in their cauliflower coral habitat (RNJ personal observation). Overall, these species diets differed significantly, with the diets of Speckled Scorpionfish being comprised mainly of domeciid crabs, while Galactic Scorpionfish largely consumed portunid crabs, xanthid crabs, and alpheid shrimps. In the COI assays, some fish DNA (Labridae: *Thalassoma* spp. and *Pseudocheilinus tetrataenia*) was recovered from Speckled Scorpionfish stomachs and from Galactic Scorpionfish collected in deeper reef areas. Recruit and juvenile

Saddle Wrasse (*Thalassoma duperrey*) and Four-Lined Wrasse (*Pseudocheilinus tetrataenia*) regularly hide within the branches of cauliflower coral (Counsell et al. 2018) and may be opportunistic prey of resident scorpionfishes. Although Harmelin-Vivien and Bouchon (1976) documented that piscivorous tendencies increased with increasing body size in the Barchin Scorpionfish (*Sebastapistes strongia*), we found that small Galactic Scorpionfish consumed some fishes while larger individuals did not.

Additionally, we found that the diets of Speckled Scorpionfish and Galactic Scorpionfish were not significantly different comparing areas where they co-occurred with one another vs. areas where each occurred alone, suggesting that these species may have evolved to consume different prey rather than actively shifting their diets over ecological time scales. This evolution of diet may or may not have resulted from past competition between these two species (Connell 1980).

Finally, we found some evidence with the metazoan prey assays that Galactic Scorpionfish may consume different prey in shallow reef areas than in deep reef areas. This pattern may indicate differences in prey availability across depths, which could explain the bathymetric segregation of Speckled Scorpionfish and Galactic Scorpionfish if the preferred prey of Speckled Scorpionfish do not occur or are rare in deep reef areas. Surveys of the resident communities associated with cauliflower corals support this possibility because coresident prey families of Speckled Scorpionfish tended to be less common (Palaemonidae) or absent (Domeciidae and Portunidae) in deeper colonies while coresident prey of Galactic Scorpionfish were found across all depths (Alpheidae). These differences, however, were not seen in the crustacean specific analyses.

Diet studies are crucial for understanding the ecological roles of species and can help predict how species will react and adapt to changing environmental conditions (Bellwood et al. 2004). Coral-associated species, such as the Speckled Scorpionfish and Galactic Scorpionfish, will be some of the first to be negatively affected by coral habitat loss. Small coral-reef fishes often act as an important trophodynamic link between reef cryptofauna and larger species (Brandl et al. 2018; Mihalitsis et al. 2022), yet detailed descriptions of these species' diets can be challenging (Longenecker 2007). This constraint often results in guilds of apparently similar species being grouped together as functionally redundant for management purposes, which may not reflect reality. As demonstrated here, novel DNA barcoding methods provide a means of documenting the gut contents of fishes, an especially valuable approach when gut contents cannot be identified visually.

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3.6 Tables

Table 1. Number of individual fish gut contents successfully sequenced and identified to taxonomic ranks using the COI (marine metazoans) and 16S (crustaceans) metabarcoding assays for Speckled Scorpionfish and Galactic Scorpionfish. The total number of detected MOTUs (percent of total MOTUs detected), filtered MOTUs removing *Sebastapistes* spp., mammals, and those that could not be identified to phylum (percent of total filtered MOTUs), and mean MOTU richness (± SE), evenness, and composite diversity (Shannon H') are also given for the COI and 16S assays.

Assay	**Species**	**Ident. Phylum**	**Ident. Class**	**Ident. Order**	**Ident. Family**	**Ident. Genus**	**Ident. Species**	**Total MOTUs**	**Filtered MOTUs**	**Richness**	**Evenness**	**Composite Diversity**
COI	Galactic	48	47	46	43	34	17	359 (69%)	148 (73%)	4.88 (0.71)	0.09 (0.02)	0.50 (0.08)
	Speckled	38	37	36	34	28	15	232 (45%)	101 (50%)	4.92 (0.80)	0.10 (0.02)	0.48 (0.07)
16S	Galactic	56	55	55	43	36	34	1320 (63%)	1041 (62%)	27.3 (5.68)	0.06 (0.01)	0.83 (0.097)
	Speckled	40	40	40	38	37	30	1054 (50%)	803 (48%)	30.2 (4.02)	0.05 (0.007)	1.09 (0.13)

Table 2. Taxa of fishes and invertebrates visually documented in cauliflower coral colonies and clusters from which Speckled Scorpionfish and Galactic Scorpionfish were collected. Families with superscript letters matched DNA found in the stomachs of Speckled Scorpionfish (S) or Galactic Scorpionfish (G).

		Percentage of central colonies occupied (all colonies in cluster)			
		Shallow (5 - 10 m)			**Deep (11 - 15 m)**
Family	**Species**	**Both**	**Speckled Only**	**Galactic Only**	**Galactic**
Mollusks					
Muricidae	*Quoyula monodonta*	20% (13%)	10% (8%)	30% (20%)	15% (20%)
Crustaceans					
Alpheidae^{S,G}	*Alpheus lottini*	80% (51%)	55% (34%)	75% (54%)	80% (76%)
Diogenidae^{G}	Hermit Crabs	0% (6%)	15% (10%)	5% (5%)	0% (8%)
Domeciidae^{S,G}	*Domecia hispida*	0% (1%)	5% (4%)	15% (6%)	0% (4%)
Palaemonidae^{S,G}	*Harpiliopsis depressa*	85% (64%)	80% (60%)	95% (66%)	30% (24%)
Portunidae^{S,G}	*Goniosupradens hawaiensis*	5% (4%)	0% (0%)	0% (0%)	0% (1%)
Thoridae^{S,G}	*Saron* spp.	15% (11%)	10% (9%)	25% (13%)	5% (5%)
Trapeziidae^{S,G}	*Trapezia bidentata*	55% (36%)	65% (38%)	40% (33%)	30% (25%)
-	*Trapezia digitalis*	30% (26%)	50% (41%)	35% (44%)	0% (0%)
-	*Trapezia flavopunctata*	30% (28%)	80% (40%)	15% (25%)	0% (4%)
-	*Trapezia intermedia*	55% (58%)	55% (49%)	65% (64%)	60% (56%)
-	*Trapezia tigrina*	65% (50%)	50% (35%)	80% (56%)	70% (79%)
Xanthidae^{S,G}	*Pseudoliomera speciosa*	5% (6%)	10% (4%)	5% (3%)	0% (0%)
Echinoderms					
Ophiocomidae	*Breviturma pica*	80% (61%)	55% (43%)	75% (60%)	90% (86%)
-	*Ophiocoma erinaceus*	0% (0%)	0% (0%)	5% (4%)	15% (18%)
Fishes					
Acanthuridae	*Acanthurus nigrofuscus*	0% (0%)	5% (1%)	0% (0%)	0% (0%)
Blenniidae	*Exallias brevis*	5% (4%)	10% (5%)	5% (3%)	10% (3%)
Cirrhitidae	*Amblycirrhitus bimacula*	30% (21%)	45% (30%)	25% (19%)	0% (0%)
-	*Cirrhitops fasciatus*	0% (1%)	0% (0%)	0% (0%)	5% (1%)
-	*Paracirrhites arcatus*	15% (16%)	25% (10%)	5% (16%)	30% (33%)
Labridae^{S,G}	*Pseudocheilinus tetrataenia*	15% (14%)	15% (9%)	5% (5%)	20% (25%)
-	*Thalassoma duperrey*	5% (4%)	5% (3%)	5% (1%)	0% (0%)
Pomacentridae	*Dascyllus albisella*	0% (0%)	5% (4%)	0% (0%)	0% (0%)
-	*Plectroglyphidodon johnstonianus*	5% (9%)	20% (9%)	0% (5%)	5% (1%)
Scorpaenidae	*Caracanthus typicus*	25% (21%)	50% (31%)	15% (26%)	5% (1%)

3.7 Figures

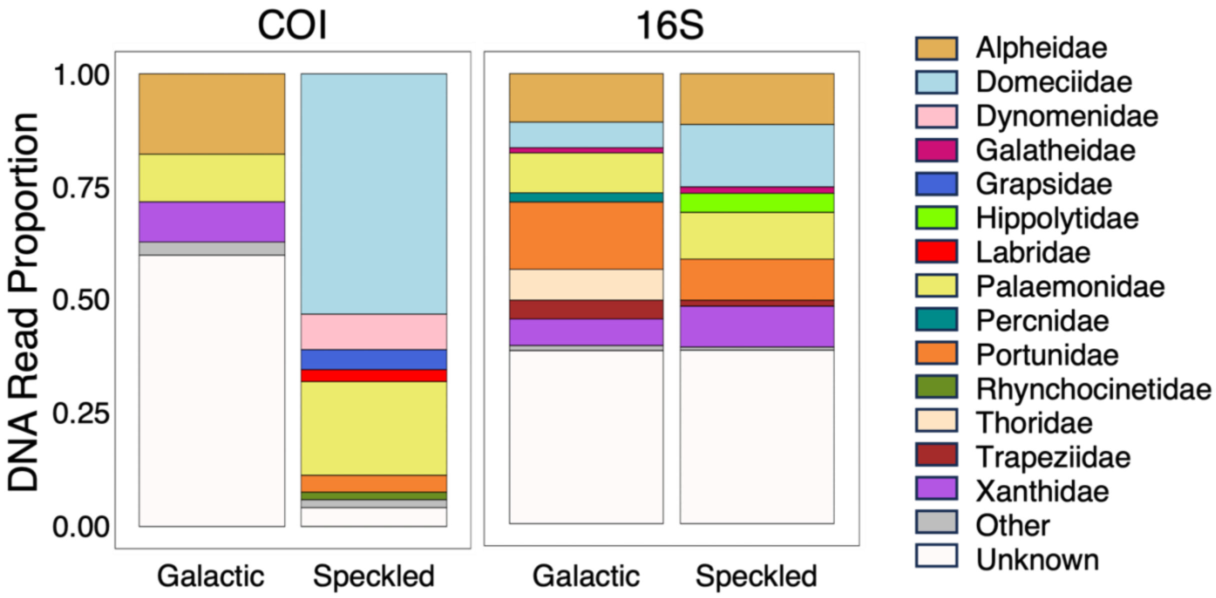

Figure 1. Relative DNA read proportions from stomach contents of Speckled Scorpionfish and Galactic Scorpionfish resolved to taxonomic family using COI (marine metazoans) and 16S (crustaceans) metabarcoding assays. DNA read proportions <0.01 were grouped as "Other" (Appendix A: SM2). DNA reads that could not be classified to the family level are marked as "Unknown". In total, there were 1,743,482 reads assigned to 518 MOTUs for the COI assay and 3,474,993 reads assigned to 2,094 MOTUs for the 16S assay.

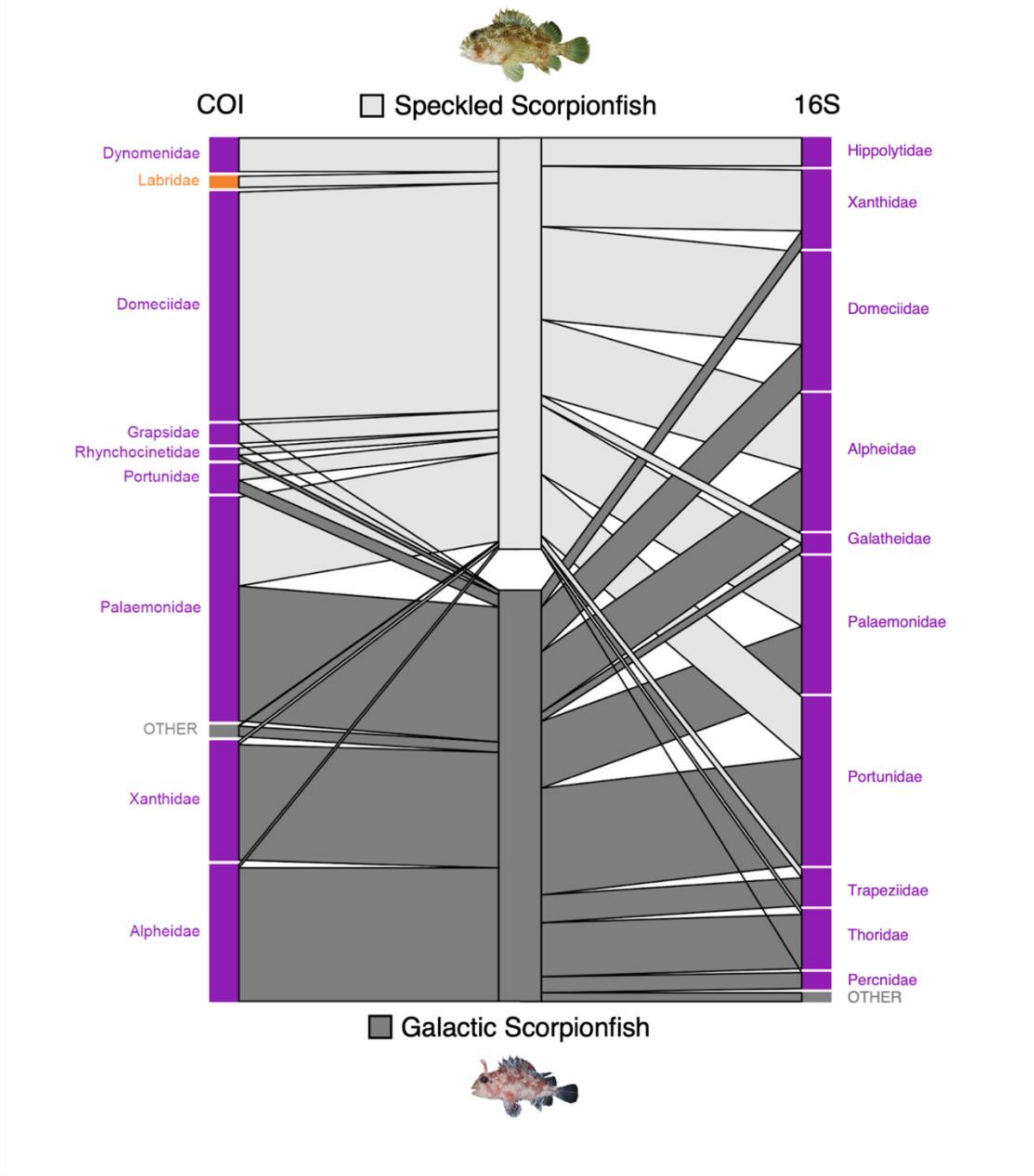

Figure 2. Bipartite plot of the diets of the Speckled Scorpionfish and Galactic Scorpionfish that could be resolved to taxonomic family using COI (marine metazoans; left) and 16S (crustaceans; right) metabarcoding assays (crustacean prey: purple; fish prey: orange). Width of connecting bars reflects the relative number of reads identified to prey family for each species. Rare taxa were grouped as "OTHER" (Appendix A: SM3).

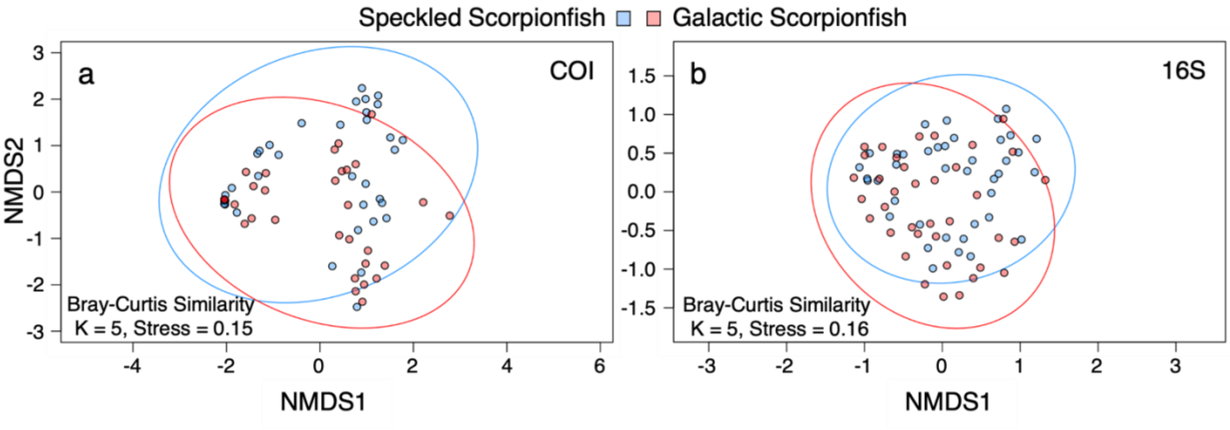

Figure 3. Non-metric multidimensional scaling ordination plot comparing the diets of Speckled Scorpionfish (blue) and Galactic Scorpionfish (red) off Waikīkī Beach on the south shore of Oʻahu, Hawaiʻi, using a Bray-Curtis distance matrix for **a** COI (marine metazoans; stress = 0.15) and **b** 16S (crustaceans; stress = 0.16). Ellipses represent 95% confidence intervals. An analysis of variance (PERMANOVA) showed a significant difference in dietary composition between species using both COI (p = 0.008) and 16S (p = 0.048) assays.

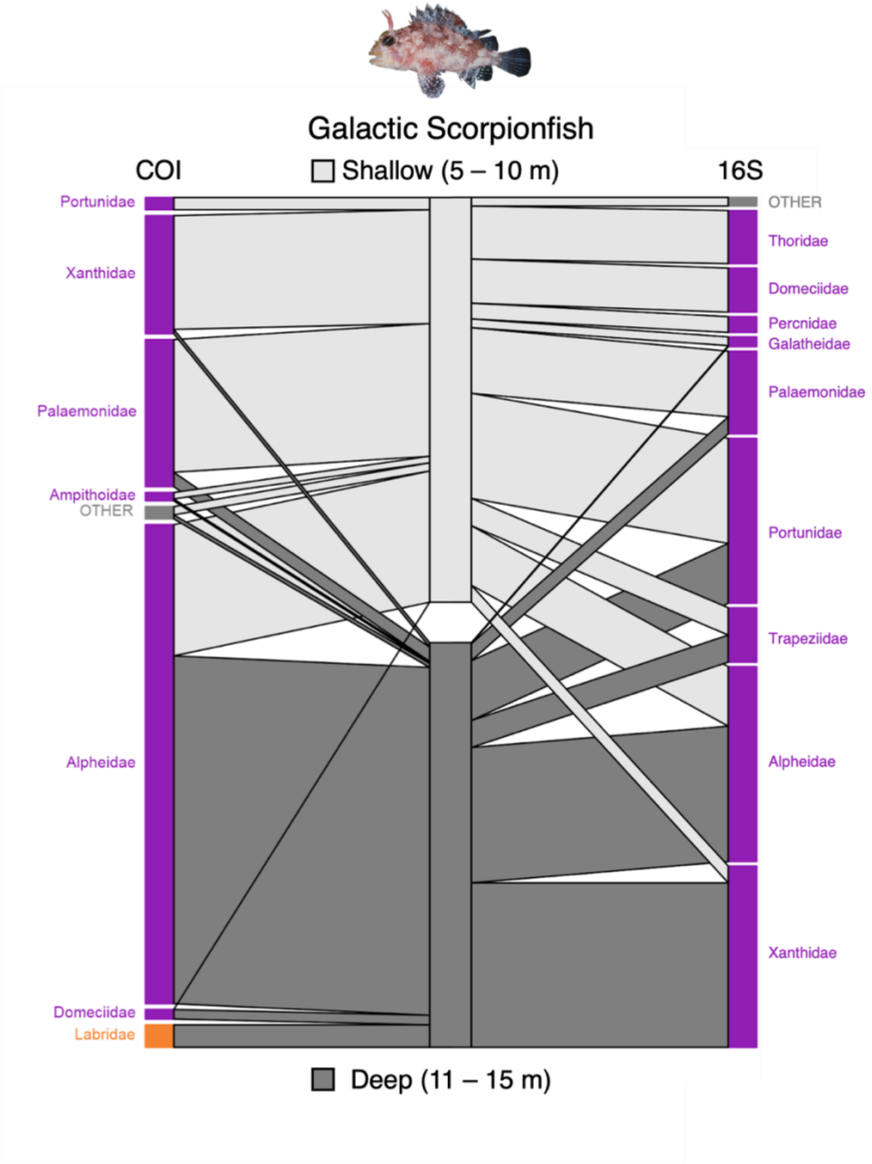

Figure 4. Bipartite plot of the diet of Galactic Scorpionfish collected from shallow reef areas (5 – 10 m depth) and deep reef areas (11 – 15 m depth) that were identified to taxonomic family using COI (marine metazoans; left) and 16S (crustaceans; right) DNA analysis (crustacean prey: purple; fish prey: orange). Width of connecting bars reflects the relative number of reads identified to prey family for each species. Rare taxa were grouped as "OTHER" (Appendix A: SM4).

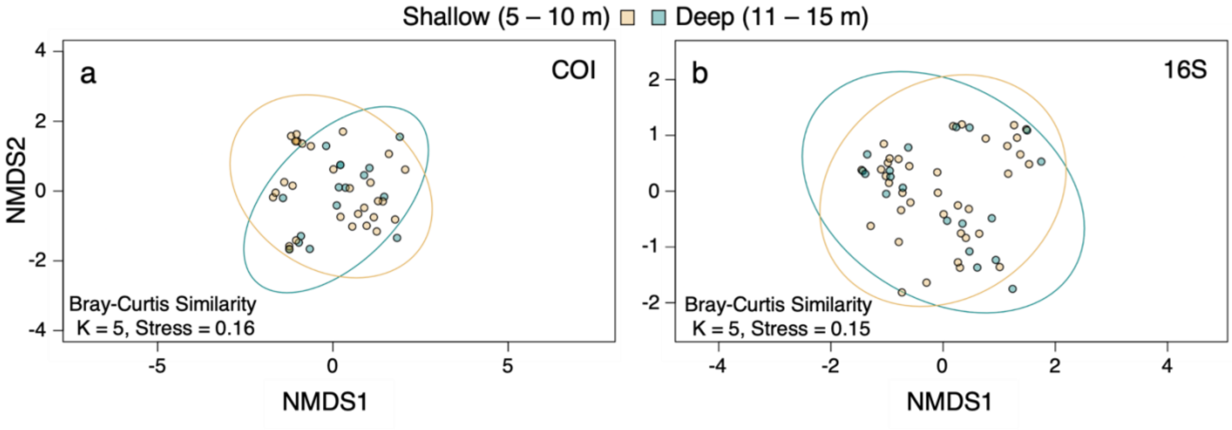

Figure 5. Non-metric multidimensional scaling ordination plot comparing the diets of Galactic Scorpionfish collected from shallow reef areas (5 – 10 m depth; yellow) and deep reef areas (11 – 15 m depth; green) off of Waikīkī Beach on the south shore of O‘ahu, Hawai‘i, using a Bray-Curtis distance matrix for **a** COI (marine metazoans; stress = 0.16) and **b** 16S (crustaceans; stress = 0.15). Ellipses represent 95% confidence intervals. An analysis of variance (PERMANOVA) showed a significant difference in dietary composition between fishes collected from the two depth zones using the COI assay (p = 0.04), however, this difference was not seen in the 16S assay (p = 0.37).

CHAPTER 4

Experimental Evidence That Coral-Dwelling Scorpionfishes Are Not Presently Limited by Competition

Ryan N. Jones, Erik G. Brush, and Mark A. Hixon

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Abstract

Competition is an important ecological interaction that can structure communities and may be especially intense for ecologically similar species. Throughout Hawaiʻi, Speckled Scorpionfish (*Sebastapistes coniorta*) and Galactic Scorpionfish (*S. galactacma*) shelter within host-coral branches of cauliflower coral (*Pocillopora meandrina*) during the day and feed in and around these corals at night. Because these species are closely related, comparably sized, appear ecologically similar, and co-occur in shallow reef areas (Galactic Scorpionfish occurring alone at deeper depths), we hypothesized that they compete for resources, thereby limiting local distribution and abundance. We used manipulative field experiments to document how each species affected the other across depths, among clusters of corals, and within individual colonies. We found that Speckled Scorpionfish did not expand their distribution to deeper reefs when Galactic Scorpionfish were removed. A recolonization experiment showed that corals were commonly reoccupied by the same scorpionfish species that occurred before removals. Finally, reciprocal removals at depths where both species occurred did not increase the local abundance of the remaining species. These findings indicate that competition for habitat is not presently occurring between Speckled Scorpionfish and Galactic Scorpionfish in Hawaiʻi, suggesting that local populations are structured more by coral habitat characteristics and/or larval supply.

Key Words

Coral reef fish, habitat use, Hawaiʻi, interspecific competition, *Pocillopora,* Scorpaenidae, *Sebastapistes*

4.1 Introduction

Interspecific competition is a major ecological process that can structure communities (Schoener 1983) and may be strongest among organisms of comparable sizes with similar ecological roles (Schoener 1974). Competition between species can be inferred observationally by comparing resource use between populations that naturally exist in both the presence and absence of a presumed competitor, assuming all other factors are the same (Diamond 1978). However, such "natural experiments" alone are insufficient for demonstrating competition. The more rigorous experimental approach is to remove a putative competitor from an area and follow the local abundance and/or niche expansion of the other putative competitor (Connell 1975). Reciprocal removals of each potentially competing species can help distinguish the fundamental niche of each species from the realized niche in the presence of a competitor (Colwell and Fuentes 1975).

Interspecific competition can be especially intense among coral-reef fishes (review by Bonin et al. 2015). Competition has been shown to limit local abundances of reef fishes via demographic density dependence (review by Hixon and Webster 2002) due to increased mortality or reduced fitness (e.g., growth rates, health, fecundity) resulting from decreased access to resources (Bonin et al. 2015). While predation is often the proximate agent of mortality, competition may be the ultimate cause (Hixon and Jones 2005). Further, reef fishes with similar ecological needs have been shown to segregate themselves spatially, typically by reef zone (Munday et al. 1997). Nonetheless, there is a lack of experimental evidence directly showing competition driving observed instances of bathymetric segregation among coral-reef fishes, though competition leading to segregation by depth has been demonstrated in congeneric species pairs of temperate fishes (Hixon 1980, Larson 1980).

Coral reefs in Hawaiʻi largely lack the diversity and structural complexity of other Pacific reefs (Grigg 1983). Consequently, refuge habitat may be in short supply for many Hawaiian reef fishes. The small, branching cauliflower coral (*Pocillopora meandrina*) is common throughout the Hawaiian Islands and supports a diverse community of fishes and invertebrates within its branches (Counsell et al. 2018). Two species that almost exclusively inhabit cauliflower coral are the Speckled Scorpionfish (*Sebastapistes coniorta*) and Galactic Scorpionfish (*S. galactacma*), named for their dark speckled patterns and milky white splotches, respectively. These nocturnal, cryptobenthic reef fishes occupy cauliflower coral colonies during the day and feed on various invertebrates and fishes in and around their host corals at night (Hobson 1974).

While Galactic Scorpionfish are more common in deeper reef areas (10 – 17 m) and Speckled Scorpionfish do not typically occur deeper than 10 m, there is a zone of overlap between approximately 4 to 10 m depth where both species occur (Appendix C: SM1). Within this depth range, these species frequently cooccur within the same general areas (i.e., adjacent coral colonies) and occasionally co-occupy the same individual coral colonies (Jones *submitted*). Given the apparent ecological similarities between these two species, competition for limited food and/or space may be strong in areas where these species cooccur (Schoener 1974). Alternatively, competition may be occurring at a very slow rate or not at all, especially if populations are maintained at low levels due to recruitment limitation (Doherty 1983), which has been suggested for the isolated archipelago of Hawaiʻi (Walsh 1987).

The goal of this study was to test experimentally whether competition between Speckled Scorpionfish and Galactic Scorpionfish was occurring on reefs in Hawaiʻi by examining what effects each species had on its putative competitor. We used manipulative field experiments to explore the effects of habitat preference, priority effects, and competition on the local distribution and abundance of each *Sebastapistes* species.

The first experiment involved a pulse removal of all *Sebastapistes* from clusters of cauliflower coral in both the shallow zone of overlap and in deeper reef areas where only Galactic Scorpionfish occur. If Speckled Scorpionfish expanded its depth distribution into deeper reef areas following the removal of Galactic Scorpionfish, then this niche expansion would be evidence for competitive exclusion. Additionally, if the composition of *Sebastapistes* spp. within a colony was determined by a random lottery (Sale 1978) and/or priority effects (Almany 2003), then the composition of *Sebastapistes* spp. that recolonized denuded cauliflower coral colonies would be unrelated to the composition prior to removals. Alternatively, if each colony possessed certain characteristics that each species of *Sebastapistes* selected when recruiting, then the composition of scorpionfishes following recolonization would be similar to the composition before removals.

The second experiment involved a press reciprocal removal of each species within the shallow zone of overlap. If Speckled Scorpionfish and Galactic Scorpionfish did compete for resources to a level that limited local abundance, then the removal of one species from an area would lead to an increase in the local abundance of the remaining species. Further, due to Speckled Scorpionfish's larger body size (Jones *submitted*), we predicted that Speckled Scorpionfish would be competitively dominant over Galactic Scorpionfish and would have a weaker response than Galactic Scorpionfish to the removal of its putative competitor. Because the foraging ranges of these species are apparently quite limited yet undocumented, removal experiments were conducted twice to test for competition at two spatial scales: 1) clusters of adjacent cauliflower coral colonies and 2) within individual colonies.

4.2 Materials and Methods

Study Site

This study was conducted across approximately 10 ha of spur-and-groove reef roughly 800 m offshore of Waikīkī Beach on the south shore of Oʻahu, Hawaiʻi (21°16'10"N, 157°50'15"W; Appendix C: SM2). Reefs at this location had sparse live coral coverage (~8%; Franklin et al. 2013) largely dominated by scattered pocilloporid coral colonies with sand-filled channels between reefs.

Recolonization Experiment

Patterns of *Sebastapistes* spp. recolonization of cauliflower coral were tested with a pulse removal experiment among 12 clusters of closely positioned cauliflower coral colonies (4 colonies each) in both shallow (5 – 8 m; n = 6) and deep (11 – 15 m; n = 6) zones. Shallow clusters were inhabited by a mixture of Speckled Scorpionfish and Galactic Scorpionfish (Fig. 1) while deep clusters were occupied by only Galactic Scorpionfish, as Speckled Scorpionfish rarely occur at these depths (Jones *submitted*). Clusters were randomly assigned to either control (i.e., no removals; n = 3 at each depth) or manipulated groups (n = 3 at each depth), which underwent a pulse removal of all *Sebastapistes* spp. Shallow control clusters were shared with a simultaneous press removal experiment (see below). Before manipulations, all coral colonies within clusters were surveyed weekly eight times between May and August 2019 (μ ± SE = 70.4 ± 1.5 days). During surveys, we identified, counted, and visually estimated the sizes of all resident fishes (total length). Redundant surveys were used to validate size estimates and abundance counts, though the complex morhphology of the colonies may have obscured some individuals.

Removals of all *Sebastapistes* within half the clusters occurred at the end of July immediately following the eighth round of surveys, coinciding with the seasonal peak of reef fish recruitment in Hawaiʻi (Walsh 1987), using a non-lethal, low-concentration mixture of clove oil (<7%) and seawater, and chopsticks to gently prod the fish into small hand nets (DAR SAP No. 2019-45). Control groups were not exposed to clove oil. Removed individuals were transported at least 1.5 km away, across two large sand channels, and released over another reef. There was no evidence of removed fish returning. We revisited each treatment colony at least once before the next round of surveys to remove any individuals we were unable to capture during initial removals. The first of eight weekly post-removal surveys began in August 2019 and continued until October. We compared the compositions of *Sebastapistes* spp. within cauliflower coral colonies immediately before removals to the compositions after recolonization using Fisher's exact probability test. All data were processed in the statistical analysis program R (R Core Team 2023).

Competition Experiment – Cluster Scale

Concurrent to the recolonization experiment, we conducted a reciprocal press removal of Speckled Scorpionfish and Galactic Scorpionfish using six additional shallow clusters of cauliflower coral and the same six shallow control clusters used in the recolonization experiment. Removal clusters were divided into two groups: Speckled Scorpionfish removals (n = 3) and Galactic Scorpionfish removals (n = 3). Colony survey methods and schedules were the same as in the recolonization experiment. However, removals in this experiment were maintained for the entire period following initial manipulation and the few colonizers of the excluded species to experimental clusters were removed following each survey.

Competition Experiment – Colony Scale

In the summer of 2020, we conducted a second reciprocal press removal experiment focusing on individual cauliflower coral colonies that were naturally inhabited by both Speckled Scorpionfish and Galactic Scorpionfish. The 21 colonies identified were equally divided into three treatments: controls, Speckled Scorpionfish removals, and Galactic Scorpionfish removals (n = 7 colonies each). Two rounds of initial surveys were conducted over two weeks from the end of May to the beginning of June. Removals in manipulated groups were conducted immediately following the second survey. Both surveys and removals used the same methods as the cluster-scale reciprocal removal experiment. Post-removal surveys were conducted every two weeks for one year following initial removals (26 surveys). The few individuals of the excluded species that recolonized experimental colonies were removed whenever observed.

Statistical Analyses for Competition Experiments

Both reciprocal removal experiments were analyzed using a Before-After-Control-Impact (BACI) framework (Smith 2002). Scorpionfish abundances were modeled with generalized linear mixed-effect models using the *glmer* function from the R package *lme4* (Bates et al. 2015). Models for both experiments used a Poisson error distribution and included treatment, before/after manipulation, and the interaction of the two as fixed effects, and survey number, survey date, and cluster/colony ID (to account for repeated measures) as random effects. Models for the cluster scale experiment included additional random effects for reef area (to account for similarities among experimental units that occurred on within similar areas) and for the number of scorpionfish within each of the four colonies in the cluster (to account for variation within an experimental unit).

4.3 Results

Recolonization Experiment

In total, we removed 104 Galactic Scorpionfish from deep treatment clusters during the pulse removal effort and 36 new individuals had recolonized by the end of the experiment. In shallow clusters, we removed 11 Speckled Scorpionfish and 51 Galactic Scorpionfish. Five new Speckled Scorpionfish, all of adult size, and 23 Galactic Scorpionfish, mostly of adult size, recolonized removal clusters by the final survey.

In both deep and shallow areas, clusters were recolonized by new individuals of the same species that occurred before removals. Clusters of coral colonies in deep reef areas were occupied by only Galactic Scorpionfish before removals (x̄ ± SE = 19.7 ± 1.9 per cluster) and were recolonized by only Galactic Scorpionfish following removals (12 ± 1.5 per cluster; Appendix C: SM3b). Shallow clusters were occupied by both Speckled Scorpionfish (2.7 ± 0.3 per cluster) and Galactic Scorpionfish (10.7 ± 10.8 per cluster) before removals and were recolonized by both species in similar ratios after removals (Speckled: 1.7 ± 0.7 per cluster; Galactic: 7.7 ± 4.2 per cluster; Appendix C: SM3a).

An analysis of the composition of scorpionfish species within individual cauliflower coral colonies in shallow clusters revealed that colonies were frequently recolonized by new individuals of the same species, or combination of species, that occupied those colonies before removals (Fig. 2). The frequency of each combination of scorpionfish species did not differ significantly before removals and after recolonization (Fisher's Exact Test: $p = 0.89$). Of the twelve manipulated colonies, eight were recolonized by the same species (66.7%), one colony was recolonized by only one of the two species that occurred before removals (8.3%), one unoccupied colony was colonized by Speckled Scorpionfish (8.3%), and two previously occupied colonies were not recolonized (16.7%).

Competition Experiment – Cluster Scale

The local abundances of both the Speckled Scorpionfish and the Galactic Scorpionfish were relatively constant throughout the experiment and were unaffected by the removal of their putative competitor (Fig. 3a, b).

Following the removal of Speckled Scorpionfish from manipulated clusters, the change in local abundance of Galactic Scorpionfish did not significantly differ between removal and control clusters over the same period (treatment × before/after manipulation: $p = 0.48$; $\chi^2 = 0.50$; $df = 1$; Fig. 3a). Eleven Speckled Scorpionfish were taken from removal clusters during the initial manipulation (3.7 ± 1.5 per cluster) and two more individuals that recolonized were removed following later surveys. In manipulated clusters, there was a model-estimated mean of 10.7 ± 1.4 Galactic Scorpionfish per cluster before removals and 10.4 ± 1.4 following removals. Control clusters had a model-estimated mean of 5.6 ± 1.3 Galactic Scorpionfish per cluster during the pre-removal period and 6.2 ± 1.4 during the post-removal period. Despite the random allocation of clusters into treatment groups, Galactic Scorpionfish abundance was significantly greater in removal clusters throughout the experiment (treatment: $p = 0.02$; $\chi^2 = 5.8$; $df = 1$).

In clusters where Galactic Scorpionfish were removed, there was no significant change in the local abundance of Speckled Scorpionfish relative to control clusters over the same period (treatment × before/after manipulation: $p = 0.58$; $\chi^2 = 0.31$; df = 1; Fig. 3b). A total of 23 Galactic Scorpionfish were captured during initial removals (7.7 ± 1.8 per cluster) and 25 were removed following later surveys (1.1 ± 0.20 per cluster per week). In removal clusters, there was a model-estimated mean of 2.7 ± 1.4 Speckled Scorpionfish per cluster before removals and 2.4 ± 1.5 after. Control colonies had a model-estimated mean of 3.8 ± 1.4 Speckled Scorpionfish per cluster during the pre-removal period and 2.9 ± 1.4 during the post-removal period.

Competition Experiment – Colony Scale

At the beginning of the experiment, all colonies contained at least one individual of each scorpionfish species, which occurred in similar abundances (Speckled: 2.0 ± 0.2 per colony; Galactic: 2.1 ± 0.2 per colony). We initially removed 15 Speckled Scorpionfish and 16 Galactic Scorpionfish from their respective treatment groups. An additional 10 Speckled Scorpionfish and 15 Galactic Scorpionfish were removed throughout the remainder of the experiment. No new Galactic Scorpionfish or Speckled Scorpionfish recolonized removal colonies after 16 and 32 weeks post-manipulation, respectively.

The change in abundance of Galactic Scorpionfish in colonies where Speckled Scorpionfish were removed did not differ from control colonies over the same period (treatment × before/after manipulation: $p = 0.95$; $\chi^2 = 0.0048$; df = 1; Fig. 3c). The model-estimated mean of Galactic Scorpionfish in Speckled Scorpionfish removal colonies over the two surveys before manipulation was 2.0 ± 1.2 and was 1.7 ± 1.1 after removals. In control colonies, there was a model-estimated mean of 1.5 ± 1.3 Galactic Scorpionfish pre-removal and 1.2 ± 1.1 in surveys post-removal. Manipulated colonies did contain significantly more Galactic Scorpionfish than control colonies on average (treatment: $p << 0.001$; $\chi^2 = 16.99$; $df = 1$) despite the random assignment of colonies to treatment groups.

There was also no significant difference in the change in abundance of Speckled Scorpionfish between control and removal colonies following the removal of Galactic Scorpionfish (treatment × before/after manipulation: $p = 0.89$; $\chi^2 = 0.021$; $df = 1$; Fig. 3d). There was a model-estimated mean of 2.1 ± 1.3 Speckled Scorpionfish in colonies before removal of Galactic Scorpionfish and 1.7 ± 1.2 after removals. Control colonies contained a model-estimated mean of 1.2 ± 1.4 Speckled Scorpionfish during pre-removal surveys and 0.8 ± 1.2 for surveys during the post-removal period. Despite the random allocation of colonies to treatment groups, Speckled Scorpionfish abundance within manipulated colonies was significantly greater on average than in control colonies (treatment: $p << 0.001$; $\chi^2 = 41.59$; $df = 1$).

4.4 Discussion

Multiple experiments at multiple temporal and spatial scales were unable to detect ongoing competition between Speckled Scorpionfish and Galactic Scorpionfish in terms of local distribution and abundance. Generally, the abundance of either species of *Sebastapistes* within cauliflower coral was unaffected by the presence or abundance of the congeneric species. Unknown habitat characteristics or perhaps limited and patchy larval supply, both at the reef scale and colony scale, appear to be the main driver of *Sebastapistes* spp. composition across depths and within cauliflower coral colonies.

Only Galactic Scorpionfish naturally occurred within cauliflower corals located in deeper reef areas (11 – 15 m deep). Following the pulse removal of Galactic Scorpionfish in these areas, only Galactic Scorpionfish recolonized, indicating that the absence of Speckled Scorpionfish in deep cauliflower coral is likely due to neither competitive exclusion (e.g., Larson 1980) nor priority effects (e.g., Almany 2003). Similarly, six of the eight coral colonies (75%) that were recolonized in shallow reef areas (5 – 8 m deep) were recolonized by the same scorpionfish species or combination of species that were present prior to removals. These results suggest that *Sebastapistes* occupation of cauliflower corals is not a random lottery (cf. Sale 1978), and coral colonies likely have specific characteristics that each species of scorpionfish select when colonizing new host colonies either as settling as larvae or as immigrating juveniles or adults.

Only four of approximately 80 individuals that recolonized manipulated colonies appeared to be recent recruits (≤ 2cmTL), all of which were Galactic Scoprionfish. Roughly three-fourths of recolonizers were of adult sizes, likely having immigrated from surrounding reef area, and may have selected corals with branch spacing that best matched their body size (cf. Hixon and Beets 1993). Jones (*submitted*) found that the larger Speckled Scorpionfish were more fequently associated with colonies with wider spacing between branches while Galactic Scorpionfish were more commonly found in colonies with more narrowly spaced branches. Additionally, Speckled Scoprionfish have occasionally been observed occupying the relatively wider spaced antler coral (*P. grandis*) in both shallow and deep areas (EGB personal observation). Further, 4 of 12 treatment colonies were not recolonized by any *Sebastapistes* by the end of this experiment. Three of these colonies were occupied by Blue-eye Damselfish (*Plectroglyphidodon johnstonianus*), a territorial resident species (Gajdzik et al. 2016) that may have prevented recolonization. No Blue-eye Damselfish were present on coral colonies that were recolonized by *Sebastapistes*. Alternatively, these colonies may have remained uncolonized if habitat space is not in short supply for these species in Hawaiʻi (Jones *submitted*), potentially due to recruitment limitation and patchy larval supply (Walsh 1987).

Reciprocal removal experiments at both the cluster and colony scale did not result in an increase in local abundance of the remaining species, as would be expected if these species were competing for limited resources (Connell 1975, Schoener 1983). The absence of a response in local abundance could suggest either that these species do not compete or that the effects of competition could not be detected by our experimental designs. One of the greatest limitations of our study was the logistic constraint of limited replication imposed by SCUBA diving. We acknowledge that the lack of power associated with our small sample sizes may have obscured weak or moderate effects of competition. Small sample sizes also likely contributed to the significantly different starting abundances between some treatement and control groups, despite random allocation of clusters and colonies into treatment groups.

Additionally, competition between Speckled Scoprionfish and Galactic Scoprionfish may occur, yet at a slower rate than detectable at the temporal scales of our experiments. Alternatively, the generally low level of juvenile fish recruitment to the Hawaiian Islands (Walsh 1987), populations of Speckled Scorpionfish and Galactic Scorpionfish may be maintained at levels where habitat resources are not limited (Doherty 2002). Another possibility is that coexistence between these species may be mediated by resource partitioning involving moderate overlap in both the use of space (Jones *submitted*) and food (Jones and Nichols *in prep*).

Competition may also occur during different phases of ontogeny (e.g., in grunts, Pereira et al. 2015), thus obscuring the effects of competition when manipulated individuals are mostly adults. Adult Speckled Scorpionfish are larger-bodied than adult Galactic Scorpionfish and may have a wider variety of prey options due to relative gape limitations of Galactic Scorpionfish. It is possible these species compete as juveniles when their body sizes are similar, or adult Galactic Scorpionfish may compete with only similarly sized juvenile Speckled Scorpionfish.

Habitat partitioning may also be occurring at finer spatial scales than were investigated in this study (Ross 1986). Though both species of *Sebastapistes* have been observed occupying all available positions within cauliflower coral colonies, in colonies where both species cooccur, Speckled Scorpionfish were frequently found in the upper, more exposed portions of the colony while Galactic Scorpionfish tended to be closer to the base of the host coral and deeper within the branches (RNJ personal observation). This pattern could be a result of competition-induced, within-colony habitat partitioning between the two species (cf. Macarthur's 1958 warblers) or may be a simple result of noncompeting individuals of different sizes occupying refuge space best suited to their body size (Hixon and Beets 1993). Future studies documenting how these species use and partition space within a colony would provide better insight into possible competition at the microhabitat scale.

Our findings suggest that populations of *Sebastapistes* in Hawaiʻi are structured more by coral habitat characteristics and/or larval supply than by competition. As coral bleaching events become more common, such coral-associated species will be among the first to be negatively affected (Feary et al. 2007). Pocilloporid corals are especially vulnerable to heat stress and are some of the earliest species to experience bleaching (McClanahan et al. 2020) including at our study site (Jones et al. 2021). Coral mortality and loss of habitat resulting from coral bleaching displaces resident species (Coker et al. 2012) and can enhance competition for remaining habitat (Pereira and Munday 2016). While it appears that habitat is not currently a limited resource for Speckled Scorpionfish and Galactic Scorpionfish in Hawaiʻi, future habitat loss due to climate change may lead to stronger competition for space.

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4.6 Figures

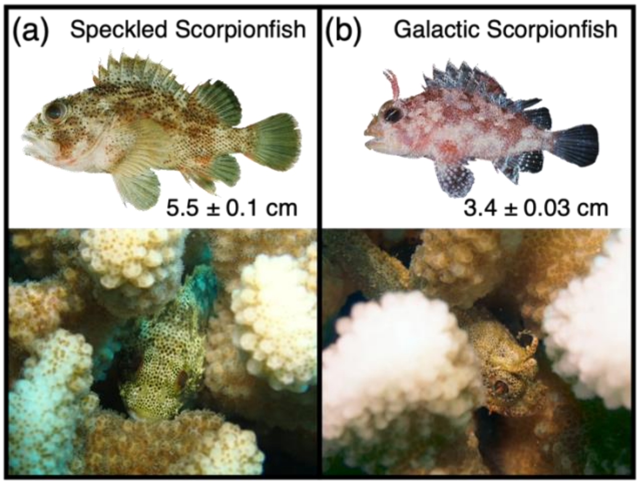

Figure 1. (a) Speckled Scorpionfish (*Sebastapistes coniorta*) and (b) Galactic Scorpionfish (*S. galactacma*). Bottom photographs show each species residing in colonies of cauliflower coral (*Pocillopora meandrina*) off Waikīkī Beach, Hawai‘i. Mean total length estimates (± SE) from Jones *submitted.* Top photographs by Jack Randall, bottom photographs by Ryan Jones.

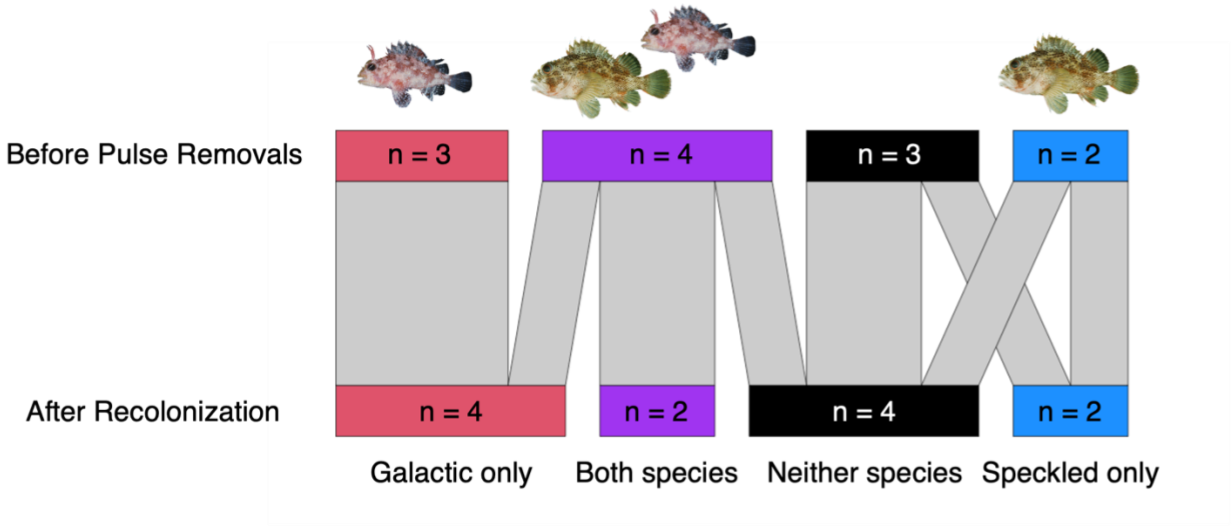

Figure 2. Number of cauliflower coral colonies occupied by Galactic Scorpionfish only (red), both species (purple), neither species (black), or Speckled Scorpionfish only (blue) in 12 colonies before a pulse removal of all scorpionfishes (top) and after recolonization eight weeks post-removal (bottom). Bars connecting the top and bottom rows indicate whether colony composition changed or remained the same after recolonization by new individuals (bar thickness is proportional to number of colonies).

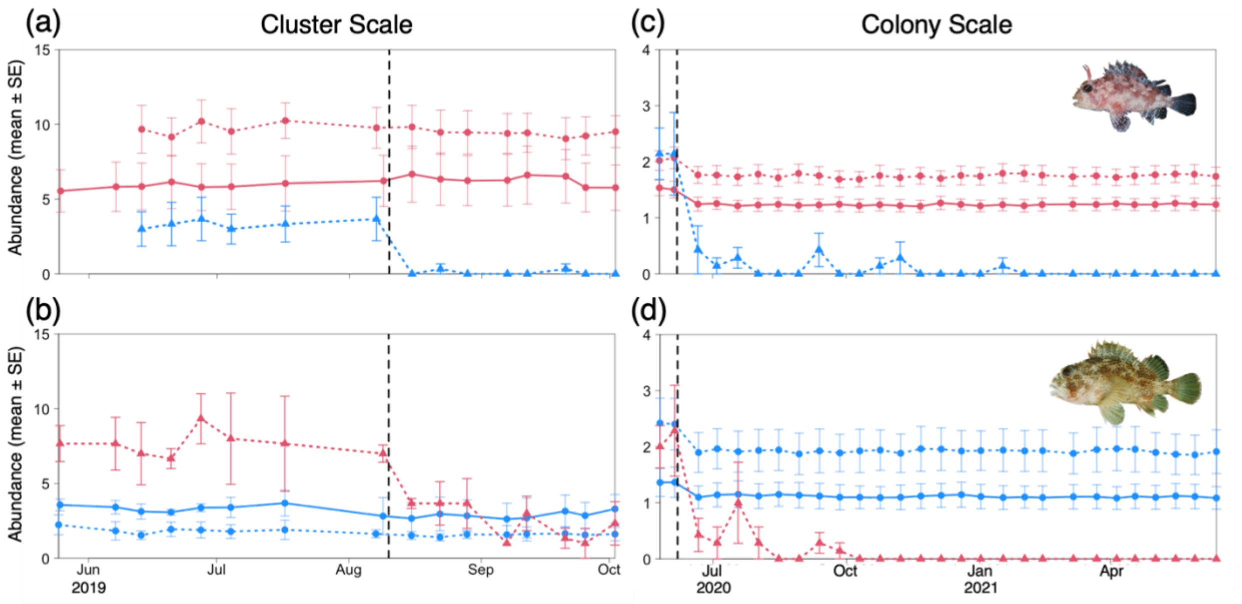

Figure 3. Response of (a, c) Galactic Scorpionfish (red) and (b, d) Speckled Scorpionfish (blue) to the press removal of the congeneric putative competitor at the scale of (a, b) a cluster of cauliflower coral colonies and (c, d) individual colonies in which the species co-occurred. Circles are estimated means of *Sebastapistes* abundance (± SE) from the models described in the text and triangles are raw means of the removed species to show the efficacy of removals. Dotted lines represent mean *Sebastapistes* abundances in removal clusters (n = 3) and colonies (n = 7) and solid lines represent control groups (clusters: n =3, colonies: n = 7). The dashed vertical lines indicate the times of removals. Clusters where Speckled Scorpionfish were removed began two weeks later than other treatment groups following the replacement of an original cluster after wave damage.

CHAPTER 5

General Conclusions

Ryan N. Jones

The Speckled Scorpionfish (*Sebastapistes coniorta*) and Galactic Scorpionfish (*S. galactacma*) are coral-associated cryptobenthic reef fishes that are commonly found occupying cauliflower corals around Hawaiʻi (Counsell et al. 2018). Despite their abundance, relatively little is known about their ecology, including how these congeneric species interact and coexist with one another. Cryptobenthic reef fishes are an integral component of coral reef trophic systems, serving as a link between macroinvertebrates and higher trophic species that are often culturally and economically important (Depczynski and Bellwood 2003; Brandl et al. 2018). Coral-associated species have been shown to provide their host coral colonies with a range of mutualistic benefits in return for the protection offered by the host coral's structural complexity (Meyer and Schultz 1985; Liberman et al. 1995; Goldshmid et al. 2004). This dissertation describes how the Speckled Scorpionfish and Galactic Scorpionfish use habitat and food resources and tests whether these species are currently competing for resources in Hawaiʻi.

The second chapter of this dissertation examined what habitat characteristics were associated with Speckled Scorpionfish and Galactic Scorpionfish presence and abundance. The most striking habitat difference between these species was their depth distributions within reefs. Speckled Scorpionfish were almost entirely found in cauliflower coral colonies shallower than 10 m deep. In contrast, Galactic Scorpionfish were observed across all depths surveyed, though were more frequently observed in corals in deeper reef areas (> 10 m). This inverse depth distribution between the two species is similar to trends observed in other species of the Family Scorpaenidae (Larson 1980). Bathymetric segregation of ecologically similar species, such as was observed here, could be due to differing gradients of food or habitat availability and/or could result from competitive interactions between the species (Hixon 2006). The depth distributions of these species were similar across all sites surveyed on the south and west shores of Oʻahu. Notably, Speckled Scorpionfish were not observed at one of the five study sites: Hanauma Bay. Despite the absence of Speckled Scorpionfish, Galactic Scorpionfish showed a similar depth distribution at Hanauma Bay compared to the other four sites, this "natural experiment" potentially indicating that Speckled Scorpionfish are not competitively excluding Galactic Scorpionfish from shallow reef areas.

Speckled Scorpionfish and Galactic Scorpionfish were also associated with host coral colonies with subtly different yet overlapping characteristics. Primarily discerning between the two species was the amount of interstitial space between the branch tips of the host coral colony. Speckled Scorpionfish, the larger of the two species, were more commonly associated with colonies with wider spacing between their branches while Galactic Scorpionfish were less common in colonies with widely spaced branches. This pattern could indicate that these species select host coral colonies whose interstitial branch spaces most closely match their body size, thereby providing the best refuge from external predators (Hixon and Beets 1993). Additionally, Galactic Scorpionfish within colony abundance was greater in larger cauliflower coral colonies than in smaller colonies, while Speckled Scorpionfish typically occurred either singly or in pairs no matter the size of their host coral.

Despite the above general differences in habitat use, Speckled Scorpionfish and Galactic Scorpionfish overlapped moderately in their use of habitat. These species cooccurred in 33.3% of reef areas and 10.8% of cauliflower coral colonies surveyed between 5 and 10 m deep, the depth range where both species occur. Additionally, unoccupied cauliflower coral colonies were common and indistinguishable from corals occupied by either species of scorpionfish. This pattern could indicate that coral habitat is not currently a limiting resource for *Sebastapistes* scorpionfishes in Hawaiʻi.

Chapter three explored how Speckled Scorpionfish and Galactic Scorpionfish use a second important resource axis: food. While attempts to use traditional visual stomach content analysis methods were unsuccessful, we found that modern DNA metabarcoding techniques were effective at describing the diets of these two reef fishes. Both species consumed prey taxa that commonly coinhabit cauliflower coral colonies with Speckled Scorpionfish and Galactic Scorpionfish (e.g., crustaceans of the families Alpheidae, Domeciidae, Palaemonidae, and Xanthidae). Speckled Scorpionfish diets were mainly composed of domeciid crabs and palaemonid shrimps, while Galactic Scorpionfish mainly consumed portunid crabs, xanthid crabs, and alpheid shrimps. Both predator species were found to have consumed some labrid fishes, likely co-resident juvenile Saddle Wrasse (*Thalassoma duperrey*) and Four-Lined Wrasse (*Pseudocheilinus tetrataenia*).

The prey DNA recovered from both predator species revealed that Speckled Scorpionfish and Galactic Scorpionfish had significantly different diet compositions generally. The amount of dietary overlap between these species for metazoan prey (Schoener's Index [SI] = 27%; Schoener 1971) was less than what is generally considered ecologically relevant overlap (SI > 60%; Wallace and Ramsey 1983), though these species highly overlapped in their use of crustacean prey (SI = 82%). We additionally found that neither species appeared to shift their diets when cooccurring with the other, indicating that these species may have evolved to consume somewhat different food resources, potentially due to past competition (Connell 1980).

During this study, we also compared the diets of Galactic Scorpionfish collected from shallow reef areas (5 – 10 m deep), where they overlap with Speckled Scorpionfish, to conspecifics collected from deep reef areas (11 – 15 m deep), where they occur alone, to determine whether their diets differed across depths. We found some evidence analyzing metazoan prey that Galactic Scorpionfish may consume different prey in shallow reef areas than in deep reef areas, potentially indicating that prey availability varies across depths. Bathymetric distributions of prey availability may explain why Speckled Scorpionfish are apparently restricted to shallow reef areas if their preferred prey do not occur in deeper areas. In summary, we found that the diets of Speckled Scorpionfish and Galactic Scorpionfish overlap moderately and that prey availability may explain the bathymetric segregation we see between the two species.

The fourth chapter reported a set of manipulative field experiments empirically testing whether Speckled Scorpionfish and Galactic Scorpionfish presently compete on Hawaiian reefs. The first experiment employed a pulse removal of all *Sebastapistes* scorpionfishes from clusters of cauliflower coral colonies from shallow reef areas, where both species were present, and from deep areas, where only Galactic Scorpionfish were present. In shallow reef areas, 66.7% of experimental colonies that were recolonized by the end of the experiment were occupied by new individuals of the same species that had previously occupied those colonies, suggesting that *Sebastapistes* occupation of cauliflower corals is not a random lottery (cf. Sale 1978) and coral colonies likely have specific characteristics that each species of scorpionfish select when colonizing new host colonies either as settling as larvae or as immigrating juveniles or adults. Additionally, 33.3% of shallow experimental corals were not recolonized by the end of the eight-week experiment, indicating that is not strong inter- or intra-specific competition for space. Finally, in deep reef areas, no Speckled Scorpionfish were observed before or after removals, and only Galactic Scorpionfish recolonized experimental colonies.

In addition to the pulse removal and recolonization experiment, we also conducted a pair of reciprocal press removals in shallow reef areas where both species were present. The first experiment focused on clusters of four closely positioned cauliflower corals that contained both Speckled Scorpionfish and Galactic Scorpionfish, and removals were maintained for eight weeks. The second experiment was conducted at the colony scale (i.e., individual cauliflower corals occupied by both species), and removals were maintained for an entire year. If competition for resources was occurring between these species, then we predicted that the removal of one species would result in an increase in the local abundance of the remaining species, and vice versa. For both species at both scales, the removal of the putative competitor did not result in an increase in the local abundance of the remaining species. This lack of response could indicate that competition is not presently occurring between Speckled Scorpionfish and Galactic Scorpionfish in Hawaiʻi or is occurring at substantially slower rates than these experiments were able to detect.

Why then might these species not be competing? First, the moderate overlap in their use of both food and habitat resources documented in this dissertation may allow these species to coexist on reefs in Hawaiʻi without competition. This partitioning of resources, however, may have arisen over evolutionary time scales as a result of past competition between these species even though Speckled Scorpionfish and Galactic Scorpionfish do not appear to be presently competing (Connell 1980). Alternatively, these species may have simply never competed and have developed their ecological niches independently of one another. Finally, food and habitat resources may not be in short supply in Hawaiʻi for Speckled Scorpionfish and Galactic Scorpionfish if their populations are recruitment limited (Doherty 2002). The Hawaiian archipelago is extremely isolated, and levels of juvenile fish recruitment have historically been shown to be low relative to other regions of the Pacific (Walsh 1985, 1987).

The high number of unoccupied cauliflower coral colonies observed in the second chapter of this dissertation may provide evidence habitat is not a limiting resource in Hawaiʻi. Climate change and ocean warming, however, may soon threaten populations of cauliflower coral, which are especially vulnerable to coral bleaching (McClanahan et al. 2020; Jones et al. 2021). While it currently appears that habitat is not a limiting resource, coral mortality and the subsequent loss of habitat may result in stronger competition for space between these species.

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APPENDIX A

Chapter 3 Supplemental Text

SM1. Some recovered DNA was biologically improbable to be actual prey of Speckled Scorpionfish and Galactic Scorpionfish. In some cases, it is plausible that metabarcoded gut DNA could be capturing secondary predation (prey of prey, e.g., Poritidae), indirect predation/incidental ingestion (e.g., Rhodomelaceae, etc.), or failing to capture local taxa that do not yet exist in sequence databases (Gold et al. 2021). For example, *Thunnus* DNA, although matched to 100%, came from a single MOTU (MOTU 375, with 2 reads) but is unlikely to be part of the scorpionfish diet. Regardless, the vast majority of matched MOTUs were consistent with taxa known to occur with scorpionfish and could reasonably be prey. Recovered mammalian DNA was of human origin, likely due to contamination during processing, and thus was excluded from analyses.

Gold Z, Curd EE, Goodwin KD, Choi ES, Frable BW, Thompson AR, Walker HJ, Burton RS, Kacev D, Martz LD, Barber PH (2021) Improving metabarcoding taxonomic assignment: A case study of fishes in a large marine ecosystem. Mol Ecol Resour 21:2546–2564

SM2. Families of rare taxa grouped as “OTHER” from relative DNA read proportion from stomach contents of Speckled Scorpionfish (SECO) and Galactic Scorpionfish (SEGA): COI: Acanthuridae (SECO 0.002%), Alpheidae (SECO 0.78%), Ampithoidae (SECO 0.03%, SEGA 0.54%), Candaciidae (SECO 0.03%, SEGA 0.006%), Cavoliniidae (SECO 0.002%), Dasyaceae

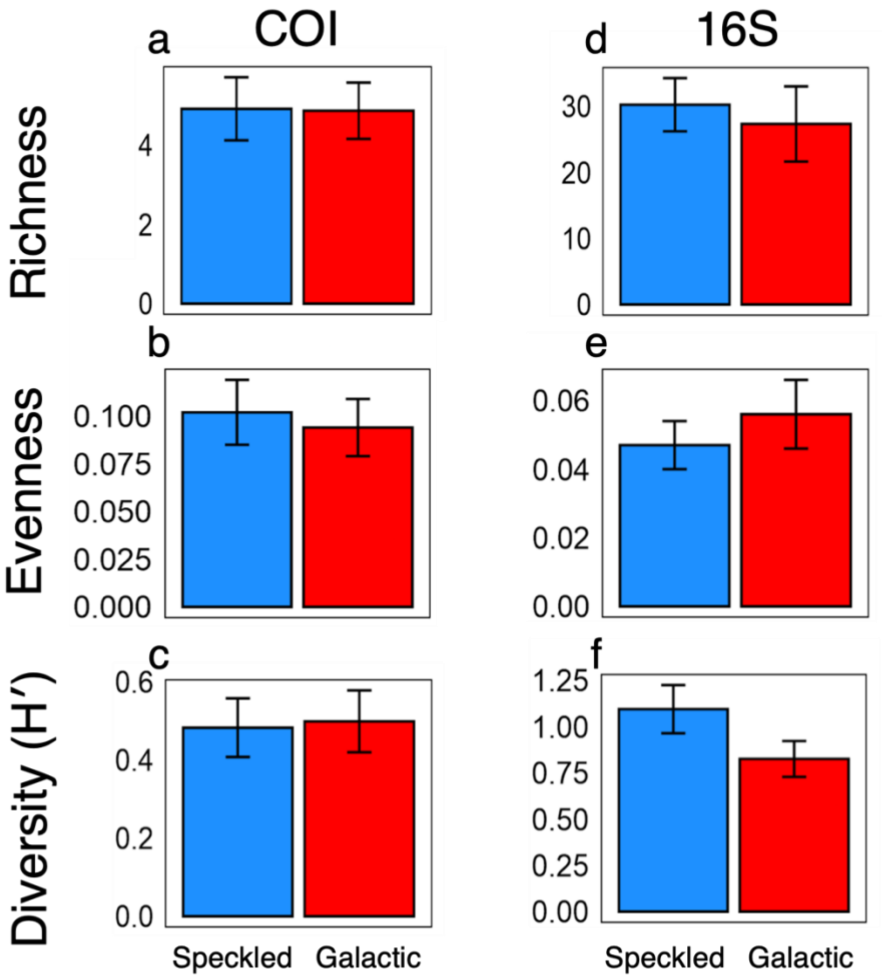
(SEGA 0.002%), Diogenidae (SEGA 0.18%), Domeciidae (SEGA 0.21%), Gelidiellaceae (SEGA 0.12%), Grapsidae (SEGA 0.006%), Halocyprididae (SEGA 0.001%), Holocentridae (SECO 0.002%), Labridae (SEGA 0.5%), Peyssonneliaceae (SEGA 0.01%), Poritidae (SECO 0.04%), Portunidae (SEGA 0.95%), Rhodomelaceae (SECO 0.004%, SEGA 0.002%), Rhynchocinetidae (SEGA 0.34%), Scombridae (SECO 0.002%), Thoridae (SECO 0.05%, SEGA 0.04%), Trapeziidae (SEGA 0.02%), Xanthidae (SECO 0.79%). 16S: Asellidae (0.001% SEGA), Diogenidae (0.01% SEGA), Oplophoridae (<0.0001% SEGA), Percnidae (<0.0001% SECO), Thoridae (0.007% SECO).

SM3. Families of rare taxa grouped as “OTHER” bipartite plot of the diets of the Speckled Scorpionfish and Galactic Scorpionfish: COI: Acanthuridae (0.001%), Ampithoidae (0.82%), Candaciidae (0.02%), Cavoliniidae (0.001%), Dasyaceae (0.002%), Diogenidae (0.3%), Gelidiellaceae (0.06%), Halocyprididae (0.002%), Holocentridae (0.001%), Peyssonneliaceae (0.016%), Poritidae (0.02%), Rhodomelaceae (0.006%), Scombridae (0.001%), Thoridae (0.09%), Trapeziidae (0.03%). 16S: Asellidae (0.13%), Diogenidae (0.94%), Oplophoridae (<0.001%).

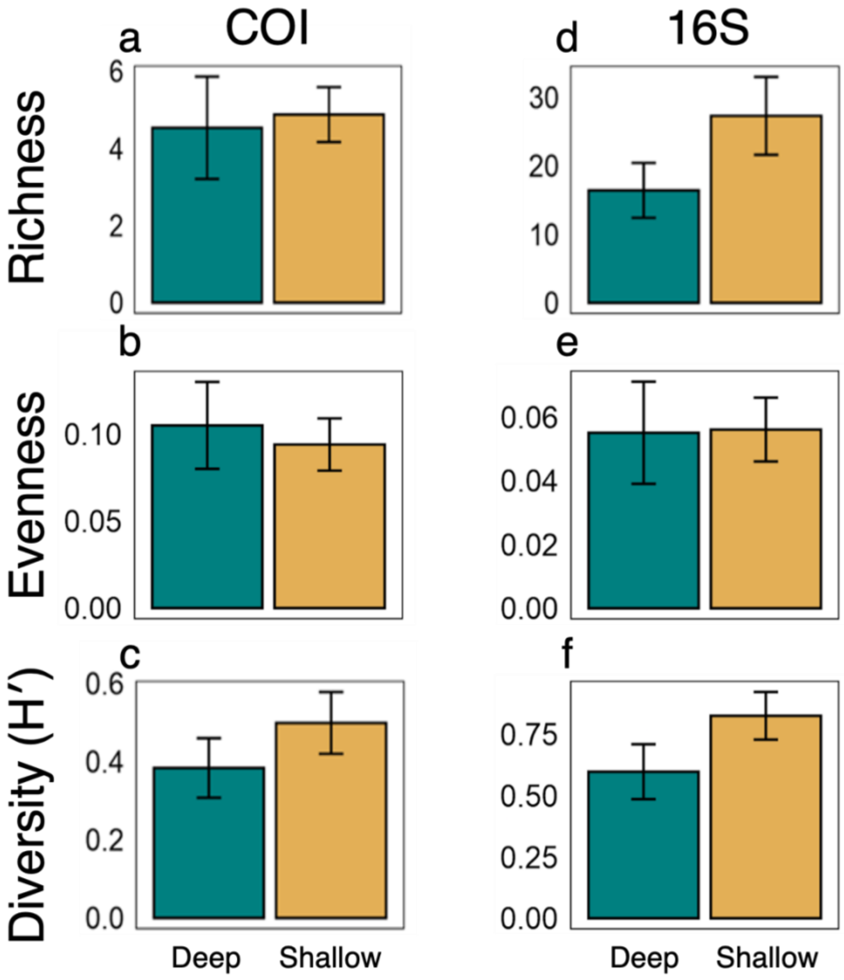
SM4. Families of rare taxa grouped as “OTHER” bipartite plot of the diets of Galactic Scorpionfish collected from shallow and deep reef areas: COI: Candaciidae (0.02%), Dasyaceae (0.008%), Diogenidae (0.29%), Gelidiellaceae (0.54%), Grapsidae (0.009%), Halocypridae (0.002%), Peyssonneliaceae (0.02%), Rhodomelaceae (0.003%), Rhynchocinetidae (0.54%), Thoridae (0.06%), Trapeziidae (0.03%). 16S: Asellidae (0.13%), Diogenidae (0.94%), Oplophoridae (<0.001%).

APPENDIX B

Chapter 3 Supplemental Figures



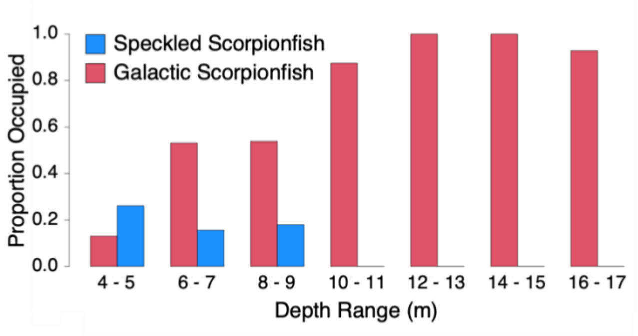
SM1. Measurements of **a, d** diet richness, **b, e** evenness, and **c, f** composite diversity (Shannon's H') for Speckled Scorpionfish (blue) and Galactic Scorpionfish (red) and based on **a-c** COI (marine metazoans) and **d-f** 16S (crustaceans) metabarcoding assays resolved to taxonomic family.



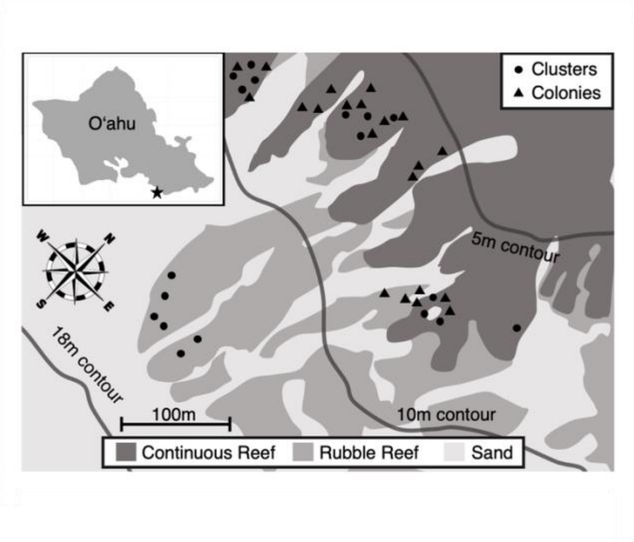
SM2. Measurements of **a, d** diet richness, **b, e** evenness, and **c, f** composite diversity (Shannon's H') for Galactic Scorpionfish collected from deep reef areas (green) and shallow reef areas (yellow) based on **a-c** COI (marine metazoans) and **d-f** 16S (crustaceans) metabarcoding assays resolved to taxonomic family.

APPENDIX C

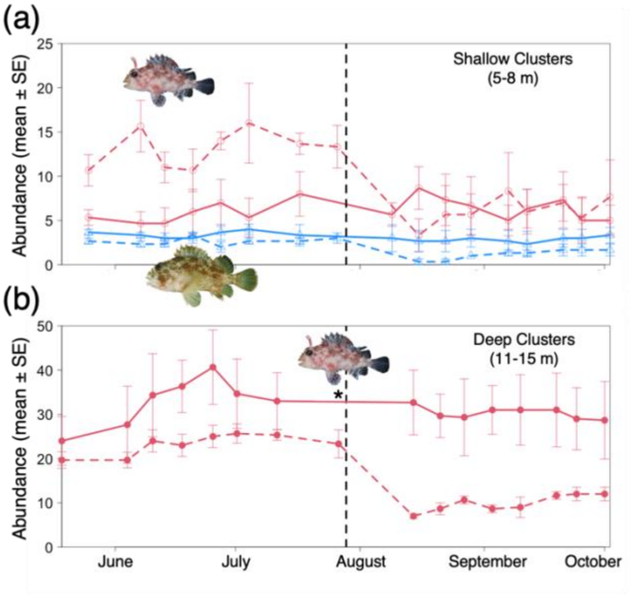
Chapter 4 Supplemental Figures



SM1. Proportion of cauliflower coral colonies occupied by Speckled Scorpionfish (blue) and Galactic Scorpionfish (red) across depths at our study site off Waikīkī Beach, Hawai‘i (data from Jones *submitted*).



SM2. Map of study site off Waikīkī Beach on the south shore of Oʻahu, Hawaiʻi. “Clusters” refer to groups of cauliflower coral colonies used in experiments in 2019, whereas “colonies” are individual coral heads used during the year-long experiment spanning 2020 to 2021.



SM3. Recolonization by Galactic Scorpionfish (red) and Speckled Scorpionfish (blue) in 2019 following a pulse removal (vertical dashed line) of all scorpionfish from clusters of cauliflower coral colonies in (a) shallow water (5-8 m deep) and (b) deep water (11-15 m deep). Dashed lines represent removal clusters (n = 3), and solid lines represent control clusters (n = 3). No Speckled Scorpionfish were present in deep cauliflower corals for the duration of this study. One week of surveys of deep control clusters was missed, as indicated by an asterisk.

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