

DIVERSITY IN A TROPICAL MARINE MUTUALISM

By  
CHRISTIAN SEABIRD HOLLIS MCKEON

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To my teachers in their many forms

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Abstract of Dissertation Presented to the Graduate School  
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DIVERSITY IN A TROPICAL MARINE MUTUALISM

By

Christian Seabird Hollis M<sup>c</sup>Keon

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Symbiosis has been described as “the most relevant and enduring biological theme in the history of our planet”. These relationships are diverse and pervasive, reaching every habitat and the great majority of life forms. Nowhere are they more important in ecological function, than on coral reefs. Coral reefs are quite literally powered by symbiotic relationships- the partnership between unicellular algae and corals allows the deposition of calcium carbonate. Yet other mutualisms are also important. In this work I describe the relationship between a single genus of coral, *Pocillopora*, and a genus of crab, *Trapezia*. The mutualism focuses on the defense of the coral from environmental stressors, such as predation, sedimentation, and algal overgrowth. Yet nuances exist. As any relationship can be viewed as “reciprocal exploitation,” the lines between mutualism and parasitism blur upon close examination.

## CHAPTER 1 DIVERSITY IN A TROPICAL MARINE MUTUALISM: INTRODUCTION AND GOALS

### **Context and Goals of this Dissertation**

Coral reef ecosystems are the most diverse of marine systems (Paulay 1997, Reaka-Kudla 1997) and scleractinian corals serve as the structural species of modern coral reefs (Veron 1995). As such, scleractinians are the basis of reef diversity, in the same manner that rainforest trees support tropical moist forest diversity (Terborgh 1990). Understanding the origin, function and implications of diversity is one of the primary goals of modern biology. Our estimation of reef diversity is incomplete, and our understanding of the function of that diversity is even more limited.

The widespread die-off of coral reef ecosystems is recognized as an ecological crisis of global proportions (Pandolfi et al. 2003). Understanding and predicting the changes that are taking place in these ecosystems is difficult because they are species rich and there are many interactions among the species. In particular, mutualistic associations are diverse and pervasive across the ecosystem. For example, the relationships between scleractinian corals and their symbiotic algae (zooxanthellae) serve as the foundation of reefs as biogenic structures by allowing reef building animals (corals) to outcompete autotrophic algae. Other coral symbioses, such as the *Trapezia-Pocillopora* mutualism, have received relatively little attention, despite evidence suggesting such relationships are important for the growth, reproduction, and survival of coral.

Corals, like other organisms, face a number of stressors in their interactions with the environment. These interactions may be modified by the presence of symbionts (Figure 1). The zooxanthaella genus *Symbiodinium* compose the primary group of photosymbionts of scleractinian corals. This association has recently received increased

attention as the phenomenon of coral bleaching, (i.e. the breakdown of the symbiosis between coral and zooxanthellae and subsequent whitening of coral colonies), has become a symptom of environmental decay. Variation in this endosymbiosis has been demonstrated (Rowan et al. 1997, Rowan 1998), with different clades of zooxanthellae found to differ in their response to stressors.

Early researchers believed that *Symbiodinium* was a single widespread taxon of limited variability (Muscatine and Hand 1958). The use of molecular methods in phylogenetics revealed an unanticipated diversity within the group, across and within host species (Rowan 1991, Rowan et al. 1997, Rowan 1998). *Symbiodinium* are now discussed as clades (reviewed in (Pochon et al. 2006). Some of these clades are more susceptible to heat induced stress than others, creating variability in the bleaching of the coral hosts (Oliver and Palumbi 2009). Such symbioses may take on new importance as threats from global climate change, sedimentation, and other forms of anthropogenic impact increase.

Previous research has demonstrated the effectiveness of crustacean symbionts as guards against corallivorous seastars (Glynn and Krupp 1986, Pratchett and Vytopil 2000) and as housekeepers - removing sediment that would otherwise smother their coral hosts (Stewart et al. 2006). The role of crustacean-coral mutualisms in mitigating these environmental stressors may be of dramatic importance in the maintenance of diversity on reefs in the face of anthropogenic change. As with the endosymbioses above, the exosymbiotic relationships of corals and crustaceans vary: species, size, and community composition differ from one coral head to another. Despite descriptive work

on the some of the beneficial effects of exosymbiotic crustaceans, these differences have yet to be evaluated in the context of the benefits offered to the host coral.

In this dissertation I present a multi-armed approach to examining the role of diversity in relationships between corals and their crustacean symbionts, and how these relationships respond to environmental stressors such as corallivory and sedimentation.

### **The Study System**

In many parts of the tropical Pacific, *Pocillopora* are numerically dominant corals (Veron 2000). They are also one of the corals most sensitive to bleaching (Mumby et al. 2001), and when unprotected, a favored food of corallivorous seastars (Glynn 1976). *Pocillopora* hosts numerous symbiotic and non-symbiotic taxa, ranging from juvenile fish (Holbrook et al. 2000), to boring bivalves (Mokady et al. 1994). As such they represent microcosms of reef diversity and are ideal for experimentation. *Pocillopora* are facultative hosts of *Trapezia* crabs and the relationship between crabs and coral has become a textbook example of mutualism (Glynn 1980, 1983, Glynn 1987). In the Red Sea, the West coast of Panama, and many regions across the Indo-Pacific, the crabs have been observed actively defending the coral from predators. The crabs are obligate symbionts - never found on dead coral, and only rarely on non-pocilloporids. Corals gain defense from the relationship, while crabs gain shelter, and direct nutrition through lipid droplets that are provided by the coral in tentacle tips (Stimson 1990). In many ways the mutualism closely resembles that of ant-plant relationships (Janzen 1966, Longino 1989).

### **Rationale and Context**

Despite superficial knowledge of their potential role in defending corals from multiple stressors, the details of the relationship between *Trapezia* and *Pocillopora* are poorly

understood. Evolutionary theory tells us mutualisms are stable when they are truly 'reciprocal exploitation' by two species (Herre et al. 1999). However, in this case, the players involved in the mutualism have been studied as genera, not species, and as such do not fit into the pairwise perspective that has defined much of our theoretical understanding of mutualisms to date (Stanton 2003). *Pocillopora* is abundant, widespread, and includes a range of species that vary in their response to bleaching (Glynn et al. 2001), sedimentation (Caley et al. 2001), predation (Glynn and Krupp 1986, Glynn 1987), as well as in the symbiotic community they support. The taxonomy of *Pocillopora* is confused, with as few as four, or as many as 20 species recognized (Veron and Pichon 1976, Veron 2000). The morphology of the corals varies tremendously over environmental gradients such as sedimentation and wave exposure (Veron and Pichon 1976), creating challenges to morphological identification. Molecular methods have not resolved the issues, suggesting hybridization and introgression across the genus (Combosch et al. 2008).

*Trapezia* species are similarly widespread. In the most recent taxonomic study of the genus, 23 species were recognized using classical morphology (Castro et al. 2004). They exhibit habitat preferences across the reef (Odinetz 1983), and compete for space within the corals (Huber 1987a). Within a coral head, *Trapezia* species are most frequently found as male/female pairs. Multiple species of *Trapezia*, and other symbiotic crustaceans, often occupy the same coral head creating complex dynamics (Preston 1971, 1973, Gotelli et al. 1985), and making generalizations about the system more difficult. Glynn (Glynn 1976, 1987) proposed that the presence of crustacean

symbionts could be a major factor in reef coral demography through the modification of corallivory, and increased survival of corals that host *Trapezia* and other symbionts.

This makes detailed knowledge of both the coral and the crab essential for understanding whether and how this symbiosis can mitigate reef stressors such as sedimentation, *Acanthaster* outbreaks, and global climate change. Our lack of understanding of symbioses, particularly in marine systems, hinders our ability to understand, let alone repair, changing reef ecosystems (Herre et al. 1999).

Underlying almost all ecological studies of shallow reef environments are questions of diversity. Many of our most broadly considered ecological models are based on assumptions regarding the interchangeability of species, yet this idea remains largely untested (Resetarits and Chalcraft 2007b). While our estimates of the biodiversity of coral reefs are still imperfect (Reaka-Kudla 1997), our appreciation of the potential role of diversity in these ecosystems is only increasing as molecular methods help to redefine the boundaries of species (Knowlton 1986).

In order to examine the ecological implications of species level diversity of *Trapezia* in the context of the *Trapezia-Pocillopora* mutualism, I sought to test the relative efficacy of different species of *Trapezia* in response to the environmental stressors of *Pocillopora*, namely corallivory and sedimentation. These comparisons could only be conducted with knowledge of the boundaries and differentiation between the species of *Trapezia*, spurring a phylogenetic analysis of the genus. A second group of questions stem from the diversity of the coral-symbiont system, centered around the potential interactions of the component species within symbiont assemblages. I examined two

interactions: between two exosymbiotic species (*Trapezia serenei* and *Alpheus lottini*), and between the endosymbiont dinoflagellate *Symbiodinium* and *Trapezia septata*.

### **An Outline**

The chapters to follow address the following topics:

- **Phylogenetics, speciation, and character evolution in *Trapezia* with *Gustav Paulay***
  - Molecular characterization using mitochondrial genes allowed for the testing of species boundaries of *Trapezia*, and tracking the evolution of ecological and morphological traits.
- **Diversity in Mutualist Services offered by Coral Crabs with *Jenna Moore***
  - While some of the ecological services of *Trapezia* have been demonstrated, differences in services provided between species and size classes of crabs have not been examined. We tested the efficacy of different species and size classes in protecting their host against three different corallivores and sediments.
- **Multiple Defender Effects Modify the Benefits of Species Stacking: Synergistic Coral Defense by Mutualist Crustaceans with *Adrian Stier* and *Shelby Boyer***
  - While previous studies have documented the abilities of *Trapezia* to protect corals against predators and from sedimentation, how interactions of multiple species impact these services within the same coral head were not previously examined. We examined the cumulative effect of protection rendered by the crab *Trapezia serenei* and the snapping shrimp *Alpheus lottini* in the same coral head against corallivores.

- **Full House: Endosymbiont-Exosymbiont Connections in Reef Corals** *with Tomas Oliver*

-The fundamental symbiosis between scleractinian corals and the endosymbiotic dinoflagellate *Symbiodinium* allows for the development of coral reefs. The response of the symbiosis to thermal stress varies depending on *Symbiodinium* clade hosted; these differences may also impact other attributes, such as growth rate. Given the provisioning of carbon by the endosymbiont to the coral, and the coral to the crab, we examined the relationship between clade of *Symbiodinium* and size of *Trapezia*.

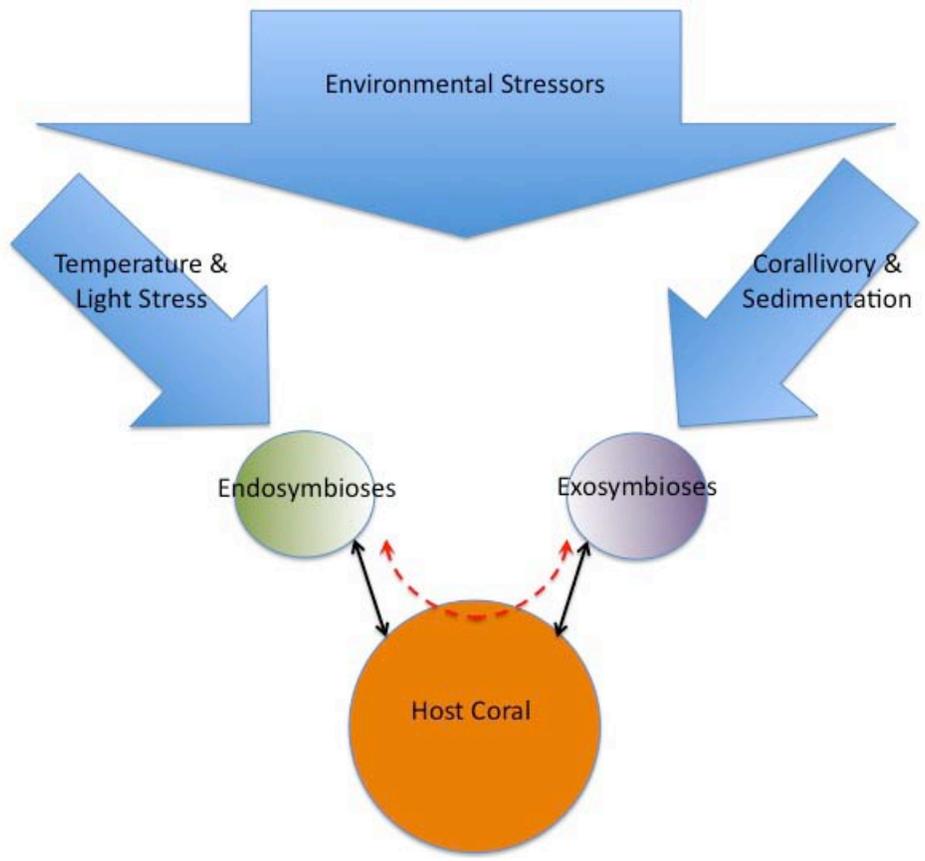


Figure 1-1. A conceptual model of coral symbioses

CHAPTER 2  
PHYLOGENETICS, SPECIATION, AND CHARACTER EVOLUTION IN *TRAPEZIA*

**Introduction**

Colorful, common, and of ecological importance, the coral crabs of the genus *Trapezia* have been known by western science since at least 1799, with the description of “*Cancer rufopunctata*” by Herbst (Castro et al. 2004). The genus continues to yield new ecological information important to our understanding of the function of coral reefs and mutualisms in general (McKeon et al. 2010). Despite this long history and continued importance, our systematic understanding of *Trapezia* is incomplete, with questions remaining at the species level, as well as regarding their deeper phylogenetic relationships within the Xanthoidea (Castro et al. 2004, Lai et al. 2009).

Morphological identification and recognition of species within *Trapezia* has been challenging because of the similarity of many species and the loss of color in preserved specimens. Closely related species are most readily, and sometimes only, differentiable by color. In the absence of data on color pattern however, earlier systematists were denied these corroborating features to the sparse selection of structural morphological characters. Furthermore, the color patterns of *Trapezia* can be quite variable within species (Castro 1997b, Sin 1999), and a long taxonomic history of confusion and misidentification persists to this day, despite the clarifying efforts of several successive generations of carcinologists (Galil 1983, Serene 1984, Galil and Lewinsohn 1985a, Galil and Lewinsohn 1985b, Castro 2000, Castro et al. 2004). As with many other groups of marine crustaceans, color may be a strong indicator of genetic differences (Knowlton 1986), though other tools, such as behavior (Odinetz 1984b), have also been used to differentiate species occurring sympatrically. The

independence of these three character sets; genetic tools, behavior and color pattern may be used to test hypotheses regarding species boundaries, and to detect the occurrence of unrecognized cryptic species.

Like many coral reef taxa, most species of *Trapezia* as currently understood have very broad geographic ranges - in some cases stretching from the East Coast of Africa to the West Coast of the Americas. This large area can be considered in its component parts: the Indo-west Pacific region (IWP), which includes the Indian Ocean (IO), and the West Pacific (WP), and the East Pacific (EP) region. The biogeography of *Trapezia* was reviewed by Castro (2000), who noted that the majority of *Trapezia* have ranges that span the IWP, with several that continue into the EP, to the west coast of Central and South America. The remaining species, such as *T. richtersi* (IO), and *T. globosa* (SE Pacific), are more geographically restricted. As our understanding of species boundaries within broad-ranging taxa has increased, many “species” have proven to be assemblages of deeply divergent populations that replace each other along biogeographic boundaries (Meyer 2003, Meyer et al. 2005, Malay and Paulay 2010).

As mutualists of scleractinian corals, the relationship between *Trapezia* and corals of the family Pocilloporidae is an important ecological factor on Indo-Pacific coral reefs. The mutualism has been invoked as modifying interactions between corals and predators (Glynn 1987), other benthic organisms (Stier et al. 2010), as well as removal of smothering sediments (Stewart et al. 2006). Host specificity beyond the family level has not been noted in the system (Garth 1964, Patton 1966, Patton 1974, Gotelli et al. 1985) (but see Sin and Lee (2000) for an alternative perspective). In contrast there is

increasing evidence for specificity in other coral symbiont groups (Mokady et al. 1994, Gittenberger et al. 2004, Malay 2010).

Molecular phylogenetics is a powerful tool for resolving difficult problems in taxonomy and systematics. Initial work by Huber (1987a) using gel electrophoresis revealed divergence within Hawaiian *Trapezia digitalis* that corresponded to differences in behavior and color. Huber also noted divergence between Hawaiian and Enewetak populations of *T. ferruginea* (a species now considered to be synonymous with *T. bidentata*) (Huber 1985), suggesting that the two populations studied may be different species. The description of *Trapezia lutea*, a very similar species in terms of coloration and morphology to *T. bidentata*, that followed shortly after (Castro 1997a) suggests he might have been correct; perhaps his study compared populations of *T. bidentata* from Hawaii with *T. lutea* from the Marshall Islands.

Phylogenetic approaches allow differentiation of species-level units readily when specimens are grouped into reciprocally monophyletic clusters by multiple independent markers. Mitochondrial DNA markers are especially useful in this regard, because they evolve rapidly, while nuclear markers, morphology, color pattern, as well as geography can provide independent corroborating markers for comparison. When two or more markers differentiate populations we refer to them as Evolutionary Significant Units (ESUs) (Moritz 1994). When ESUs occur sympatrically, they represent unequivocal biological species, because they are exposed to potential crossing. When they are distributed allopatrically their reproductive status is not readily assessed, but they still meet the definition of phylogenetic species.

We used Cytochrome Oxidase I sequence data together with morphological characters, color pattern, and geographic distribution to delineate ESUs in *Trapezia* and to construct a phylogenetic hypothesis for the genus. By extensively sampling across species and geography, we were able to test genetic and distributional boundaries for most known *Trapezia*. The distribution and level of genetic divergence between sister ESUs was used to define Evolutionary Significant Events (ESEs sensu Malay and Paulay (2010)), effectively phylogenetic speciation events. We also explored the evolution of color patterns and ecological traits by mapping them on the resulting phylogeny. We specifically address the following questions:

- Are recognized species of *Trapezia* valid? Do presently unrecognized taxa exist?
- Are striking color patterns, like dots or reticulate patterns, convergent or monophyletic? What are the dynamics of color evolution in this group?

H<sub>0</sub>: Color patterning in *Trapezia* is not homoplastic.

H<sub>1</sub>: Color patterns, such as spots, reticulated markings, or a plain carapace, have evolved repeatedly.

- Are *Trapezia* host specific beyond the family level? If so, how has host specificity evolved?
- Is the mode of speciation predominantly allopatric?

H<sub>1</sub>: Allopatric sister species pairs are genetically more similar (and thus likely younger) than sympatric sister species pairs.

- Is there evidence for mediated coexistence between sympatric sister species through potential character displacement?

H<sub>1</sub>: Sympatric sister species pairs differ more strongly in habitat or morphology than allopatric sister species pairs.

## Methods

### Collection Methods

Specimens were collected from numerous sites from Madagascar to Hawaii using snorkel and SCUBA. Specimens were preserved in 75-95% ethanol. When possible specimens were photographed alive or fresh and ecological information, including host coral, depth and habitat were recorded. All but two of the 23 described species of *Trapezia* were collected, exceptions being *T. corallina*, endemic to the Pacific Central America, and *T. cheni*, known from a limited number of specimens from Taiwan (Galil 1983). Extensive sampling at the type locality of *T. cheni* was unsuccessful at securing any specimens.

Outgroups included other brachyurans, including species of *Tetralia*, *Domecia*, and *Cymo* within the Xanthoidea, and *Thalamitoides quadridens* (Portunidae), *Carpilius convexus* (Carpiliidae), *Potamon fluviatile* (Potamidae), *Varuna litterata* (Varunidae), and *Pilumnus sp.* (Pilumnidae).

### Extraction and Sequencing

DNA was isolated from muscle tissue taken from the leg or body. Two protocols were employed for extracting, cleaning, and amplifying DNA. Some specimens were processed at the University of Florida, following protocols given by Meyer (2003). Others were processed at the Smithsonian Institution's Laboratories of Analytical Biology (LAB). There, DNA was extracted by digesting tissue overnight in 150 ul M2 buffer and 150 ul M1 + proteinase K buffer. This digestion was done at 56.5 °C while samples were agitated at 50 rpm on a Labnet Vortemp 56. Extraction was done on an AutoGenprep 965 Automated DNA Isolation System using phenol-chloroform. PCRs were performed on an ABI 2720 Thermal Cycler or MJ Research PTC-225 Peltier

Cycler. The PCR profile used for amplifying COI was as follows: initial denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation at 95°C for 30 sec, annealing at 48°C for 30 sec, elongation at 72°C for 45 sec, and a terminal elongation at 72°C for 5 minutes. The PCR products were cleaned using ExosapIT (from USB). Sequence data was collected for the mitochondrial marker CO1 using the primers dgLCO (5'-GGT CAA CAA ATC ATA AAG AYA TYG G-3') and dgHCO (5'-TAA ACT TCA GGG TGA CCA AAR AAY CA-3') (Meyer 2003); all samples were sequenced in both directions. This generated a sequence of approximately 650base pairs long.

### **Sequence Analysis**

Chromatograms were cleaned, assembled, and aligned using the program Geneious (Drummond et al. 2006), and checked by eye. Cleaned sequences were checked for stopcodons and indels suggesting NUMTs. In all analyses, characters were unordered, and gaps were treated as missing data in a GTR+ $\Gamma$  model with rates estimated from the data. Maximum likelihood analyses and bootstrapping were performed using the program RAxML (Randomized Axelerated Maximum Likelihood)(Stamatakis 2006). We calculated pairwise genetic distances for each sister species pair identified in our phylogenetic trees using PAUP\*, using Kimura's(1980) K2P distance metric to facilitate comparison with earlier studies.

### **Speciation**

We labeled sister relationships between ESUs, Evolutionarily Significant Events (ESEs); these had to be supported by a bootstrap of at least 50. These are to speciation, what ESUs are to species (Malay and Paulay 2010). ESEs were labeled primary when they were between two ESUs, and secondary when they were between one ESU and a clade composed of more than one ESU. Nodes with multiple ESEs in

each branch were not considered. The spatial and temporal boundaries between ESEs were assessed using the distribution of the component clades and their K2P genetic distances.

### **Morphology and ecology**

Character traits gleaned from specimens and collection data were mapped using the program MacClade (Maddison and Maddison 1992). Descriptors of color pattern, size, host specificity, occurrence with sister taxa were coded as unweighted characters. Nodes with scores less than 50 were collapsed into polytomies for character analysis.

## **Results**

The aligned dataset contained 214 sequences of 601 characters. No indels were present, so alignment was trivial. One species, *Trapezia punctimanus*, failed to sequence and is excluded from further analysis.

### **Phylogeny and Species Boundaries**

The phylogenetic analyses differentiated 21 ESUs within *Trapezia* (Appendix A, Table 2), each reciprocally monophyletic for COI with concordant support from morphological identification, color pattern, and/or geographic distribution.

The deepest divergence within *Trapezia* is between a branch with *T. rufopunctata* and *T. flavopunctata* and the rest of the genus. These are the two largest species, and share a number of morphological characteristics which have led previous researchers to consider the two crabs closely related (Galil and Lewinsohn 1985b). Within the CO1 phylogeny, the branch is linked with one of the outgroups; an unidentified pilumnid crab, *Pilumnus sp.*, though this linkage is weakly supported (B= 40). We believe this to be an artifact of the poor resolution of CO1 at determining deeper nodes, and expect other

genes and gene regions will support *Trapezia* as a monophyletic genus. Preliminary runs of 16S support this hypothesis.

The next clade that branches in the phylogram is the *Trapezia digitalis* group. *Trapezia digitalis* as currently delineated forms a paraphyletic complex with *T. areolata* thus forming three allopatric ESUs: Pacific *T. digitalis*, Indian Ocean *T. digitalis*, which are both uniformly dark, and *T. areolata*, a SE Polynesian endemic with a reticulated color pattern. Though distinctive in coloration, the body shape and ecological preferences of these two named species are very similar. The sister taxon to this *T. digitalis* species complex is *T. globosa*, a small specialist of “crash zone” areas of reefs in the South Central Pacific, though this was poorly supported (Bootstrap 52).

*Trapezia bella*, forms a shallow, but well supported clade within a paraphyletic *T. formosa*. Sequenced *T. bella* form a clade that differs 1.7-3.0% (K2P) from *T. formosa* specimens. There is no clade-level geographic differentiation within *T. formosa*, a species with 0.2-3.0% (K2P) intraspecific variation in COI. Despite a lack of reciprocal monophyly at CO1, these two species are supported as ESUs by both differences in color pattern and allopatric ranges in addition to genetic differentiation.

Four of the 21 ESUs in the phylogeny are not currently recognized as valid species *T. lutea* (IO), *T. cymodoce* (PO), *T. digitalis* (IO), and *T. cymodoce* (IO) (Castro et al. 2004). One currently recognized species *T. neglecta*, recently described from Guam (Castro 2003), is genetically indistinguishable from *T. intermedia*, previously thought to be a Hawaiian endemic. This result has since been morphologically corroborated by Peter Castro (in litt, 2009) who agrees that these two populations are not specifically distinct. A second species pair, *T. speciosa* and *T. garthi*, also proved genetically

indistinguishable, though are visually distinct, and have partially overlapping ranges. We have considered these two forms distinct taxonomic species in the following analyses, though we cannot corroborate them as ESUs due to lack of more than one character group showing divergence. Further research may demonstrate them to be morphotypes of the same species.

### **Evolution of color patterning**

*Trapezia* are colorful crabs, but while some are solidly colored, others show striking patterning with spots or reticulations (Figure 4). Patterning is evolutionarily labile, with 6 transitions, 2 losses, 1 gains, and 2 ambiguous, in the phylogeny. Of the ten geminal ESU pairs supported in the phylogenetic analysis, five had one ESU with a plain carapace, the other with a complex color pattern (Figure 1).

### **Host Specificity**

Host specificity beyond the family level does not commonly occur in *Trapezia*, with only three ESUs limited to a subset of taxa (Table 1). Host branch width, rather than taxonomy appears to be the main factor limiting occurrence in these species. *T. rufopunctata* and *T. flavopuncta* both prefer large branched pocilloporid corals (*P. eydouxi*, *P. woodjonesi*, *Stylophora* sp.), while *T. guttata* prefers thin branched corals (*P. damicornis*, *Seriatopora hystrix*). The two large-branch-preferring species form a clade, indicating two total transitions among *Trapezia* from generalist to more specialized host preference (Figure 2).

### **Geographic differentiation**

Of the nine primary ESEs resolved in *Trapezia*, seven are comprised of allopatric sister taxa, and two (*rufopunctata-flavopunctata*, *septata-guttata*) are between sympatric ESUs. One of the secondary ESEs retains allopatry, while three show sympatric ranges.

K2P distances between allopatric sister taxa ranges 4.86 – 11.50%, while between sympatric sisters it ranges 1.39 – 5.89%, the two values are significantly different (t-test,  $p < 0.005$ ).

Six of the 8 ESEs separate taxa between the western Indian Ocean and the Pacific ocean. We lack sampling in the eastern Indian Ocean to further localize this break. The remaining two ESEs differentiate taxa in SE Polynesia from taxa in the western Pacific; in both sampling constrains the ESE boundary to lie between Samoa and the Society Islands. Of the currently recognized species of *Trapezia* that range across the Indian and Pacific oceans, four (*T. lutea*, *T. bidentata*, *T. cymodoce*, *T. digitalis*) demonstrate differentiation at the level of reciprocal monophyly in COI between the two ocean basins. K2P distances between PO and IO pops range from 1-6%. Subtle difference in color patterns corroborates divergence in all but *T. digitalis*, which has quite high levels of divergence (4-5% K2P), and uniform coloration across the range. The *T. digitalis* complex shows further differentiation in SE Polynesia with the endemic *T. areolata*, sister taxon to Pacific *T. digitalis*, despite dramatically different coloration and pattern (figure 1). The shallowest divergence is found in *T. cymodoce*, which displays only 1-2% (K2P) divergence between Indian and Pacific populations.

The remaining pair of allopatric sister taxa the recognized species *T. serenei* (PO) and *T. richtersi* (IO) which also are divided by Pacific – Indian Ocean basins. Similar to the taxa above, the *T. serenei* and *T. richtersi* differ from 2-3% (K2P).

Four sister taxa show sympatric ranges (Figure 4). Sympatric species pairs with resolved taxonomy show 4-10% (K2P) divergence, while the *T. garthi*-*T. speciosa* clade demonstrates 1-2% (K2P) divergence.

## Discussion

### Species Boundaries and Taxonomic Implications

The genetic diversity encompassed within *Trapezia* appears to be both under- and over-recognized in the current taxonomy. Indian vs. Pacific Ocean *T. cymodoce*, *T. lutea*, and *T. bidentata*, are ESUs that are genetically differentiated but have not in the past been recognized as species. These taxa show distinct color differences from their Pacific sister taxa and have allopatric ranges. Similar levels of divergence have been also been seen in sister ESUs that are currently accepted as species such as *T. serenei* and *T. richtersi*. These designations of species status are subjective, as allopatry does not allow for direct testing of the BSC criteria. Boundaries between two pairs of currently recognized species: *T. speciosa*-*T. garthi* and *T. formosa*-*T. bella* are poorly established in the COI phylogeny, despite distinctive color pattern differences. These two complexes may have different underlying causes. *T. garthi*-*T. speciosa* are indistinguishable genetically, despite morphological/color differences, and co-occur, at least in Taiwan. Alternative explanations for this include very rapid speciation and sympatry (including potential sympatric speciation), introgression of mitochondrial DNA between the two taxa, or unrecognized intra-specific color polymorphism. Sequencing additional markers, scoring multiple morphological characters, and field observations and lab experiments on pairing could be used to test among these alternatives.

In contrast *T. formosa* and *T. bella* are allopatric. The phylogenetic analysis suggests that *T. bella* is a divergent ESU nested within the variable '*T. formosa*'. As the range of *T. bella* is peripheral to that of *T. formosa*, it is possible that *T. bella* originated through founder speciation in SE Polynesia.

## Color Patterns

In general color evolves more rapidly than structural morphology in decapods (Knowlton 1986). Recognition of this is changing operational species criteria in carcinology as it becomes more widely appreciated. In the majority of sister ESUs, color pattern provides a visible means of distinguishing these most closely related forms. Sister ESUs are often remarkably different. Pairs where one ESU has a spotted or reticulated pattern while the other shows more uniform coloration is observed repeatedly: in *Trapezia tigrina* - *T. plana*, *T. guttata* - *T. septata*, *T. areolata* - *T. digitalis*, *T. serenei* - *T. richtersi*. (Figure 1). In contrast the most uniformly colored species, *T. digitalis*, does not show obvious color differences despite geographic and genetic structure.

The presence or absence of complex color patterns was largely uninformative of relationship. For example, *T. rufopunctata*, *T. bella*, and *T. tigrina* are all pale crabs with reddish spots, but this pattern appears to have evolved independently in each (Figure 1). In contrast sister taxa are frequently divergent in color and pattern. *Trapezia tigrina* is a large spotted crab with grey eyes, superficially similar to *T. rufopunctata*; its sister *T. plana* is a much smaller animal, with a uniform orange-brown carapace and striking blue eyes. Similar contrasts are seen between the spotted *T. richtersi*, and its sister, the solid pink *T. serenei*. The evolution of complex color pattern appears to occur very rapidly. Both *T. digitalis* (PO) and *T. areolata*, and *T. garthi* - *T. speciosa* demonstrate dramatic differences in color despite very close relationships. *T. digitalis* (PO) and *T. areolata* are separated by 1.9-2.4% (K2P) divergence, similar to the 1.8-2.6% (K2P) divergence within the *T. garthi*- *T. speciosa* complex.

## Host Specificity

We did not find evidence of host specificity beyond the family level in *Trapezia* species. Family level host specificity binds all currently recognized species of *Trapezia* to scleractinian corals of the family Pocilloporidae. However, as in the work of Sin and Lee (2000), observable preferences are notable in habitat choice among host species, but even this is uncommon. *T. guttata* has a distinct preference for fine branching pocilloporids such as *Pocillopora damicornis* and *Seriatopora hystrix*, while both of the largest species of *Trapezia* (*T. flavopunctata* and *T. rufopunctata*) typically inhabit only the largest species of pocilloporids, *Pocillopora eydouxi*, *P. woodjonesi*, and robust forms of *Stylophora*. To some degree this may be due to the relationship between crab size and interbranch width, though this remains untested.

Host specificity plays a central role in the speciation of parasitic symbiotic lineages, with increased specialization resulting from the escalation of 'arms races' between host and symbiont (Ehrlich and Raven 1964, Van Valen 1973). How this dynamic plays out in mutualisms and other forms of symbiosis is less clear (Bronstein and Hossaert-McKey 1996, Holland et al. 2004, Ness et al. 2006). If mutualists derive fitness benefits from a breadth of partner availability, then we can expect selection for both the crab and coral mutualists at intermediate levels of specificity where the relationship is sufficiently stable to assure a valid partnership, but open enough to allow for flexibility in symbiotic opportunities for both parties. The continued supply of benefits accrued by both symbiotic partners are central to this argument (Herre et al. 1999, Palmer and Brody 2007). As Stimson (1990) noted that the lipids provided to *Trapezia* by *Pocillopora* are 'shut off' in the absence of the crab, while the services provided to the coral by the crab are also variable (McKeon and Moore 2010), so the possibility of cheating in the system

exists. Cheaters external to the mutualism, or lack of control over benefits may explain the diversity of commensal organisms found on pocilloporids (Patton 1966, Patton 1974)- it is possible that the coral may be “robbed”, “tricked” into providing nutrition for non-defensive symbionts, or these symbionts may parasitize the coral-crab mutualism, though this needs further investigation into the ecological ‘function’ of symbiont species. Investigation into the evolution of the mutualism may be facilitated by the study of coral-associated crustacean groups that demonstrate a broader range along the parasitism-mutualism continuum.

### **The development of sympatry, microsympatry, and geographic ranges in *Trapezia***

The majority of geminal taxa in *Trapezia* are geographically isolated from each other, yet tend to have adjacent ranges, supporting allopatric speciation as the main mode of diversification. This is particularly true of “young” sister taxa with divergences of less than 3% (K2P). In contrast, sympatrically occurring sister taxa that are supported as ESUs display K2P divergences greater than 4%. Speciation events cluster in two locations, between the western Indian and Pacific ocean basins (5 ESEs), and between the western Pacific and SE Polynesia (2ESEs). Both these locations are well known as important biogeographic boundaries driving speciation across numerous taxa (Kay 1984, Randall 1988, Williams and Reid 2004) Two major biogeographic boundaries are encompassed between the western Indian and Pacific ocean, either of which may be responsible for the divergence of *Trapezia* species. One separates the western and eastern halves of the Indian Ocean basin, the other lies between the Indian and Pacific Ocean basins.

It is also notable that sympatric sister species pairs show evidence of some degree of ecological separation in all four cases in *Trapezia*. *T. rufopunctata* - *T. flavopunctata*

share the same range in the Pacific basin and co-occur on reefs. However they do not tolerate each other in the same coral, despite tolerating up to 4 other species of *Trapezia* within coral heads they occur in (McKeon et al. 2010). *T. tigrina* and *T. plana* are more poorly understood ecologically, but *T. tigrina* is a much larger crab, almost double the size of *T. plana*. *T. septata* and *T. guttata* are sister taxa that co-occur over much of their range, however they rarely occur in the same corals; while *T. septata* is a host “generalist”, *T. guttata* has a preference for thin branched corals (*P. damicornis*, *Seriatopora hystrix*, etc. )(Sin 1999). This heteropatry may have allowed the sympatric evolution of the two species, or have facilitated the development of secondary sympatry following another form of divergence.

The *T. garthi-speciosa* species complex consists of two genetically indistinguishable (with CO1) forms, with divergent color patterns and broadly overlapping geographic ranges. Both forms co-occur in individual coral heads. The information available at this point cannot differentiate between introgression, color polymorphism, or rapid speciation. The role of coloration in the mating systems of *Trapezia* have yet to be fully explored, but preliminary studies are suggestive of a direct role in mate choice (Huber 1987b). As the mutualism with pocilloporids is obligate it restricts habitat availability of closely related and thus potentially ecologically or reproductively competing forms. Rapid differentiation of color pattern may allow for the coexistence of sympatric species through heteropatric processes– if coloration directs assortative mating. In his work with Hawaiian *T. digitalis* Huber (1987b) noted that two slightly different colorations would pair assortatively in the lab, though not under field conditions, and was also associated with a significant increase in heterozygosity (Huber 1985). Direct tests of behavioral

patterns, such as those conducted by Odinetz (1984b) on the response of *Trapezia* to crabs of a different species that were similar in morphology, but different in color, may be illustrative of the processes at work.

The preliminary work on the molecular phylogeny of *Trapezia* presented here supports the idea that this is an ideal model system for the study of coral reef crustaceans. As with many other marine invertebrates, the genus is under-described in comparison to morphological assessments alone, but has remaining mysteries such as the *T. garthi-speciosa* complex, which are currently differentiated solely on the basis of color pattern. Complex color pattern appears to have multiple origins within the genus. Most sister ESUs within the genus have allopatric distributions, while the few exceptions each showing notable ecological or behavioral differentiation that may facilitate sympatry. In this respect the patterns found in *Trapezia* follow general phylogenetic patterns found in reef decapods, while the ecology and behavior of *Trapezia* is relatively well known. In tandem, the two areas of study, systematics and ecology, may allow for greater insight into the evolutionary processes that drive patterns of abundance, geographical distributions, and speciation in this and other reef-associated taxa.

Table 2-1. *Trapezia* ESUs

ESU	Number of Specimens Sequenced	Patterning	Host Specificity
<i>Trapezia rufopunctata</i>	5	Patterned	Large Branched Pocilloporids
<i>Trapezia flavopunctata</i>	6	Patterned	Large Branched Pocilloporids
<i>Trapezia globosa</i>	2	Solid	Pocilloporids
<i>Trapezia digitalis (IO)</i>	5	Solid	Pocilloporids
<i>Trapezia digitalis (PO)</i>	7	Solid	Pocilloporids
<i>Trapezia areolata</i>	3	Patterned	Pocilloporids
<i>Trapezia cymodoce (IO)</i>	5	Solid	Pocilloporids
<i>Trapezia cymodoce (PO)</i>	7	Solid	Pocilloporids
<i>Trapezia lutea (IO)</i>	5	Solid	Pocilloporids
<i>Trapezia lutea (PO)</i>	3	Solid	Pocilloporids
<i>Trapezia intermedia</i>	5	Patterned	Pocilloporids
<i>Trapezia bidentata (IO)</i>	6	Solid	Pocilloporids
<i>Trapezia bidentata (PO)</i>	15	Solid	Pocilloporids
<i>Trapezia septata</i>	8	Patterned	Pocilloporids
<i>Trapezia guttata</i>	12	Solid	Small Branched Pocilloporids
<i>Trapezia speciosa</i>	3	Patterned	Pocilloporids
<i>Trapezia garthi</i>	2	Patterned	Pocilloporids
<i>Trapezia formosa</i>	9	Solid	Pocilloporids
<i>Trapezia bella</i>	4	Patterned	Pocilloporids
<i>Trapezia cheni</i>	0	Solid	Pocilloporids
<i>Trapezia corallina</i>	0	Solid	Pocilloporids
<i>Trapezia tigrina</i>	17	Patterned	Pocilloporids
<i>Trapezia plana</i>	2	Solid	Pocilloporids
<i>Trapezia punctimanus</i>	0	Solid	Pocilloporids
<i>Trapezia richtersi</i>	6	Patterned	Pocilloporids
<i>Trapezia serenei</i>	10	Solid	Pocilloporids

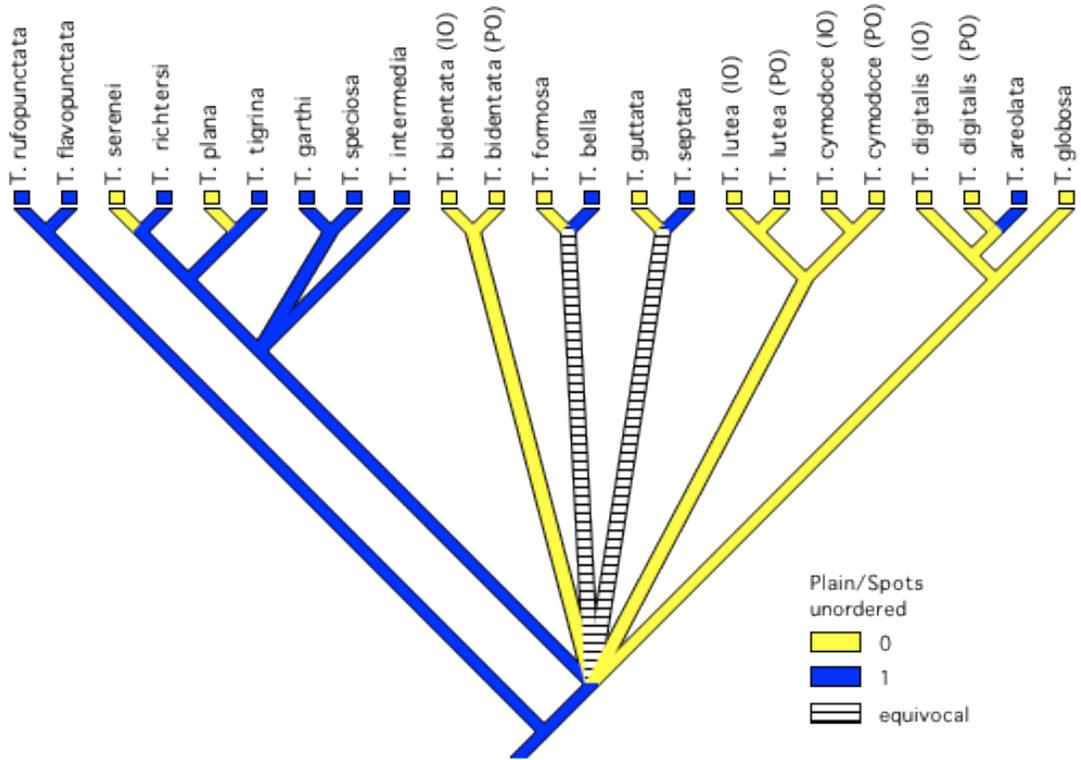


Figure 2-1. Character Map of Color Pattern in *Trapezia*. Species with a plain carapace coded as '0', species with a patterned carapace coded as '1'.

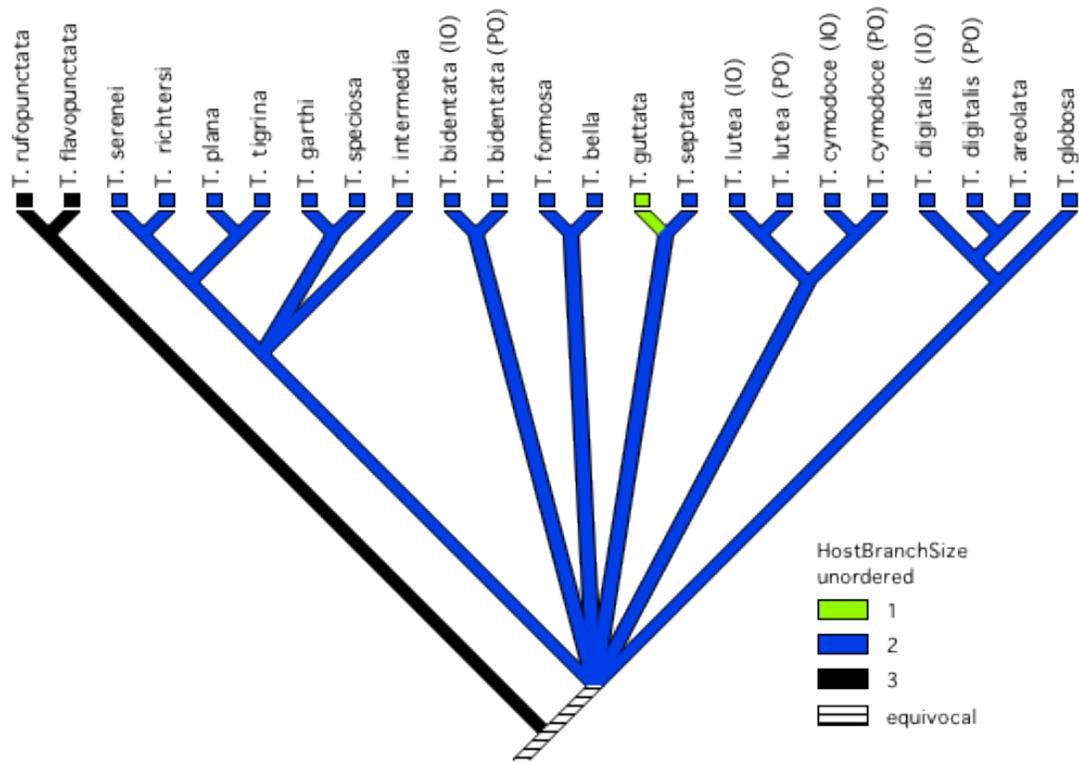


Figure 2-2. Character Map of Host Specificity in *Trapezia*. Preference for large branched pocilloporid species coded as '3', no preference coded as '2', preference for small branched pocilloporids coded as '1'.

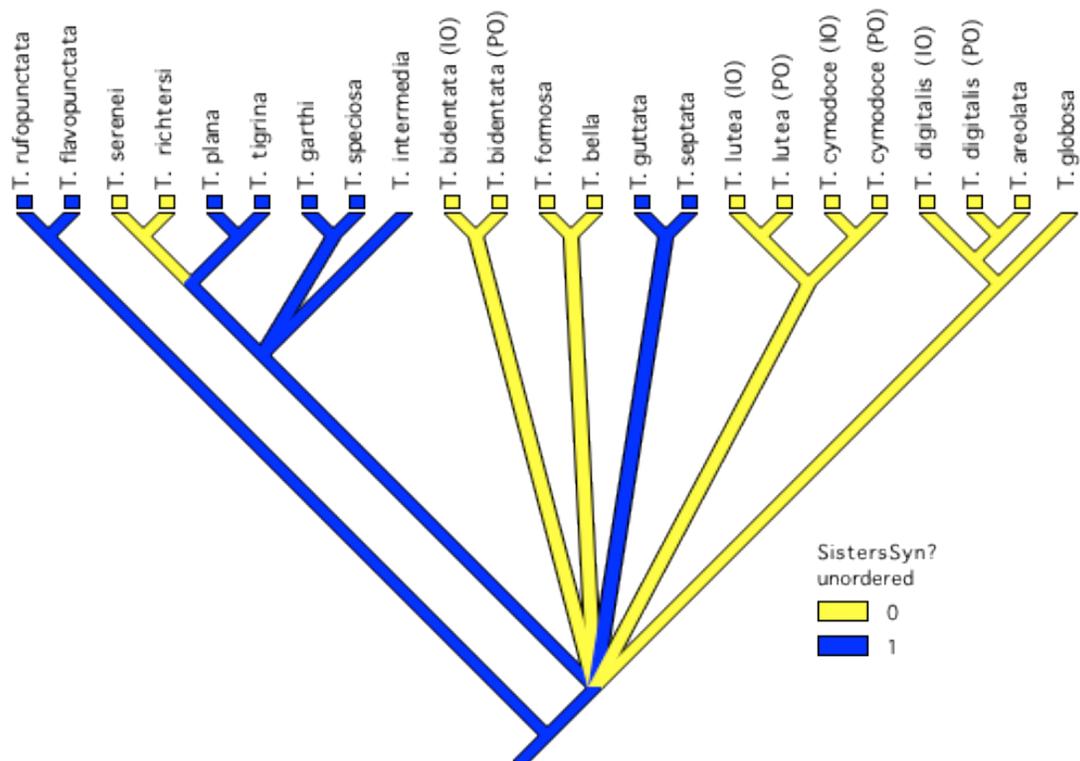


Figure 2-3. Character Map of Sympatric Sister-Species Pairings in *Trapezia*. ESUs found sympatrically with their nearest sister are coded as '1'. ESUs found to be allopatric with their sister taxon are coded as '0'.



*T\_areolata.jpg*



*T\_bidentata (IO).jpg*



*T\_digitalis.jpg*



*T\_flavopunctata.jpg*



*T\_formosa.jpg*



*T\_globosa.jpg*



*T\_guttata.jpg*



*T\_intermedia.jpg*



*T\_lutea (IO).jpg*



*T\_lutea.jpg*



*T\_plana.jpg*



*T\_punctimanus.jpg*



*T\_rufopunctata.jpg*



*T\_septata.jpg*



*T\_serena.jpg*



*T\_speciosa.jpg*



*T\_tigrina.jpg*

Figure 2-4. Examples of coloration and pattern in *Trapezia*

## CHAPTER 3 DIVERSITY IN MUTUALIST SERVICES OFFERED BY CORAL CRABS

### **Introduction**

The creation and maintenance of biological diversity inspire fundamental questions in ecology. Nowhere are these questions more strikingly apparent in the sea than in the hyperdiversity of coral reefs. Ultimately, diversity is the result of speciation rates exceeding extinction rates. Ecology influences extinction rates, and thus diversity, via biotic interactions such as competition, predator-prey interactions, and ecosystem engineering. Two emergent questions in ecology are how are diversity and species coexistence maintained in the face of such interactions, and what are the ecological consequences of diversity?

Understanding coexistence in the face of competition has a long history in ecology. Hutchinson (Hutchinson 1961) posed the “paradox of the plankton”: how can a limited range of resources support within-guild diversity given the expectation of competitive exclusion? He proposed a number of mechanisms for coexistence, including variable environmental conditions, differential susceptibility to predators, or mutualism as factors that may promote within-guild coexistence. These factors, and others added since, may be grouped into two general categories: those that lead to differences in niches (potentially through character displacement), and those that are a function of stochastic variability.

Interspecies interactions form the basis of coexistence and have evolutionary consequences. Predator-prey relationships can promote both speciation (ecological character displacement facilitating reproductive character displacement), and extinction through ‘arms races’ (Van Valen 1973). Competition may result in competitive

exclusion, reducing diversity (McNaughton 1985), while mutualisms should have a direct positive effect on diversity through two routes: 1) reduction of antagonism between host and symbiont, thus no arms races that could end in extinction, and 2) the survival benefits conferred between pairs of mutualists may buffer the species involved from stressors external to the relationship.

A third route is the potential for emergent survival benefits in systems that possess complementary species assemblages of mutualists. Community diversity has been largely overlooked, despite its relevance to many mutualist systems (Howe 1984). Greater numbers of mutualists may confer greater survival benefits to the host species than individual mutualists (Stachowicz and Whitlatch 2005, Baskett et al. 2009). Whole communities may have net beneficial effects that can be considered mutualistic in nature (Bracken et al. 2007, Stachowicz et al. 2008). Studies on the interactions between mutualist species are underrepresented in the literature (Morris et al. 2007), but relevant and central to understanding diverse systems (McKeon et al. 2010). Groups of co-occurring mutualists meet the definition of both alpha (based on resource use) and beta (based on environmental conditions) guild types (Wilson 1999), as well as functional groups.

The diversity or redundancy of ecological roles in similar species has become a focal point in our understanding of the way that communities are assembled and diversity is maintained (Harris 1995, Loreau et al. 2002, Loreau 2004). The concept of 'functional groups' has been used as a tool to simplify diversity into ecological units, but studies testing for the equivalence of species within functional groups have showed mixed results (Chalcraft and Resetarits 2003). Despite this, assumptions of functional

equivalence of species within functional groups have remained a central, if challenged, premise in much of theoretical ecology, from Neutral Theory (Hubbell 2005) to Food Web Theory (Menge and Sutherland 1987, Leibold and McPeck 2006).

The contrasting view of functional diversity, and resulting concept of the complementarity of species within communities (Barnes 2002), also have underpinnings in ecological theory, despite their direct opposition to functional equivalence. The limited studies to date that compare the ecological functions of closely related species under the same environmental conditions have supported the idea that functional diversity, rather than equivalence, may be the norm (Reserits and Chalcraft 2007b).

Testing for functional diversity and equivalence may be most tractable and applicable in clades of closely related or morphologically similar species (Leibold and McPeck 2006). Crabs of the genus *Trapezia* differ in size, but remain morphologically very similar. *Trapezia* have been described as defensive mutualists of their pocilloporid coral host (Pearson and Endean 1969, Weber and Woodhead 1970, Glynn 1976, 1987), despite lingering questions regarding the effectiveness of this defense against the most well-known of corallivores, the crown-of-thorns seastars of the *Acanthaster planci* complex (Bonito 2002). These crabs have been implicated in providing a number of ecological services to their host corals: repulsion of corallivores (Glynn 1987, Pratchett and Vytopil 2000, Pratchett 2001a), removal of sediment (Stewart et al. 2006), and alleviation of the impacts of vermetid snails (Stier et al. 2010). Quantification of these services has been limited, and despite hypotheses regarding the importance of the mutualism to the demography of reef corals (Glynn 1976, Glynn 1983, Glynn 1987), little work has explicitly examined the survival of corals from which *Trapezia* have been

removed. Despite its familiarity as one of the most well known of marine mutualisms, the differences between the species in the genus have never been examined in detail.

### **Study system**

*Trapezia* occur within branching pocilloporid corals across their entire range, across the Indo-Pacific region, from the east coast of Africa to Central America. The crabs form a monophyletic assemblage, and are further related to several genera (such as *Tetralia*) within the largest radiation of crabs, the Xanthoidea that are also cnidarian (often coral) symbionts (Castro et al. 2004). The crabs range in adult size from 4 mm to more than 20 mm carapace width. Colors and patterns are distinctive and elaborate, but have at times confused rather than clarified species level identifications within the group across its range (Castro et al. 2004, Lai et al. 2009).

Of the 23 described species, 13 occur in French Polynesia, and 12 in American Samoa. In both areas *Trapezia* are dominant members of an assemblage of specialized associates of pocilloporid corals. Community assembly varies with coral species, growth form, reef zone, and community membership (Odinetz 1983); pers. obs.), with the maximum size of the *Trapezia* notably constrained by interbranch width (Adams et al. 1985, Huber and Coles 1985). In many reef environments sampled to date more than 90% of pocilloporid colonies host *Trapezia* (Stewart et al. 2006, McKeon et al. 2010). In the Central Pacific, coral colonies are typically occupied by a single pair of a given *Trapezia* species, though these colonies can host multiple species as well as additional juveniles. A small coral head may shelter a single pair of crabs, while larger coral heads may have pairs of up to 5 species of *Trapezia*. In addition to providing shelter, some *Pocillopora* corals have been shown to produce lipids that are

sequestered in enlarged tentacle tips and eaten by the crab. In the absence of *Trapezia*, *Pocillopora* stop producing these lipid stores (Stimson 1990).

*Pocillopora* species make up a large percentage of reef cover in lagoonal and fore reef systems in the Central Pacific. The bulk of this is composed of coral colonies with morphologies in three coarse groupings. The *Pocillopora verrucosa* – *P. meandrina* group, referred to in this manuscript as “*Pocillopora verrucosa*”, are mid-sized pocilloporids, rarely exceeding 15 cm in colony height and typically occupied by small- to medium-sized *Trapezia* species. A second morphological group, the *P. eydouxi* – *P. woodjonesi* group (hereafter “*Pocillopora eydouxi*”), is larger in stature, commonly exceeding 60 cm in colony height, and have substantially broader spaces between their branches where crabs are sheltered. These corals host the entire size range of *Trapezia* species; from the smallest at branch junctures and the colony's base, to the largest, which actively transit the openings between branches.

The third morphological grouping is composed of species currently assigned to *Pocillopora damicornis*, a finely branched morphotype that exhibits extreme environmental variation across reef microhabitats (Veron and Pichon 1976), and is referred to as “*Pocillopora damicornis*” in the following experiments.

Species-level distinctions across the geographic range of *Pocillopora* remain unresolved at morphological, genetic, and taxonomic levels (Veron and Pichon 1976, Veron 2000, Combosch et al. 2008). As such, we chose to use internally consistent morphological groupings in the experiments explained below, but do not have further identification of the entities involved.

Three species of corallivores were used in the studies: two sea stars: *Acanthaster planci* (a species complex, (Vogler et al. 2008) crown-of-thorns seastar, hereafter “*Acanthaster*”), *Culcita novaeguinae* (hereafter “*Culcita*”), and the muricid gastropod *Drupella cornus* (hereafter “*Drupella*”). These species are common coral predators on Indo-Pacific reefs. *Acanthaster* predation of reef corals is well documented, as are its population booms (Birkeland 1982, 1989). Large numbers of *Acanthaster* are capable of eating the complete coral assemblage on a reef, though they do have feeding preferences (Pratchett 2007, Pratchett et al. 2009). *Culcita* has been documented to feed on a variety of sessile prey, including corals (Glynn and Krupp 1986). *Drupella* is a specialized corallivore second only to the Crown-of-Thorns seastar in its impact on reef corals (Turner 1994).

During 2008 - 2009, Moorea experienced an outbreak of *Acanthaster*. By the fall of 2008, the majority of live coral on the surface of the reef had been eaten, with the notable exception of *Pocillopora eydouxi*. *Pocillopora* has previously been ranked among the favored corals in the diet of *Acanthaster* (Pratchett 2007). The smaller *Pocillopora verrucosa* had been consumed in near totality, and least-favored taxa such as *Porites* and soft corals were also being eaten.

## **Objectives**

The objectives of this study were to examine the extent of functional redundancy vs. ecological complementarity among *Trapezia* species in their ability to protect their host corals from predation and sedimentation, and to explore the emergent properties of this coral-symbiont microcosm. We examined the efficacy of various combinations of crab species and sizes in protecting *Pocillopora* against *Culcita*, *Drupella*, and sedimentation

in the lab, and the impact of *Acanthaster* on *Pocillopora-Trapezia* communities in the field, during an *Acanthaster* outbreak. Our studies targeted the following questions:

- Do species of *Trapezia* differ in the protective services they offer to corals through defense against various corallivores and sedimentation?
- Are small *Trapezia* species, and small individual crabs of larger species functionally equivalent?
- Does the effect of *Trapezia* mutualism extend beyond the host coral to the larger reef environment?

## Methods

### Study Locations

Studies on coral defense were conducted in Moorea, French Polynesia, at the Gump Research Station of the University of California, utilizing the flow-through seawater system. Studies on sedimentation were based out of the park field station of the Ofu Field unit of the American Samoa National Park, using a temporary flow-through seawater system (Smith et al. 2007a, Barshis et al. 2010). The two locations are similar in terms of the number of *Trapezia* species present, although Moorea has two endemic species not found outside of French Polynesia.

### Removal of Sediment

Sediment removal trials were conducted in a small flow-through sea water system on Ofu. Sediment from the local fringing reef comprised largely of biogenic CaCO<sub>3</sub> was collected, then separated into size classes with geological sieves. Two size classes (1 – 2 mm and 3 – 4 mm) were kept for experimental use. These were washed, dried using a drying oven, and divided into 50 g units. *Pocillopora damicornis* colonies were collected from 75 cm deep water in the lagoonal pools of the immediate area. Corals in this experiment had an average size of 389 cm<sup>3</sup> (n = 43, sd = 153). Unwanted *Trapezia*

species were removed from the coral using bamboo skewers. Male-female pairs of three species (*T. serenei*, *T. septata*, and *T. guttata*) with carapace width (CW) of 7-10 mm were utilized, along with a control treatment of no crabs. All three species of crabs were used in trials with the larger sediment size (3 – 4 mm), while only *T. serenei* and *T. septata* were used in trials with the smaller sediment size (1 – 2 mm). Corals were positioned on a supportive plastic grid over a two-layered catch tray, allowing for an initial measurement of sediment that fell from the coral before the start of the trial, without disturbing the colony. 50 g of sediment were sprinkled onto the coral, and the amount that fell off was removed from the experiment, dried, and weighed to calculate the amount of sediment on the coral at the beginning of the trial. After 24 hours, the additional sediment that fell from the coral was dried and weighed.

Data were analyzed using a linear model approach. The effectiveness of crab species in removing sediment was evaluated along with a control group in a linear model (ANOVA) framework. ANOVA/Tukey's HSD were applied in the statistical programming environment R (2006).

### **Effectiveness against corallivores**

#### **Effectiveness against *Drupella***

For experiments testing the effectiveness of *Trapezia* in repelling the corallivorous snail *Drupella*, two 135 L aquaria served as experimental chambers. Two sets of experiments were conducted: one comparing the effect of small individuals of *Trapezia serenei* and *T. punctimanus* in repelling *Drupella* from their small coral hosts, the other examining the effect of larger sized *T. serenei* in repelling *Drupella* from larger coral hosts. *Pocillopora verrucosa* were gathered from the back reef environment on the day

of the trial. The small coral colonies had an average volume of  $260 \pm 136 \text{ cm}^3$ , while the larger coral colonies had an average volume of  $3706 \pm 1057 \text{ cm}^3$ .

The desired experimental symbiont community was established through removal of symbionts and other coral-associated animals. Animals were removed from each coral by chasing or flicking them out with wooden skewers. Treatments were: control (no symbionts), *T. serenei* (two size classes:  $n = 22$ , 4 – 6 mm Carapace Width (CW);  $n = 19$ , 9 – 10 mm CW) and *T. punctimanus* ( $n = 22$ , 4 - 6 cm CW) pairs. *T. punctimanus*, is a small species with a mean carapace width (CW) of 5 mm, while *T. serenei* has mean CW of 13 mm, but often attains reproductive maturity at much smaller sizes in small corals.

Forty *Drupella*, with 15 – 17 mm aperture lengths were collected from the fore reef, housed in a glass aquarium with a flow-through sea water system and starved for  $\geq 72$  hours prior to beginning experiments.

One *P. verrucosa* colony with a pair of *Trapezia* was placed into the center of one randomly assigned tank, and a coral without symbionts was placed into the center of the other tank. Three *Drupella* individuals were lined up on the bottom, 3 cm from the downstream rear edge of the aquarium to ensure the availability of a chemosensory signal from the coral to the snails. Three *Drupella* were used so that a measurable feeding scar would be produced during the experimental period. The experiment was repeated with new coral colonies and crabs each night. Coral treatments were alternated between tanks each day so that no treatment would be in the same tank for two consecutive trials. *Drupella* were reused after being starved for  $\geq 72$  hours between trials. After ten days, new *Drupella* were used.

After an experimental run of 19 – 24 hours, the length, width, and height of corals were measured. Responses measured included the number of snails found on the coral and the size of the feeding scars where tissue had been removed from the coral skeleton. Proxies of coral volume and feeding scars created by *Drupella* were calculated as the volume of an ellipsoid using the formula:  $V = 4/3\pi abc$ , where a, b, and c are radii. These data were relayed together as coral tissue lost. After each trial the aquaria were scrubbed, drained and refilled.

The defensive efficacy of the crab treatments was compared to appropriately sized controls; the differences in host size does not allow for comparison between the efficacy of small crabs in small corals and larger crabs in larger corals.

### **Effectiveness against *Culcita***

The effectiveness of defense against *Culcita* by different sizes and species of *Trapezia* was assessed in flow-through plastic pools of approximately 2,670 l volume. *Pocillopora verrucosa* were gathered from the back reef environment on the day of the trial. “Small” coral colonies had an average volume of 519 cm<sup>3</sup> (n = 17, sd = 175), while “Larger” coral colonies had an average volume of 3354 cm<sup>3</sup> (n = 63, sd = 979). As above, the desired experimental symbiont community was established through removal of symbionts and other coral-associated animals. Trials testing the efficacy of “medium” sized *Trapezia* in larger corals included male/female pairs of *T. bidentata* (n = 20) and *T. serenei* (n = 20) pairs where each individual was between 9 – 11 mm carapace width, along with controls with no symbionts present (n = 20). Treatments of “small” sized *Trapezia* in small corals included pairs of *T. serenei* where each individual was between 4-6 mm carapace width (n = 9), and controls (n = 8).

*Culcita* (n = 37) were collected from the backreef, held in flow-through cattle tanks, and starved for at least 48 hours before being used for a trial. Corals were positioned in the middle of tanks with the draining standpipe positioned to the North. *Culcita* were placed directly on top of the experimental corals at sundown, and feeding was evaluated the following morning, after ~15 hours. Proxies of both coral size and the size of feeding scars were calculated using the formula for the volume of an ellipsoid, as noted above, the latter related as 'coral tissue lost'. The differences in defense provided to the coral host by two species of *Trapezia* (*T. serenei*, *T. bidentata*) were evaluated using a linear model (ANOVA) approach. As all small corals with small crabs were consumed in the second set of experiments, no statistical test was needed.

### **Response to *Acanthaster* Outbreak**

Several studies have suggested that *Trapezia* may be able to repel *Acanthaster* corallivory (Glynn 1987; Pratchett 2001). These studies have either been observational or conducted as lab experiments. A large outbreak of *Acanthaster* on Moorea beginning in 2007 allowed us to assess the efficacy of protection rendered by *Trapezia* to *Pocillopora* in a natural setting.

To assess the impact of the outbreak on the coral community we conducted surveys of live coral cover using 45 haphazardly placed 1 m<sup>2</sup> grid photo quadrats between 5 and 10 m depth in the spur-and-groove zone of the fore reef of the North coast of Moorea during the fall of 2008 and again in fall 2009. The majority of corals that survived the outbreak were *Pocillopora eydouxi*.

To assess protection rendered by *Trapezia flavopunctata*, the largest species of *Trapezia*, to these *P. eydouxi* colonies, we removed this species from 45 haphazardly selected colonies, while maintaining a second set of 45 colonies as controls, in the

same area. Each coral head was visually surveyed for exosymbiont species before the removal experiment began, and the removals were maintained for one month. Other symbiotic species of fish and arthropods, including other species of *Trapezia*, were left in the coral head. Every 48 hours all corals were checked for loss (feeding scars), and measured as described above.

Additional surveys were conducted around 90 live and 90 dead *P. eydouxi*, for live corals that remained in the immediate proximity of *P. eydouxi* colonies. *Pocillopora eydouxi* is one of the large, arborescent species on the fore reef, and even prior to the *Acanthaster* outbreak, arched as a canopy over neighboring smaller corals. Living coral colonies encountered under the canopy and to 1/2 of colony diameter beyond the canopy margin were counted and identified to genus.

Species identification of crabs was made by the first author, relying on Castro et al. (2004), initial descriptions of the species involved, and molecular methods (McKeon and Paulay 2010)

## **Results**

### **Effectiveness against Sedimentation**

#### **Three Species and Large-grain Sediment**

All crab species were significantly more effective at removing sediment than the coral alone (Tukey HSD,  $p > 0.005$ ). Two of the crab species (*T. septata* and *T. guttata*) had effect ranges that were significantly different from each other (Tukey HSD,  $p > 0.005$ ), while the effect of the third species (*T. sereneri*) could not be distinguished from the other two (Tukey HSD,  $p > 0.1$ ; Figure 1).

## Two Species and Small-grain Sediment

Sediment removal did not differ significantly between the two species of crab (*T. septata* and *T. guttata*) and control treatments (ANOVA, Tukey HSD,  $p > 0.1$ ; Figure 2).

## Defensive efficacy against corallivores

### Trapezia vs. Drupella

Corals without crab symbionts were preyed upon in 91% of the trials, while corals containing a *T. serenei* pair (CW = 5 mm) were preyed upon in 36% of the trials. Tissue loss was significantly higher in *P. verrucosa* containing no symbionts (13.9 cm<sup>3</sup>,  $x = 0.087$ ) than in corals containing *T. serenei* pairs (2.9 cm<sup>3</sup>,  $x = 0.013$ ; ANOVA  $p < 0.05$ , Figure 3).

*P. verrucosa* containing *T. punctimanus* (CW = 5 mm) were preyed upon in 41% of the trials, while corals containing no symbionts were preyed upon 91% of the time. Coral tissue loss was significantly higher in corals containing no symbionts (22.9 cm<sup>3</sup>,  $x = 0.057$ ) than in corals containing *T. punctimanus* pairs (2.88 cm<sup>3</sup>,  $x = 0.0061$ ; ANOVA  $p < 0.05$ ; Figure 3). There was not a significant difference of tissue loss between treatments with the two crab species (ANOVA  $p > 0.05$ ).

In the experiments with larger *T. serenei* (CW = 10 mm), tissue loss was not significantly different in corals containing *T. serenei* (11.67 cm<sup>3</sup>,  $x = 0.0031$ ) from corals containing no symbionts (13.40 cm<sup>3</sup>,  $x = 0.0036$ ; Student's t-test,  $p > 0.05$ ).

### Trapezia vs. Culcita

The average amount of tissue lost to *Culcita* differed among treatments; it was 19% in medium-sized corals hosting large (~10mm) *T. serenei* ( $n = 5$ ), 37% in corals with comparable sized *T. bidentata* ( $n = 10$ ), and 52% in controls without crabs ( $n=20$ ; ANOVA,  $p < 0.01$ ). Differences between crab treatments and controls, and

between *T. serenei* and small (~5 mm) *T. bidentata* were also significant (Tukey HSD,  $p < 0.001$ ,  $p < 0.05$ , Figure 4).

Small corals with (n=9) and without (n=8) *T. serenei* were completely consumed by *Culcita*.

## **Response to *Acanthaster* Outbreak**

### **Live Coral Cover**

Live coral cover in 2006, as estimated from photos of the study area, approached 80%, with approximately 10% of the cover comprised of *Pocillopora eydouxi*. The fore reef of Moorea had strikingly high and rich coral cover in the early and mid 2000's, dominated by acroporids and pocilloporids (Adjeroud et al. (2002)). By the fall of 2008, coral cover had plummeted in N Moorea as a result of *Acanthaster* predation. In the study area, total coral cover has decreased to 3.4% (se = 0.97, n = 45), with 100% of recorded corals being *P. eydouxi*. Coral cover changed little by fall 2009, with estimated cover at 3.2% (se = 0.75, n = 45), of which 100% was *P. eydouxi*. It is noteworthy that other species of *Pocillopora*, mainly *P. verrucosa*, which were more abundant on the reef prior to the outbreak than *P. eydouxi* was, were virtually absent from the reef after the *Acanthaster* outbreak. While *Pocillopora eydouxi* hosts the two largest *Trapezia*, vis. *T. flavopunctata* and, on Moorea less common, *T. rufopunctata*, these large crabs are generally absent from *P. verrucosa*.

### ***T. flavopunctata* and *Acanthaster***

Removal of *T. flavopunctata* from remaining live *P. eydouxi* led to an increased rate of attack and tissue loss in hosts. Over the two-week trial, 29 of 45 of these less defended corals, compared with 8 of 45 corals with *T. flavopunctata*, showed evidence of attack (Binomial proportions test,  $p < 0.005$ ; Figure 5). Loss of coral tissues

averaged 22% in undefended vs. 2% in defended corals (Binomial proportions test,  $p < 0.005$ ; Figure 6). Thirty-four corals from which *T. flavopunctata* were removed still possessed a complement of other *Trapezia* species (as well as other symbiotic taxa). When we compared attack rate between *P. eydouxi* that had both *T. flavopunctata* and smaller *Trapezia* species present (10 of thirty-three), to corals from which *T. flavopunctata* had been removed, but still had smaller *Trapezia* species (24 of 34), corals which did not have the larger crab species were at a significantly greater risk of attack (Binomial proportions test,  $p < 0.005$ ).

### **Associational Defense**

Of the 90 live and 90 dead *P. eydouxi* surveyed, live *P. eydouxi* alone sheltered living corals of other species under and near its canopy. Thirteen species of corals were found sheltered around 64% (Binomial proportions test,  $p < 0.005$ ) of the live *P. eydouxi* surveyed (Table 1). These species were otherwise lost from the reef and not encountered away from *P. eydouxi*, although they were common across the reef surface prior to the *Acanthaster* outbreak. None of the 90 dead *P. eydouxi* sheltered live coral colonies.

### **Discussion**

The efficacy of different *Trapezia* species in protecting their host coral against sedimentation and three different corallivores varied substantially. *Trapezia* species of comparable size, morphology, habitat, and geographic range (Table 2) demonstrated different efficacy in removing large-grain (but not small-grain) sediments from host corals. Corals possess a variety of sediment removal abilities (Bak and Elgershuizen 1976, Abdel-Salam et al. 1988, Acevedo et al. 1989), and are better able to reject small-grain sediment than large-grain sediment (Lasker 1980). Stafford-Smith and Ormond

(1992) found that *Pocillopora damicornis* was able to move small grain sediment using its tentacles, but attributed most sediment clearing in this and other branching corals to skeletal morphology and water motion. Larger grains, such as those used in our treatment, were not tested in the Stafford-Smith and Ormond study. The small grain size we used may fall into a range that the corals were capable of clearing actively or passively, without the additional presence of the crabs.

Efficacy to defend against corallivores differed among *Trapezia* species as well as among size classes. Thus small (CW = ~5 mm) *T. punctimanus* and *T. serenei* were comparably effective in defending against *Drupella*, while large (CW = ~10 mm) *T. serenei* were ineffective, albeit in a slightly different setting (see below). Large (CW = ~10 mm) *T. serenei* however were effective in defending against *Culcita*, while small ones (CW = ~5 mm) were not. Further, large (CW = ~10 mm) *T. serenei* and comparably sized *T. bidentata* significantly differed in the protection they rendered their host against *Culcita* attack. Finally, in an *Acanthaster* outbreak only corals hosting the largest *Trapezia* species, i.e. *T. flavopunctata* or *T. rufopunctata* survived; smaller *Trapezia* species were unable to protect their hosts against this greatest of the three predators, and were quickly attacked when these (but not the other) *Trapezia* species were removed from them.

These results suggest substantial ecological complementarity, as well as a hierarchy of defense effectiveness among different species and sizes of *Trapezia*. Small crabs effectively defend against the small predator *Drupella*, but fail against larger predators. Medium sized crabs are effective against *Culcita*, with effectiveness varying among species, but do not defend against *Drupella* or *Acanthaster*, (at least in an outbreak

setting). The largest crabs defend actively against *Acanthaster*, their efficacy against the other coral predators remains untested. Thus a coral harboring all these symbionts would be defended against all three corallivores, but one with a lesser complement of *Trapezia* species may be vulnerable to some predators. An additional aspect of 'species stacking' is that several species may create additional synergistic defensive effects even against the same coral predator (McKeon et al. 2010).

These results suggest that the characteristics of the mutualism between *Trapezia* and *Pocillopora* may shift as the resident crabs and/or coral hosts increase in size. Because the feeding scars of the corals without symbionts in the small *T. serenei* and *T. punctimanus* experiments were nearly the same size as the feeding scars in both groups of the large *T. serenei* experiments, it may be possible that the threat to smaller crabs and their correspondingly smaller hosts by *Drupella* predation is proportionally greater. This may explain why larger crabs did not respond as aggressively as the smaller crabs to *Drupella*. Glynn (1980) suggested that another small species of *Trapezia*, *T. formosa* may not play a role in defending their host corals against *A. planci*. However, smaller species of *Trapezia* previously thought to be ineffective guards, because they were only tested against *Acanthaster*, play important defensive roles against other corallivores.

Crab defense against *Culcita* varied by the size classes of the corals and/or the size and species of *Trapezia*. Small *Pocillopora* were consumed entirely by *Culcita*, regardless of the presence or absence of small *Trapezia*. The crabs apparently flee the coral, or are consumed along with the host.

The efficacy of protection provided by two species of *Trapezia* (*T. serenei*, *T. bidentata*) against *Culcita* differed under controlled conditions. Both significantly reduced the rate of attack and volume of tissue consumed by *Culcita*, but *T. serenei* was more effective despite the same size range and habitat. *T. serenei* is also the more common of the two species in the backreef habitat where *Culcita* populations are the most dense, while *T. bidentata* is more common in fore-reef environments where *Culcita* is less frequently encountered in Moorea.

Both attack rate and the amount of coral tissue consumed by *Acanthaster* were significantly reduced by the presence of the largest *Trapezia* in the system, *Trapezia flavopunctata*. In contrast *P. eydouxi* were eaten by *Acanthaster* when no *Trapezia flavopunctata* were present. It is of note that species of *Pocillopora* that do not host *T. flavopunctata* were completely consumed by *A. planci* in the area of reef studied, despite occupancy of other *Trapezia* spp. greater than 90% (Stewart et al. 2006, M<sup>c</sup>Keon et al. 2010).

The protective impact of *T. flavopunctata* appeared to extend beyond the host coral, providing protection for a microcommunity of reef corals in the vicinity of their host. 61 colonies of 13 other reef coral species, which had been eaten from the surface of the reef in all other areas by *Acanthaster*, were found sheltering in the vicinity of 90 *P. eydouxi*-symbiont microcosms surveyed. This “associational defense” is only possible in the presence of the largest species of *Trapezia*.

The sister species of *T. flavopunctata*, *T. rufopunctata* is also present in the forereef of Moorea where the removal experiments took place. Several live *P. eydouxi* were found with *T. rufopunctata* present during the time period of the study, but numbers of *T.*

*rufopunctata* were insufficient to directly compare the defensive efficacy of the two species. It is of note that unlike the other species of *Trapezia*, *T. rufopunctata* and *T. flavopunctata* are not found to inhabit the same coral heads.

The results of these experiments raise many questions about the *Trapezia* – *Pocillopora* mutualism. Glynn (1980) demonstrated that *Trapezia* respond aggressively to chemical signals produced by *Acanthaster*, but whether *Trapezia* are able to detect and respond to chemical signals produced by other corallivores such as *Drupella* remains unknown. During an analysis of coral population dynamics in Australia, Hughes and Connell (1987) found that 39% of *Pocillopora damicornis* corals measuring less than 10 cm<sup>2</sup> died over the course of one year, while only 8% of *P. damicornis* measuring between 10 and 50 cm<sup>2</sup> suffered the same fate. While differential mortality is expected with changes in colony size, impacts of mutualists on different sizes of corals should be examined. The frequency of occurrence of *Trapezia* in medium-sized *Pocillopora* colonies is well documented (Huber and Coles 1985, Sin and Lee 2000, Stewart et al. 2006) but our understanding of the occurrence of *Trapezia* in very small corals is limited. Further investigation into the role of small crabs of the genus *Trapezia* in the survival of very young *Pocillopora* colonies is needed.

While the largest crab species provide the greatest defensive efficacy against *Acanthaster*, the frequency of *Acanthaster* outbreaks on Moorea has been approximately on a 30-year cycle (Faurea 1989). Attacks by seastars may be relatively rare events in the life of a coral. Removal of sediment, and deterrence of smaller corallivores such as *Drupella*, may be more common needs for coral, even if the size of their impact on a colony may not be as severe as that resulting from *Acanthaster* attack.

This spectrum of needs may be sufficient “motivation” for a coral to maintain the nutritive benefits that are provided to the crabs (Stimson 1990).

The idea that parasitism is a driver of diversity has been a mainstay of evolutionary thought (Summers et al. 2003): it is in the interest of the host to defend against assault, and in the interest of the parasite to overcome this, leading to increased specialization to the host and typically corresponding restriction of host range (Ehrlich and Raven 1964). Mutualisms may also select for specialization (Bronstein and Hossaert-McKey 1996) , but the feedbacks may be less specific and in many cases, such as that of *Trapezia-Pocillopora*, it is in the best interests of both the host and symbiont to remain generalized with respect to host range. If all *Trapezia* are beneficial, a coral will most benefit by being able to host any species of *Trapezia* that settle on it. The same can be said of the crabs: availability of host should be maximized through generalization. Thus while both the crabs and corals are specialized with regard to their mutualistic mode of life, there is limited incentive to narrow host range as long as host-symbiont communication can be maintained. While coral-parasitic mollusks and crustaceans tend toward host specificity at the species level and may co-speciate with their host (Gittenberger et al. 2004, Malay 2010), species-level specialization and co-speciation appears to be limited in *Trapezia* (Sin 1999, McKeon and Paulay 2010). Nevertheless the *Trapezia – Pocillopora* system does show specialization in other dimensions, such as to reef habitat and topography (Odinetz 1983).

The experiments we conducted suggest that functional diversity exists in the *Pocillopora-Trapezia* mutualism at a species level. Variation within the mutualism was demonstrated to impact coral host survival on a landscape scale. Interactions between

the species and size classes are likely an integral part of the mechanisms that promote the existence of the *Pocillopora-Trapezia* mutualism, but have yet to be explored in depth. Variation in the services provided by mutualists has been recorded in a wide variety of systems (Addicott 1986, Bronstein and Hossaert-McKey 1996, Del-Claro and Oliveira 2000, Fenster and Dudash 2001). This makes the idea of functional groups more coarse than generally noted in the literature and of questionable utility in understanding diverse mutualist systems.

The positive relationship between biodiversity and the maintenance of marine ecosystem function has been well documented (Worm et al. 2006, Stachowicz et al. 2007). Ecological mechanisms contributing to this effect include:

1. complementarity in the use of resources among species and their responses to environmental variation;
2. positive interactions among species; an increased likelihood of key species being present when species diversity is high;
3. and redundancy, which can provide biological insurance against changes in ecosystem function because species that are similar in many ways may be differentially susceptible to environmental change.

Our studies suggest that the *Trapezia-Pocillopora* mutualism possesses the three mechanisms outlined above. We also found that taxa range in response to environmental factors, and that measuring a diversity of response variables is important in assessing the level of functional diversity present in a system. Our results demonstrate the importance of the *Trapezia-Pocillopora* mutualism in response to the key environmental factors of sedimentation and corallivory, including an outbreak of *Acanthaster*. A diverse commensal fauna provides more resilience to host corals than individual species do. Moreover, these benefits cascade beyond the holobiont and may have far-reaching impacts on the reef, in the form of altering the demography of small

corals, structuring the surviving communities of corals and offering associational defenses and refuge to nearby corals during catastrophic predator outbreaks.

Table 3-1. “Coral” species sheltered by the *P. eydouxi*-*T. flavopunctata* mutualism.  
Taxonomy following Veron (2000).

Fungiidae	<i>Herpetolitha limax</i>	1
	<i>Fungia</i> sp.	4
Poritidae	<i>Porites rus</i>	16
	<i>Porites</i> (massive)	3
Acroporidae	<i>Acropora hyacinthus</i>	4
	<i>Acropora valida</i> complex	7
	<i>Montipora</i> sp.	2
Pocilloporidae	<i>Pocillopora verrucosa</i> complex	7
Agariciidae	<i>Pavona varians</i>	3
	<i>Leptoseris incrustans</i>	3
	<i>Gardineroseris</i> <i>planulata</i>	10
Milleporidae	<i>Millepora</i> sp.	1

Table 3-2. *Trapezia* species used

Species	Experimental Use	Mean Carapace Width	Localities present	Host Preference
<i>Trapezia serenei</i>	Sediment removal Response to <i>Drupella</i> (small individuals) Response to <i>Drupella</i> (large individuals) Response to <i>Culcita</i> (small individuals) Response to <i>Culcita</i> (large individuals)	13mm	Samoa, Moorea	Pocilloporid corals
<i>Trapezia punctimanus</i>	Response to <i>Dupella</i>	5mm	Moorea	<i>P. verrucosa</i>
<i>Trapezia guttata</i>	Sediment removal	14mm	Samoa, Moorea	Fine branched pocilloporids ( <i>P. damicornis</i> , <i>Stylophora hysterix</i> )
<i>Trapezia septata</i>	Sediment removal	15mm	Samoa	Pocilloporid corals
<i>Trapezia bidentata</i>	Response to <i>Culcita</i>	14mm	Samoa, Moorea	Pocilloporid corals
<i>Trapezia flavopunctata</i>	Response to <i>Acanthaster</i>	26mm	Samoa, Moorea	Large pocilloporid corals ( <i>P. eydouxii</i> , <i>Stylophora pistillata</i> )

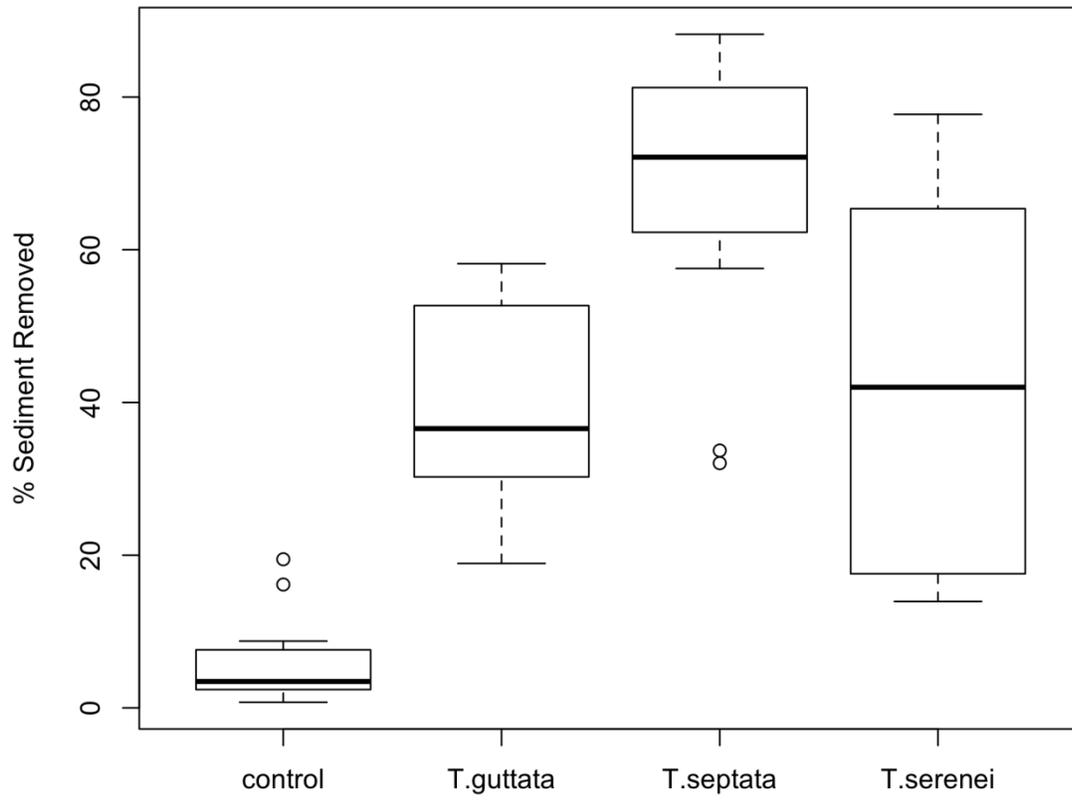


Figure 3-1. Percentage of large grain sediment cleared by three species of *Trapezia*.

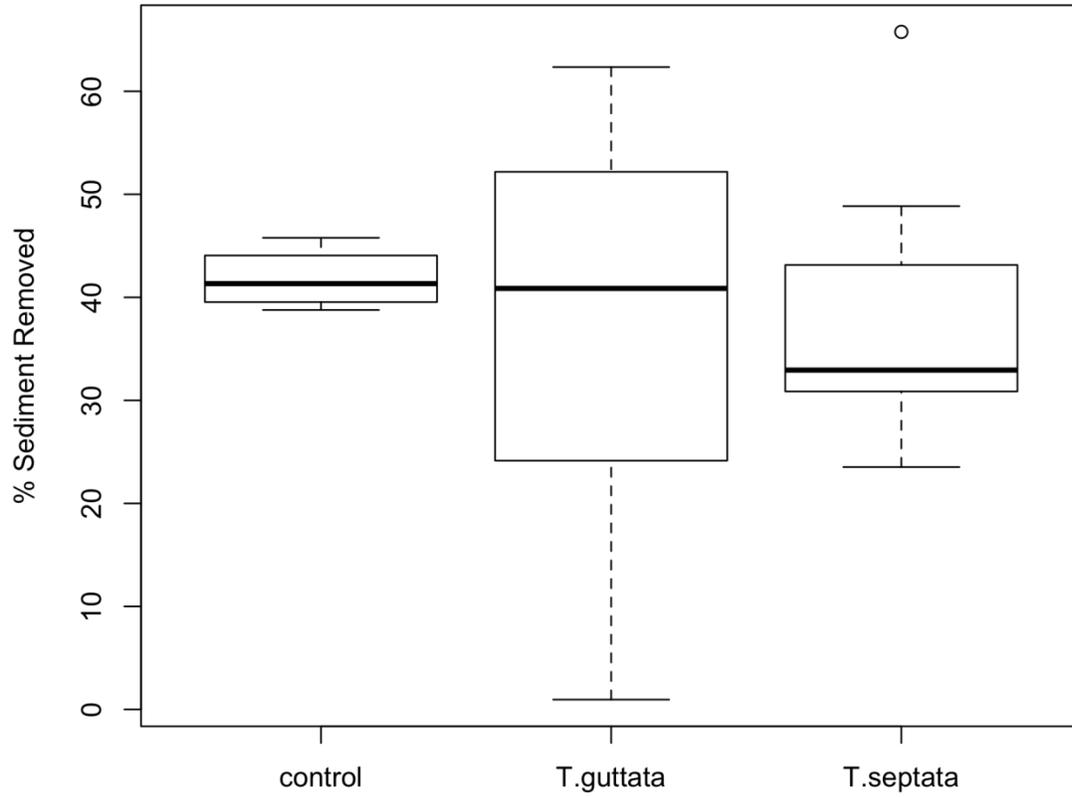


Figure 3-2. Percentage of small grain sediment cleared by two species of *Trapezia*

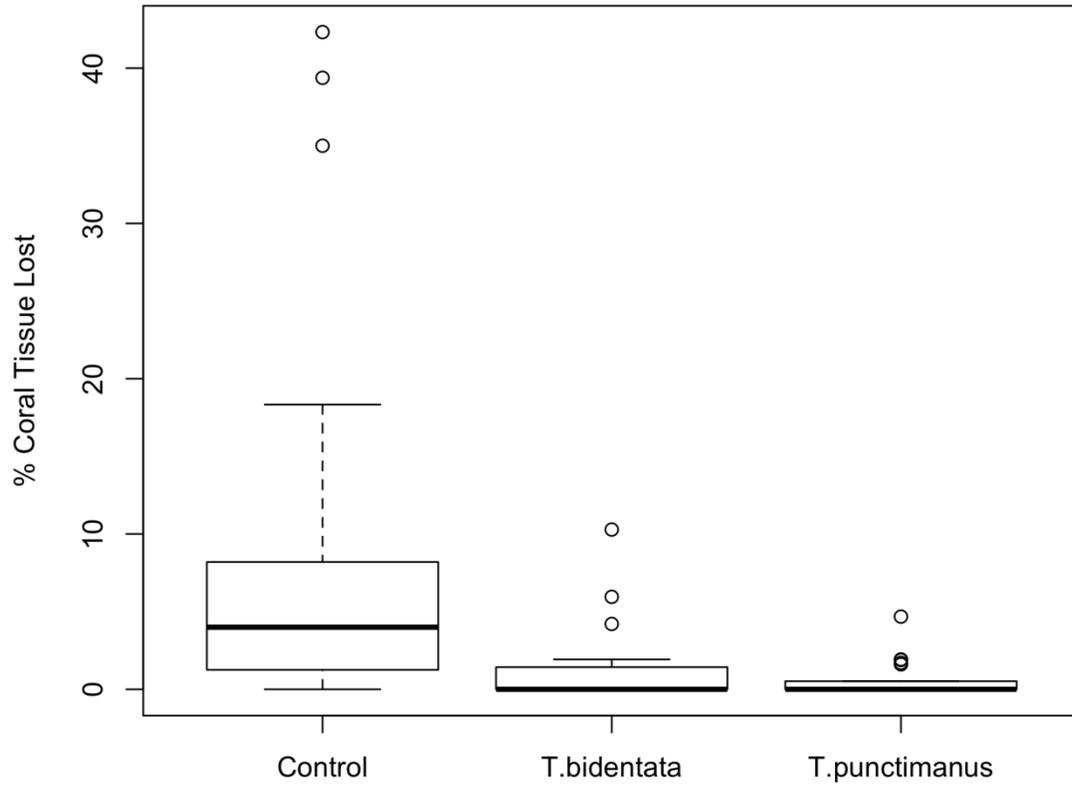


Figure 3-3. Amount of coral tissue consumed by *Drupella cornus* in corals hosting *Trapezia serenei* and *Trapezia punctimanus*

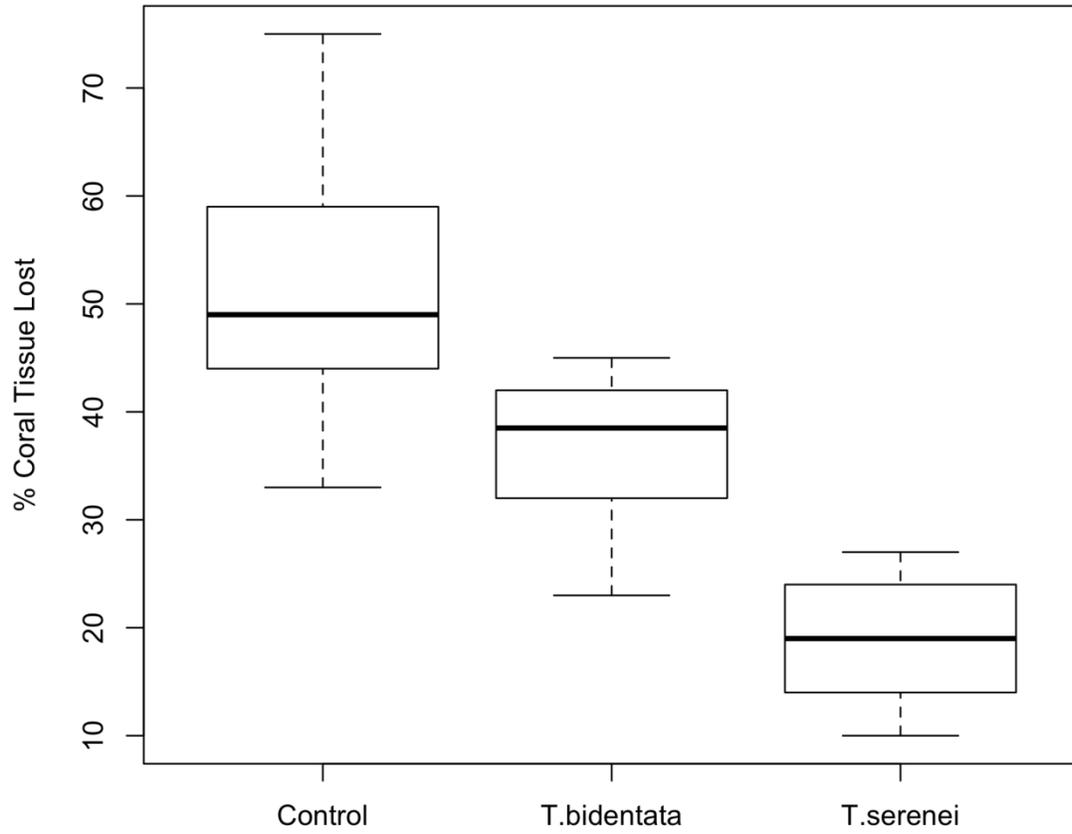


Figure 3-4. Percent of coral lost to *Culcita novaeguinae* in corals hosting *Trapezia bidentata* and *T. serenei*

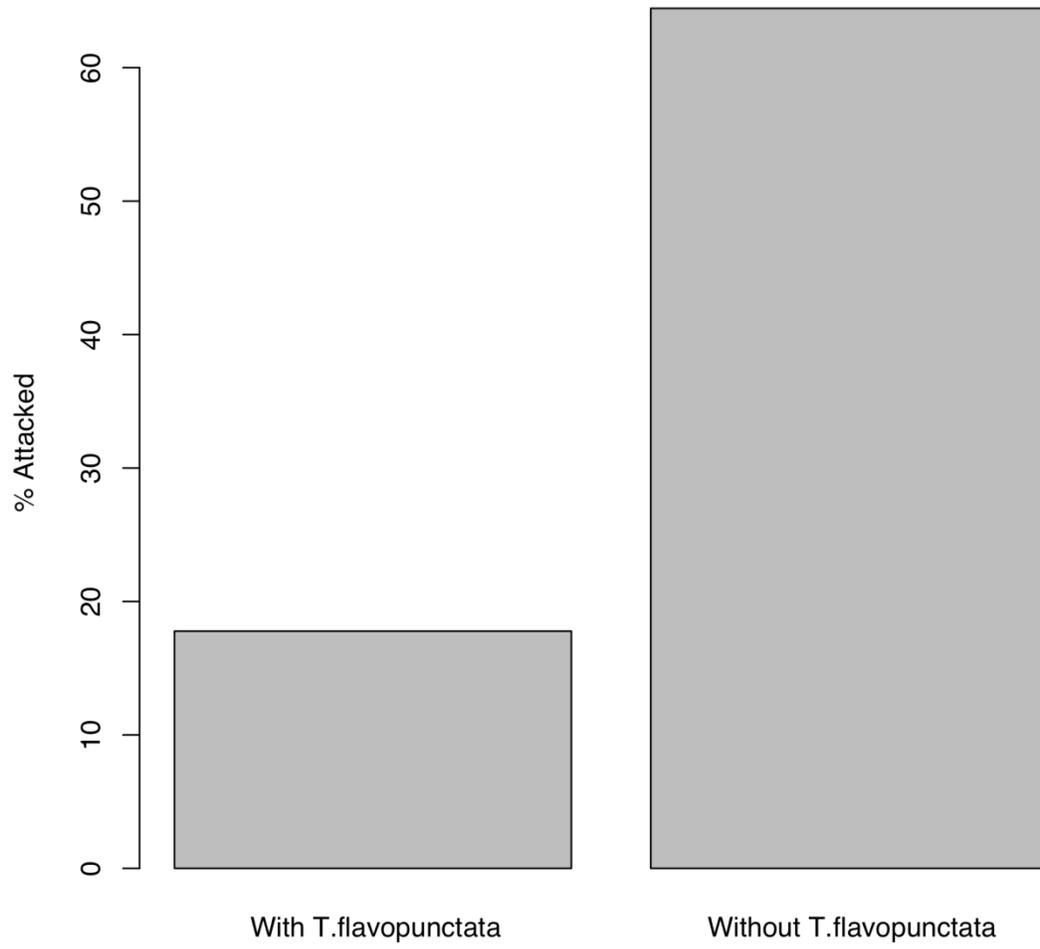


Figure 3-5. Rates of attack in defended and undefended *P. eydouxii*

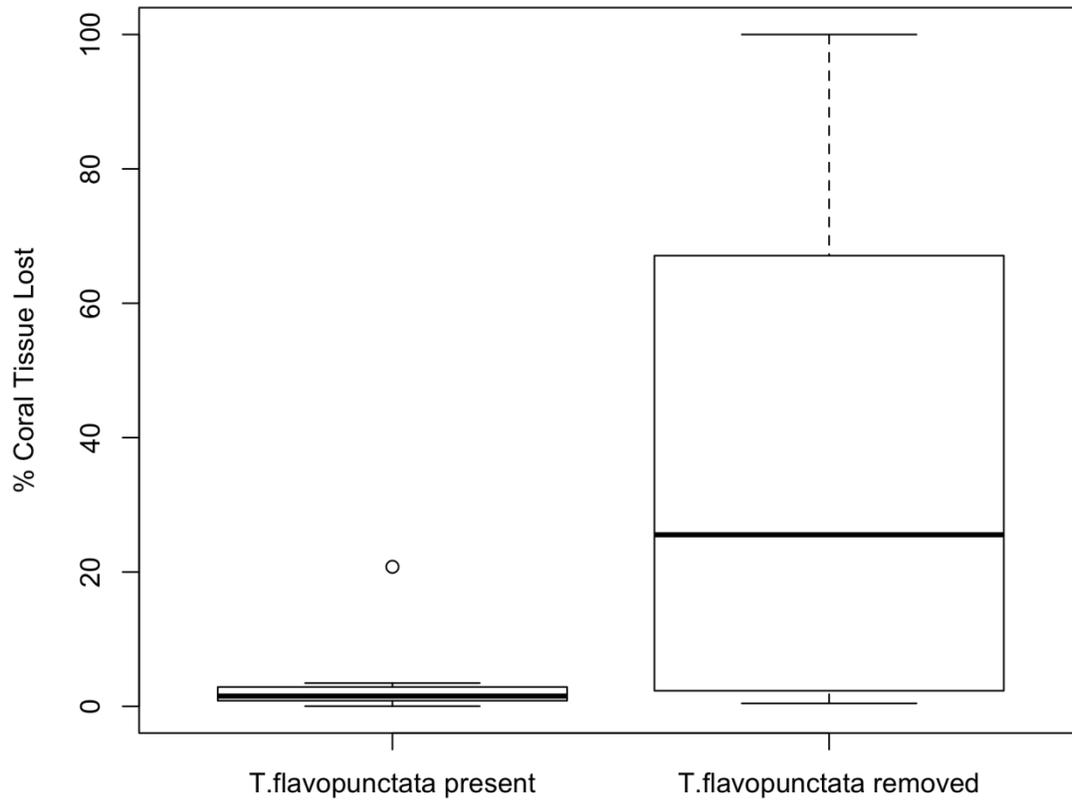


Figure 3-6. Amount of coral tissue consumed by *Acanthaster* in defended and undefended *P. eydouxi*

CHAPTER 4  
MULTIPLE DEFENDER EFFECTS MODIFY THE BENEFITS OF SPECIES  
STACKING: SYNERGISTIC CORAL DEFENSE BY MUTUALIST CRUSTACEANS

**Introduction**

Individual species within a guild of mutualists may exhibit differences in efficacy of the services provided to their host species. This pattern has been observed both within the defensive mutualisms such as ant-plant systems (Palmer and Brody 2007, Palmer et al. 2008) pollination syndromes (Holland et al. 2004), and corals (Little et al. 2004, Mieog et al. 2009). Intraguild competition or cooperation among mutualists can lead to reduced or enhanced mutualist efficacy relative to that which is expected though summing the independent pair wise effects of single species of mutualists in isolation (Stachowicz et al. 1999). However, few studies have attempted to go beyond estimating the differences in the effects of individual mutualists, which can be achieved through the factorial manipulation of multiple mutualists. Indeed, a recent meta-analysis by Morris *et al.* (2007) on independent and interactive effects of natural enemies and mutualists on plant performance found that of 160 studies on interactive effects (enemy-enemy, enemy-mutualist, and mutualist-mutualist) only 10 studies (reviewed from 5 different papers) exist on mutualist-mutualist interactions, while 114 studies focused on enemy-mutualist dynamics, and 36 focused on enemy-enemy dynamics. There was, however, no consistent effect of intraguild mutualist interactions on partner performance; across ten mutualist-mutualist studies, the authors found evidence for independent, antagonistic, and synergistic effects of groups of mutualists on hosts.

The study of higher-order interactions that lead to a non-independent response is not new to the field of ecology (Billick and Case 1994). Indeed, the study of intraguild dynamics among predators is now commonplace in a number of systems (Griffen 2006,

Vance-Chalcraft et al. 2007). An experimental design and analytical framework for testing higher order interactions within guilds has been developed in these multiple-predator studies. Tests for multiple-predator effects are often conducted using a factorial manipulation of predators (crossing the presence or absence of predator A with the presence or absence of predator B (Exlöv 2000, Warfe and Barmuta 2004)). The expected survival probability of prey in the presence of both predator A and B ( $P_{A+B}$ ) follows the following multiplicative risk model:

$$\hat{P}_{A+B} = \frac{(P_A)(P_B)}{(P_{NP})}, \quad (4-1)$$

where  $P_i$  is the survival probability in the presence of different predator treatments (A: predator A, B: predator B, and NP: no predator) (Soluk and Collins 1988, Sih et al. 1998, Vonesh and Osenberg 2003). Expected survival probability in the presence of two predators assumes a multiplicative risk model because predation events by each predator species are dependent upon predation by the other species (i.e., conditional probability - a single prey item cannot be eaten twice). Because two factor ANOVA (the appropriate analysis for a factorial design) assumes an additive model, the multiplicative risk model above can be log transformed to an additive form for analysis:

$$\ln(P_{A+B}) = \ln(P_A) + \ln(P_B) - \ln(P_{NP}). \quad (4-2)$$

Initial studies testing for multiple-predator effects did not log-transform their data, or did so to meet assumptions of ANOVA rather than to match the assumptions of the underlying biological model to the statistical model. This approach led to erroneous inference by interpreting statistical interactions as biological interactions after assuming an additive model on the arithmetic scale rather than log-additive risk model (Sih et al. 1998). In our study detailed below we test for intraguild higher-order interactions among

mutualists, a term we define as emergent multiple defender effects (MDEs), the analog of emergent multiple predator effects (MPEs).

Here, we focus on combined effects of mutualistic symbioses in coral reefs. Symbioses on reefs are common, and are exemplified by the well-known relationship between scleractinian corals and endosymbiotic algae (zooxanthellae) (Rowan 1998, Hoegh-Guldberg 1999, Mumby et al. 2001), a mutualism thought to be the fundamental factor allowing coral reefs to persist in the oligotrophic tropics. Though guilds of different endosymbionts are known to provide variable amounts of support to corals and respond differently to heat stress (Baker et al. 2004, Fabricius et al. 2004, Rowan 2004), their interactive effects are poorly studied (Rowan 1998). By comparison, exosymbiotic crustaceans have received relatively little attention; despite potential importance for the growth, reproduction, and survival of coral (Glynn 1983, Stachowicz and Hay 1999, Stewart et al. 2006), and the potential large-scale effects of these mutualisms on reef dynamics (Glynn 1987).

We conducted a series of experiments and behavioral observations on two crustacean species (*Trapezia serenei* and *Alpheus lottini* 'stripes', hereafter referred to as *Trapezia* and *Alpheus* respectively) to assess how the independent and combined effects of these exosymbionts contribute to defense of a host coral, *Pocillopora* cf. *meandrina* (hereafter *Pocillopora*) from the coral-eating seastar *Culcita novaeguineae* (hereafter *Culcita*). Our study was designed to answer the following questions: (1) Do exosymbionts effectively defend the coral? (2) Are the defensive responses of both exosymbionts equal? (3) Does a doubling in exosymbiont density increase the defensive efficacy, and (4) are the effects of multiple defensive exosymbionts

independent, antagonistic (less than predicted under the assumption of independence), or synergistic (better than expected)?

## Methods

### Study Species

All species of the coral genus *Pocillopora* are facultative hosts of *Trapezia* (trapeziid crabs) and *Alpheus lottini* complex (a species complex of alpheid snapping shrimps). Both *Trapezia* and *Alpheus lottini* are most commonly found in heterosexual pairs (C.S. M<sup>o</sup>Keon, unpublished data) which aggressively defend their territory from conspecifics (Huber 1987a). Glynn (1976) described the defensive reactions of crustacean symbionts to attack of their coral host by corallivorous seastars (*Acanthaster* and *Culcita*). Work within the system to date has shown the importance of exosymbiotic communities of corals in influencing the feeding of corallivorous seastars (Pratchett and Vytopil 2000, Pratchett 2001b) and the possibility of communication among co-occurring symbionts (Lassig 1977, Vannini 1985). The twenty-two species of *Trapezia*, along with 2-3 species of the *Alpheus lottini* complex, can be considered an interacting guild of coral-defending crustaceans. Potential costs to the coral host are largely unstudied, with evidence of provision of lipids by the coral host to *Trapezia* documented by Stimson (1990). The extent to which each potential symbiont species can be considered beneficial to its host is unknown, but given the high frequency of co-occurrence of *Trapezia* and *Alpheus lottini* (134 of 139 *Pocillopora* surveyed in the area of this study, S. M<sup>o</sup>Keon pers. obs.), and the evidence for communication among symbionts, there exists a strong potential for interactive effects between these two groups of mutualists.

## Experimental design

Colonies of *Pocillopora cf. meandrina* were collected from shallow water habitats near the Richard B. Gump Research Station, Moorea French Polynesia. We selected colonies that were roughly hemispherical in shape, and of moderate size (average maximum horizontal circumference = 61 cm,  $n = 80$ ). Volume of corals used in deterrence trials were  $20,083 \pm 2,035 \text{ cm}^3$  (mean  $\pm$  95% CI). We measured the length, width, and height of each experimental coral colony. Colonies were carefully surveyed for exosymbionts, and all had at least one pair of *Trapezia serenei* and one pair of *Alpheus lottini*. We then removed animals to yield the four treatment groups: 1) No Exosymbionts, 2) pair of *Alpheus lottini* only ('Alpheus') 3) pair of *Trapezia serenei* only ('Trapezia') 4) pair of *Alpheus lottini* and pair of *Trapezia serenei* ('Alpheus + Trapezia'). Corals were then positioned in 3m of water in the field to maintain the corals between lab trials and maximize survival.

We collected *Culcita novaeguineae* from shallow water habitats close to the research station. Though both large and juvenile individuals were found in the area, only medium sized *Culcita* (mean diameter of  $16 \pm 1.4 \text{ cm}$  (mean  $\pm$  SD),  $n = 64$ ) were used for the experiments in this study. *Culcita* were housed and fed *ad libitum* in flow-through seawater tanks for the duration of the study. Food was withheld for 24 hr before an individual was used in a trial. Individual *Culcita* were haphazardly assigned to trials and no individual was used in consecutive trials.

## Deterrence trials

We conducted choice experiments by allowing a *Culcita* to choose between two *Pocillopora* colonies in large (~150 L) flow-through aquaria. The coral colonies were

placed in opposite corners of the aquarium. A *Culcita* was placed equidistant from the two *Pocillopora* at dusk. Ten replicates were made for *No Exosymbionts vs. Alpheus + Trapezia*. The following morning we recorded which of the two corals was eaten by *Culcita* by examining corals for feeding scars, easily identifiable as the area of the colony where the coral tissue was eaten (i.e. bare white skeleton).

### **Test for Emergent Multiple Defender Effects**

We used feeding trials to quantify the efficacy of defense for four exosymbiont treatment groups: *Trapezia + Alpheus*, *Trapezia*, *Alpheus*, and *No Exosymbionts*. Twenty replicates in total were conducted for each exosymbiont treatment in a temporally blocked design with two replicates in each of ten temporal blocks. Trials were conducted in a large octagonal flow-through seawater tank approximately 0.5m deep and 2 meters across. The tank was divided into 8 equal sections using plastic screening. Each section was provisioned with a seastar refugium constructed from concrete blocks. We placed *Pocillopora* colonies into the tank from the field during mid- to late-afternoon. *Culcita* were placed directly on top of the coral colony to ensure seastars were exposed to the available prey. The following morning, we measured the coral size (length, width, height) and the feeding scars left by the *Culcita* (length, width, depth). We calculated volume consumed as an ellipsoid ( $\frac{4}{3}\pi abc$ , where  $a$  is  $\frac{1}{2}$  the length  $b$  is  $\frac{1}{2}$  the width and  $c$  is  $\frac{1}{2}$  depth)

### **Behavioral observations**

In a separate study we used behavioral observation methods based on those of Glynn (1980) to measure the reaction of *Pocillopora* exosymbionts to *Culcita* presence at different vertical locations on the colony. A coral colony with both *Alpheus* and

*Trapezia* exosymbionts was placed on an elevated pedestal within a large seawater aquarium. *Culcita* was presented to the coral sequentially in two different experimental positions, where *Culcita* was held against the side or the top of the coral colony. Each trial alternated starting position, and lasted three minutes with a five-minute rest period between treatments. Defensive behaviors were scored within three minutes in each for proximity and degree of contact of the exosymbionts with the *Culcita*. If the *Trapezia* showed any response to the presence of the *Culcita*, usually adjusting its position while remaining deep within the branches of the coral, it was given a score of 0.25. If the *Trapezia* advanced further, to within 2 cm of the *Culcita*, it was given a score of 0.5 points. One point was added each time the *Trapezia* attacked by snapping at the body or tube feet of the *Culcita*, and an additional 2 points were given if the *Trapezia* attack resulted in a cut to the *Culcita* or removal of tube feet. If the *Trapezia* retreated and then reengaged, the scoring system was restarted and all bouts were summed. The defensive snap of *Alpheus* involves a distinctive snapping sound, each of which was given a score of 1 point.

### **Data analysis**

Deterrence trials were evaluated statistically using a binomial proportions test (Crawley 2007). In the multiple defender effects experiment we evaluated three measures: (1) frequency of predation, (2) volume of coral consumed (only for the corals that were attacked-zeros were removed), and (3) the location of feeding scars. Using a generalized linear mixed model (fixed effect: exosymbiont treatment; random effect: temporal block) with a binomial distribution and logit link (lme4 package R 2.7.2) we evaluated the effects of the different exosymbiont treatments on the frequency of predation with a one-way analysis of deviance (ANODEV). Similarly, a linear mixed

model (log transformed ( $\ln(x)$ ), lme package R 2.7.2) with the same parameters was fit and evaluated with a one-way ANOVA to test the effects of exosymbiont treatments on the volume of coral consumed. For models of both frequency and volume we conducted three orthogonal contrasts using Wald  $t$ -tests to evaluate: 1) the effect of symbionts (*No Exosymbionts vs. Alpheus, Trapezia, and Alpheus + Trapezia*), 2) the differences in defensive efficacy between *Alpheus* and *Trapezia* (*Alpheus vs. Trapezia*), and 3) the difference between one and two symbiont pairs (*Alpheus, Trapezia vs. Alpheus + Trapezia*). Analysis in one-factor ANOVA format facilitated orthogonal contrasts, which quantify the symbiont effects, differences between symbionts, and density effects; however, none of these contrasts explicitly test for an emergent MDE.

We assume a multiplicative defense model in calculating the expected defensive efficacy of both exosymbionts, because the probability of defense in the presence of a given species of exosymbiont is conditional upon the defense by another exosymbiont. The expected probability of *Culcita* attack with independent defensive behaviors for the combined effect of the exosymbiont treatment (*Alpheus + Trapezia*),  $\hat{\Phi}_{A+T}$ , following a multiplicative defense model can be derived from the independent defensive effects of *Alpheus* and *Trapezia*:

$$\hat{\Phi}_{A+T} = \frac{(\Phi_A)(\Phi_T)}{(\Phi_{NE})} \quad (3)$$

This equation can be log-transformed into an additive form for analysis,

$$\ln(\hat{\Phi}_{A+T}) = \ln(\Phi_A) + \ln(\Phi_T) - \ln(\Phi_{NE}) \quad (4)$$

where  $\Phi_i$  is the probability of attack in the presence of exosymbiont treatment  $i$  ( $i = A$ : *Alpheus*,  $T$ : *Trapezia*,  $A+T$ : both *Alpheus* and *Trapezia*, and  $NE$ : no exosymbionts). The

expected volume consumed by *Culcita*  $\hat{\Omega}_{A+T}$  can be similarly derived from independent effects of *Alpheus* and *Trapezia* as a multiplicative model:

$$\hat{\Omega}_{A+T} = \frac{(\Omega_A)(\Omega_T)}{(\Omega_{NE})} \quad (5)$$

Where  $\Omega_i$  is the volume consumed by *Culcita* in the presence of exosymbiont treatment *i*. This expected model can be similarly log-transformed into an additive form for analysis as described above for  $\hat{\Phi}_{A+T}$ . Note that our assumptions of a multiplicative defense model do not necessarily mean that all mutualist-mutualist interactions operate on a log-additive scale, but rather that it is important to consider on which scale a given system operates.

We statistically tested for a non-independent MDE using a two-factor Analysis of Deviance (ANODEV) and ANOVA (Billick and Case 1994, Wooten 1994) on the log transformed frequency of predation and log (volume consumed) response variables respectively (main effect of *Alpheus*, main effect of *Trapezia*, interaction between *Alpheus* and *Trapezia*, and a random effect of temporal block). ANODEV can be loosely described as the statistical analogue to ANOVA that allows for alternative assumptions about the underlying variance (i.e. binomial error rather than normal error) and conducts a series of likelihood ratio tests to hypotheses for main and interactive effects (Bolker 2008). A significant interaction in the two-factor analysis indicates an emergent MDE (i.e., either risk enhancement or risk reduction driven by non-independent exosymbiont interactions). We therefore re-analyzed both frequency and volume data with a two-factor ANOVA. Assumed underlying error distributions, random effects, and fixed effects estimates were synonymous to the one-factor ANODEV and ANOVA models for the

frequency of predation and volume response variables respectively, with the exception that for the frequency of attack data we used a log rather than a logit link.

We separately evaluated whether the location of the feeding scars in the multiple defender effects experiment feeding differed between corals with and without symbionts using a Pearson's Chi-squared test with Yates continuity correction to test whether the location of feeding scars differed.

We tested whether the defensive behaviors of *Trapezia* and *Alpheus* differed between the two experimental positions (top and side) using a Multivariate Analysis of Variance (MANOVA). Data was square root transformed to reduce heterogeneity of variance. We used the statistical programming environment R 2.7.2 for the computation of all statistics (R Development Core Team 2008; <http://www.R-project.org>).

## Results

### Deterrence trials

Corals with exosymbionts were less likely to be attacked than unprotected corals. In 90% of trials, *Culcita* fed upon undefended *Pocillopora* colonies, but not defended colonies, when given the choice. The volume of coral consumed was lower where exosymbionts were present (with exosymbionts: 2.76 [3.10, 17.67 cm<sup>3</sup>], and without: 184.03 [145.48, 680.49 cm<sup>3</sup>], (mean [lower CI, upper CI])).

### Emergent Multiple Defender Effects

Feeding trials were used to evaluate three variables: frequency of predation, location of predation, and the amount of tissue consumed. Orthogonal contrasts on predation frequency revealed that the presence of symbionts significantly reduced the predation frequency by 41%, but that there was no difference between *Alpheus* and *Trapezia* or between one and two exosymbiont pairs (Table 1, Fig. 1a). Qualitative effects of

exosymbiont pairs differed between predation frequency and volume consumed. The presence of exosymbionts reduced the volume of coral consumed by *Culcita* by 31% relative to controls (Figure 1b, Table 1). There was no significant difference between volume of coral eaten by *Culcita* between *Alpheus* and *Trapezia* treatments (Table 1); however, the presence of two exosymbiont pairs reduced coral predation by 72% compared to one exosymbiont pair (Table 1, Figure 1b).

Evidence for Multiple Defender Effects differed between analysis of the frequency of predation and volume consumed response variables. The frequency of predation did not significantly decrease with an increase in exosymbiont density and diversity (Table 1a), i.e. there was no evidence for an MDE in the frequency of predation (Table 2a). We did, however, find evidence for a significant MDE in the volume consumed response variable, where the combined exosymbiont treatments far exceeded the defensive efficacy of the symbionts when acting alone (table 2b)

Corals without exosymbionts ( $n = 16$ ) were equally likely to be eaten on the side or top of the coral colony. There was no statistically significant effect of exosymbionts on feeding location (top or side;  $n = 28$ ), despite a trend for corals to be more frequently eaten on the side (21) rather than on the top (7) ( $\chi^2 = 1.83$ ,  $P = 0.18$ ).

### **Behavioral observations**

Position of exposure affected the defensive behavior of exosymbionts ( $F_{1,8} = 22.3$ ,  $P < 0.001$ ). Placement of *Culcita* on the side of the colony induced significantly lower defensive behavior than placement on top, for both *Trapezia* ( $F_{1,8} = 33.5$ ,  $P < 0.001$ ) and *Alpheus* ( $F_{1,8} = 5.8$ ,  $P = 0.04$ , Fig 2). Both *Alpheus* and *Trapezia* displayed increasing aggressive behavior over the three-minute time period.

## Discussion

It is well known from comparative studies that the efficacy of different species of mutualists can vary significantly within a guild (Little et al. 2004, Mieog et al. 2009); however, these studies rarely examine interactions between mutualists within a guild or quantify the consequences of these interactions on the host (but see (Palmer et al. 2003)). Mutualist-mutualist interactions are of particular interest in diverse systems like coral reefs, where a number of species can co-occur on single hosts, creating a complex network of possible higher order interactions (e.g., cooperative, competitive, or intraguild predatory interactions) that may non-independently modify the benefit a host receives by supporting multiple mutualists. Our study tested for an emergent MDE from two species of crustacean exosymbionts that cohabit a common scleractinian coral and collectively defend the coral from predators.

In the central Pacific, *Trapezia* and *Alpheus lottini* form heterosexual pairs and will not tolerate the presence of other conspecifics. Other species of crustacean mutualists, however, may be tolerated, and effectively increase the ‘defense force’ of the coral head. In initial studies in the summer of 2006 we observed up to 5 species of *Trapezia*, along with *Alpheus lottini*, occupying a single *Pocillopora* colony at the study site. This ‘species stacking’ of mutualists may allow the colonization of symbionts of different defensive abilities and create intra-guild dynamics that facilitate *Pocillopora* survival (C.S. M<sup>c</sup>Keon, unpublished data). Our study system using two common species of guard crustaceans is a first step toward understanding this diversity, and represents a state frequently found in low diversity lagoonal environments.

Effects of *Alpheus* and *Trapezia* in reducing predation frequency were independent. However, we found evidence for synergy among exosymbionts in their ability to reduce

the volume of coral tissue consumed. This emergent MDE was largely driven by the poor defensive efficacy when either exosymbiont was alone, but increased efficacy in the presence of both exosymbionts (Fig. 1b). These differences in the observation of MDE depending on the response variable measured, suggests that the response of *Culcita* to exosymbiont defenses may substantially differ between initial interactions and during feeding events. The behavioral data document similar reactions of *Alpheus* and *Trapezia* to *Culcita* threats. With simulated predator presentation, *Alpheus* and *Trapezia* begin to immediately snip and push at the *Culcita*. Alone, neither exosymbiont is capable of reducing volume consumed, but together the two species drastically reduce the volume consumed. While these symbionts are not completely successful in warding off coral predation by *Culcita* all of the time, aggressive defense of the top and inner branches of the coral may be sufficient to maintain a core of surviving coral polyps sufficient for regeneration of the colony after predation. This is supported by our behavioral data in which *Trapezia* and *Alpheus* more aggressively defended against *Culcita* attacking from the top of the coral compared to *Culcita* attacking from the side—where habitat may be less critical.

### **Study Limitations**

Our ability to draw inference about intraguild dynamics is limited by our experimental design: we applied only an additive design (2 *Alpheus*, 2 *Trapezia*, 2 *Alpheus* + 2 *Trapezia*) rather than an additive and substitutive design (2 *Alpheus*, 2 *Trapezia*, 2 *Alpheus* + 2 *Trapezia*, 1 *Alpheus*, 1 *Trapezia*, 1 *Alpheus* + 1 *Trapezia*). This limits our ability to differentiate between density and diversity effects (Griffen 2006).

The degree to which certain mutualists can be labeled as the “best defenders” may be dependent on the spatiotemporal scale of observation (i.e., individual species that

are highly abundant but only mediocre in their benefit to the host may actually be *better* than those mutualist species that are highly effective but exhibit recruitment limitation). For example, work in ant-acacia systems (Palmer et al. in review), has demonstrated the potential value of an abundant, yet inferior mutualist defender, within the ontogeny of an individual host tree. Similar dynamics may operate where coral mutualists share space within a host. Marine organisms are known to have highly variable recruitment in space and time (Gaines and Roughgarden 1985, Roughgarden et al. 1988). Certain species of coral exosymbionts may differ in recruitment and efficacy in coral defense, and this variation in recruitment may alter the frequency of interspecific interactions within the exosymbiont community. Though we cautiously interpret interspecific variation in efficacy between the two mutualists in their benefits we observed, we must acknowledge that these differences as well as the strength of the MDE may vary with host ontogeny, temporal scale of the experiment, and the degree to which species vary in recruitment.

### **Conclusion**

The consideration of intra-guild relationships is of general importance in the study of mutualisms (Morris et al. 2007). Interactions between symbionts have the potential to dramatically alter the relationship with the host, and may provide insight into why hosts may maintain multiple mutualists despite the potentially negative effects of one individual species within a symbiont guild. Here we observed differences in the efficacy of defense two coral mutualists provide. We also describe how the benefit exosymbionts provide can shift due to synergism between mutualists, suggesting the importance of higher order interactions in modifying observed benefits and costs of multiple

mutualists. Lastly, we found that the positive benefit of intraguild species interactions on mutualisms can depend on the response variable measured.

A number of foundation species depend on diverse suites of mutualists for reproduction, defense, and nutrient acquisition. However, the importance of intraguild species interactions and functional diversity is poorly studied despite the fact that shifts within guilds may fundamentally alter the magnitude of ecosystem services that mutualisms provide (Resetarits and Chalcraft 2007a). Our study adds evidence to a growing literature suggesting the benefits of guard exosymbionts in maintaining the health of coral reefs and the importance of exosymbiont diversity in enhancing these benefits. Such benefits have broad implications in an ecosystem where the foundation species (corals) provide services such as ecotourism, subsistence fishing, and protection from storm damage.

Table 4-1. Results of orthogonal contrasts testing the effect of different symbiont treatment combinations on two response variables: (A) predation frequency (one-way Generalized Linear Mixed Model), and (B) volume consumed (mixed effects one-way ANOVA), both with Wald *t*-tests. \*\* represents  $P < .05$ .

Orthogonal Contrast	Estimate	SE	<i>t</i> -value	<i>DF</i>	<i>P</i>
<i>A. Predation frequency</i>					
Intercept	1.628	1.186			
No symbionts vs. symbionts	1.156	0.328	3.525	27	0.006**
<i>Alpheus</i> vs. <i>Trapezia</i>	-0.2944	0.566	-0.520	27	0.616
2 symbionts vs. 4 symbionts	0.487	0.328	1.487	27	0.149
Block SD	3.435				
<i>B. Volume consumed</i>					
Intercept	6.716	0.161			
No symbionts vs. symbionts	-0.46	0.114	-3.312	27	< 0.001**
<i>Alpheus</i> vs. <i>Trapezia</i>	-0.096	0.111	-0.865	27	0.019**
2 symbionts vs. 4 symbionts	-1.298	0.122	-10.630	27	< 0.001**
Block SD	0.465				

Table 4-2. Results tests for Multiple Defender Effects in two response variables (A) predation frequency and (B) volume consumed (mixed effects two-way ANOVA), P-values for predation frequency response variables were with Wald *t*-tests. \*\* represents  $P < .05$ .

Source of Variation	Estimate	SE	<i>t</i> -value	DF	<i>P</i>
<i>A. Predation frequency</i>					
Intercept	5.096	1.560			
<i>Alpheus</i>	-4.431	1.462	-3.030	27	0.005**
<i>Trapezia</i>	-3.842	1.400	-2.744	27	0.011**
<i>Alpheus X Trapezia</i>	2.674	1.744	1.533	27	0.137
Block SD	3.436				
<i>B. Volume consumed</i>					
Intercept	6.716	0.161			
<i>Alpheus</i>	-0.376	0.114	-3.312	27	0.002s
<i>Trapezia</i>	-0.096	0.111	-0.865	27	0.392**
<i>Alpheus X Trapezia</i>	-0.826	0.164	-5.031	27	< 0.001**
Block SD	0.465				

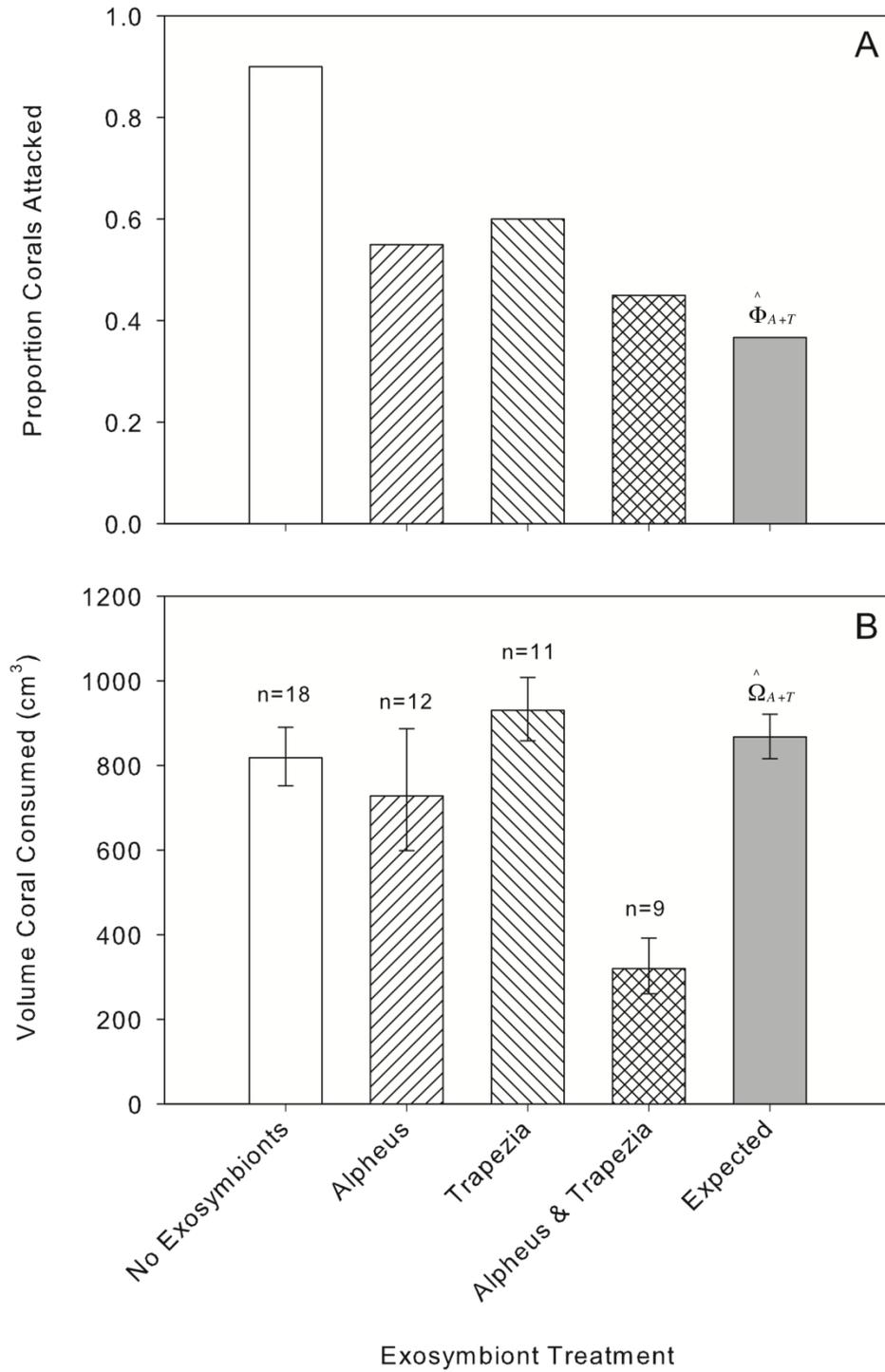


Figure 4-1. (A) Effect of symbionts on the frequency of *Culcita novaeguineae* attack ( $n = 20$  / treatment). (B) For trials in which predation occurred, the volume of tissue removed by *Culcita novaeguineae*.

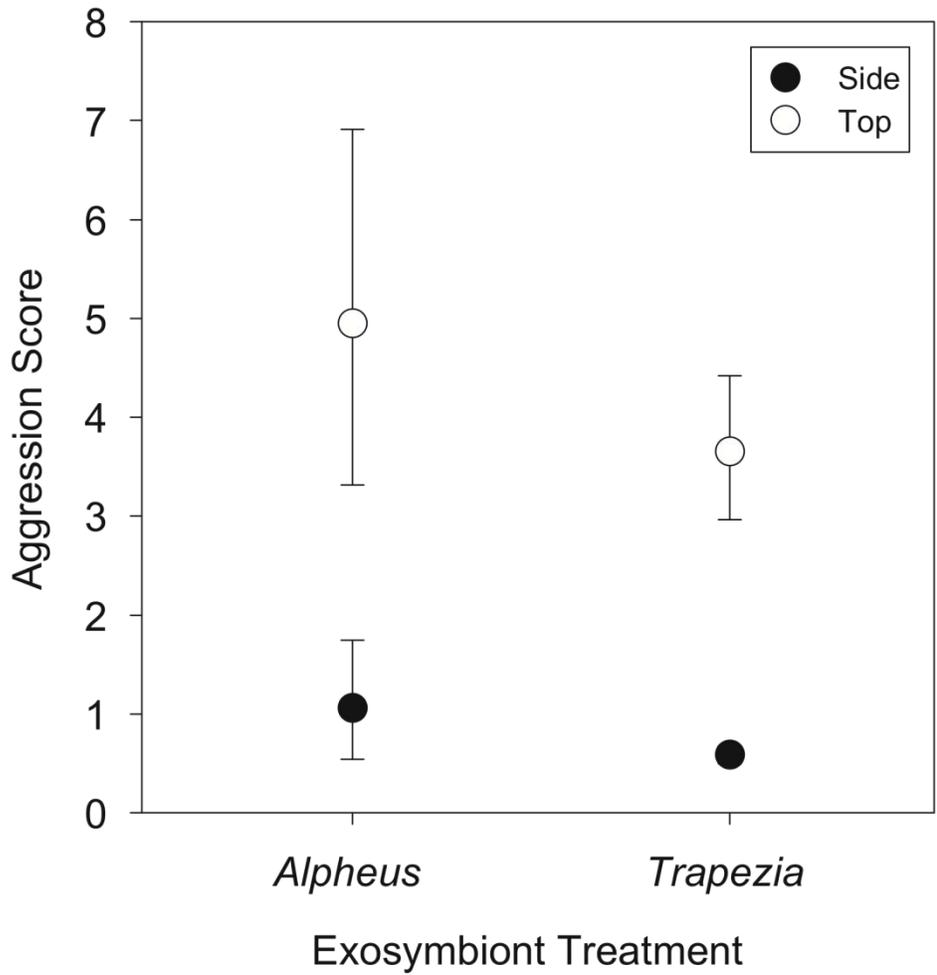


Figure 4-2. Aggressive defensive behavior of *Pocillopora* exosymbiont pairs in response to coral predator *Culcita novaeguineae* presented on either the top or side of the coral head (backtransformed mean  $\pm$  1 SE,  $n = 5$  for each pairwise combination).

CHAPTER 5  
FULL HOUSE: ENDOSYMBIONT-EXOSYMBIONT CONNECTIONS IN REEF  
CORALS

**Introduction**

The symbiosis between dinoflagellates of the genus *Symbiodinium* and scleractinian corals is fundamental to the creation and function of modern shallow water coral reefs (Veron 1995). Modern reefs formed by zooxanthellate corals are notable in their size, rate of growth, and longevity (Veron 1995). Reefs are important on both geological and biological scales; creating atolls (Darwin 1839) and hosting biodiversity thought to be unparalleled in the oceans (Paulay 1997, Reaka-Kudla 1997). The mutualism between corals and zooxanthellae is thought to have facilitated both the diversification of the scleractinian corals in the Tertiary, and led to mass extinction in the changing environment of the Cretaceous (Cowen 1988).

Photosynthesis of *Symbiodinium* efficiently provide fixed carbon for corals (Muscatine and Hand 1958), supplying up to 95% of the carbon demands of the host (Muscatine 1990). This translocated carbon is a product of photosynthetic pathways, and abundant enough that previous researchers have suggested that the coral is faced with excess, which is released into the environment as mucus (Davies 1984).

Such photosymbioses have come under increased scrutiny as their breakdown, manifested in coral bleaching, has come to be considered a symptom of global warming. Bleaching occurs when *Symbiodinium* are expelled from the coral host, with consequent loss of golden-brown pigment of the algae from the coral tissue. The symbiosis is very sensitive to stressors such as changes in salinity, water quality, excess UV and temperature, and an increase of about 1°C over summer maximum can be sufficient to initiate expulsion of zooxanthellae (Goreau and Hayes 1994), and

subsequent bleaching. The resultant whitening is a striking and ominous environmental indicator, and can lead to the death of the coral if prolonged. Bleaching has been recorded from all major tropical areas (Hoegh-Guldberg 1999), and frequency of bleaching appears to be increasing with global warming (Pandolfi et al. 2003).

Genetic studies have shown photosymbiotic *Symbiodinium* in corals (and other tropical hosts) to be more diverse than previously thought based on limited variation in their simple morphology (Trench 1987, Rowan 1991). The diversity is cryptic, of uncertain magnitude. This diversity has been categorized into a number of alphanumerically designated clades based on ITS sequences, (Pochon et al. 2006, Stat et al. 2006, Coffroth et al. 2007), of which five (A,B,C,D,F) are known to occur symbiotically with scleractinian corals. Further genetic differentiation has been demonstrated and categorized within clades, although species limits are poorly understood. These clades have been demonstrated to vary among (Stat et al. 2009) and within (Rowan et al. 1997, Baker and Romanski 2007, Smith et al. 2007b) host species, can show predictable differentiation across the landscape of some colonies (Rowan et al. 1997), and also vary across geography (Oliver and Palumbi 2009).

*Symbiodinium* clades vary in their susceptibility to temperature-induced bleaching (Rowan 2004). These differences have become the basis of the “adaptive bleaching hypothesis” (Buddemeier and Fautin 1993)- suggesting that differential susceptibility of different clades of zooxanthellae to thermal stress allows holobionts to adapt to a changing thermal environment. One group, clade “D”, has demonstrated greater resistance to thermal stress in surveys and in laboratory experiments than others (Rowan 1998, Glynn et al. 2001, Baker et al. 2004). Other ecological differences

among clades have not received the same degree of attention, though some data exists to support the idea that hosts containing different clades of *Symbiodinium* show different growth rates (Kinzie and Chee 1979, Little et al. 2004). For example, work by Little et al. (2004) demonstrated greater growth in *Acropora tenuis* that hosted the *Symbiodinium* strain C1 in comparison to those hosting clade D.

Corals host a number of symbionts beyond zooxanthellae, including bacteria, protists, algae, fungi, and diverse metazoans. Fish, worms, gastropod and bivalve mollusks are well-represented among coral symbionts. The largest numbers though are crustaceans, with decapods being particularly well-represented both as parasites and mutualists. These exosymbioses range from parasitic to mutualistic, and vary in level of host specificity.

Of particular importance to the survival of coral hosts are the mutualistic crab families Trapeziidae, Tetraliidae, and the snapping shrimp family Alpheidae. *Trapezia* were first recognized as obligate associates of pocilloporid corals by Knudsen (1967). Since that time, researchers have described several ways in which these crabs benefit the coral host, including: defense from corallivores (Glynn 1976, 1987), removal of sediment (Stewart et al. 2006), and cleaning of vermetid gastropod mucus nets (Stier et al. 2010). The nature and level of these protective benefits vary with size and species of *Trapezia* (McKeon and Moore 2010). Benefits to the crab include shelter and a reliable food source. The diet of *Trapezia* has been recorded as 'coral mucus' and tissue (Odinetz 1984a, Kropp 1986). Linkages between the health of the coral and the crab have been provided by Glynn et al. (1985), who documented decline in lipid levels in *Trapezia* that occupied stressed and bleached corals. Further evidence of trophic

transfer was provided by Rinkevich et al. (1991) who documented pathways between the host coral and *Trapezia* using radioisotopes. Stimson (1990), provided evidence for the provisioning of lipids by the coral to the crab through specialized, lipid-packed tentacle tips, describing a system much like that of ant-plant mutualisms (Longino 1989). The lipid supply is correlated with the presence of the crab; corals from which the crabs have been removed stop producing lipid-packed tentacles.

The connection between the health of the coral and health of the crabs leads to interesting questions regarding potential relationships between the endosymbiotic dinoflagellates and exosymbiotic crustaceans. If endosymbiont photosynthesis is providing the coral with much of its organic carbon, then endosymbionts may be indirectly subsidizing the coral's transfer of carbon in support of defensive crustacean mutualists. As the crabs have a notable effect on the survival of the coral host (Glynn 1983), potential variation among *Symbiodinium* clades in the amount and type of organic carbon they transfer to the host may impact exosymbionts (and thus the entire holobiont) as this carbon is further translocated to the crabs. In this paper we set out to test the hypothesis that size of defensive crustacean mutualists is correlated with clade of *Symbiodinium* of their host.

## Methods

All surveys and collections were conducted in the Ofu field unit of the American Samoa National Park. Ofu is unusual in that it has a series of natural lagoonal pools that have different heating and cooling regimes due to differences in size, and depth. Several species of coral are present in these pools, including species of *Pocillopora*. The taxonomy of *Pocillopora*, as of many corals, is poorly resolved at the species level (Veron and Pichon 1976, Veron 2000, Combosch et al. 2008); however, three coarse

morphological groupings, matching the descriptions of *P. 'damicornis'*, *P. 'verrucosa'*, and *P. 'eydouxi'*, are readily differentiable in the field. Corals used in this experiment belonged to single morphotypes of *P. 'damicornis'* and *P. 'verrucosa'*, and were selected from the same depth (0.5 m) within the pools.

Previous work by Oliver and Palumbi (2009), had demonstrated difference in the prevalence of *Symbiodinium* clades C1 and D across these pools. Using this information, we selected *P. damicornis* from pools 300 and 400, and *P. verrucosa* from pool 300 as most likely to give us sufficient numbers hosting each *Symbiodinium* clade for a meaningful comparison of *Trapezia* size within and between pools.

Sixty *P. damicornis* were selected from pool 300 (n=30) and pool 400 (n=30). Corals were surrounded by a plastic bag and separated from the substratum. Length, height and width of each colony was measured, and the volume of the coral estimated as an ellipsoid using the formula  $\frac{4}{3}\pi abc$ , where a, b, and c are radii. Collected corals had a mean size of 769 cm<sup>3</sup> (n = 60, sd = 273). The corals were surveyed for mollusk, vertebrate, and arthropod exosymbionts. Forty-seven *P. damicornis* contained *Trapezia septata*. Female *Trapezia septata* were collected and preserved in 75% Ethanol. The crabs were measured across the broadest part of the carapace ("carapace width", hereafter CW) using digital calipers. Eggs were removed from ovigerous females (n = 45) using gentle irrigation, and counted using a hemocytometer. Coral tissues samples from each host were preserved for genetic analysis in 95% Ethanol and DMSO-salt buffer.

Fifty *P. verrucosa* were selected from pool 300. As above, corals were collected, measured and surveyed for exosymbionts. *P. verrucosa* had an average size of 30148

cm<sup>3</sup> (n = 50, sd = 5392). Forty-seven corals contained *Trapezia septata*. Both genders of *Trapezia septata* were collected and preserved in 75% ethanol, and measured using digital calipers in the lab. Coral tissues were preserved in 95% Ethanol or DMSO-salt buffer for genetic analysis.

Molecular methods followed Oliver and Palumbi (2010). Genomic DNA of *Symbiodinium* was extracted from all samples using Nucleospin columns (Clontech), and then PCR amplified using cp23s forward and reverse primers: cp23s1: GCTGTA ACTATAACGGTCC; cp23s2: CCATCGTATTGAACCCAGC. After PCR, the samples were cleaned using SAP and EXO and cycle-sequenced using BigDye di-deoxy sequencing chemistry (USB, Cleveland, OH; ABI, Foster City, CA). Labeled samples were ethanol precipitated and capillary sequenced on an ABI 3100 (ABI, Foster City, CA). Cycling conditions are given in Pochon et al. (2006). All *Symbiodinium* samples were sequenced at chloroplast 23s rDNA and compared to existing databases of sequences from Pochon et al. (2006) and Jones et al. (2008). Samples were assigned major clade identities A, C or D if they clustered within those groups as defined by Pochon et al. (2006). As we did not perform bacterial cloning or qPCR (Ulstrup and Van Oppen 2003, Apprill and Gates 2007), we cannot address the proportional composition of a mixed symbiont community within a single sample. We simply report the dominant sequence present in a sample. However, as cp23s shows length variation across major clades (A/C/D), if a sample showed multiple bands on a gel, those bands were separately extracted and sequenced, and any coral showing such variation was scored as half of the one *Symbiodinium* type and half of the other.

All statistics were performed in the Statistical Programming environment R (2006). Colonies that hosted crabs and were successfully sequenced for *Symbiodinium* were used. Haplotypes of *Symbiodinium* for which we had insufficient data for comparison were excluded from the analysis. Statistical comparisons were made using Wilcoxon tests. Linear regression was used to evaluate the relationship between fecundity and size in *Trapezia*.

## Results

Complete data was available for 30 crab-coral-zooxanthellae associations in *Pocillopora damicornis*. *Trapezia septata* from *P. damicornis* with clade C1 zooxanthellae (n=11) were smaller than crabs from clade D hosts (n=19) (Figure 1; Wilcoxon test  $p < 0.0005$ ). This relationship was confounded with pool, as crabs from pool 300 (n = 17) and pool 400 (n = 13) also have statistically different sizes (crabs in pool 300 were larger, Wilcoxon test  $p = 0.02250$ ) reflecting the paucity of samples in which corals hosted clade D in pool 400 (n = 2) and clade C in pool 300 (n = 0).

Complete data was available for 48 crab-coral-zooxanthellae associations in *Pocillopora verrucosa*. *Trapezia septata* from *P. verrucosa* with clade C1 *Symbiodinium* (n = 29) were smaller than crabs from clade D coral (n = 19) (Figure 2; Wilcoxon test  $p = 0.02095$ )

Number of eggs brooded by female *T. septata* (n = 45) increased with carapace width of female (Figure 3;  $r^2 = 0.5689$ ).

## Discussion

Our study shows a direct correlation between clade of endosymbiotic zooxanthellae hosted by a coral and the size of exosymbiotic crabs on it. Body size in crabs is important with respect to predator avoidance through gape limitation (Kim and O'Connor

2007), mate selection (Adams et al. 1985) and fecundity. We show that fecundity scales with linear body size in *T. septata* (Hines 1982). This is similar to previously documented patterns in *Trapezia* (Finney and Abele 1981, Gotelli et al. 1985). Size of the *Trapezia* is also important for the host corals. Larger crabs may be more energetically expensive to maintain as they may require greater provisioning by the host. This energy subsidy from the coral may however be compensated for by the delivery of nutrients from crabs to coral (Holbrook et al. 2008).

Crab size also appears to be related to the efficacy of defense provided by crabs for the host. In other studies (McKeon and Moore 2010), the ability of *Trapezia* to repel three size classes of corallivore was strongly influenced by crab size and species, with only the largest crabs being able to effectively defend their coral host from the most voracious predator, the “Crown of Thorns” seastars (COTS, *Acanthaster planci* complex). With COTS being a major source of coral mortality, the questions of what influences the size, species composition, and abundance of *Trapezia* take on greater interest.

Despite the importance of the coral-*Symbiodinium* relationship to the health and function of reef building corals, connections between endosymbiotic algae and the rest of the coral holobiont have been little explored. The differences in heat and light preference and tolerance among clades of *Symbiodinium* are well studied, but other differences and the consequences of these are poorly known. In contrast previous authors have shown strong links between crab exosymbionts and the health of their coral host (Glynn 1983, Rinkevich et al. 1991). Our work documents that differences in *Symbiodinium* clade are reflected in the size of guard crustaceans.

Trade-offs between thermal tolerance and the production of photosynthates are an important part of the adaptive bleaching hypothesis (Buddemeier and Fautin 1993, Rowan 2004), but have yet to be demonstrated broadly. Little et al. (2004) found that *Acropora* corals that hosted the C1 strain of *Symbiodinium* had greater growth rates than those that hosted the thermal tolerant strain D. In contrast we found that corals that hosted clade D *Symbiodinium* had larger *Trapezia* than those that hosted strain C1.

Several hypotheses may explain the opposite trends of coral growth rate and crab size in *Pocillopora* with C1 and D *Symbiodinium*. The study site in this experiment, the lagoonal pools of Ofu, are a thermally stressful environment (Oliver 2009, Barshis et al. 2010), and it is possible that the clade D corals sampled were at suboptimal health due to temperature, though no external differences were noted between the two study groups. Corals with different endosymbionts may show differential susceptibility to corallivores, and correspondingly invest more in an exosymbiotic 'army'. Differences in excess carbon release may also be part of the strategy of thermally tolerant coral/endosymbiont partnerships, leading to greater energy available for *Trapezia*. Finally *Pocillopora* in the Ofu pools may show a different relationship between *Symbiodinium* clade and coral growth rate than observed by Little et al (2004) in *Acropora tenuis* in Australia. Additional work is needed to explore these alternative hypotheses.

The links between endosymbiotic zooxanthellae and defensive crustacean exosymbionts have implications for the demography of reef corals. Both symbiont groups have differential responses to stressors, making survival in an ocean environment where temperatures, sedimentation, and corallivory are increasing into an

ever more difficult card game. For corals to survive, they will need the right combination of endo- and exosymbioses, a full house, to make it to the next hand.

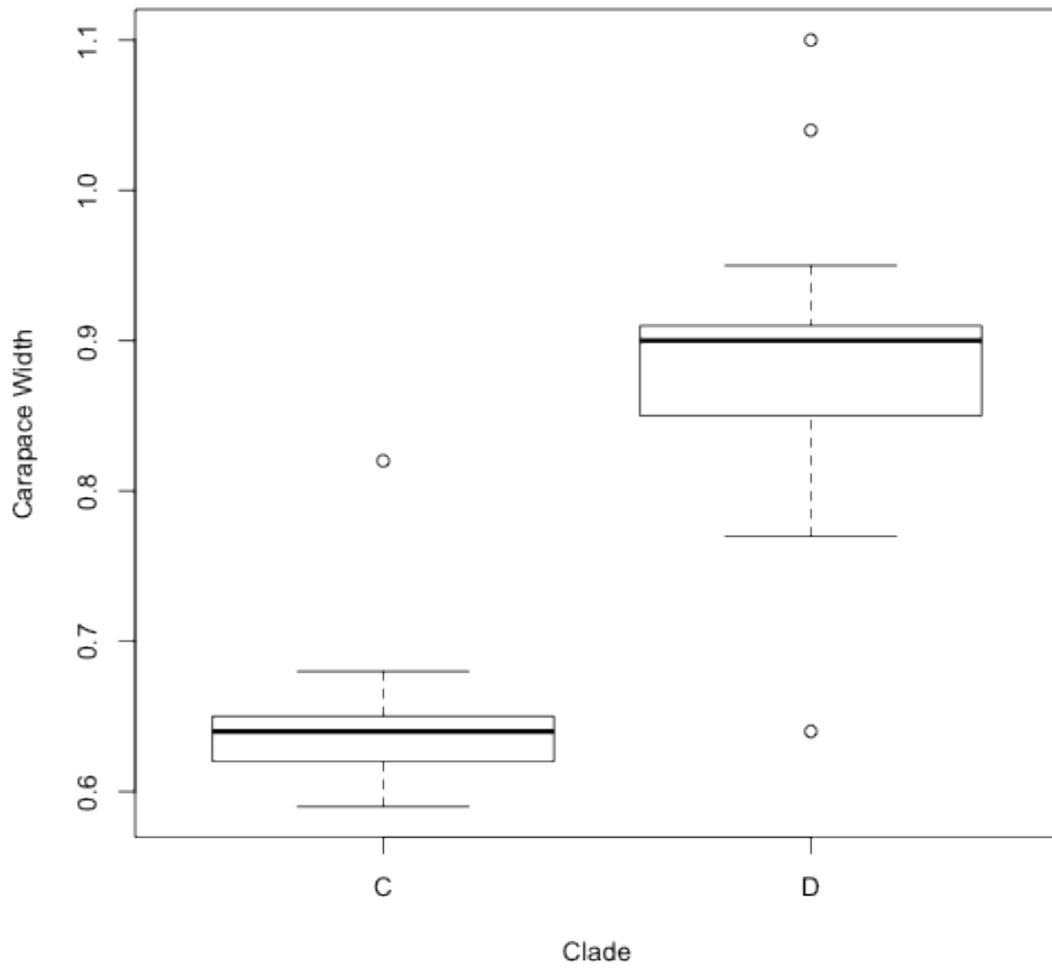


Figure 5-1. Carapace Width of *T. septata* in *P. damicornis* by clade of *Symbiodinium*

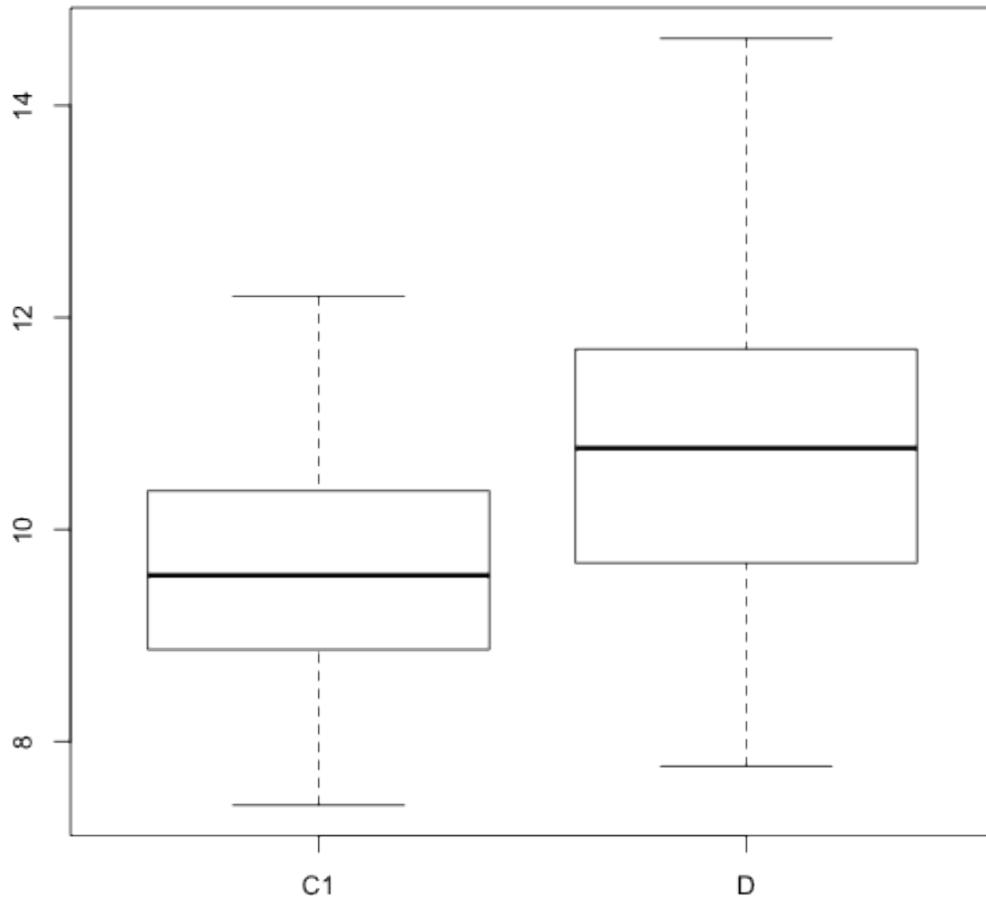


Figure 5-2. Carapace Width of *T. septata* in *P. verrucosa* by clade of *Symbiodinium*



CHAPTER 6  
CONCLUSION: DIRECTIONS AND IMPLICATIONS FOR THE STUDY OF THE  
TRAPEZIA-POCILLOPORA MUTUALISM

The study of *Trapezia* and *Pocillopora* has a long history and notable contributions have been made by many previous researchers. From Panama to South Africa, Natural Historians and Biologists have contemplated the diversity, biogeography, and ecology of this symbiosis. I have used this solid base of information, "stood on the shoulder of giants", to complete this dissertation. With that in mind, I cautiously offer ideas as to the future study of this symbiosis.

**Phylogeny, Speciation, Color and Pattern**

My work with the phlogenetics of *Trapezia*, though revealing, raises more questions than answers. My use of CO1 alone does not resolve relationships beyond the species level. Increasing resolution of deeper nodes should be attainable with additional markers. As suggested by Lai *et al.* (2009), the Trapeziioidea does not appear to be a monophyletic assemblage, with the Tetraliidae and Trapeziidae appearing to derive from different points within the greater cluster of Xanthoid lineages. Greater genetic sampling within the Xanthids should allow for resolution of the origin of coral crab clades.

The emergence of the of the *T. rufopunctata* - *T. flavopunctata* clade as the sister group to all remaining *Trapezia*, makes it's inclusion in the genus suspect. As several morphological features differentiate this species complex from the remaining *Trapezia*, this species complex may be significantly different from the remaining *Trapezia* and worthy of consideration as a sub-genus or genus.

Our efforts using CO1 were successful in differentiating between sister populations of several species of wide-ranging *Trapezia*. Divergence in some of these taxa is

sufficient to support the recognition of several "new" species of *Trapezia*. Questions remain with regards to populations of these wide ranging taxa that were not sampled. My sampling along one of the proposed regions of speciation, the Eastern Indian Ocean, was limited in this study. Additional sampling from peripheral regions is also needed. For example Huber (1987b) documented assortative mating in *T. digitalis* from Hawaii, suggesting the possibility of multiple species in one location. Peripheral ranges are often the location of speciation (Meyer et al. 2005), and despite our efforts, are undersampled in this study. The eastern Pacific and Red Sea in particular, are poorly sampled on a genetic level and may be host to several unrecognized taxa.

In some cases CO1 was insufficient to clarify the relationship within a species complex, despite divergent color patterns and biogeography. This illuminates the speed at which color, and perhaps species, are diverging within the *Trapezia*. Understanding of the evolutionary processes at play within the *T. speciosa* – *T. garthi* species complex will require an even faster genetic tool- such as the development of a primer specific to this group, or the use of multi-species population genetics tools.

### **Functional Diversity, Multiple Symbiont Effects, and the *Pocillopora* Community**

Different species and sizes of *Trapezia* provide different services to their host corals. In situations where a single species of *Trapezia* is hosted by *Pocillopora* the ecological consequences of this diversity may be intuitive. These situations are rare, and the majority of examples demonstrate a diverse community with pocilloporids both of *Trapezia* species, and a large number of other symbiotic taxa. The interactions between these players remain largely unknown, despite our efforts to examine the non-independent nature of the relationship between *Trapezia serenei* and *Alpheus cf. lottini*.

The sum role of the symbionts on the coral host is an interesting idea, strengthened by the work of Pratchett (2001a) and Braken et al. (2007). A first step may be the consideration of the total community of *Trapezia* species. We now have evidence of role differentiation between *Trapezia* species, but little knowledge of how these roles apply in a community context. For example- can the presence of multiple individuals of a smaller species of *Trapezia* equate to the defensive capabilities of a larger one? While *Trapezia* are most frequently found as pairs in the central Pacific, these 'rules' do not seem to apply in the western Pacific near Taiwan, and perhaps other locations (Figure 1).

Host diversity is also important to consider in this context. The genus *Pocillopora* is thought to be a basal member of it's family, with *Seriatopora* and *Stylophora* representing more derived lineages. Across this diversity symbiont host specificity appears to be limited (with the possible exception of the fish genus *Paragobiodon*), though preferences are noted (Sin and Lee 2000). Differential response of host coral species to individual species of *Trapezia* may be telling. Unfortunately, initial experiments on this subject matter were completely destroyed during Typhoon Morakot, 2009. The question remains open, and of significant interest.

*Pocillopora* is thought to be the last coral to have gone extinct in the Carribbean following the closure of the Panamanian isthmus, potentially taking *Trapezia* with it (Schweitzer 2005). The existence of similar communities on *Acropora* and other genera of corals may demonstrate a broader range of relationships, and serve as relevant model to understanding the evolution of coral exosymbioses.

## **Connections between *Trapezia-Pocillopora* and the reef environment**

The connections between coral exosymbionts and endosymbionts as described in this paper are understudied. Even our understanding of the transfer of food from the coral to the crab is based on a single paper (Stimson 1990). More research needs to be done on the topic. My efforts to trace lipid levels in crabs and corals that host different clades of zooxanthella, failed, despite following established protocols (Grottoli-Everett 1995, Grottoli et al. 2004). Direct linkages will be useful for future examinations of the subject. These limitations understood, the correlation between clade of *Symbiodinium* and the exosymbiotic *Trapezia*, is a first step in understanding how the fundamental coral-zooxanthellae symbiosis varies with regards to reef processes independent of bleaching. The information adds context and complexity to the integration of reef biodiversity and thermal tolerance. Future investigations should examine the role of *Symbiodinium* clade on corallivory and parasitism, as well mutualism such as featured here.

## **Conclusion**

As we have come to more fully appreciate the importance of coral reefs on biodiversity, ecosystem function, and fisheries, we have struggled to understand anthropogenic influences on reefs. Understanding the *Trapezia-Pocillopora* mutualism may allow us some predictive capacity with regards to reef stress, while careful manipulation of positive relationships, such as the *Trapezia-Pocillopora* mutualism, may eventually be in the toolkit of managers seeking to restore or stabilize declining reefs. This will not be possible unless we understand these relationships in detail: without an understanding of process we will fail to preserve pattern.

The *Trapezia-Pocillopora* mutualism has direct bearing on the conservation of coral reefs in the face of changing marine ecosystems. At least three major sources of reef decline can be linked to, and studied through the symbiosis. Global climate change, sedimentation, and corallivore outbreaks dramatically reduce reef diversity and health. Understanding the *Trapezia-Pocillopora* mutualism and its potentially ameliorating effects on reef stressors, should be of primary interest to managers and stakeholders in coral reef ecosystems and economies.

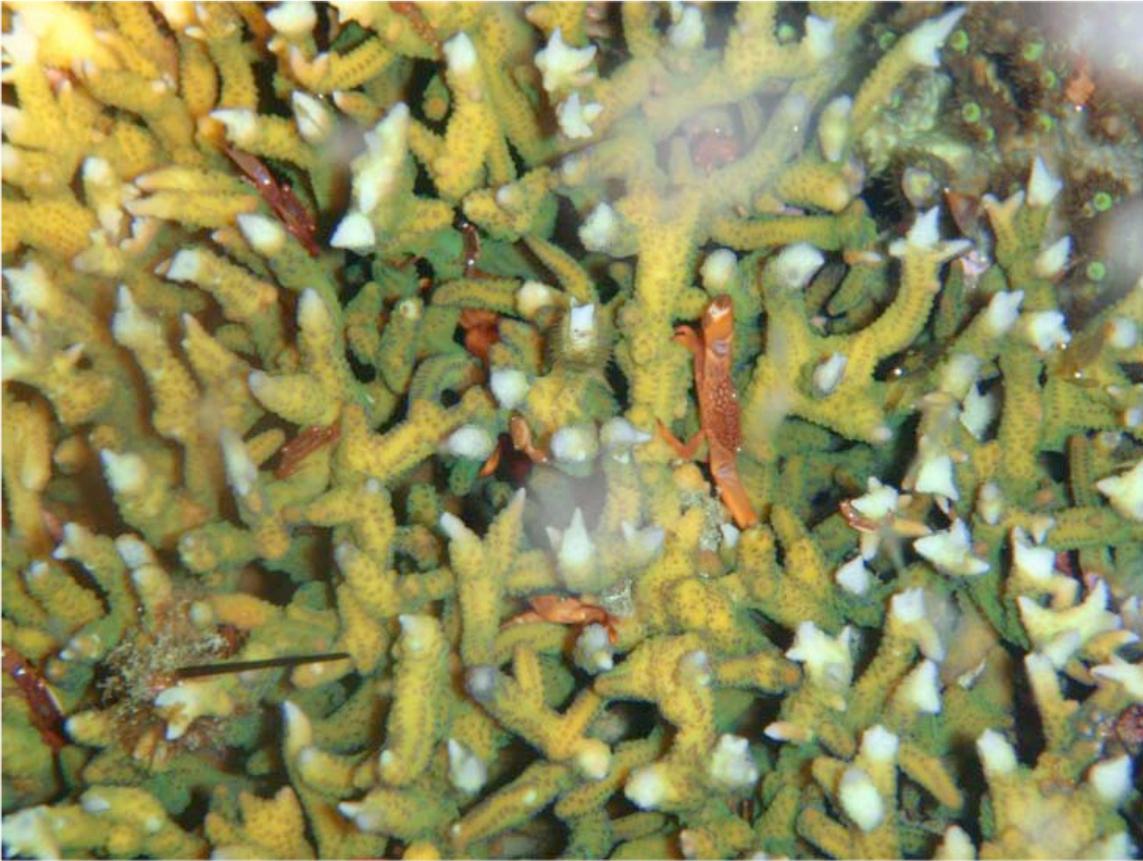
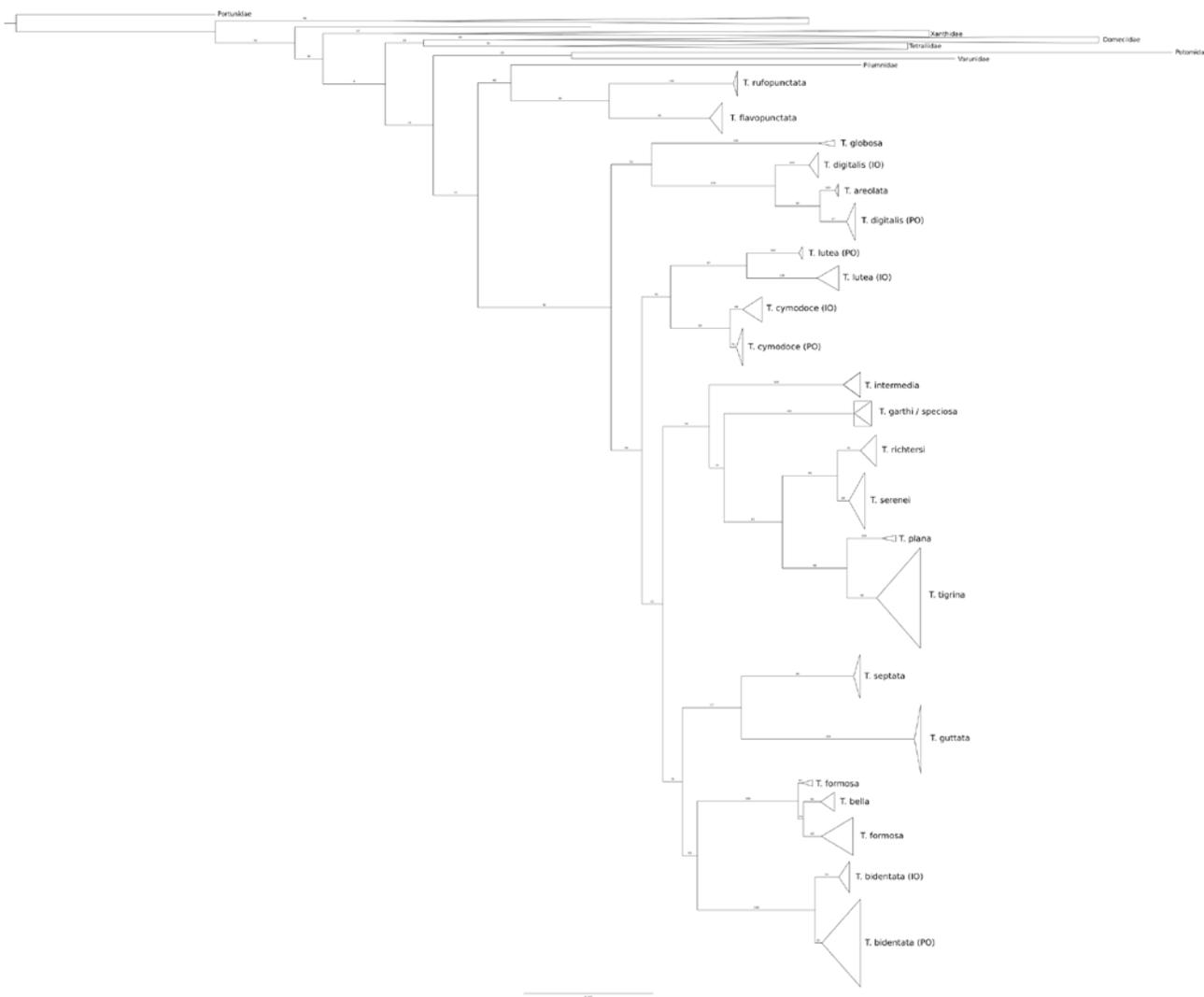


Figure 6-1. Multiple individuals of a single species of *Trapezia* may be found in some coral heads in the Western Pacific.

APPENDIX A  
MAXIMUM LIKELIHOOD PHYLOGRAM OF CO1 IN *TRAPEZIA*



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## BIOGRAPHICAL SKETCH

C.S. M<sup>c</sup>Keon was born in Athens, Greece. He has had the good fortune to find himself in amazing places surrounded by extraordinary people. His work to date has focused on using the systematic relationships among organisms and ecological experimentation to understand evolutionary processes, such as shifts from mutualism to parasitism, or from one reproductive strategy to another, in taxa as diverse as carnivorous plants of the genus *Utricularia* and the *Periclimenes* anemone shrimps. He's thinking about taking up watercolor, or maybe sailing around the world.