# Biology and Geomorphology of Tropical Islands

## Integrative Biology 158 :: Environmental Science, Policy and Managment 107

## Student Research Papers :: Fall 2002

Richard B. Gump South Pacific Biological Research Station :: Moorea, French Polynesia

University of California, Berkeley

# Biology and Geomorphology of Tropical Islands

## Moorea Student Research Papers

## Volume 11

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Integrative Biology 158; Environmental Science, Policy, and Management 107
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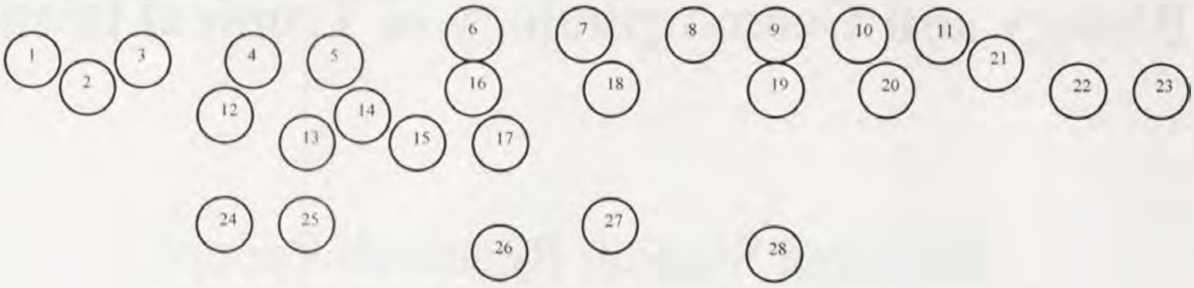
**Front Cover**: In the legend of the old name of Moorea, "Aimeho", the demigod octopus that lived on the marae at Papetoai was banished from there and became Mt Rotui. You can see it clearly from the marae, head with eyes and mantle, and its arms running down the ridges of the mountain. The octopus was the guardian of knowledge and the term "aimeho" means to hide something while eating it, just as an octopus does with its mantle. Legend courtesy of Frank Murphy of Moorea. Photo by Jere Lipps.

**Back Cover**: Artwork by Brooke Keeney, February 2003



1. Brent Mishler (professor), 2. Nicole Woodling (GSI), 3. Brent Vickers, 4. Nick Schmidt, 5. Chris DiVittorio, 6. Chris Berkson, 7. Tyler Reynard, 8. Keefe Reuther, 9. Steve Carlson, 10. Pete Oboyski (GSI), 11. Hannah Stewart (GSI), 12. Gregg Mendez, 13. Nicole Santos, 14. Marissa Hirst, 15. Alice Nguyen, 16. Brooke Keeney, 17. Christine Hara, 18. Jay Nagtalon, 19. Stephanie Porter, 20. Lara Cushing, 21. William Wood, 22. Rosie Gillespie (professor), 23. Jere Lipps (professor), 24. Michelle Ochomogo, 25. Mary Sorensen, 26. Jamie Lipps, 27. John Holt, 28. Parissa Vassef

Taking notes at Tettiaroa

Halloween
at the
Gump Station

Learning Tahitian dance

The stream transect

Class at Bodega Marine Lab
Including professors Carole Hickman (2nd from top left) and George Roderick (top right)

Hiking at the Belvedere

Returning from Tettiaroa

Dinner

On the road again


CALIFORN
BERKELE

**Gump Station on the edge of Cook's Bay, Moorea with Mt. Mouaputa in the clouds. Photograph by Jere Lipps.**

# The Moorea Class 2002

## Introduction

Moorea is a very special experience—a tropical paradise with so much geology, oceanography, anthropology and biology visible all around. For the Berkeley undergraduates who have taken the course on the "Biology and Geomorphology of Tropical Islands" there, Moorea changed their lives. The purpose of the course is to make these students into professional biologists, geologists or whatever, through the creation, design and implementation of their own original research project. Many unique research opportunities exist on Moorea, from the reefs to the tops of the spectacular mountains. This book records the diverse results of those efforts. Berkeley students and faculty are indeed fortunate that Richard P. Gump donated land and facilities to the campus years ago, so that these experiences could be available to undergraduate and graduate students.

The Departments of Environmental Sciences and Policy Management in the College of Natural Resources and Integrative Biology in the College of Letters and Science—give the course at the Richard P. Gump Biological Research Station on Cook's Bay, Moorea. A diverse team of faculty and Graduate Student Instructors from both departments teaches the course. Some of the team gives lectures at Berkeley while others provide instruction and assistance on Moorea. Five faculty took part in the field instruction for two or three weeks each, and three GSIs lived and worked with the class for the entire nine weeks.

The course has been immensely successful since its inception in 1991. Nearly 200 students have taken the course, done research on their own original projects, presented their results in the annual Symposium on the Biology and Geomorphology of Tropical Islands, and have written scientific papers published in this series of books. These books have been widely distributed to libraries and personnel at Berkeley, on Moorea and Tahiti, as well as elsewhere. Many of the students' papers have also been published in regular scientific journals.

The Moorea course is a complete research experience, just like that of professional scientists. Each student is involved completely in the scientific preparation, logistic planning, equipment and supply selection, and the development of their own research project. The course began with 3-1/2 weeks of work in Berkeley absorbing the science background and figuring out the logistics and supply needs of the class. Then the entire group of 21 students, several faculty and three Graduate Student Instructors moved with much excitement to Moorea. On Moorea and Tahiti the students took part in field trips on the reefs, in the streams and grottos, up the mountains, and down the valleys, each experiencing the intricacies of these tropical islands. After a week of this preparation, the students were ready to develop their own scientific project. They learned also that safety, logistics, cost, equipment and supplies are critical aspects of each project that requires detailed planning as well. For the next eight weeks, the students pursued their own research on Moorea, returning to Berkeley to deliver an oral presentation at the annual Symposium and the written report contained in this volume.

At the end of the fall semester, we realized that everyone--students, GSIs and faculty--learned many new things, had new and different experiences, and enjoyed most every minute. Once again this year, the student projects were enormous. Perhaps, more than anything else and as in all intensive field research, they and we learned a good deal about one another and people in general. It was a thoroughly enjoyable, if at times stressful, research trip.

Jere H. Lipps, Professor, Integrative Biology. Course Coordinator.
Rosie Gillespie, Professor, ESPM
Carole Hickman, Professor, Integrative Biology
Brent Mishler, Professor, Integrative Biology
George Roderick, Professor, ESPM
Hanna Stewart, Graduate Student Instructor, Integrative Biology
Peter Oboyski, Graduate Student Instructor, ESPM
Nicole Woodling, Graduate Student Instructor, ERG

# Acknowledgments

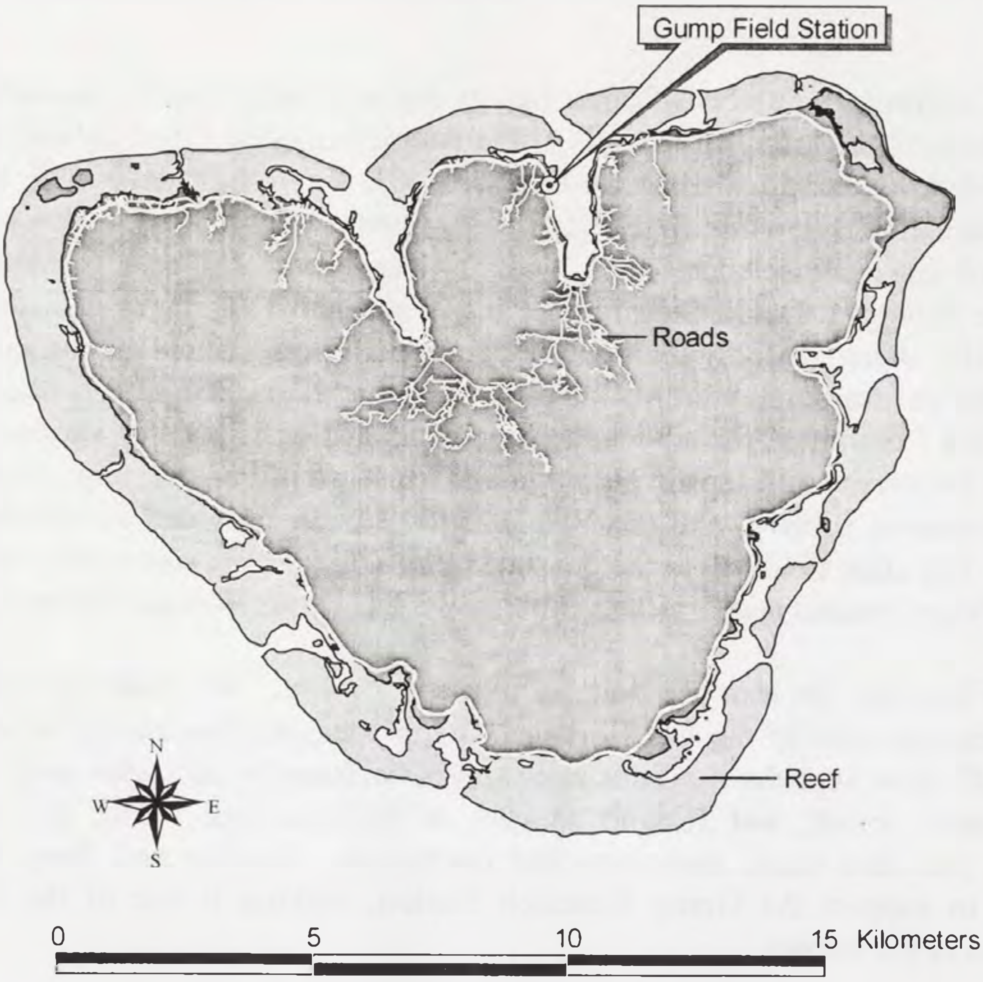
Any field expedition is enormously complex and the Moorea course is no exception. We thank the students, faculty, Graduate Student Instructors, and our colleagues at Berkeley and on Moorea who made the 2002 class hugely successful. We all appreciated and remember with wonderful feelings the good times, camaraderie and excitement we shared with the students. Those of us in the field with the students also learned a good deal from each of them, as they developed and explored their own research.

In California, a number of other faculty and staff helped us by presenting lectures and workshops on a variety of topics. These people included Roy Caldwell (Integrative Biology), Mark Frieberg and Brigette Bankay (UCB Environmental Health and Safety), Jim Hayward (UCB Diving Safety Officer), Carole Hickman (Integrative Biology), Norma Kobzina (Bioscience and Natural Resources Library), David Lindberg (Integrative Biology), David Smith and Pang Wai Chan (VLSB Visualization Center). We especially thank Dorothy Tabron (Integrative Biology) and Gleoria Bradley-Sapp, who kept the course going administratively, and Don Bain (Geography) and the Animal Use and Care Committee for advice, arrangements and assistance of various sorts. The staff of the University and Jepson Herbaria, the Museum of Paleontology, Bioscience and Natural Resources Library, and the Visualization Center provided assistance with their resources. The class field trip to the coast of Central California was made possible by the efforts of Peter Conners and Lisa Valentine of the UC Davis Bodega Marine Laboratory.

On Moorea, we also are grateful to many people. We thank our colleague Dr. Neil Davies, manager of the UC Berkeley Gump Research Station on Moorea and the Station staff, who kept the facilities operating so efficiently and who made our stay so very pleasant. Frank and Hinano Murphy of Moorea and Tahiti, and many other Mooreans, provided much assistance and interaction. Gordon and Betty Moore have continued to support the Gump Research Station, making it one of the finest field laboratories in the tropics.

Without all this help of such various kinds, our students would not have had this wonderful opportunity to learn about science, a new culture and, especially, themselves. They have been changed forever!

Island of Mo'orea, Society Islands, French Polynesia



# Biology and Geomorphology of Tropical Islands

Moorea Student Papers

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## CONTENTS

Biol. Geomorph. Trop. Is. (2002) 11:1-8
UC Berkeley, IB 158 / ESPM 107

# A survey of tree and forest architecture in Mo'orea

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ABSTRACT. As the inherited plan for a tree's structure, an architectural model markedly affects the shape of a tree. This study assigned architectural models to 13 species found in two common forest types on Moorea, *Inocarpus*-dominated mid-elevation forest and *Hibiscus*- and *Cocos*- dominated coastal forest. Trunks and crowns of every tree taller than 3 meters were plotted on top-down drawings and profiles of five 50 $m^2$ plots from each forest type. The total area covered by each species and architectural model was determined for each plot, and the two forest types were found to be dominated by trees with different architectural models. Light penetration was measured in each forest type, but no significant difference was found between penetration of the two forest types.

KEYWORDS: tree architecture, Moorea, forest architecture, light penetration, architectural model.

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## Introduction

Because trees are stationary light-harvesting organisms, the shape of a tree plays a large role in determining a tree's fitness and how it affects neighboring trees. Factors that determine the shape of trees include space availability, direction and abundance of sunlight (Poorter 1999), inherited architecture, disturbances caused by animals or diseases, damage from falling rocks or neighboring trees (Halle et al. 1978), and climactic conditions. The genetic material of a tree determines the rules for how a tree may react to external factors. Generalized patterns of the expression of this material are grouped by Halle, Oldeman, and Tomlinson in their 1978 textbook (hereafter referred to as "Tropical Trees") into 23 architectural models (Halle et al. 1978). These models are characterized by several factors, including: the habit of branches (growing vertically vs. horizontally), origin of flowers (arising from terminal vs. lateral meristems), and the timing of branch and trunk growth (rhythmic vs. continual). These characters are subject to change to some extent within a species or even an individual, due to mutations or ecology (Halle 1978).

This study's goal is to examine some of the ways tree architecture translates into morphologies and to gain further insight into the ecological importance of architectural models. Is knowing a tree's architectural type enough to predict interactions it will have with other trees, or are there too many variables responsible for a tree's shape for such a prediction to be accurate? A tree's shape translates to the amount of light it can intercept. If an architectural model allows a predictable pattern of light to slip through a tree's branches, branches of trees with different architectural models might tend to overlap more with each other than those of the same model. Also, forests with more architectural diversity may intercept more light than less diverse forests. This could be important to islands, where species diversity is low, in order to predict a forest's susceptibility to invasion by foreign species, for example. This study starts by describing, somewhat quantitatively, two forest types on Mo'orea.

## Materials and Methods

Two prominent forest types were chosen: "Coast" and "Belvedere" (Figures 1 and 2). The "Coast" type was studied near the Gump station. The area chosen was an old

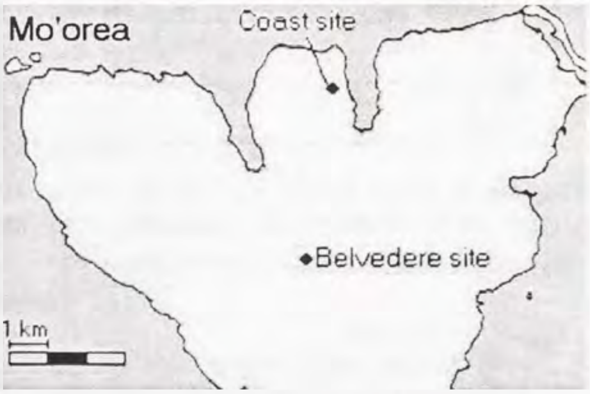


**Figure 1.** Locations of the two forest types studied.

coconut plantation that has not been used for many years, where several species have invaded. The "Belvedere" type starts about 200 meters west of the Belvedere parking lot, and is *Inocarpus*-dominated forest that consists mostly of trees that were probably introduced by the Polynesians several hundred years ago. Within each habitat type, five plots were studied. These plots measured 5 x 10 meters and were chosen for maximum representation of the surrounding area, diversity, and accessibility.

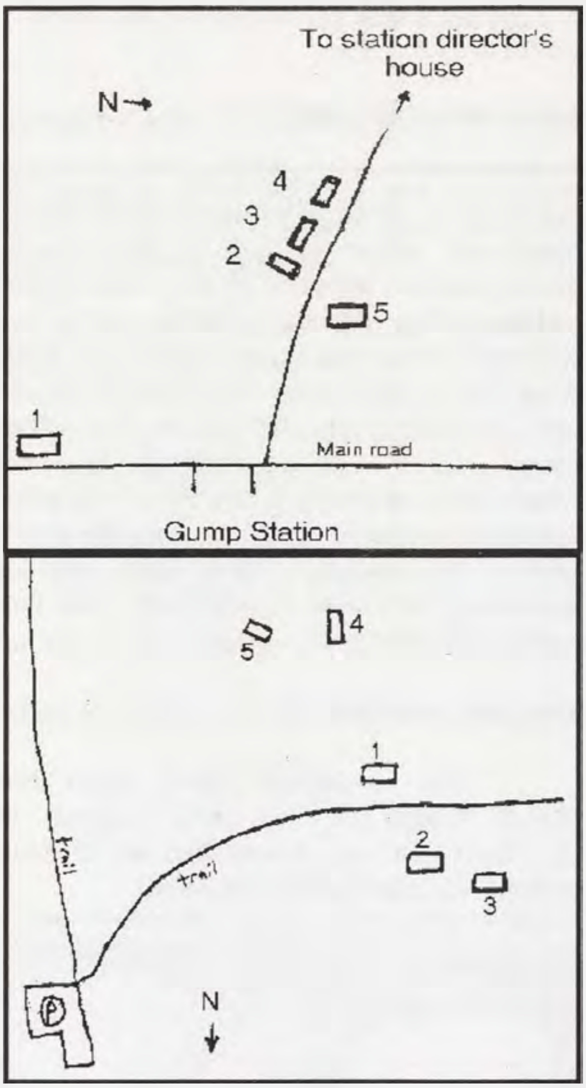


**Figure 2**. Maps to the plots at the Coast site (GPS 06 k 0199675 vtm 8063950), top, and Belvedere site, bottom. 1 cm ≈ 80 m.

*Model assignments*

Architectural models were assigned for each species found within the plots, using the key found on pages 84-97 of "Tropical Trees". Several individuals of each species were analyzed to recognize key characters, including those mentioned in the introduction. In the text, Halle assigns models for *Hibiscus*, *Spathodea*, *Albizzia*, *Cocos*, *Miconia*, *Mangifera*, and *Syzygium*.

*Forest diagrams*

Within each plot, the positions of the trunks of every tree taller than 3 meters (sometimes less) were plotted on a top-down-view drawing of the area. Then their crowns were roughly plotted on this drawing, measured both by an observer standing under the edge of each tree's crown and by looking at the overlaps and relative positions of each crown. When a tree was too tall for an accurate estimation, a densitometer was used to find the edge of its crown. Next, a profile of the plot was drawn, showing the edges of each tree's crown and their main trunks. Heights were measured with either a 9 or 12 meter graduated length of bamboo, and double checked by noting the relations between all the trees in the plot. Heights taller than the bamboo were estimated, using the bamboo as a reference. On both drawings, all crowns of trees taller than 3 meters were drawn, even overhanging branches of trees whose trunks are not located within the plot (see Appendix B for example diagrams from each forest type).

Once these plots were completed, the total cover of each individual was tallied, and from this, percent cover by each species and each model was determined.

*Light penetration*

In order to measure light penetration, three photographs were taken with a Nikon Coolpix 990™ digital camera along the middle of the 10-meter length of each plot, one at 2.5 meters from each end and one in the middle. The camera was pointed straight up from a height of 1-2 meters, and was meant to capture the canopy represented on the plots, so in some cases branches from understory plants were moved aside. These photos were analyzed using Adobe Photoshop™ 6.0 as follows: First, the greens were selected and changed to black, representing light intercepted by the canopy. Then, the blues were selected and changed to white, representing light that has penetrated the canopy. The histogram feature was then used to measure the percentage of pixels that are at least 50% white (levels 128-255 on the luminosity histogram). This percentage represents approximate light penetration of the canopy.

| Species | Model |
|---|---|
| *Albizzia falcata* | Troll's |
| *Aleurites molucanna* | Stone's |
| *Cocos nucifera* | Corner's |
| *Hibiscus tiliaceus* | Scarrone's |
| *Inga feuillei* | Champagnat's |
| *Inocarpus fagifer* | Troll's |
| *Mangifera indica* | Scarrone's |
| *Miconia calvescens* | Leeuwenberg's |
| *Neonauclea forsteri* | Massart's |
| *Spathodea campanulata* | Chamberlain's/ Leeuwenberg's |
| *Syzygium* | Mangenot's |
| *Timonius polygamus* | Massart's |
| Unidentified species | Massart's |

**Table 1**. Architectural model assignments for every species encountered in the Coast and Belvedere forest types on Mo'orea. Species identification: *Albizzia*, *Syzygium* by Peter Oboyski; *Aleurites*, *Neonauclea*, *Spathodea* by Lara Cushing (her voucher specimens are in the Jepson Herbarium); *Timonius* and *Inga* by Dr. Brent Mishler (voucher specimens are in the Jepson Herbarium).

## Results

### *Species assignments*

Thirteen species representing nine architectural models were found in the two forest types (Table 1).

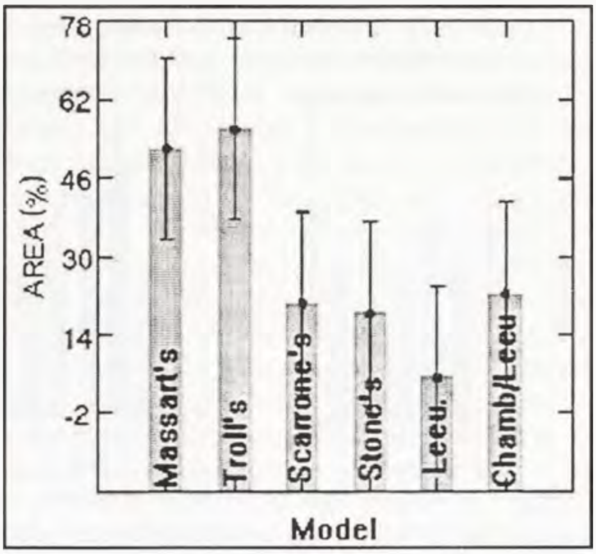
### *Forest structure*

Eight species and six out of the 23 possible models were found in the Belvedere forest type plots, and seven species and six models were found in the Coast plots. A total of nine different models and 12 species were found between the two forest types. Three models were found in both forest types.

### *Light penetration*

There is no significant difference between light penetration levels in the two sites (P = 0.629).

**Belvedere**



**Coast**

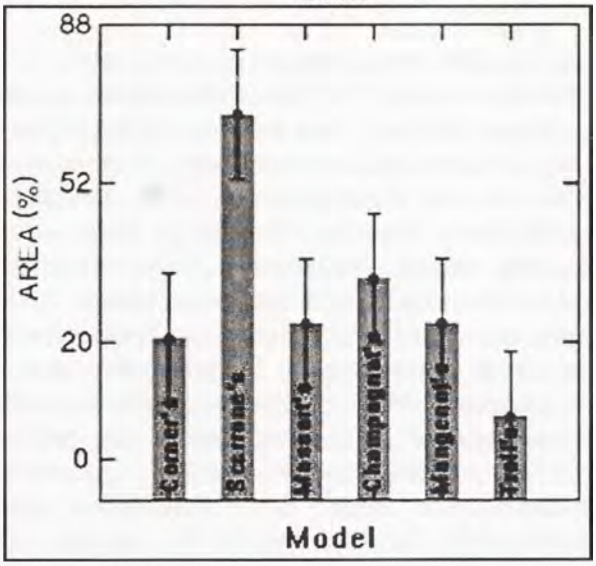


**Figure 3.** The composition of each forest type by percent cover of each architectural model. Areas of individual trees were summed, so percentages add up to more than 100% due to overlap.

## Discussion

### *Model assignments*

As Halle and Oldeman stated in a 1975 essay,

> "The analysis of tree architecture cannot be conducted successfully in the field, unless on a very abundant species and one where organization is not very complex."
> (Halle & Oldeman 1975)

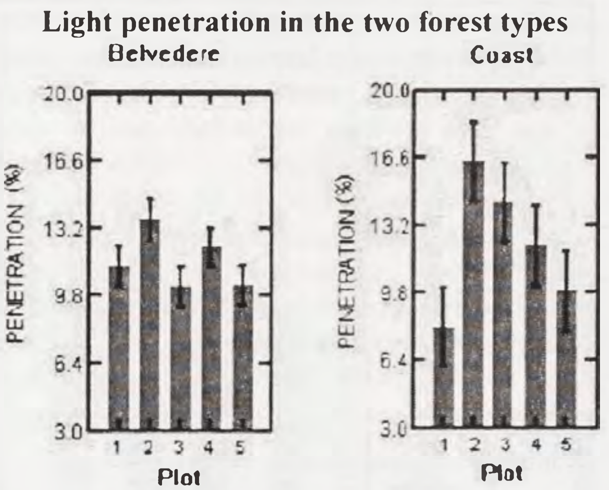


**Figure 4.** Percent light penetration measured by analyzing digital canopy pictures with the Adobe Photoshop™ 6.0 histogram feature.

Being able to assign trees architectural models, however, makes the job a little easier for the amateur student of tree architecture, but fitting a tree to a model is not always easy or completely accurate for several reasons. It is sometimes difficult to describe branches as being either orthotropic or plagiotropic. "Tropical Trees" describes plagiotropic shoots as "shoots which are more or less horizontal with dorsiventral symmetry," meaning the leaves are displayed in a plane parallel to the branch, which is usually accomplished by the bending of the petioles and/or twisting of the branch. Orthotropic shoots "are erect, with essentially radial symmetry" (Halle et al. 1978), meaning the leaves are arranged perpendicularly to the branch. In both branch orientations, the leaves are arranged to intercept as much light as possible. In reality, however, branches often grow diagonally and the petioles are bent slightly to orient the leaf perpendicularly to the light, so there is a spectrum between orthotropy and plagiotropy, and obviously not every branch in a tree looks the same. Further complicating the matter, a third branch orientation is referred to as "mixed," meaning the branch changes directions at some point during its growth, often contributing to part of the trunk and a lateral branch. Sometimes this shift in orientation is caused by gravity bending down a branch with an orthotropic meristem, as in Champagnat's model. Another source of ambiguity, in the case of *Aleurites* and *Hibiscus*, is the distinction between rhythmic (growth cycle includes a period of rest) and continuous growth. Because tropical trees experience no winter season and many are not deciduous, they often do not form buds that remain dormant for part of the year before giving birth to a branch segment, which is one telltale feature of rhythmic growth. In some cases, in order to be sure which growth pattern a tree follows, observations for a whole year would be necessary. Also due to the short time available for the study, flowering or fruiting was not observed for some trees (*Aleurites*, *Inocarpus* and *Neonauclea*). In some individuals, flowers or fruit may have been present high in the tree where it was difficult to note whether it was produced by a terminal or lateral meristem. In recognizing Troll (*Inocarpus*) and Massart's (*Neonauclea*) models, however, this doesn't matter.

Having said this, most of the model assignments were fairly straightforward. *Albizzia* trees clearly have plagiotropic branches, some of which straighten after aging and form a sympodial trunk. *Aleurites* has all orthotropic meristems, appears to have terminal flowers (sympodial growth), and though it was difficult to say for sure from the individuals short enough to observe, appears to have continual growth. There is no doubt in assigning a model to *Cocos nucifera*, the coconut palm, as it clearly does not branch and has lateral inflorescences. There was some uncertainty as to whether *Hibiscus* exhibits continuous or rhythmic trunk growth. This growth form is usually characterized by branches that originate from the same vertical point on the trunk, similar to how whorled leaves cluster together on a stem; due to the unusual habit of *Hibiscus* trees described later, this pattern was rarely observed. "Tropical Trees" assigns Scarrone's model to *Hibiscus*, and this is what has been used in this study. *Inocarpus* seems to be a clear example of Troll's model. Plenty of trees of every age were present, and all branches exhibited plagiotropic growth from the beginning, with basal segments straightening to form an erect trunk. Plenty of individuals, young and old, of *Mangifera* or mango, were present in the Coast forest, and the tree was easily keyed out as Scarrone's model, agreeing with "Tropical Trees". Unlike *Hibiscus*, mango trees clearly branch rhythmically, often with three or four branches coming out of the trunk within a single centimeter. Halle has pointed out, however, that *Mangifera* actually follows Leeuwenberg's model, which is similar to Scarrone's, except that it lacks a main vertical trunk, when it is in direct sunlight (Halle 1978). All individuals examined on Mo'orea were in a shaded environment. *Miconia* was straightforward, with equivalent branches arising at the base of the obvious terminal inflorescences. Many *Neonauclea* trees, young and old, were present. The old trees are difficult to use for model assignment, as their crowns are too high for details to be visible, where the high-order branches appear to be a nonsensical tangle. Trees 3-4 meters in height, though, clearly show the main orthotropic axis and rhythmic plagiotropic branching characteristic of Massart's model. *Spathodea* trees seem to be a single monopodial trunk until they reach a height of several meters, at which point it is difficult to distinguish the long compound leaves from small branches. Only a few young trees were present in the Belvedere forest, and the fully mature trees were much too tall to discern the branching pattern. This seems to be an example of one of the trees to which Halle and Oldeman refer whose organization is too complex to study in the field. "Tropical Trees" has placed *Spathodea* between Chamberlain and Leeuwenberg's models, because, unlike in *Miconia*, sometimes only one branch arises from the base of a terminal inflorescence. The growth pattern of *Syzygium* is distinguished by its meristems' initial vertical growth later changing to horizontal or even downward growth. The unidentified tree, a short tree that has large leaves with dark green and yellow patches; exhibits a monopodial, orthotropic main trunk with plagiotropic, monopodial branches born rhythmically from the trunk; characters consistent with Massart's model. *Timonius* shows an architectural form very similar to this tree. *Inga*'s growth is initially orthotropic, with branches bending apparently due to gravity as they get larger, and the trunk is a sympodium.

This is the first time a model has been assigned to all the species not mentioned in "Tropical Trees", because it is usually not possible to identify a tree's model from herbarium specimens, and surprisingly little research has been done on macroscopic tree architecture (Donaghue 1981). Once the models were identified, their ecological significance could be examined.

The architectural model alone does not dictate the habit of a tree. For example, *Hibiscus* and *Mangifera* are both described by Scarrone's model, but they appear to have completely different shapes and seem to have different ecological roles. *Hibiscus* often reproduces asexually, sending reiterative branches up to fill holes in the canopy. Branches appear to grow quickly until they bend under their own weight and in turn, produce new reiterative branches where light is available. Because branches are extremely long and rely on neighboring trees for structural support, *Hibiscus* trees sometimes look like vines. Additional research focusing on the growth habit of *Hibiscus* in response to different light conditions would be useful in further illuminating the architecture- morphology relationship. In contrast to *Hibiscus*, Mango trees usually grow with one main trunk and a comparatively symmetrical crown, so the tree is shaped more like a mushroom than a vine.

### *Descriptions of forest types*

The diagrams of the plots in this survey can be used as a general description of the two forest types. Within the Belvedere forest type, *Inocarpus* is the dominant species. The species seems to be well established there: mature trees cover some portion of every plot, and saplings appear to be abundant throughout the forest. Fallen *Inocarpus* leaves also cover much of the forest floor throughout this forest type. Even though this species is the only representative of Troll's model in the Belvedere forest type, Troll's still covers more area than any other model. The second most abundant model is Massart's, which is represented by three species: *Neonauclea*, the unidentified species, and *Timonius*. The other four models are each represented by just one species. *Timonius* and unidentified trees were small, either because the trees are limited genetically to only a few meters in height or they are not well established either because of time or competition from other trees.

There were very few instances of major overlap of branches between species, except between canopy and understory trees. The most surprising exception was a *Timonius* trunk growing only a few centimeters away from the trunk of the unidentified species, both trees nearly the same height, with their branches intertwined and with apparently similar density or foliage. Since these trees are of the same architectural model, this example could be interpreted to suggest that the model doesn't meaningfully predict the pattern of light absorbed by a tree.

The Coast type is dominated by *Hibiscus* trees, which, especially in plots with shorter trees, easily extend reiterated branches above surrounding trees, which then branch profusely to form the canopy. Usually *Hibiscus* cover appears to be fairly thin, leaving an abundance of light for understory trees. Mango and *Syzygium* trees were the largest in the area, growing solidly and much more symmetrically than *Hibiscus*. This forest type, and especially *Hibiscus*, *Mangifera*, *Albizzia*, *Cocos*, and *Timonius*, dominates much of the land near the main road of the island.

*Light penetration*

One point that backs up the validity of these light measurements is that the density of cover of the two forest types, calculated by dividing the sum of cover by every individual by the total area, was almost the same (Belvedere = 1.74, Coast = 1.81). It would make sense that forests with similar density of cover would have similar light penetration values. Both types had species from six models, and there was no significant difference between the two in the amount of light penetration through their canopies. Thus nothing can be said conclusively about the correlation between architectural diversity and light penetration. However, since density, architectural diversity, and light penetration were similar in both forest types, the data from this experiment does not conflict with the hypothesis that diversity might relate to light penetration, and so future studies might measure light penetration in forests with differing levels of architectural diversity.

## Conclusion

This survey was conducted as a preliminary study to look for trends in the distribution and cover of trees of different architectural models. Additional field work in a variety of habitats would be useful in speeding the understanding of the mechanisms trees use to engineer themselves and the interactions between trees in all habitats. Some studies have been recently done to describe models as they relate structure to carbon fixation and thus photosynthesis, using molecular methods (Escobar-Gutierrez et al. 2001) and high-tech imaging techniques. In one study, a complete 3-D model of an entire peach tree was digitized once initially, and again after four weeks of growth (Diaz-Espejo et al. 2002). It is exciting to think that one day the growth of an entire tree might be predicted by mathematical models. Knowing the specific function of a tree's genes that control its structure could have applications to biologists, farmers, horticulturists, and foresters.

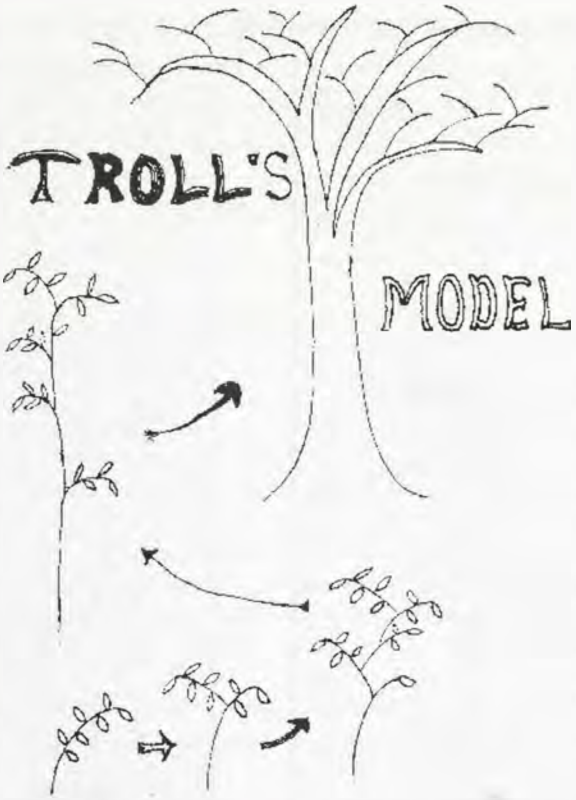


**Figure 5.** A diagram of the growth pattern of trees described by Troll's model

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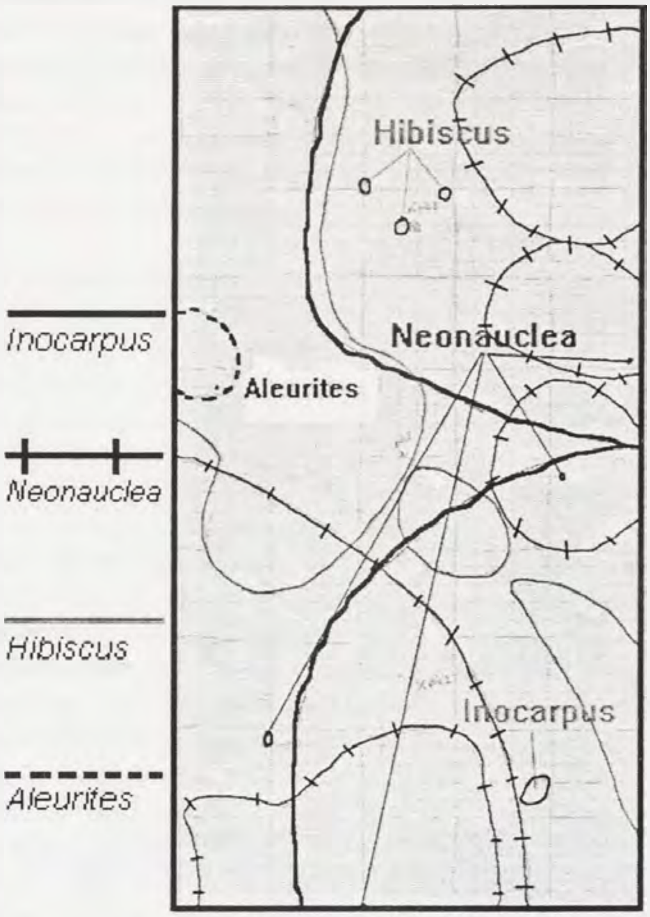
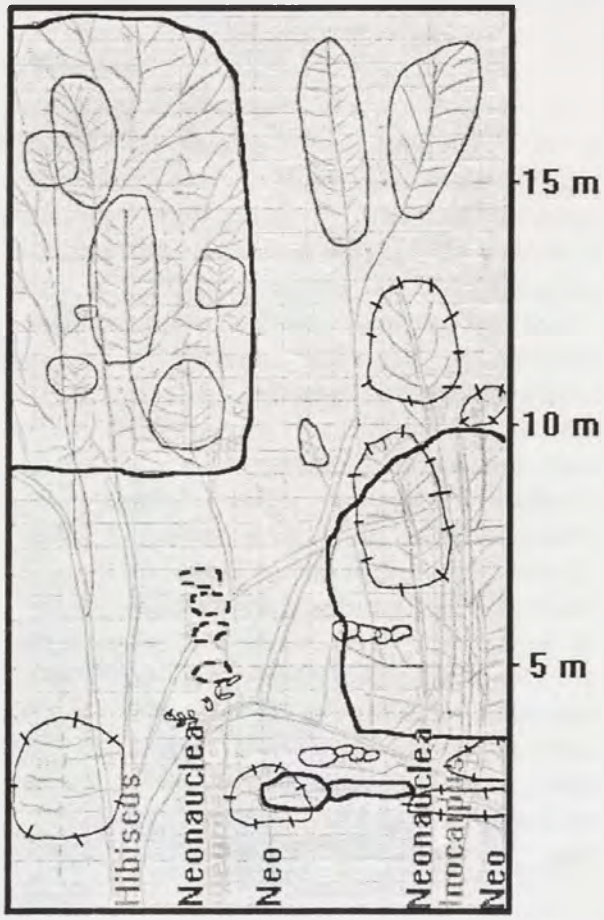
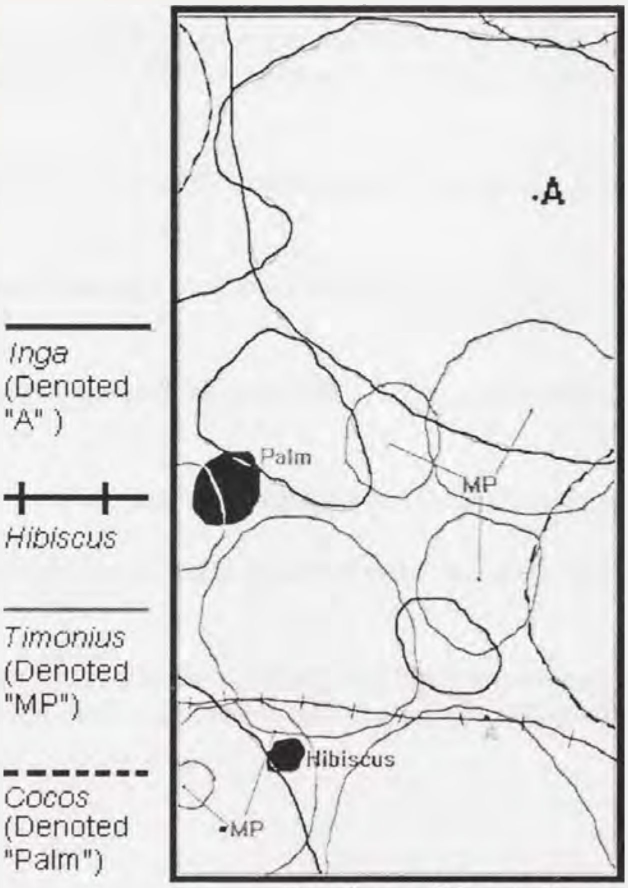
**Appendix A: Examples of diagrams of the forest plots**



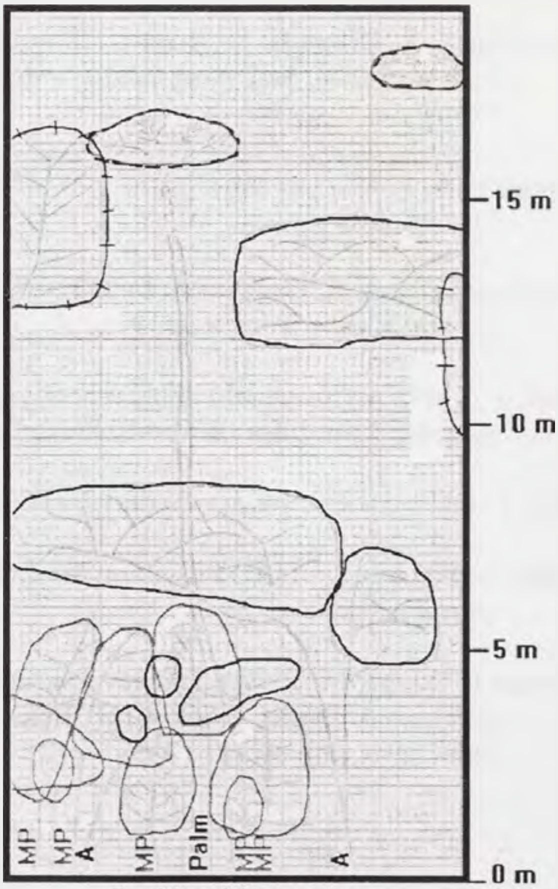
Top-down of Belvedere site



Profile of Belvedere site



Top-down of Coast site



Profile of Coast site

Biol. Geomorph. Trop. Is. (2002) 11:9-14
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# Flower Visitors of *Metrosideros collina* (Myrtaceae) on Moorea

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ABSTRACT. Island ecosystems are fragile assemblages of unique organisms. Moorea is such an assemblage, with many unique plants, animals, and species interactions. However, like many islands, Moorea is increasingly inhabited by invasive species that compete with and often drive to extinction the native species. The indigenous tree *Metrosideros collina* has small red bottlebrush-like flowers grouped in inflorescences of approximately 10-20 flowers. Red color, abundant nectar, and an open shape are thought to be characters of bird pollinated flowers, and indeed in Hawaii and New Zealand *Metrosideros* species have been shown to be bird pollinated. On Moorea the pollinators of *M. collina* have been little studied. Through observation this study found that *M. collina* on Moorea is visited most often by European honeybees (*Apis mellifera*) and white-eye birds, and is additionally visited by three species of wasp, two species of butterfly, and a skink lizard. Many specimens were captured and analyzed for pollen presence. *M. collina* flowers are generalist, offering nectar and pollen rewards accessible to many nectivorous animals. A generalist strategy facilitates both colonization of new islands and persistence on islands with high rates of species turnover. Future study could investigate pollinator efficiency and pollinator community composition over an elevation gradient.

Keywords: *Metrosideros collina*, Moorea, Pollination, flower morphology, self compatibility

## Introduction

The genus *Metrosideros* has about fifty species, representatives of which are present on islands throughout the entire Pacific. *M. collina* (the species found on Moorea) can be found throughout the Pacific in a 60-degree latitude on most large islands (Carpenter 1976) including the Societies, Hawaii, and the Marquesas. Recent genetic analysis (Wright et al. 2000) has found that the same genetic lineage of *Metrosideros* trees is present in New Zealand, Raratonga, Tahiti, and Hawaii. The study proposes that the dispersal from New Zealand to the remote Polynesian islands did not occur until relatively recently, during the Pleistocene, and speculates that dispersal was facilitated by El Nino-like changes in climate and wind patterns. *Metrosideros* has been able to disperse to distant locations because of its tiny windborne seeds that can be lifted by gusts of only 5-19 km per hour and can survive a month in seawater (Corn 1972). Many species of *Metrosideros* are adapted to colonize disturbed patches such as lava flows (Hatfield et al 1996) a valuable trait for island plants. In Hawaii *Metrosideros collina* and *Metrosideros polymorpha* are the dominant canopy forming trees in the rainforest ecosystem (Stemmermann 1983), and both species have been fairly well studied. In Hawaii it has been shown that *Metrosideros* is pollinated primarily by birds, though it is also visited by some insects (Carpenter 1976). In New Zealand *Metrosideros excelsa* is also pollinated primarily by birds and also by insects (Schmidt-Adam et al. 1999).

The small flowers of *Metrosideros collina* are arranged in inflorescences (Fig 1). The inflorescence is composed of a variable number of flowers. Each flower is like a small eucalyptus flower with rudimentary petals and a single ring of long stamens around a nectar cup from which protrudes a single long stigma. Buds may open all at once or sequentially. The bud opens by first extending the style (Carpenter 1976, personal observation). The flower lasts several days, then the stamens and petals drop off, followed several days later by the stigma. Essentially there is a hermaphrodite phase proceeded and followed by a female phase. What remains on the tree after flowers senesce are small green fruits. The fruits are dehiscent capsules with three compartments of small (2-3mm) airborne seeds. The capsules grow to full size within a month but do not turn brown and dehisce for four months to a year (Wickland 2001).

It is thought that flowers exhibit pollinator "syndromes," combinations of characters that vary depending on what the primary pollinator of a particular plant is. Because of its red color, flower dimensions, copious and easily accessible nectar, low sugar concentration in nectar (Carpenter 1976), and lack of smell, *Metrosideros* flowers display a bird-pollinator syndrome. However, most

Moorean birds are extinct due to introduced predators and competitors, habitat loss, and predation by humans (Lobban and Schefter 1997).

On Moorea, *M. collina* (called Puerata in Tahitian) is distributed from an elevation of approximately 100m to the tops of the highest mountains. It grows in small stands, primarily in exposed areas, where it has a shrub like form. In areas where it is more sheltered it takes on a taller more tree like shape. Other aspects of morphology are also variable. The leaves can be round or ovate, and the leaves and stems can be glabrous or pubescent. A previous study showed that *M. collina* on Moorea is showing low recruitment: very few juvenile trees are present (Wickland 2001). A possible explanation for low recruitment is the absence of fertile seed due to absence of pollinators. In addition, as a native tree *M. collina* may attract remaining species of native pollinator. The bird species that pollinate *Metrosideros* in Hawaii and other islands are not present on Moorea. Indeed, birds currently found in Moorea are recent European introductions, as is most of the insect fauna. *M. collina* pollination on Moorea is relevant to issues of native plant and insect conservation. Native species are precious because of their link to the past and importance to native ecosystems. The pollination system of *M. collina* on Moorea is not known to have been previously investigated in detail.

**Figure 1.** The inflorescence structure of *Metrosideros collina*, including (from top to bottom) buds, flowers, and fruits.

## Methods and Materials

Though *M. collina* is not uncommon on Moorea, it is predominantly found in steep, difficult to get to areas. Located approximately one half-mile west of the Belvedere lookout (17° 32' 34" S 147° 49' 34" W) is another lookout area with a stand of *M. collina* on relatively flat ground. Additionally, this stand was flowering during the study period and included some shorter trees and trees with accessible branches. This location was ideal for observing flower visitation; therefore all of my data was collected at this site.

The role of pollinators differs between plants that are self-fertile or self-infertile. If a plant cannot self fertilize, pollinators are vital to reproduction. However, if a plant can self-fertilize then the role of the pollinator is to facilitate outcrossing and enhance genetic diversity. To examine self-compatibility, I created mesh bags and used them to enclose seven unopened inflorescences. Once the buds opened, I could be sure that they had never been visited by pollinators. I self-pollinated the newly opened flowers using a clean Q-tip for each inflorescence to transfer pollen from all the stamens of the inflorescence to all of the pistils. I chose to test for compatibility at the level of inflorescence rather than within a single flower or within a plant since pollen transfer in nature could occur through wind or plant movement at the inflorescence spatial level. I repeated the Q-tip procedure on several separate days until all of the stamens had dropped off of an inflorescence. I removed the bags after all of the styles dropped off several days later. For comparison I marked several control inflorescences of similar age. I intended to harvest the seeds to assess the ratio of filled (fertilized) to unfilled seeds.

To verify that the plant could not produce seed without any pollen at all, another bagging experiment was performed without replication. I bagged a single unopened inflorescence. When the flowers began to bloom I removed the anthers from all of the unfurling stamens.

I used three trapping methods to attempt to assess possible pollinator insect species present in the study area. The first trap was a commercial sticky trap designed for mice consisting of a piece of cardboard approximately 16cm by 9cm with a sticky substance coating one side. I suspended two such traps from study trees for two weeks. The second trap was a traditional Malaise trap about two meters high. This trap was left in the study area for a week.

Lastly, I constructed two intercept traps (Fig 2) that were left in the study area for two weeks.

The bulk of this study was direct observation of flower visitation. To attempt to eliminate some observer bias I established observation protocols. The observation periods had to last fifteen minutes or more. Anywhere from one to ten inflorescences were observed, but all inflorescences had to be near enough to each other that they could be seen simultaneously. Observations were conducted between 9am and 3pm. Each visitation was recorded and, when possible, the visitor was captured for pollen analysis. In addition, opportunistic observations of visits to non-target inflorescences were recorded separately.

Insects captured in the field were brought back to the Gump Field Station and frozen for at least twenty-four hours. The insects were dabbed on the head, mouthparts, underbelly, and legs with an insect pin dipped in Vaseline. The insect pin was wiped on a clean microscope slide and the slide examined at 10x and 40x. *M. collina* pollen was distinguishable from other pollen by its distinct triangular shape. Data was recorded only as pollen presence/absence without quantifying pollen load.

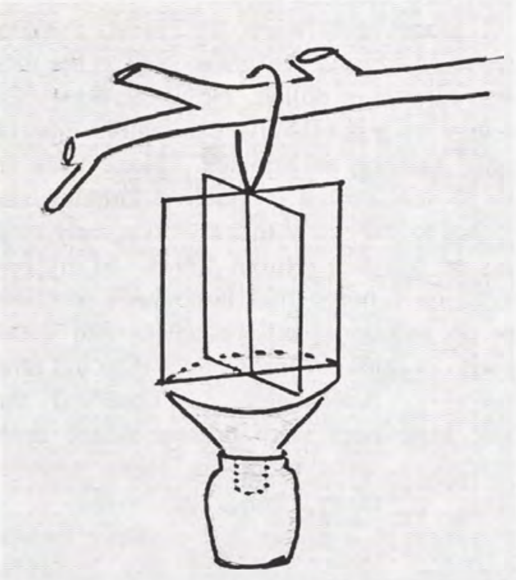

**Figure 2.** An intercept trap. This trap design was unsuccessful in capturing pollinators.

## Results

The duration of the study was not long enough to observe seed set in the bagged flowers, but the green fruits were collected and dissected. No difference was observed between dissected bagged and control flower heads. Both contained many filamentous strands presumed to be the developing seeds.

In the inflorescence in which the stamens were removed, every fruit in the inflorescence abscised soon after blooming was completed.

All three trapping methods were ineffective. Very few insects were collected and none of the insects captured were potentially flower-visiting taxa.

Ten insect species, a bird, and a skink were observed to visit *M. collina* at the study site. (Table 1). Of these, the Italian honeybee (*Apis Mellifera*) was the most frequent visitor. Statistical analysis of visitation by the eight most common visitors yielded a Chi-squared value of 410.160 (degrees freedom=6, prob= .000), indicating that the visitation by all species was not the same. The two visitor species that were not included in statistical analysis were observed only once each, and no specimens were captured. A chi squared analysis indicated that honeybees visited flowers significantly more often than the other seven visitor species combined (chi squared=6.48, degrees freedom=1, probability=.011).

Pollen analysis showed that some visitors carry pollen but that pollen is not always present after flower visitation (Table 2). Specimens of six of the species observed visiting flowers were collected. Sample size for most species was small, so for statistical purposes pollen presence on honeybee specimens was compared to pollen presence on all other specimens. Honeybees were shown to carry pollen significantly more frequently than all other specimens (chi squared=9.821, degrees freedom=1, probability=.002) and more frequently than the other three species of hymenoptera (chi squared=9.972, degrees freedom=1, probability=.002). Honeybees were qualitatively observed to carry the most pollen in addition to carrying pollen the most frequently. On honeybees pollen was packed into balls carried on the hind legs and was visible under a low-power microscope, whereas on other specimens pollen was not detectable without the compound microscope. The wasps carried pollen less frequently, and the one captured skink was found to carry pollen. White-eye birds were not captured.

| Species | Total Flower Visits Observed |
|---|---|
| *Apis mellifera* | 118 |
| Vespidae 1 | 14 |
| *Polistes spp.* | 3 |
| Vespidae 2 | 17 |
| Nymphalidae | 9 |
| Danaidae | 1 |
| Skink | 15 |
| White Eye | 23 |

**Table 1.** List of *Metrosideros collina* flower visitors and number of times each species was observed visiting a flower.

**Discussion**

Results indicate that *M. collina* is self-fertile, as are many island plants. The first colonizing individual of a self-fertile species can produce seeds by pollinating itself, thereby initiating a sexually reproducing population. As an early colonizer of newly formed islands *M. collina* would not need a pollinator to reproduce. Wind action and subsequent plant movement would probably be sufficient to transfer pollen within an inflorescence. Flower visitors are nevertheless important to self-fertile plants. Flower visitors to self-fertile species facilitate outcrossing by carrying pollen from faraway and genetically diverse individuals. Additionally, flower visitors facilitate self fertilizating by carrying pollen from anther to stigma within a flower, an inflorescence, and a plant.

When anthers were removed from the flowers of one inflorescence, all fruits abscised soon after blooming was completed. Though unreplicated and inconclusive, this event suggests that *M. collina* may abscise all fruits containing infertile seed. Similar abscission was noted by Carpenter (1976). Such a mechanism would save the energy that would have been necessary for the fruit to grow to maturity. If indeed such a mechanism is present, fruit abscission could be used in pollination study as a measure of successful or unsuccessful pollination of *M. collina*.

It is unknown why the three trapping methods were so ineffectual. The sticky traps' texture was somewhat altered by the high humidity but was still strongly adhesive. It is possible that some chemical scent repulsed insects, or it is possible that densities of insects and of traps were not high enough that insects would randomly fly into the trap. The malaise trap caught many small insects such as moths and flies but did not catch suspected flower visiting taxa. However, this trap was not left for very long. The two intercept traps I constructed caught not a single insect in a week. It has been observed that flower-visiting insects in this study were exceptional fliers. Visitors frequently had to maneuver in high wind and were remarkably effective in doing so. Insects may have been adept at maneuvering out of traps and avoiding capture by the methods used.

| Species | Number with pollen | Number without pollen |
|---|---|---|
| *Apis mellifera* | 15 | 2 |
| Vespidae 1 | 4 | 9 |
| *Polistes spp.* | 2 | 1 |
| Vespidae 2 | 1 | 2 |
| Nymphalidae | 0 | 2 |
| Danaidae | 1 | 0 |
| Skink | 1 | 0 |
| White Eye | 0 | 0 |

**Table 2.** *Metrosideros collina* flower visitor specimens analyzed for pollen presence.

Honeybees were the most common visitors to *M. collina* flowers as well as the most frequent carriers of pollen. However, honeybees alone may be less effective pollinators than the currently existing assemblage. Honeybees are known to stay near a productive foraging site, sometimes to the extent that a worker only visits flowers on one tree (Huryn 1997). In my own observations I noted that honeybees remained longer on individual inflorescences and within areas with multiple inflorescences than did other pollinators. Additionally, I observed that multiple honeybees were present where many inflorescences were blooming close together, resulting in many honeybee visits to a inflorescence in a group, but a single isolated inflorescence rarely received any honeybee visits. Further study could test this observation with more observations and quantitative statistical analysis.

Of the three Hymenopteran wasp species, *Polistes spp*. And vespidae 1 behaved very similarly in the field. The two species were very abundant in the study area, and were often observed flying up and down branches and trunks of trees, seemingly examining all visible surfaces. These wasps were most likely foraging for arthropod prey. The two wasp species were observed to alight on or near flowers without taking nectar, but occasionally were seen to prod nectar cup with their heads, assumed to be a nectar feeding behavior. These wasps probably do not need nectar but are facultative nectivores, drinking nectar when it is convenient and accessible. The third wasp species, vespidae 2, was not as common in the study area. However, when the third wasp was found it was most often on or near flowers. This species may need or prefer nectar as a food source.

Butterfly (Lepidopteran) visitors were observed less frequently than other species, but may nonetheless be very valuable pollinators of *M. collina*. Schmitt 1980 points out that butterflies forage over a much greater distance than some other pollinators and can therefore provide a genetic link for spatially distant populations.

Skink lizards were seen to seek out inflorescences and prod the nectar cups with their snouts. Possible hypothesis for this behavior are that the skinks are drinking the nectar as a food source, drinking nectar as a water source, or searching for insect prey within the flower. Whittaker (1987) found that two species of gecko regularly visit and take nectar from *Metrosideros excelsa* in New Zealand, and carry pollen on a uniquely textured patch of skin on the throat area. Further study could elucidate the purpose of this skink behavior as well as the efficiency of pollen transfer by the lizards.

As predicted, *M. collina* flowers were visited by a bird, the White-eye, an introduced bird on Moorea. White-eye observation was difficult as the birds were frightened of my presence, but I observed white eyes opportunistically and recorded number of visits witnessed and qualitative impressions. White-eyes seem to visit a few times a day as part of a larger flock of birds. The flock passes through with each bird visiting several flowers, and then moves on. Though no birds were captured and examined for pollen, it is likely that the texture of feathers provides a ready attachment surface for pollen grains. Pollen was found on the throats of birds in a study of the pollination of Hawaiian *Metrosideros* (Carpenter 1976). The feeding behavior of the White-eyes on *M. collina* in Moorea certainly brings their throat area into contact with anthers and pollen.

Having many and variable visiting/pollinating species is advantageous to the spread and persistence of *M. collina* on islands. Johnson and Steiner (2000) found that plants in areas where the availability of pollinator species varied from year to year favored a generalist strategy. When first arriving on a new island, *M. collina* would attract visitation by almost any nectivorous animal. After establishing on an island, *M. collina* can persist even if its pollinator community goes extinct. On Moorea, *M. collina* is visited by primarily recent European-introduced animals. It is likely that the animals that pollinated *M. collina* before European presence on Moorea are now absent from the island or confined to high elevation sites. If *M. collina* had a tight relationship with a single pollinator species it would be doomed to dissapear along with its pollinator. Island ecosystems are inherently unstable, so even in the absence of human disturbance, it is valuable for plants to possess a generalist pollination strategy to maximize visitation regardless of the specific species available at that time. Though honeybees may be the most frequent visitors, *M. collina* likely benefits as a generalist because honeybees effect local pollination and other organisms effect long-distance pollination and outcrossing.

Seemingly any animal with a taste for nectar or pollen has the potential to be a *M. collina* visitor. However, pollen-carrying ability varies among species depending on morphology and behavior. Honeybees have special hairs and body structures that enable them to carry large quantities of pollen. Wasps have a smoother external surface and are not adapted to carry pollen, yet captured specimens did carry pollen, which could probably be transferred to the stigma of another flower. Some wasps approach the nectar cup from underneath and to the side of a flower, avoiding contact with anthers. The generalist strategy of *M. collina* allows the plant to take advantage of all available pollinators, both more and less efficient.

Future studies could investigate the extent to which flowers are self-compatible and could quantify and compare seed set and germination in crossed or selfed flowers. To further study pollination, flowers could be experimentally exposed to a pollinator of a given species and seed set and germination quantified. A useful measure of pollination may be flower abscission, as suggested by the stamen removal experiment. Many flower heads in the Q-tip treatment fell off, but the number of these events was not recorded. Percent of abscised fruits could be used as a measure of pollination efficiency. Pollinator community variation at different elevations should be considered due to the high incidence of native insects at high elevations on islands.

As a colonizer of new islands, *M. collina*'s generalist flower design encourages pollination by any nectivorous animals. In the absence of any pollinators, *M. collina* can likely reproduce via wind-facilitated self-fertilization. Once established, *M. collina* is adapted to withstand characteristically high species turnover found on islands. If *M. collina* as a species exists on an island longer than the species that pollinate it, it is advantageous to be a generalist Then *M. collina* does not depend on any one pollinator to continue its existence. In addition, *M. collina* can reproduce by self-fertilization, ensuring persistence even in the absence of pollinators, with pollinators enhancing the genetic diversity of the population when they are present.

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# Epiphyte community composition on three non-native and three indigenous tree hosts

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ABSTRACT. I examined the epiphyte community composition on the three indigenous trees *Metrosideros collina* (Myrtaceae), *Neonauclea forsteri* (Rubiaceae), and *Pandanus tectorius* (Pandanaceae) and the three non-native tree species *Paraserianthes falcatoria* (Fabaceae), *Psidium cattleianum* (Myrtaceae), and *Spathodea campanulata* (Bignoniaceae) on the island of Mo'orea, French Polynesia. Host characteristics hypothesized to relate to epiphyte abundance were also examined. *Paraserianthes* and *Psidium* were found to support less epiphyte cover overall. Epiphyte community composition was distinct between host species, and *Metrosideros* and *Neonauclea* support significantly more cover and richness of mosses and vascular species than the other trees. These two species also have higher percent cover of loose flaking bark and high bark water-absorption capacity, suggesting that bark stability and moisture content are important for epiphytic mosses and vascular plants. These results suggest that invasion by non-native trees has implications for epiphyte conservation, and consequences will likely be specific to the tree species.

KEYWORDS: epiphyte, invasive species, Mo'orea

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## Introduction

Epiphytes make up approximately 10% of the world's flora (Kress 1986) and constitute an important component of forests, where they play important roles in primary production (McCune 1993), hydrology (Brown & Bates 1990) and nutrient cycling (Coxson & Nadkarni 1995, Nadkarni & Matelson 1992, Hsu *et al.* 2002). Epiphytes also create important habitat for arthropods and nesting animals, and may moderate the harsh microclimates of tree canopies for other organisms (Stuntz *et al.* 2002). Epiphytic lichens are well known bio-indicators of air pollution (Geebelen & Hoffmann 2001), and Nadkarni & Rodrigo (2002) suggest that because of their response to manipulations in cloud water, epiphytes may also be good indicators of climate change in tropical forests. Considering these roles, their diversity and abundance, epiphytes are comparatively understudied. Because of the lack of information regarding their ecology, information about threats to their populations and their conservation is also lacking.

Factors threatening the health of epiphyte populations may include habitat loss, alteration of habitat through land use change and introduction of invasive species. Land use and habitat fragmentation affect population dynamics of epiphytic lichens (Dettki & Esseen 1998) and orchids (Williams-Linera *et al.* 1995). Native and an alien species of tree fern support different assemblages and abundances of vascular epiphytes in Hawaii (Medeiros *et al.* 1993), suggesting that invasion by non-native plant species may also have important consequences for epiphytes.

Island ecosystems are especially vulnerable to damage by the introduction of non-native organisms. This can be in the form of native species extinctions and altered ecological functioning (Vitousek *et al.* 1987, Michelle *et al.* 2001). Islands are thought to be more easily invaded because their paucity in species has resulted in a native biota that is not adapted to compete with the types of organisms historically absent from the island. Small population sizes and the lack of refuge space also likely contribute to the high rate of extinction on islands (Simberloff 1995). Hotspots for invasion and species extinctions, islands are thus ideal for focusing our efforts on understanding invasive species biology and its consequences for conservation.

Several host characteristics may determine epiphyte host preferences and help predict the consequences of non-native tree invasion for epiphyte populations. Tree age may be important in determining which epiphytes have had a chance to become established. Structural complexity and bark roughness may provide important sites for propagule adhesion.

Canopy effects on light, bark stability and bark moisture content have all been shown to correlate with epiphyte host preferences (Callaway *et al.* 2002). Likewise, epiphyte abundance may also be related to chemistry of water throughfall and host bark (Callaway *et al.* 2002, Frei and Dodson 1972).

This study was conducted in the tropical forest of the island of Mo'orea, French Polynesia, in the South Pacific Ocean (17° S, 149° W), and sought to address two broad questions there:

1. How do non-native and indigenous tree species differ in the epiphyte communities they support?

2. What host tree characteristics are important in structuring epiphyte communities?

In order to address these questions, I compared the epiphyte communities on three alien and three indigenous tree species. I also compared various tree characteristics in an attempt to understand which host qualities are important in structuring epiphyte abundance and diversity.

## Methods

### *Study species and location*

Origin, date of introduction and growth habit of the six tree species studied are listed in Table 1. *Paraserianthes falcatoria* (L.) I. C. Nielsen (syn. *Albizia falcatoria*, Fabaceae), Strawberry guava (*Psidium cattleianum* Sabine, Myrtaceae), and the African tulip tree (*Spathodea campanulata* P. Beauv., Bignoniaceae) are all considered invasive in French Polynesia and have become naturalized on the islands of Tahiti and Mo'orea (J. -Y. Meyer, pers. comm. 2002). I compared their quality as epiphyte hosts to the indigenous trees *Metrosideros collina* (J.R. & G. Forst.) A. Gray (Myrtaceae), *Neonauclea forsteri* (Seem.) J. Merr. (Rubiaceae), and *Pandanus tectorius* Parkinson ex J.P. du Roi (Pandanaceae). These three species are considered indigenous to the Society Islands, although *Pandanus* is a useful plant thought to have been carried by Polynesians to other Pacific islands (Cox and Bananack 1991). Voucher specimens of these six trees are housed at the Jepson Herbarium, University of California, Berkeley. Nomenclature follows Stanley 1998. I will hereafter refer to them by genus name.

My six host trees occur together in much of the mid-elevation tropical forest on Mo'orea. All sampling for this study was conducted at one study site (the Belvedere); UTM coordinates S 17°32.434' W 149° 49.602'. All data were collected from a stratified random sample of ten of each host tree species. Trees were selected in an area defined by the range of the least common species (*Paraserianthes*) in order to control for effects of location. In an attempt to encompass any variation between hosts due to age, trees were chosen to include at least 2 individuals in each of three approximate size classes based on tree height and diameter at breast height (DBH = 1.37 m). The size classes represent the range of sizes observed at the study site and are specific to each species. In the case of *Pandanus*, a range of sizes was not observed in the field, and all sample trees have a similar DBH.

### *Epiphyte community*

The epiphyte community on each tree was sampled from 0.5 to 1.5 m above the ground on the main trunk. Three 1 m vertical transects were placed at compass bearings of 0, 120 and 240° and length of each epiphyte along the transect was recorded. Epiphytes were identified by morphotype to get an estimate of

**Table 1.** Origin, date of introduction and growth habit of the six study trees. Nomenclature follows Stanley 1998. Origin and introduction dates are from J. -Y. Meyer, pers. comm. 2002.

| Study tree | Origin | Approx. date of introduction to Society Islands | Growth habit |
|---|---|---|---|
| *Metrosideros collina* | indigenous | - | Shrub or tree |
| *Neonauclea forsteri* | indigenous | - | Canopy tree |
| *Pandanus tectorius* | indigenous | - | Small tree |
| *Paraserianthes falcatoria* | Asia | 1936 | Canopy tree |
| *Psidium cattleianum* | Tropical America | 1854 | Shrub or tree |
| *Spathodea campanulata* | West Africa | 1932 | Canopy tree |

species richness. Because it was not feasible to conduct sampling under a microscope and some taxa are more easily distinguished in the field, higher plants may have more taxonomic resolution than lichens and liverworts. The percent cover of each epiphyte was calculated as its length along the transects divided by 300 cm (the cumulative length of all three transects). Because several layers of epiphytes occurred along some transects, the total percent cover may exceed 100%. The Shannon-Wiener index was used to quantify the diversity of epiphytes on each tree. This measure of diversity weights species richness with evenness and is calculated as follows:

$$H = \sum_{i=1}^{s} [(p_i)(\log_2 p_i)]$$

where $s$ = number of species
$p_i$ = proportion of total cover belonging to $i$th species

This measure was chosen because my species richness sampling procedure was not exhaustive, and the Shannon-Wiener index gives more emphasis to rare species than, for instance, Simpson's index (Krebs 1989).

The number of epiphytic vascular plant species on the entire tree was also surveyed visually from the ground. I observed that many vascular species occurred higher up on the trunks, and the visual survey was done in an attempt to catalog the diversity of vascular plants more comprehensively for the entire tree.

### *Host characteristics*

DBH was measured for each tree host and used as a proxy for tree age. Branches were counted in the lower 2 m of each trunk in order to examine local structural complexity. Only branches larger than 2 cm diameter at the base were counted. Canopy cover was measured using a 1.75 in. diameter densiometer (gridded convex mirror). This method encompasses both the nature of each host tree's canopy, and that of the surrounding microenvironment in which it grows. In an attempt to document the stability of bark as a substrate for epiphyte establishment and growth, the length of loose flaking bark was measured along the same transects used to sample epiphyte cover.

Two samples of 2.5 x 2.5 cm square segments of bark were cut from sample trees at eye and knee level in order to measure bark water-holding capacity. Segments were haphazardly chosen and combined into one sample per tree. Only bare sections of bark were chosen (sometimes this required sampling from heights other than eye and knee level). The bark was then examined under 10X magnification and any epiphyte tissue or wood was removed. Samples were dried at 60°C for 48 hrs. and weighed to obtain dry weight. After soaking overnight in water, bark samples were weighed wet, hung to dry in envelopes for 24 hrs., and then weighed again. Absorption capacity was calculated as the difference between wet and dry weight. Water retention capacity was calculated as the difference between 24 hr. and 0 hr. wet weights. Bark pH was also measured with litmus paper dipped into the water in which bark samples were left to soak.

### *Analysis*

Data on epiphyte cover were transformed using an arcsin root transformation for proportional data (Sokal and Rohlf 1981). ANOVA and post-hoc Bonferroni pair-wise comparisons were used to compare diversity (H), species richness, entire-tree vascular species richness, and the percent cover of lichens, liverworts, mossees, all bryophytes, vascular plants, and all epiphytes among hosts. Regressions of total epiphyte cover against DBH were conducted to look for effects of host age within each tree species. Host characters were compared using ANOVA and Bonferroni tests (proportional data were again transformed). Because bark water-holding and retention capacity data were non-normal, they were analyzed using the Kruskal-Wallis non-parametric test and Wilcoxon rank sign test for pair wise comparisons. All statistical analysis was performed using SYSTAT 8.0 (SSPS, Inc. 1998).

## Results

### *Epiphyte community*

Figure 1 shows the epiphyte community composition of the six host trees. The total percent cover of epiphytes was significantly higher on the natives *Metrosideros* and *Neonauclea* than on the non-native *Paraserianthes* and significantly lower on the non-native *Psidium* than on all other trees ($\alpha$=0.05). The cover of specific taxonomic groups is also differed significantly between trees (Figure 2a), with *Metrosideros* and *Neonauclea* supporting greater vascular epiphyte cover than the other hosts. *Metrosideros* and *Neonauclea* also support greater moss cover than

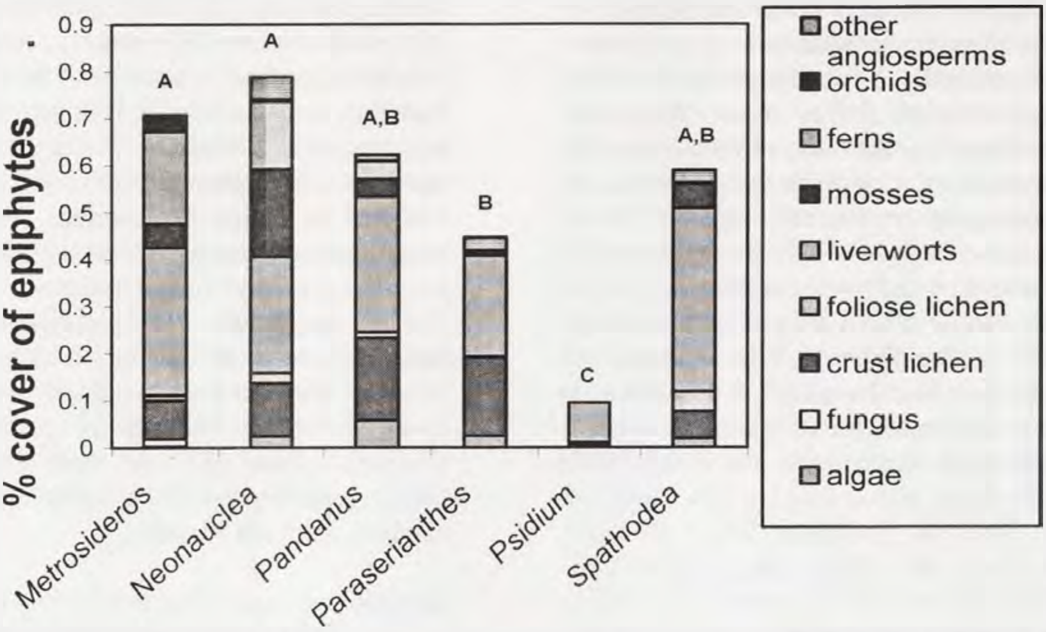


**Figure 1.** Epiphyte community composition on the lower trunk of six tree species in Mo'orea, French Polynesia. The three left columns are indigenous host trees and the three right are non-native human introductions. Bars represent the mean for each host (n=10). Columns sharing the same letter are not significantly different from each other in total percent cover of epiphytes ($\alpha=0.05$).

all other trees (data not shown, $p \leq 0.013$) while *Spathodea* has greater liverwort cover than *Metrosideros*, *Paraserianthes* and *Psidium* ($p \leq 0.045$). Species richness measures are compared in Figure 2b: Total epiphyte richness and diversity (H) on the lower trunk are significantly lower on *Paraserianthes* and *Psidium* than on all other hosts, and *Neonauclea* and *Metrosideros* support greater richness of vascular species overall than all other hosts.

*Host characteristics*

Simple linear regression indicates that DBH does not predict total epiphyte cover well across host species. Within species, only *Metrosideros* and *Paraserianthes* show a strong trend towards higher epiphyte cover with increasing DBH ($r^2=0.83$, $p<0.001$ for *Metrosideros* and $r^2=0.49$, $p=0.02$ for *Paraserianthes*; see Figure 3). *Metrosideros* had a significantly higher number of branches in the sampled lower 2 m, but this measure does not correlate well with cover or diversity of epiphytes across hosts. Percent cover of loose bark was significantly higher for *Metrosideros* and *Neaonauclea* ($\alpha=0.05$: Figure 4a), the two hosts with the greatest cover and richness of epiphytic mosses and vascular plants.

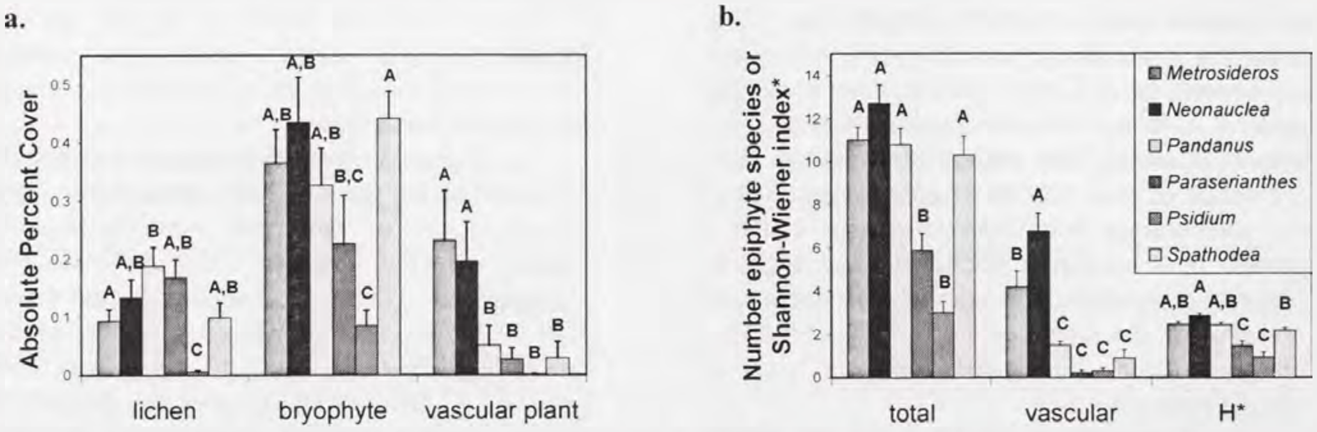


**Figure 2a-b.** Mean absolute percent cover (**a**) and diversity (**b**) of epiphytes on the lower trunk of six host trees (n=10). Solid columns are trees indigenous to Mo'orea, French Polynesia, and striped columns are non-native human introductions. Error bars are +1 standard error. Columns sharing the same letter are not significantly different at $\alpha=0.05$.

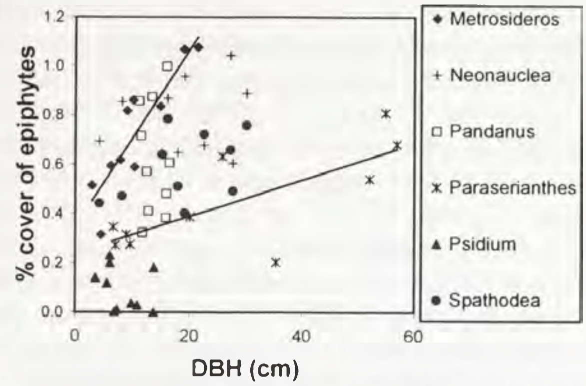
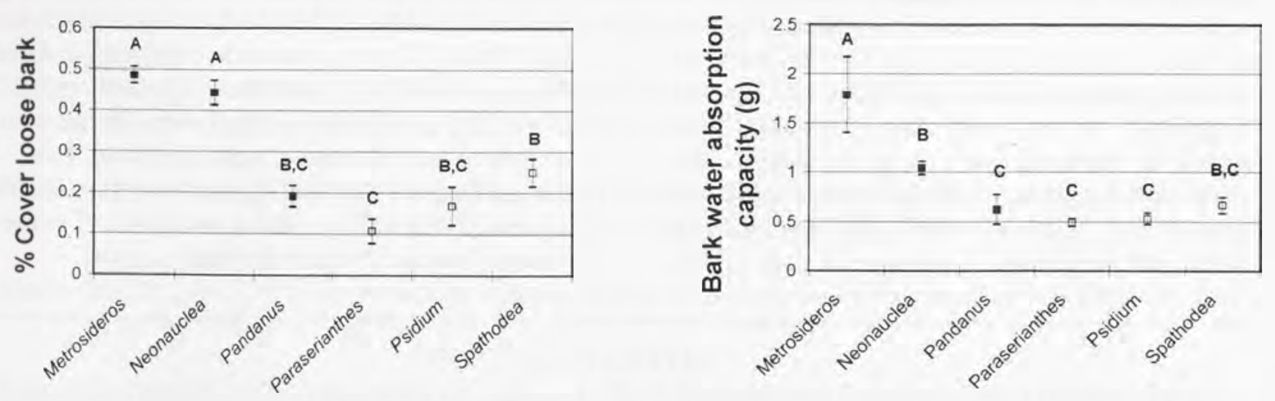


**Figure 3.** Total percent cover of epiphytes with increasing diameter at breast height for six host trees. The two simple linear regression lines shown are for *Metrosideros* ($r^2$=0.83, $p<0.001$) and *Paraserianthes* ($r^2$=0.49, p= 0.02).

*Metrosideros* had higher bark water-absorption capacity than all other trees; *Neonauclea* bark water-absorption capacity was significantly higher than *Pandanus*, *Paraserianthes* and *Psidium* (Figure 4b). Canopy cover, bark water-retention capacity and bark pH were not significantly different between hosts.

## Discussion

In this study, host tree species was the single most predictive factor examined for total percent cover of epiphytes. Epiphyte community composition is quite distinct between hosts (see Figures 1 and 2a). The alien *Paraserianthes* and *Psidium* are especially poor hosts for epiphytes, and support very little vascular epiphyte cover. Non-native *Spathodea* and the native *Pandanus* are intermediate in terms of total cover and diversity, with *Spathodea* supporting an abundance of liverworts while *Pandanus* supports an abundance of lichens. The native *Metrosideros* and *Neonauclea* support substantially greater cover and diversity of mosses and vascular epiphytes. These distinct epiphyte assemblages could be the result of several interacting factors including host specificity, competition or facilitation between epiphytes, and dispersal. Others have documented strong species-specific host preferences by epiphytes (Callaway *et al.* 2002) and I observed strong host specificity with some of my epiphytes: the two orchid species encountered only occurred on *Metrosideros* and *Neonauclea*, and filmy ferns (Hymenophyllaceae) where only observed on *Neonauclea*, which supported three species. Both positive and negative interactions have been observed between epiphytes (Callaway *et al.* 2001), illustrating that epiphyte communities may be structured by biotic interactions beyond host preference. This could include interactions with mycorrhiza, a possible mechanism for the orchid host-specificity I observed that warrants further investigation. In addition, dispersal may be an important factor determining the distribution of some epiphytes, as the spatial aggregation of epiphytic bromeliads observed by Vandunné (2002) suggests. Transplant experiments were also conducted as part of this study, but not included in the analysis because negligible differences in growth or survival rates were observed between tree species in the five weeks available for observation. That the transplanted moss (*Ectropothecium* sp.) and fern (*Pachypleuria* sp.) survived on hosts on which they did not occur naturally suggests that barriers to dispersal and recruitment may be important in


a.

b.

**Figure 4a-b.** Mean percent cover of loose, flaking bark (**a**) and bark water-absorption capacity (**b**) of the lower trunks of six trees. Means indicated with a hollow box are non-native tree species, while solid boxes represent tree species indigenous to Mo'orea, French Polynesia. Error bars are ±1 standard error. Points sharing a letter are not significantly different from each other at $\alpha$=0.05.

explaining the distribution of these epiphytic species.

Surprisingly, a strong relationship between DBH and total percent cover of epiphytes was not observed (Figure 3). This could be because DBH does not correlate well with tree age for these species. Although I included the entire range of sizes observed in the field, age may not have been comparable between tree species. It is likely that the native individuals are older than the introduced species, and age may be a more important factor in predicting epiphyte cover and community composition than I could observe using DBH. If mosses and vascular epiphytes are late successional species, their preference for *Metrosideros* and *Neonauclea* may be better explained by age than host specificity. Other factors may also interact to mediate trends in epiphyte cover with age; for instance, *Psidium* trees have less peeling bark with increasing DBH. *Metrosideros* and *Neonauclea*, the two hosts with the greatest cover and diversity of mosses and vascular epiphytes, have greater percent cover of loose bark (Figure 4a). The peeling bark may provide surface heterogeneity and sites for epiphyte establishment. Along with surface heterogeneity, more loose bark also implies quicker bark turnover and the opening up of free substrate space. Both of these factors may be important in maintaining diversity.

The fact that *Metrosideros* and *Neonauclea* also have a high bark water-absorption capacity (Figure 4b) implies that epiphytic mosses and vascular plants have a higher moisture requirement than liverworts or lichens. However, *Spathodea* bark was not significantly different in water-absorption potential from *Neonauclea*, so other factors must control why few mosses and vascular epiphytes were observed on *Spathodea*. Although I was not able to observe differences in pH between host bark leachates, the specific chemistry and "leakiness" of bark and canopy which I was unable to measure may help to explain the observed epiphyte distribution patterns. Specifically, *Spathodea* bark has well known medicinal properties (Amusan *et al.* 1995, Makinde *et al.* 1998) and its unusual bark chemistry may affect which epiphytes grow on it. *Psidium* is thought to have allelopathic properties (Wagner *et al.* 1990), and throughfall chemistry may be an important mechanism behind its low epiphyte cover. *Paraserianthes* was planted in part to amend soil with its nitrogen-rich leaf litter (J. -Y. Meyer, pers. comm. 2002), and thus may also be expected to have different throughfall chemistry than the other trees as well.

Because the host characteristics I measured did not correlate well with lichen or liverwort cover, the mechanisms behind their differential host preferences remain unclear, and warrant more detailed study. Experimental research would be useful in determining more rigorously which host characters are important for specific epiphytes.

## Conclusions

This study indicates that invasion of native forests on Mo'orea by introduced tree species will indeed be a concern for epiphytes, especially mosses and vascular plants. While overall the non-native trees supported less epiphyte cover and diversity, the differences between all tree species implies that the ability of epiphytes to grow on alien trees will be species-specific. Bark stability and water-absorption capacity may be useful in predicting the suitability of trees as hosts for mosses and vascular epiphytes. However the complexity of interacting host characteristics will likely make it difficult to accurately predict the consequences of non-native tree invasion for epiphytes.

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# The Bioactivity and Ethnobotany of Lichens, Mo'orea, French Polynesia

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ABSTRACT. Based on previous findings of bioactivity in lichens and documented ethnomedical use in other parts of the world, information on the ethnobotany and bioactivity of the lichens of the Society Islands was sought on the islands of Mo'orea and Tahiti. Approximately 25 species of lichens were collected. Twelve of these species were presented in interviews with indigenous people to gain information on ethnotaxonomy and traditional medicinal uses. Six of the lichens were tested for antimicrobial activity using *Streptococcus thermophilus* and *Lactobacillis bulgaricus* and for cytotoxicity using *Artemia salina*. One to two general Ma'ohi (Tahitian language) names are used for the lichens, based on gross morphology, but two specialized names are used for the species *?Parmeliella brisbanensis* depending on the age of the specimen. Two species of *Ramalina* and two species of *Usnea* are used to treat internal tumors and all four of these species tested significantly cytotoxic. *?Parmeliella brisbanensis, ?Siphula sp*., and *Cladonia subsquamosa* are used to treat infected wounds and ulcers, however these lichens were not included in the antimicrobial tests because the ethnobotanical information was late in coming. No antimicrobial activity was shown for those lichens tested. Most of the ethnotaxonomic data, and all of the ethnomedical data came from the islands of Huahine, Taha'a, and the Australs. The ethnobotanical knowledge of the lichens on Mo'orea and Tahiti is fragmentary, which may be the result of regional differences, higher levels of occidental cultural influence on Mo'orea and Tahiti, and/or stricter application of colonial laws forbidding Ma'ohi language and medical practice in the islands.

KEYWORDS: lichens, tropical, Polynesia, ethnobotany, Tahiti

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## Introduction

Natural products have been the source of many commonly used pharmaceuticals. In the United States, between 1959 and 1980, twenty five percent of medical prescriptions written were for drugs derived from natural sources (Farnsworth *et al.* 1985). Sixty two percent of anticancer drugs developed through 1994 came from natural sources (Cragg *et al.* 1997).

In searching for natural products that show pharmaceutical activity, ethnobotanically guided selection has been shown to be more effective than random collection. In a study by Balick (1994), 15% of plants used by a Belize Mayan healer showed pharmaceutical bioactivity as compared to only 1.6% of plants that were collected randomly.

In one famous example, *Catharanthus roseus*, a known cytotoxic plant, was being used by indigenous healers in Madagascar to treat what western doctors recognized as Hodgkin's Disease. The active compounds vincristine and vinblastine were isolated from *Catharanthus roseus* and found to have the ability to bind to the protein tubulin thereby inhibiting microtubule formation, preventing mitotic spindle formation, and effectively stopping cell division at metaphase. These compounds are now successfully used to treat a variety of cancers. The most dramatic example has been the treatment of acute childhood leukemia. Since the introduction of these plant derived drugs, the five year survival rate has jumped from 15% to 85% (Carlson per.com. 2001).

Among natural products, lichens appear to be a promising source of bioactive chemicals. Recent studies have shown lichens to have higher levels of cytotoxicity and antimicrobial activity than bryophytes or vascular plants (Lorimer *et al.* 1996). *Usnea capillacea* and *Usnea ciliifera* have been found to be specifically cytotoxic to leukemia cells (Perry et al. 1999). In bioassays run on a collection of plants from Mo'orea, another University of California undergraduate found two genera of lichen, *Ramalina sp.* and *Usnea sp.*, to be more cytotoxic than *Catharanthus roseus* indicating potential pharmaceutical value (Achrekar 1995).

In addition to scientific findings, ethnobotanical data from several areas of the world indicate potential pharmaceutical value of the lichens. In Europe *Cladonia* species have been used as a source of usnic acid to produce topical antibiotics, and *Cetraria islandica* is still used as an expectorant (Hale 1979). Other lichens used ethnomedically include *Lobaria* *pulmonaria* for respiratory illness, *Usnea plicata* for epilepsy, *Parmelia sulcata* for headache, *Peltigera canini* for rabies, and *Pertusaria albescens* or *Pertusaria amara* as antipyretics (Van Haluwyn & Lerond 1993). In Polynesian culture, the Maori of New Zealand have long used *Usnea* species for feminine hygiene (Galloway 1985, Riley 1994). Grepin (1984) made the only reference found for medicinal use of a lichen, *Usnea barbata*, in the Society Islands, but no information was given on preparation or medical application. The most complete published work on Tahitian traditional medicine known today, by Paul Petard (1986), does not include information on medicinal use of lichens.

Lichens are divided into paraphyletic groupings based on general growth forms. Crustose lichens grow very close to the substrate in a crust-like form, foliose lichens are named after their leaf-like form that clings to the substrate, and fruticose lichens grow from a single attachment in long filamentous hair-like tufts. (See the index for photos.)

The goals of this study were to assemble a lichen collection for the island of Mo'orea, to record any current or historic medicinal uses for lichens, and to run biological tests to determine if some of the lichens show cytotoxic, antibacterial, or antifungal activity.

## Materials and Methods

### *Collection*

Lichen samples were collected on Mo'orea, French Polynesia (17.30' S, 149.50'W). Specimens were collected from the coastal road around the island, the Opunohu and Vaiare Valleys, Vaiare Pass and Mount Rotui. Collecting was primarily limited to fruticose and foliose lichens because most lichens used medicinally are of this form. Additionally, foliose and fruticose lichens are more identifiable and pose fewer difficulties in removing from their substrate than crustose lichens. To free the thallus, its rhizines were cut away with a knife. During dry weather, a spray bottle was used to wet the lichens making them easier to remove without damage to the thalli. A large number of lichens were available by climbing lower tree branches and using a ladder when available. Recently fallen wood was also sought in order to access specimens from higher branches because I was unable to climb into the canopy. At the end of the study, some additional collecting was done on Tahiti-iti at the *Plateau de Taravau*, and on Tahiti-nui on the south shore of the island, however, these lichens were not entered into the ethnobotanical or the bioassay portions of the study.

### *Curation*

Specimens were soaked in water to make them pliable, cleaned of foreign material under a dissecting microscope, pressed, then dried for four days. Tentative identifications were made to genus level, and each specimen was split in two. One voucher collection containing half of each lichen specimen was sent to the Jepson Herbarium at the University of California, Berkeley for species identification by Isabelle Tavares. The second half of the voucher collection was mounted on cards for requesting ethnobotanical information by interview, and for use as a reference collection in order to gather sufficient mass for laboratory bioassays. A voucher collection remains available in the Jepson Herbarium at the University of California, Berkeley.

### *Ethnobotanical Information*

A mounted collection of 25 specimens, representing 12 species of lichens plus *Tillandsia usneoides*, was laid out in front of the interview respondents in random order. For some species, multiple specimens were included to see if the respondents would make subdistinctions within the same species. The specimen cards were marked only with randomly assigned numbers written discretely in pencil. The informants were asked if they could offer the *Mao'hi* names for any of the specimens and/or any medicinal uses, historic or current. They were then asked if they would group the specimens in any particular order, based on any criteria they chose. Seven *Tahu'a* (traditional Tahitian healers), three elders, and four knowledgeable lay persons were interviewed in Mo'orea and Tahiti between October 12th and November 27th, 2002.

### *Plant Extracts*

Six lichen species and *Tillandsia usneoides*, an angiosperm, were selected for bioassays based on suspected potential for bioactivity, availability of sufficient mass, and an interest in including species that I was not aware of having been tested before. *Catharanthus roseus* was added as a positive control for the cytotoxicity tests. Individual lichens used to make extracts were randomly selected from bags organized by species. Because identities were tentative, half of each lichen individual used for extract was packaged and carried back to Berkeley for later verification of species identification. The plants' dry masses were taken. To soften the tissues, they were soaked for a half hour in individual covered beakers in the quantity of Vaimato spring water that would be used to make the extract in a ratio of 0.1g/mL. The plants were each ground by hand in a mortar and pestle with 2g. of course grinding sand. The ground material was allowed to mascerate for three hours. The contents were centrifuged at high speed for 5 min. and the supernatants were removed to glass vials. A number was assigned to each extract and they were stored in a freezer while not in use.

*Cytotoxicity Tests*

*Artemia salina* (brine shrimp) were hatched in a 500mL beaker of sea water using a bubbler. Using a 1 mL. dropper, fifty shrimp were counted into each 10mL. test vial previously labeled with numbers 1-8. Using a syringe, equal amounts of the corresponding extracts were placed into the test vials and the vials were filled to 10mL. with sea water. *Catharanthus roseus*, a known cytotoxic plant, was used as a positive control and Vaimato spring water was used as a negative control. The number of shrimp dead after 19 hours was recorded for each of six trials. Six replications were made and an ANOVA test was run in Excel to see if any of the extracts showed significant cytotoxicity compared to the negative control.

*Incubator Construction*

A hole was cut into a 24x26x28 cm. cardboard box such that the globe of a 60 Watt desk lamp fit just outside the opening. A thermometer was placed in five different locations within the box to test the consistency of temperature. Additional holes were cut into the box at time intervals until the interior of the box reached a stable temperature of 37°C.

*Antimicrobial Tests*

Sixty X 15mm Petri dishes were filled with 30 mL. of agar. For the antibacterial tests, the Petri dishes were swabbed with a combination of *Streptococcus thermophilus* and *Lactobacillis bulgaricus* culture dissolved in UHT (*Ultra-Haute Temperature*) sterilized whole milk that was boiled then cooled to 37°C. For the antifungal test, the Petri dishes were swabbed with commercial Baker's Yeast dissolved in sugar water at 37°C. One drop of each extract was placed directly onto the plates. Antibacterial ointment was used as a positive control for the antibacterial tests, a gel containing 1% Econazole nitrate was used as a positive control for the antifungal tests, and Vaimato spring water was used as a negative control for all tests. The plates were incubated for 48 hours at 37°C. Qualitative observations were made under a light microscope to compare relative levels of activity. In Table 2, the activity is reported as: 0 = no lysed cells; + = 1%-34% lysed cells; ++ = 35%-69% lysed cells; +++ = 70%-100% lysed cells.

**Results**

The lichens found on Mo'orea as of October 4, 2002 and identified to species level are listed in Table 1. Additional lichens are waiting to be identified.

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**Table 1. Lichens Collected and their Traditional Therapeutic Uses.** Species with an asterisk were presented in interviews.

| Species | Traditional Use |
|---|---|
| **Cladonia subsquamosa* | Antibiotic |
| *Coccocarpia palmicola* | |
| *Erioderma sp* | |
| **Heterodermia japonica* | None found |
| *Leptogium austroamericanum* | |
| *Leptogium ballatulum* | |
| *Leptogium propaguliferum* | |
| **Parmeliella brisbanensis* | Antibiotic |
| **Parmotrema cristiferum* | None found |
| *Pertusaria thiospoda* | |
| *Physcia fragilescens* | |
| *Physma chilense* | |
| **Pseudocyphellaria argyracea* | None found |
| **Pseudocyphellaria crocata* | None found |
| **Ramalina leiodea* | None found |
| **Ramalina nervulosa* | Internal tumors |
| **Ramalina peruviana* | Internal tumors |
| Rimelia reticulata | |
| *Siphula sp. | Antibiotic |
| *Usnea baileyi | Internal tumors |
| *Usnea societasis | Internal tumors |

Ten of 14 respondents gave only one name, *Remu ha'ari* (= Coconut algae), for all lichens (Table 2). Four of the respondents made additional nomenclature distinctions by separating all fruticose lichens into a category they called *Huru huru ta'a* (= Beard) while two of the respondents made the same distinction for the group of fruticose lichens but could not recall the Ma'ohi name. (These two respondents were categorized as not giving more than one Ma'ohi name for the lichens.)

**Table 2. Variation in Traditional Knowledge.** Interview data is organized by island: Tahiti (Ta), Mo'orea (Mo), Huahine (Hu), Taha'a (Ta), Australs (Au)

| **Island** | Ta | Mo | Hu | Ta | Au |
|---|---|---|---|---|---|
| **# of respondants** | 4 | 6 | 2 | 1 | 1 |
| **# of healers** | 2 | 2 | 2 | 0 | 1 |
| **# giving >1 name** | 1 | 2 | 0 | 1 | 0 |
| **# of med. uses** | 0 | 1 | 2 | 0 | 2 |

The four respondents who gave the name *Huru huru ta'a* for the fruticose lichens, also made distinctions among the foliose lichens, with the addition of *?Siphula* and *Cladonia*, into the following subcategories:

1) *?Siphula sp. and Cladonia subsquamosa*
2) *?Parmeliella brisbanensis*
3) *Heterodermia japonica*
4) *Pseudocyphellaria crocata*
5) *Parmotrema cristiferum & Pseudocyphellaria argyracea.*

No names were given for subgroups 1, 3, 4, or five, however subgroup 2, consisting of *?Parmeliella brisbanensis* alone, was further subdivided into the two following groups according to age: A) young *?Parmeliella brisbanensis* called *Tari'a iore* (= mouse ears) and B) old *?Parmeliella brisbanensis* called *Pa'a ha'ari* (= coconut crust).

No medical uses for lichens were given by any of the four respondents who showed fine taxonomic distinctions even though one of them is a healer in her fifties who grew up in Pape'ete.

One final distinction in the ethnotaxonomy is for *Tillandsia usneoides*, which is an introduced angiosperm resembling fruticose lichens in its gross form and habit. It is a popular plant cultivated in many gardens and is called *Hina hina* (= white or grey hair) by locals (*Académie* 1999).

Three healers from the "less developed" islands of Huahine and the Australs were interviewed on Mo'orea. Two young healers from Huahine referred to all the lichens as *Remu ha'ari* yet told of uses for the fruticose lichens in the treatment of cancer and as a treatment for a wide range of illnesses upon which they did not elaborate. They recounted the treatment by a senior healer of a family member on Huahine for colon cancer. The young healers were given a list of plants to collect that included fruticose lichens from coconut trees.

The use of fruticose lichens for cancer treatment was reiterated by the healer from the Austral island chain. She separated out the fruticose lichens during the interview, but only gave the general name *Remu ha'ari* for all of the lichens. She uses the fruticose lichens *Ramalina peruviana, Ramalina nervulosa, Usnea societasis*, *Usnea baileyi* and possibly *Ramalina leiodea* for *Ra'au Pu'a Roto*: *Pu'a* = fibroma (a tumor of connective tissue); *roto* = inside or interior (*Académie* 1999). This treatment is used for five different cancers of the internal organs.

Also from the specimen display, the healer from the Australs selected *Cladonia subsquamosa, ?Siphula sp.*, and *?Parmeliella brisbanensis*. These three species are used for *Ra'au Pe A'ai*: *ra'au* = medicine; *Pe* = rotten; *A'ai* = 'eating' (*Académie* 1999). The healer explained that *Ra'au Pe A'ai* is typified by symptoms that may include pus, swelling, pustules, foul odor, blackening, or other sign of infection. She included a young specimen of *?Parmeliella* in the group used medicinally, but not an old specimen even when specifically asked if the old specimen could be used.

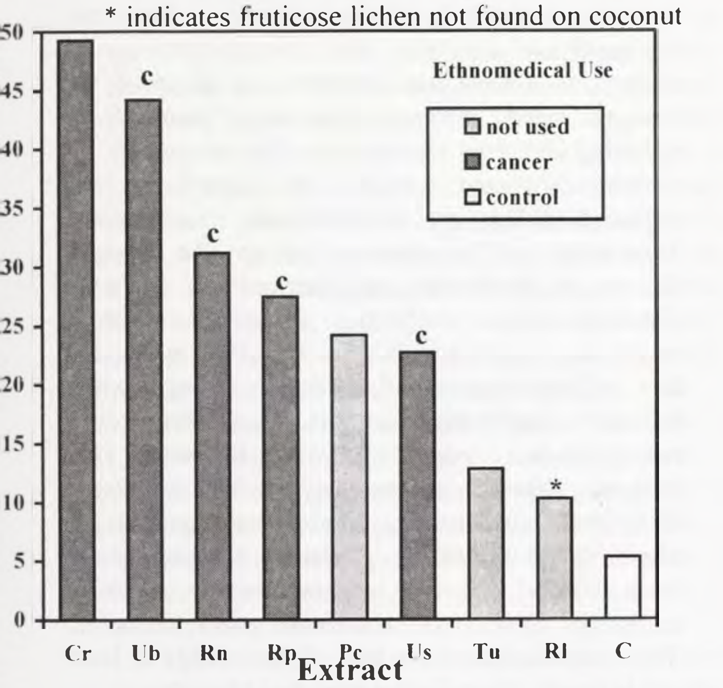
Additional information to come from the interviews included almost universal accounts of laws forbidding traditional medical practice and any use of Ma'ohi language, as well as cultural expressions including some dance forms that the colonists found contrary to the maintenance of colonial public order.

The cytotoxicity results (Figure 1) show that the most cytotoxic extract was the positive control, *Catharanthus roseus* ($p=0$), with an Average Death Rate (ADR) of 49.3 out of 50 *Artemia* killed.

In Table 1, fruticose lichens that were found on coconut trees are marked with the letter C, and the one fruticose lichen that was not found on coconut (*Ramalina leiodea*) is marked with an asterisk. *Usnea baileyi* ($p=0$, ADR=44.16), *Ramalina nervulosa* ($p=0$, ADR=31.16), *Ramalina peruviana* ($p=0$, ADR=27.5), and *Usnea societasis* ($p=0$, ADR=22.67) are all significantly more cytotoxic than the negative control. *Ramalina leiodea* ($p=0.051$, ADR=10.33), which was not found on coconut and is possibly not used medicinally, was not found to be significantly more cytotoxic than the negative control.

**Figure 1. Cytotoxicity.**
The extracts for *Catharanthus roseus* (Cr), *Usnea baileyi* (Ub), *Ramalina nervulosa* (Rn), *Ramalina peruviana* (Rp), *Parmotrema cristiferum* (Pc), *Usnea societasis* (Us), *Tillandsia usneoides* (Tu), and *Ramalina leiodea* (Rl) are on the x-axis.

C indicates fruticose lichen found on coconut
* indicates fruticose lichen not found on coconut



The foliose lichen that was tested, *Parmotrema cristiferum* (p=0, ADR=24.16), was significantly cytotoxic as was the angiosperm *Tillandsia usneoides* (p=0.0127, ADR=12.83).

The antimicrobial results are presented in Table 3. None of the extracts showed any antibacterial activity (AB) as compared to the positive control which showed 100% lysed cells. In the antifungal tests, only Parmotrema cristiferum and Usnea baileyi showed slight activity with about 2% cell lysing for each extract.

**Discussion**

Twenty-one species of lichen have been identified from this study and potentially five more species may be identified. In contrast, 217 species of lichen have been catalogued for the Society Islands (Cramer 1998). Collection for this study was done on only one of the Society Islands and excluded crustose lichens with few exceptions. Even with these considerations, my collection only represents a very small portion of the lichens and are probably biased toward those that are more easily collectible. However this bias may not be a significant problem in the current study since lichens that are used in medicine must be fairly abundant and attainable by the local people. It would be interesting to make more thorough lichen collections from a larger number of the islands and to obtain the corresponding ethnobotanical information from a wider geographical base.

**Table 3. Antimicrobial Results**
Antibacterial results (AB) and antifungal results (AF) for eight plants tested. Positive controls were commercial antibacterial and antifungal ointments . Symbols: 0=no activity, +=slight activity, ++=moderate activity, +++=strong activity.

| Species | AB | AF |
|---|---|---|
| *Catharanthus roseus* | 0 | 0 |
| *Parmotrema cristiferum* | 0 | + |
| *Ramalina leiodea* | 0 | 0 |
| *Ramalina nervulosa* | 0 | 0 |
| *Ramalina peruviana* | 0 | 0 |
| *Tillandsia usneoides* | 0 | 0 |
| *Usnea baileyi* | 0 | + |
| *Usnea societasis* | 0 | 0 |
| *Antibacterial ointment* | +++ | N/A |
| *Antifungal ointment* | N/A | +++ |

Although not statistically testable because of small sample sizes, there appears to be a trend of greater knowledge of the lichens coming from islands that are increasingly isolated from Tahiti, a center of colonization in Polynesia (Table 2). Stricter application of colonial law on Tahiti, and possibly to a similar degree on nearby Mo'orea, may account for reduced levels of ethnobotanical knowledge as compared to information from the Australs far to the South-East or from Huahine and Taha'a to the North. A similar trend was found by

Dr. Vincent Lebot (1991) who did a study of the distribution of *Kava kava* (*Piper methysticum*) over 49 Polynesian islands for periods before and after European colonization. Before colonization, *Kava* was very widely cultivated, but after a campaign by Europeans to eliminate its use, it disappeared from overt cultivation in the Society Islands, the Marquesas, and the Cook Islands. A superficial search uncovered no written laws specifically forbidding the practice of traditional medicine, but nearly every interview respondent reported that *Ra'au Tahiti* (Traditional Tahitian medicine) was effectively illegal during most of their lives and that, in the past, many practitioners spent time in prison for practicing. Even today, healers are cautious about what they say because they can still be subject to legal difficulties in some cases. For example they may use the word "*thérapie*" (= therapy), but they cannot use the word "*médecine*" (= medicine) according to a healer in Pape'ete.

In addition to forbidding medical practice, the Ma'ohi language was forbidden until as late as 1979 according to nearly every respondent interviewed. Since cultural knowledge is based in the language that is adapted to the conditions of a specific environment, and since botanical knowledge is a culturally and environmentally specific tradition, this knowledge is likely to have suffered losses when the use of Ma'ohi language was impeded from being used for the transmission of traditional knowledge. Interview respondents over the age of 30 consistently reported corporal and psychological punishments used against them, beginning at a very young age, for speaking their native language. I have not located any written laws specifically forbidding the use of Ma'ohi language, however it was not until 1981 that Tahiti was added to the *Loi Deixonne* of 1951 that allows up to one hour per week of native language instruction to be given by French State approved instructors at the primary levels. The French Government's explicitly stated goal for this plan is to improve the success of French language instruction (Reux 2000).

The variations in ethnobotanical knowledge might also be explained by regional differences that have existed in the islands since pre-colonial times, however some of the data would not support this hypothesis. One of the healers, born in Pape'ete and living in Mo'orea, made distinctions among the lichens that parallel groupings for medicinal uses made by the healer from the Australs. This could be coincidence in the case of *?Siphula* and *Cladonia* because they have a distinct and similar gross morphology (see index), however the distinctions made between young and old *?Parmeliella brisbanensis* are not likely due to chance. In independent interviews, *?Parmeliella brisbanensis* was separated into two classifications either based on names only, with no medicinal uses given, or on medicinal use only, with no names given. The names may have been forgotten, may have never been known or may have been considered unimportant by the healer from the Australs. In the case of the healer from Pape'ete, the medicinal uses may have been forgotten, may never have been known or were not divulged for fear of legal problems that may result from speaking about the internal use of a toxic plant.

Another case opposes the hypothesis that regional differences can account for varying knowledge of the lichens across the Society Islands. A non-healer octogenarian from Mo'orea discussed only one lichen when asked about medicinal uses. He picked up *Ramalina nervulosa* and said he remembered his family using it when he was young to treat some illnesses, but he could not remember specifically what illnesses. This indicates that lichen uses may well have existed historically on Mo'orea. The respondent told me about *Ra'au Tahiti* being practiced clandestinely because the colonial government ran a long campaign against the traditional plant therapies. This may account for a loss of knowledge at least in his family if not for the island of Mo'orea.

The cytotoxicity tests varied from the 1995 results found by Achreknar. He found *Ramalina sp.* and *Usnea sp.* to be more cytotoxic than *Catharanthus roseus*, however not significantly, while the tests in the current study showed *Catharanthus roseus* to be more cytotoxic than any of the lichens tested (Fig. 1).

The overall cytotoxic strength of the lichen extracts was stronger than in the Achrekar study. He used 0.2mL of extracts prepared to concentrations of 0.1g/mL. This concentration was the starting point for the current study, but dosage and concentrations had to be reduced to 0.05ml of extract at a concentration of 0.025 g/mL. The primary difference in extraction methods between the two studies was that Achrekar used 70% ethanol and this study used spring water. This alone should not account for the difference in cytotoxicity. The masceration time or possibly more thorough grinding might be explanations for the much higher overall cytotoxicity of the extracts in the current study.

Although not statistically verifiable, some interesting coincidences were found between the cytotoxicity results and the ethnobotanical data. Two young healers from Huahine were instructed by a senior healer to collect "coconut algae" of the fruticose form for a colon cancer treatment performed in Huahine. In collecting lichens, *R. nervulosa, R. peruviana, U. societasis*, and *U. baileyi* were found on coconut trees. *Ramalina leiodea* was found on Banyan and native Hibiscus, but never on coconut. The four fruticose lichens found on coconut, indicated by arrows in Figure 1, all tested significantly cytotoxic and are used in traditional medicine while *R. leiodea*, indicated by an asterisk, was not found to be significantly cytotoxic (p = 0.051) and its use in traditional medicine is questionable. This might suggest that the nomenclature "coconut algae" is based in a category of lichen with significance as a traditional medicinal plant. The ethnographic data for this is very thin, at best, so a careful study should be made on the distribution of the fruticose lichens and on Tahitian collection practices before any conclusion can be made regarding ethnobotanical classification based on bioactive properties.

Although *Artemia salina* are commonly used in research as an initial screening tool, it would be more diagnostic of potential anticancer activity if comparisons were made between the extracts` cytotoxicity to cultured mammalian cancer cells as compared to their cytotoxicity on healthy mammalian cells.

Unfortunately, the ethnomedical uses were not found until very late in this study. As a result, the lichens used as antibiotics were not included in the bioassays. A priority for follow-up is to test for antimicrobial activity the three lichens used as antibiotics.

The same lichens selected for the cytotoxicity tests (predominately fruticose lichens) were also tested for antimicrobial activity based on ethnobotanical uses of the fruticose lichens for infections of the skin and lungs (Hale 1979, Van Haluwyn et al. 1993) and on other studies that have found antimicrobial properties in some species of fruticose lichens (Perry et al. 1999, Calder 1986). The results of the current study differed dramatically from previous studies and from expectations. None of the lichens showed any antibacterial activity and only *Usnea baileyi* and *Parmotrema cristiferum* showed slight antifungal activity with about 2% cell lysing observed for each extract (Table 3). The methods used are in doubt because they were so different from what would have been expected. The tests should be done again and the results would be made more interesting by using pathogenic microbes.

The distinction between young and old ?Parmeliella brisbanensis might indicate an ethnotaxonomic division that is not made in biological taxonomy. The first significant problem with making this, even tentatively, is that the botanical identification has a degree of uncertainty. Additionally the sample size for the ethnotaxonomic and ethnomedical data is four interview respondents who made the distinction based on name and one respondent who made the distinction based on medicinal use. Special attention should be paid to ascertaining more precise botanical identification of the *?P. brisbanensis* specimens in the voucher collection to be sure there is not more than one species present. It would be very interesting to test the two age categories of ?P. brisbanensis to see if any significant differences exist in antimicrobial properties that the specimens may contain.

## Conclusion

As in most of the world, Polynesian culture has a long tradition of using natural products to treat illness. Lichens are still used in the Society Islands for traditional medicine, however much of this knowledge and practice has been lost since European contact There has been a revival of interest by Ma'ohi people in their traditions as well as a recent interest that has been expressed by foreigners. This interest may possibly lead to a reaffirmation of traditional knowledge expressed through reestablishment of verifiable therapeutic uses.

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# Preferential Breeding Habitats of Mosquitoes (*Aedes aegypti, Aedes polynesiensis, Culex quinquefasciatus, Culex atripceps, Culex roseni, Culex annulirostris, Toxorhynchites amboinesis)* in Mo'orea, French Polynesia

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ABSTRACT. To ensure success in controlling populations of mosquitoes that are vectors for known diseases such as dengue fever, and lymphatic filariasis, a better understanding of their ecology and behavior is necessary. This study aims to explore the preferential breeding behavior of *Aedes aegypti, Aedes polynesiensis, Culex quinquefasciatus, Culex atripceps, Culex roseni, Culex annulirostris,* and *Toxorhynchites amboinesis* of Mo'orea in French Polynesia. Location, inland or coastal, as well as container type was hypothesized to influence ovipositioning behaviors of mosquitoes. Sets of traps consisting of four different treatments were placed in ten different sites on Mo'orea. Of the sample sites, half were inland and half were relatively coastal. An ANOVA test showed that container type was more influential in determining where mosquitoes breed than location. Chi-square tests showed that only *Ae. aegypti* showed preferential breeding behaviors in coconuts. Rainfall is another factor affecting the distribution and abundance of mosquitoes. There was a lag time between days of rain and increases in number of mosquitoes. These results suggest that coconuts are the preferred larval habitat despite location. It is suggested that a control program cleaning up fallen coconuts or altering the chemistry of the water in the coconuts with additives should be implemented. Monthly surveys of the island should also be done to monitor the progress of the proposed program.

KEYWORDS: *Aedes aegypti, Aedes polynesiensis, Culex quinquefasciatus, Toxorhynchites amboinesis,* breeding habitats, mosquito preference, rafts.

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## Introduction

Viruses isolated from mosquitoes account for about half of all known virus species, despite the fact that only a tiny portion are arboviruses. Of special importance are larval stages of the mosquitoes that serve as possible vectors for diseases such as dengue fever (*Aedes aegypti*), and lymphatic filariasis (*Aedes polynesiensis* and *Culex quinquefasciatus*). The susceptibility of mosquitoes for carrying various diseases is partly determined by the extrinsic incubation period (EIP). This incubation period is affected by environmental factors including temperature, larval nutrition, and the infective dose of the virus (Black et al. 2002, Hardy 1988). By understanding the ecology and behavior of the mosquitoes on the island of Mo'orea, vector control programs can be established to reduce the number of breeding habitats which in turn reduce the public health risks that these organisms pose.

One attempt to control *Aedes* is the introduction of *Toxorhynchites amboinesis* Doleschall to Mo'orea in 1975 (Riviere 1979). In its larval stage, *T. amboinesis* is predatory and feeds on other mosquito larvae. However, no further studies have been done to assess the success of this intervention. A thorough investigation of mosquito breeding sites, their ecology and behavior would add to the current knowledge and information on these specific species found on Mo'orea, a necessary step to launch of a successful vector control campaign.

While some of the mosquito species on the island of Mo'orea are indigenous, others were introduced by varying methods. The indigenous species include *Culex atriceps* and *Culex roseni*; five other species have been introduced either by native Polynesian people, by Europeans and other travelers, or as biological control agents (*Toxorhynchites amboinesis*). Studies have been done which address the issue of the distribution of particular species of mosquitoes but there has not been an inclusive study of all species present on the island (Becker 1992, Wettach and Smith 1994). Different species of mosquitoes require different conditions in order to survive. Availability of breeding habitats for gravid females is not the same for all species because each has different preferences including tree holes, stream pools, crab holes, and artificial water containers of water (Belkin 1962). This study therefore addresses the following question: In what kind of breeding habitats do these seven species of mosquitoes prefer to oviposit?

## Materials and Methods

### *Sample sites*

The Society Islands in French Polynesia comprise six oceanic islands. Of those six islands, this study sampled the island of Mo'orea exclusively. Survey rounds were conducted from October 21, 2002 to November 19, 2002.

There were a total of ten sample site;, five were inland sites and five of which were coastal sites. Inland and coastal sites were grouped in five different locations, Ha'apiti, Gump station, Oponuhu, Paopao, and Viaire. Paopao and Viaire sites were sampled on either sides of the Viaire-Paopao Pass (Figure 1 and Table 1)

### *Sample treatments*

Four different treatments were tested at each site. These treatments included: clean water in a coconut, clean water in bamboo, clean water in an artificial container, and dirty water in an artificial container. All containers had a volume of at least 500mL. The bottoms of empty 1.5L water bottles were cut out and used in this study as the artificial containers at each site. Bamboo was cut horizontally down to make containers with the nodes serving as the base of the container. The dirty water that was used in the fourth treatment type was a mixture of water left in a coconut for a few days and detritus collected from the bottom of Paopao River on the west side of Viaire-Paopao pass.

Each site contained five sets of containers and each set of containers included one of the four different sample treatments. A total of two hundred containers were distributed within the ten sampling sites on the island.

### *Sampling method*

Containers of waters at each site were checked and replaced (if needed) twice a week. During each visit to a site, all containers were scanned for rafts, eggs, pupae, and larva. If any of these were found, a sample was taken back to the lab for identification. During the first two weeks, if nothing was found in containers other than the dirty water treatment, the water was replaced with clean water. The methodology was altered after this initial period by keeping the water even if nothing was found in the container.

Sample sites

| Site # | Site Name | Description | UTM Coordinates |
|---|---|---|---|
| 1 | Viaire Inland | Inocarpus Forest | E 202849<br>N 8060527 |
| 2 | Viaire Coastal | Grass by a stream | E 203642<br>N 8060369 |
| 3 | Paopao Inland | Bamboo Forest | E 202329<br>N 8060985 |
| 4 | Paopao Coastal | Inocarpus by a stream | E 201017<br>N 8060956 |
| 5 | Ha'apiti Inland | Coconut and Mango trees | E 195729<br>N 8056516 |
| 6 | Ha'apiti Coastal | Hibiscus Forest | E 195246<br>N 8055722 |
| 7 | Belvedere | Inocarpus | E 199947<br>N 8058314 |
| 8 | Oponuhu | Hibiscus | E 197459<br>N 8060846 |
| 9 | Gump Station Inland | Hibiscus | E 199711<br>N 8063590 |
| 10 | Gump Station Coastal | Hibiscus, and coconut trees | E 199845<br>N 8063798 |

**Table 1.** Table of sites, location, and description of the vegetation in the area.

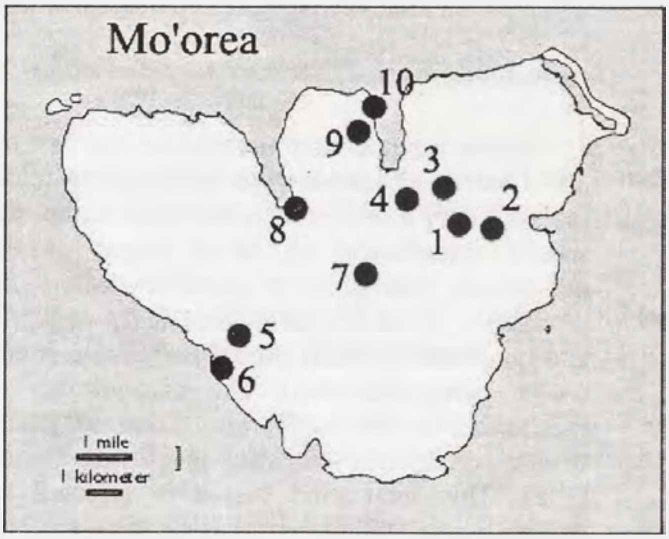

**Figure 1.** Overview map of sample sites on Mo'orea courtesy of the University of California at Berkeley's Geography map lab. Site 1 is the Viaire inland site, 2 is the Viaire coastal site, 3 is the Paopao inland site, 4 is the Paopao coastal site, 5 is the Ha'apiti inland site, 6 is the Ha'apiti coastal site, 7 is the Belvedere site, 8 is the Opunohu site, 9 is the Gump Station inland site, and 10 is the Gump Station coastal site.

This would account for any eggs that could not be easily detected with the naked eye. Specimens were kept in 250mL plastic bottles.

Specimens collected from the field were identified by reference to the keys of Merritt and Cummins (1984) and Belkin (1962). Specimens were placed in clear plastic cups with mesh top or plastic lids with holes. This allowed the specimens to live longer (relative to the collection bottles) in addition to keeping samples of different stages of mosquitoes. Samples that were kept in the lab were checked frequently for *T. amboinesis* and pupated adults. Adults were collected, identified and compared to the larvae and pupae present of the same sample. Rafts of eggs were collected from samples separately and placed on filter paper to prevent desiccation. Rafts were placed in the aforementioned plastic cups in an attempt to induce hatching which would facilitate identification.

### *Data Analysis*

Data collected on the relationship between the number of habitats occupied and by what species of mosquitoes was analyzed using the Chi-square goodness of fit test. A similar Chi-square analysis tested was conducted for rafts found in the four treatments. Differences in abundance of mosquitoes amongst the four treatments were analyzed for each species separately with a Chi-square analysis. Data collected on the 17 sampling days (24th , 25, 28, 29, 30, and 31 of October, 2002 and 1st, 4, 5, 7, 8, 11, 12, 14,15, 16, and 19 of November, 2002) were analyzed by a repeated measures ANOVA test. All statistical analysis was done using Systat 7.0 (SPSS Inc © 1997).

Voucher specimens were deposited in Essig Museum of Entomology (University of California at Berkeley, 94720).

## Results

### *Distribution of mosquito larvae and rafts*

For the 200 containers that were in the field for the course of the study, there were 1326 sampling opportunities. In 103 opportunities a container was dry leaving only 1218 viable sampling opportunities. Larvae were found to inhabit 192 while rafts (one or more) were found in 18 of the viable sampling opportunities. Additionally, only five (*C.. quiquefasciatus*, *C. roseni*, *A. aegypti*, *A. polynesiensis*, and *T. amboinesis*) of the seven described mosquitoes for Mo'orea were found in the traps.

Due to mixture of viable and non-viable containers at a site on any given sampling day, the number of mosquito larvae and rafts were averaged by day within sites (Figure 3,4). Compared to daily rainfall (Figure 2), there is a two to four day lag in the increase of abundance of both mosquito larvae and rafts.

Figure 5 shows that 70% of the mosquitoes were collected in coconut containers regardless of location. Among both inland and coastal sites, 70% of mosquitoes and 93% of rafts were found in coconuts (Figure 6). The majority (52%) of mosquitoes found were *A. polynesiensis* (Figure 7).

Inland sites rafts were found only in coconuts whereas in coastal sites, rafts were found in coconuts, bamboo, and artificial containers with dirty water.

### *Statistical analyses*

Results from a Chi-squared test on the number of mosquitoes in different locations and treatments yielded a p-value of 0.871 (df=2) suggesting no significant interaction between the two factors. Similarly, a Chi-square test was performed on abundance of rafts, comparing location and type of treatments, which generated a p-value of 0.888 (df=3). This p-value suggests that there is no statistically significant interaction between the distributions of rafts in both locations or in any of the four treatments.

When comparing the number of different mosquito species via a Chi-square test, a p-value of 0.183 (df=6) was obtained. Again, this indicates that there was no significant difference in the abundance of different species.

Chi-square tests were done for each species of mosquitoes collected on types of treatments and location (Table 2). Results from the test suggest that there was only a difference in distribution of mosquitoes among location and container type for *A. aegypti*.

A repeated measures ANOVA test (Table 3) on the number of mosquitoes, with containers and location as key variables, indicated that there was a significant difference in the numbers of mosquitoes found in containers ($p < 0.05$) versus location or the combination of the two. This type of statistical analysis also looked at the interaction between factors (location and container) over days (the repeated measures). The ANOVA test showed that number of mosquitoes varied significantly by day regardless of location or container type ($p<0.05$).

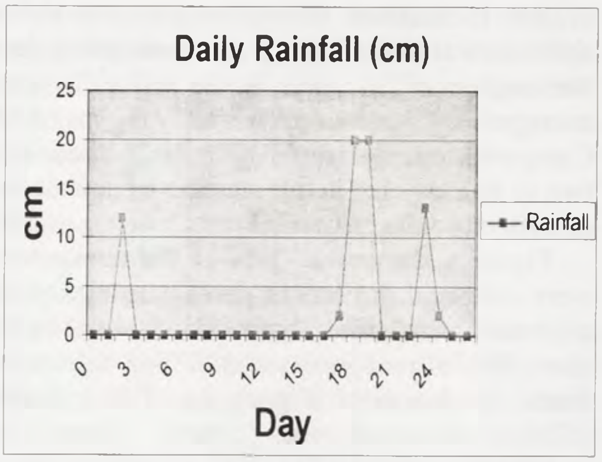

**Figure 2.** Amount of daily rainfall (cm) from October 24, 2002 (day 0) -November 19, 2002 (day 26), courtesy of the weather station at the Gump Research Station in Mo`orea.

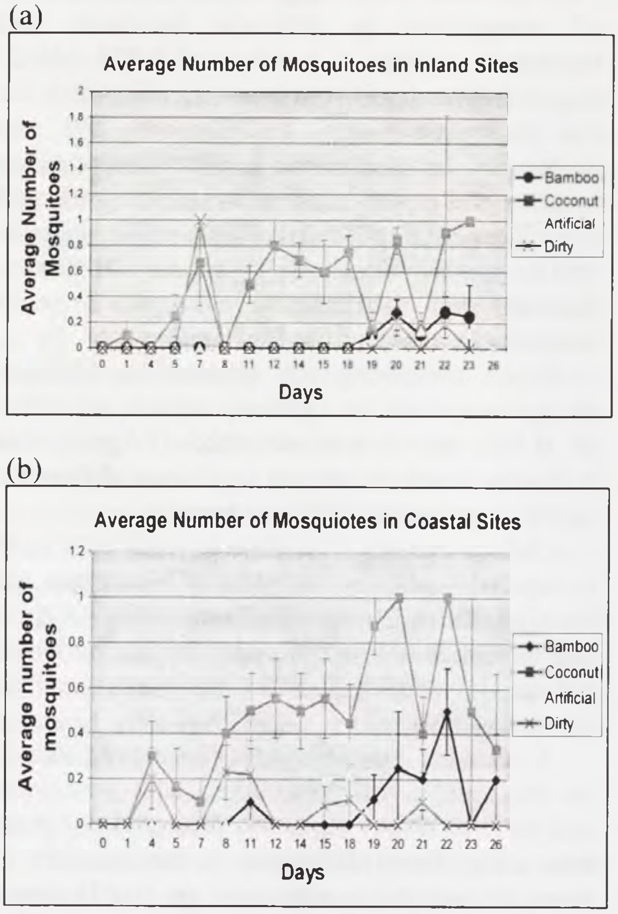

**Figure 3.** Number of mosquitoes from October 24, 2002 (day 0) -November 19, 2002 (day 26) in (a) inland sites and (b) coastal sites.

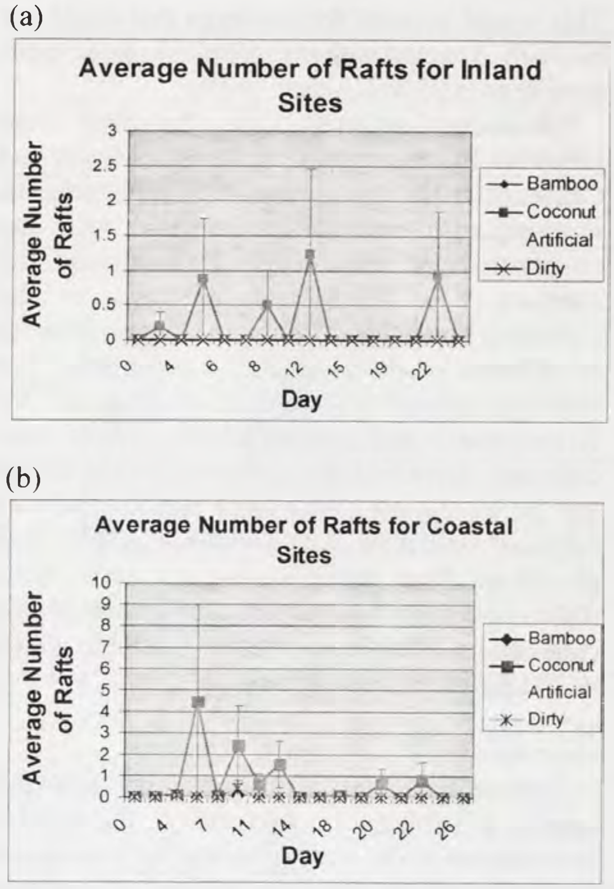

**Figure 4.** Number of rafts from October 24, 2002 (day 0) -November 19, 2002 (day 26) in (a) inland sites and (b) coastal sites.

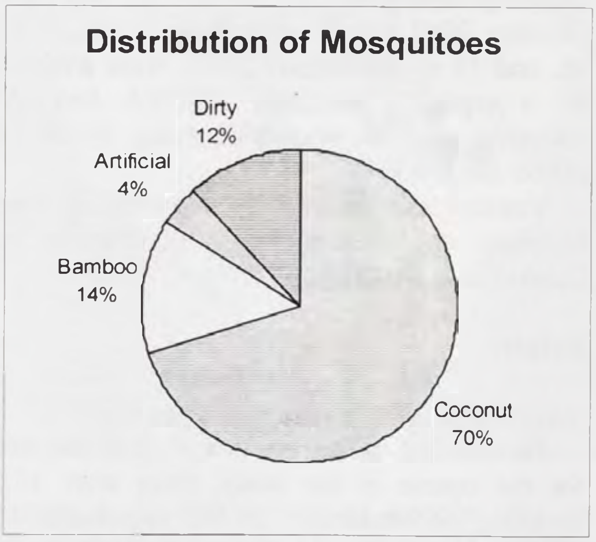

**Figure 5.** Distribution of mosquitoes in different containers.

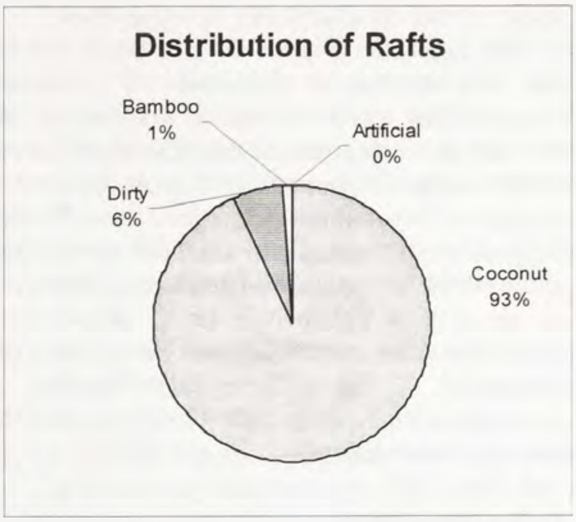

**Figure 6.** Distribution of rafts in different containers.

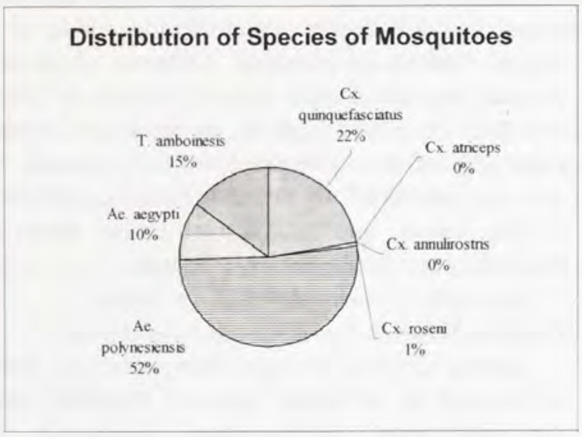

**Figure 7.** Distribution of different mosquito species among the ones collected.

Comparison of Mosquitoes amongst different locations and different treatments

| Mosquito Species | df | P-value ($<0.05$) |
|---|---|---|
| *C. quinquefasciatus* | 1 | No |
| *C. roseni* | 3 | No |
| *A. aegypti* | 2 | Yes |
| *A. polynesiensis* | 3 | No |
| *T. amboinesis* | 3 | No |

**Table 2.** A table of p-values from a Chi-square goodness of fit test between location and different treatments for each species of mosquitoes found.

Comparison of Mosquitoes between different location, treatments, and days.

| Factors | SS | df | F | P |
|---|---|---|---|---|
| Day | 1.337 | 6 | 7.444 | 0.000 |
| day & location | 0.116 | 6 | 0.019 | 0.646 |
| day & container | 1.662 | 18 | 0.092 | 3.084 |
| day, container, location | 0.328 | 18 | 0.018 | 0.608 |
| location | 0.060 | 1 | 0.418 | 0.523 |
| container | 8.899 | 3 | 20.557 | 0.000 |
| location & container | 0.036 | 3 | 0.083 | 0.969 |

**Table 3.** A table of results from repeated measures ANOVA comparing different factors and interactions among these factors.

Furthermore, the number of mosquitoes was significantly different and changed by day ($p<0.05$).

## Discussion

### *Distribution of mosquitoes*

Results from this study show that mosquitoes on Mo'orea display preference towards coconuts as breeding sites. Location of containers plays a less important role in choosing a suitable breeding site than the containers themselves. Samarawickrema et al. (1993) found that the five main larval habitats for both *A. polynesiensis* and *A. aegypti* were 200 liter water-storage drums, discarded tins, bottles, tree holes, and coconuts. Among the five habitats, tree-holes and water drums held the highest number of mosquito larvae.

Becker (1995) suggested that canopy cover was an important factor in influencing selection of mosquito ovipositioning sites. The study notes that container type was a covariant with canopy cover for *C. quinquefasciatus.* This may explain why the highest number of rafts were found at site #10 (Gump station), a coastal site with no canopy cover. However, results from the present study (Chi-square test for rafts comparing location and containers df = 3, $p<0.05$) contradicts Becker's (1995) theory that suggests a relationship between canopy cover and artificial containers.

*T. amboinesis* was found in both natural and artificial containers by Becker (1995).These results are supported by findings from this study suggesting that this species does not exhibit preference between containers or location.

Many studies agree that coconuts are of major importance as mosquito breeding sites in the South Pacific region. Succession in coconuts may be a key factor in influencing the ovipositioning behavior of mosquitoes on Mo'orea. After the coconut has fallen to the ground a series of biological changes take place as a result of the microflora and microfuana present. The first species of mosquito to colonize a decaying coconut is *C. quinquefasciatus*, followed by *A. polynesiensis* (Riviere et al. 1998). This suggests that some mosquitoes use chemical cues to locate ideal breeding sites. This would also explain why *C. quinquefasciatus* and *A. polynesiensis* are the dominant species found in coconuts. Perhaps other treatments of this study were not as successful as coconuts in attracting mosquitoes because they lack chemical cues found in coconut water. It may be that the 4th treatment (dirty water in an artificial container) attracted some mosquitoes because the water used was a mixture of water obtained from a coconut and from the stream bottom. Skinks that have fallen into the containers and died could have also altered the chemical composition of the water which would in turn affect the attraction of mosquitoes to those particular containers. Rafts were also primarily found in coconuts (e.g. site #10 October 29,2002 had 27 rafts). This further suggests the importance of chemical cues in selection of ovipositioning sites.

Another explanation for not collecting more mosquitoes especially *C. atriceps* and *C. annulirostris* is the lack of natural breeding habitats and the drying up containers from the drought. The absence of adequate breeding sites may have forced some species to recede to isolated regions of the island resulting in reduced population size, while others maybe have adapted to the coconuts as breeding habitats to survive. *A.. aeqypti* perhaps has readily made the shift in preference in response to abundance in coconuts on the island.

No difference was observed in number of mosquitoes in coastal or inland sites. Mo'orea is only 130 km$^2$. The island could be too small to observe any significant differences in physical or environmental factors (i.e. ocean breeze, salt water, wind, and exposure) between coastal and inland sites that would have a big influence on mosquito breeding behaviors.

### *Affect of rain on abundance of mosquitoes*

The lag time (Figure 2-4) between the last rain and increase in abundance of mosquitoes suggest that there exists a correlation. An increase in rainfall was observed towards the end of the study which correlated to an increase in number of mosquitoes. Combined these findings suggest that there is a seasonal distribution amongst the mosquitoes of Mo'orea. Therefore, an absence in *C. atriceps* or *C. annulirostris* could be due to the time the study was conducted. The difference in number of mosquitoes found over days furthers support this theory of seasonality.

### *Policy implications*

It is important to understand the ecology of mosquitoes both in times of drought and storm (i.e. El Nino), to be able to establish a successful vector control program. *A. aegypti* eggs can stay dormant until floods which would result in a sudden influx of possible vectors. A sound control program should include cleanup of fallen coconut in inland forests as well as coastal coconut stands. Frequent surveys should be done in search of all possible breeding habitats on the island, and samples of larvae taken at these sites to test for various diseases.

## Conclusions

During times of drought mosquitoes are more influenced by different types of breeding sites available than by location, especially in coconuts. Mosquitoes may use chemical cues exuded from the coconuts to locate breeding sites. The succession that takes place after a coconut has fallen and started to decay changes the chemical cues present differentially attracting species of mosquitoes. Monthly surveys of the island are also recommended to monitor the progress of the program which would allow one to make adjustments where necessary to improve it.

## Acknowledgements

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# Factors Influencing the Grouping Behavior of the Spider *Pholcus ancoralis* (Araneae: Pholcidae)

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ABSTRACT. Many behavioral models are based on the premise that animals should avoid the presence of competing individuals. Although spiders are territorial by nature, some species of spiders are known to live in groups. This investigation studies solitary versus grouped distributions of the sheet-weaving spider *Pholcus ancoralis* (Araneae: Pholcidae) on the island Moorea, French Polynesia. The study focuses on five potential causal factors of grouping: habitat, prey capture, egg protection, spider size, and substrate on which the webs were constructed. The results of a habitat sampling demonstrated that habitat, prey capture, and egg protection did not have a significant influence on the solitary versus grouped distribution of spiders. Size of spider showed significance in grouping distributions. Large spiders (0.8-0.9cm) on average were found more often in groups while small spiders (<0.5 cm) were more often solitary. Spiders were also captured, marked, and released to test if individuals showed a preference for solitary versus grouped living. Released spiders more often formed groups on *Inocarpus fagifera* trunks, rocks, and *Angiopteris* ferns, while spiders on leaves and branches were more likely to be solitary. Solitary or grouped behavior prior to capture was not a good predictor for whether spiders would be solitary or grouped post-release.

KEYWORDS: Pholcidae, grouping, web site selection, habitat selection, spiders, Moorea.

## Introduction

Deciding where to settle affects nearly all of an individual's later choices in life. The ability to distinguish and select among habitats is an important factor in the life history of an organism, it determines the selective environment encountered by individuals within a population (Peit & Peit 1996). Organisms tend to pick the most suitable environment in which to settle. Organisms first choose a general place to live and then make subsequent decisions on how to use the area (Orians & Wittenberger 1991). Some organisms decide to live in groups while others decide to live alone.

Many behavioral models are based on the premise that animals should avoid the presence of competing individuals. The most current theories of animal aggression are based on models that assume "winner takes all" when animals compete for resources (Stamps & Krishnan 2001). Some spiders, although territorial by nature, tend to aggregate and live in groups (Jakob et al. 1999). Multiple studies have been conducted to determine why this grouping occurs within such a territorial order but the cause is still unclear (Jakob et al. 1999, Schuck-Paim & Alonso 2001).

Factors such as habitat, prey capture, maternal care, size, and substrate on which the web was built are important variables for the selection of a web site by a spider. This suggests that these factors may also be significant in the grouping behavior of some spiders.

Spiders expend much of their energy constructing webs. Silk production is energetically costly and spiders must redirect energy from growth and reproduction to construct their own webs (Jakob et al. 1999). Some spiders may group because they would rather live on another spider's web than expend energy building their own. The high cost of web construction in spiders may contribute to the grouping of spiders (McNett & Rypstra 2000).

Habitat is a key determinant of where a spider will build its web. Moist habitats along rivers, ponds, and springs often provide a high insect abundance (Barclay 1991 & Holloway, Barclay 2000). Some spiders construct their webs in areas where they may have an increased chance of capturing prey. A "suitable" microhabitat must provide a supply of prey to the spider (Riechert & Gillespie 1986). Microclimate and prey availability may determine spider microhabitat choice (Ward & Lubin 1993). A higher prey density may cause the population of spiders in a given area to increase and may therefore contribute to the grouping of spiders.

The size of the spider may also contribute to the choice of website. The fighting ability of many spiders increases with body size (Jakob 1994). Spiders that are larger may be more likely to survive in a greater range of locations because they are better suited to cope with exposure to danger. This may also be the case with spiders carrying eggs. Spiders that are actively attending their eggs may have a reduced ability to defend their territory (Li et al. 1999) this may factor in grouping behavior.

The structural complexity of an environment can influence the abundance and diversity of species in an area. It is also a contributing factor to the behavior of the organisms inhabiting the area (McNett & Rypstra 2000). A more complex structure increases the abundance of spiders in an area (Stratton et al. 1979). This increased abundance may play an important role in the grouping behavior of spiders.

Learning more about the grouping behavior of a species can be a key factor in gaining knowledge about the ecology of a species. This study was conducted in Moorea, French Polynesia to test the hypotheses that habitat, prey capture, presence of eggs, size, and substrate on which the spider web was built were important factors in the grouping behavior of *Pholcus ancoralis* (Araneae: Pholcidae). Pholcids are a family of sheet-weaving spiders who faculatively live in groups. Group membership is flexible and individuals move frequently among groups. Groups are generally small and spiders of all sizes are found living alone and in groups (Jakob et al. 1999). There is little information about the factors influencing the grouping behavior of this species.

Such a study is useful because *P. ancoralis* has a large population on the island of Moorea. It is unclear if this is a native or introduced species although, the large population densities of *P. ancoralis* in disturbed habitats leads experts to believe that this species is introduced (Gillespie et al. 2000). Due to the high density and introduction of this species to the island of Moorea, *P. ancoralis* may be displacing native species. Therefore, knowledge of its basic biology will be useful to conservation biologists.

## Materials and Methods

### *Study Sites*

The study was conducted on Moorea, Society Islands, French Polynesia (17° 30'S, 149° 50'W), from October 7th to November 17th, 2002. Sixteen different sites representing four different habitat types were sampled. The four habitats studied were riparian *Inocarpus* forests, non-riparian *Inocarpus* forests, riparian non-*Inocarpus* forests, and non-riparian non-*Inocarpus* forests. *Inocarpus fagifera* (Fabaceae) trees were chosen because their buttress-like trunks provide a suitable attachment for pholcid webs, this may be an important factor in the grouping behavior of *P. ancoralis*. Riparian areas were defined as being within 2-3 meters of a stream. Study sites included Vaiare, Belvedere, and Afareaitu (Fig. 1). Mark-recapture data were only collected at Belvedere because this location had areas representing all four habitats being studied as well as a large population of pholcids.

### *Habitat Sampling*

Data were recorded in four 15 x 3 meter plots in each of the four different habitats types, making a total of 16 plots. Five variables were measured: 1) Number of spiders per web. Spiders were considered to be members of the same group if they were on the same web sheet and were in vibratory contact of one another. Because vision in the family Pholcidae is very poor and spiders cannot recognize each other visually they communicate through web vibration (Jakob et al. 1999). I verified that spiders were in vibratory contact with one another by poking one of the spiders in the group. This caused the spider to perform a

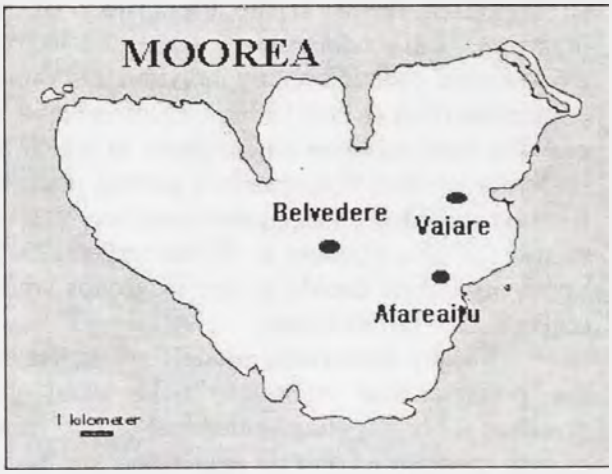

**Figure 1:** Map of Moorea showing study sites. UTM coordinates of general sites as follows: Afareaitu E 202,048.27 N 8,057,626.81; Belvedere E 199,915.98 N 8,058,177.32; Vaiare E 203,128.56 N 8,060,539.65. Summary of plots taken at each site. Riparian *Inocarpus* forests: 1 Afareaitu, 2 Belvedere, and 1 Vaiare. Non-riparian *Inocarpus* forests: 2 Belvedere and 2 Vaiare. Riparian non-*Inocarpus* forests: 1 Afareaitu and 3 Belvedere. Non-riparian non-*Inocarpus* forests 4 Belvedere.

"whirling" motion (Jakob 1999). Adjacent spiders were determined to be in vibratory contact and therefore members of the same group if they also began to "whirl." 2) Substrate to which the web was attached. Substrates were divided into the following categories; *Inocarpus* trunk, rock, branch, leaf, and *Angiopteris* ferns, and the distribution of grouped to solitary spiders per each substrate type was recorded. 3) Size of spider. Due to the difficulty of obtaining exact measurements of these fragile spiders in the field, spiders were grouped into small, medium, and large general size categories. Thirty large and 30 medium spiders were collected randomly to determine actual sizes of spiders in these three categories. Small spiders were too small to capture and measure therefore anything smaller than 0.5cm ± 0.065cm was considered to be in the small category. Spiders were measured from the tip of the abdomen to the tip of the cephalothorax to the nearest mm. Medium spiders had a range of size of 0.5cm-0.7cm (mean 0.570cm ± 0.065cm). Large spiders had a range in size of 0.8cm-0.9cm (mean 0.833cm ± 0.048cm). 4) Prey captured. If there was any evidence that the spider had gathered a prey item, the type and location of the prey was recorded when possible. 5) Presence or absence of eggs.

All statistical analyses were done using SYSTAT 7.0. A one-way ANOVA was used to test for the effect of habitat type on spider grouping. A one-way ANOVA was also used to test if presence of eggs or presence of prey influenced the grouping of the spiders. A chi-squared test was preformed to determine if size affected grouping.

### *Mark-Recapture*

A mark-recapture experiment was conducted to test if habitat and/or substrate affected grouping of spiders. Spiders of all sizes were collected randomly from the Belvedere. Three releases were conducted at non-*Inocarpus*/*Inocarpus* boundaries in both riparian and non-riparian habitats.

For each mark-recapture experiment 100 spiders were collected and marked for re-release. Spiders were taken to the lab in individual vials 3.5cm in diameter. The vials containing spiders were placed on ice to slow down the metabolism of the spider. Spiders were then marked using Jordana Holiday Red toluene and formaldehyde free nail polish. Solitary spiders were marked with a single dot of nail polish on the dorsal side of the abdomen. Spiders that were originally collected from a group were marked with two dots of nail polish. Spiders were stored at 22°C overnight. Spiders were then taken to the field. A site was selected where *Inocarpus* and other vegetation where both equally present.

Because the presence of other webs could improve the structural suitability of a site for the construction of a new web (Schuck-Paim & Alonso 2001) and because of the energetic cost of building a web, spiders without webs are more likely to settle in locations that already have a web (Jakob et al. 1999). Therefore, prior to the release of the marked spiders all spiders and webs were removed from a 7 x 7meter area around the release point. A flag was placed in the middle of the area equidistant from the different types of vegetation. At the flag, 100 spiders were then released simultaneously. Of these 100 spiders 16 were grouped and 84 were solitary. These proportions were chosen because this is the natural proportion of grouped to solitary spiders found in the field.

Spiders were left overnight to settle and find a place to build their webs. After 24 hours a 7 x 7 meter area was searched for marked spiders. Data on five variables were collected in this portion of the study: 1) the original grouping of the spider, 2) the new grouping of the spider, 3) web attachment substrate, 4) size of spider (small, medium, and large), and 5) prey captured. If there was evidence of prey, the type and location of the prey was recorded. The area was searched for spiders until five minutes had passed with no marked spider recaptured.

One-way Pearson's Chi Squared tests were done to test if habitat, prior grouping behavior, and/or substrate affected the post-release grouping behavior.

Specimens identified by information in Procter 1992 and Gillespie et al. 2000. Voucher specimens have been deposited at the ESSIG Museum of Entomology at the University of California, Berkeley.

## Results

### *Habitat Sampling*

Habitat, prey, and presence of eggs were not significant factors in the grouping distribution of *P. ancoralis* (Table 1). There was a significant difference between grouping and spider size ($\chi^2$= 8.996, df = 2.000, p = 0.011). Groups tended to consist of large spiders. Solitary spiders were more likely to be small (Fig. 2).

**Table 1:** Results of ANOVA on affects of *Pholcus ancoralis* grouping by independent variables: habitat, presence of prey, and presence of eggs.

| Factor | Σ squares | df | F-ratio | p |
|---|---|---|---|---|
| Habitat | 1391.125 | 3 | 1.574 | 0.222 |
| Prey | 28.375 | 3 | 0.946 | 0.434 |
| Eggs | 6.344 | 3 | 0.902 | 0.455 |

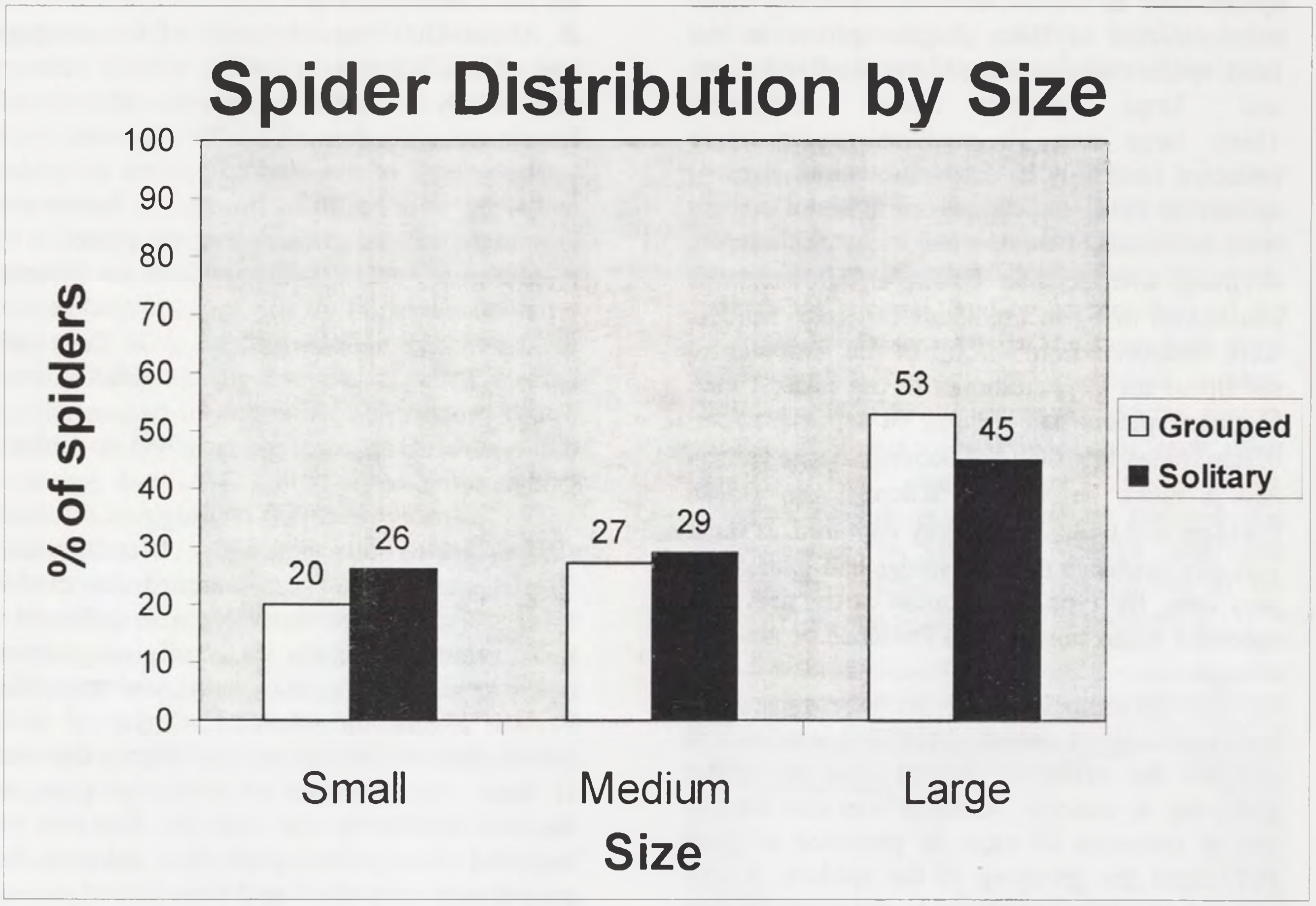

**Figure 2:** Distribution of grouped and solitary spiders by size across all habitat types. Total percent of grouped spiders equals 100%. Total percent of solitary spiders equals 100%.

**Table 2:** Results of one-way Pearson's Chi Squared Tests comparing habitat, prior grouping, and substrate to spider's grouping behavior. P-values less than 0.05 indicate that this factor significantly affected whether post-release spiders were found grouped or not.

| Factor | $\chi^2$ Value | df | p |
|---|---|---|---|
| Habitat | 1.200 | 1.000 | 0.273 |
| Prior Grouping | 1.143 | 1.000 | 0.285 |
| Substrate | 2.000 | 2.000 | 0.006 |

*Mark-recapture*

Habitat and previous grouping behavior had no significant effect on post-release spider grouping. However, there was a significant difference between grouping and the substrate on which a spider was found (Table 2, Fig. 3). Grouped spiders tended to be found on *Inocarpus*, rocks, and *Angiopteris* while on leaves and branches it was more common to find solitary spiders.

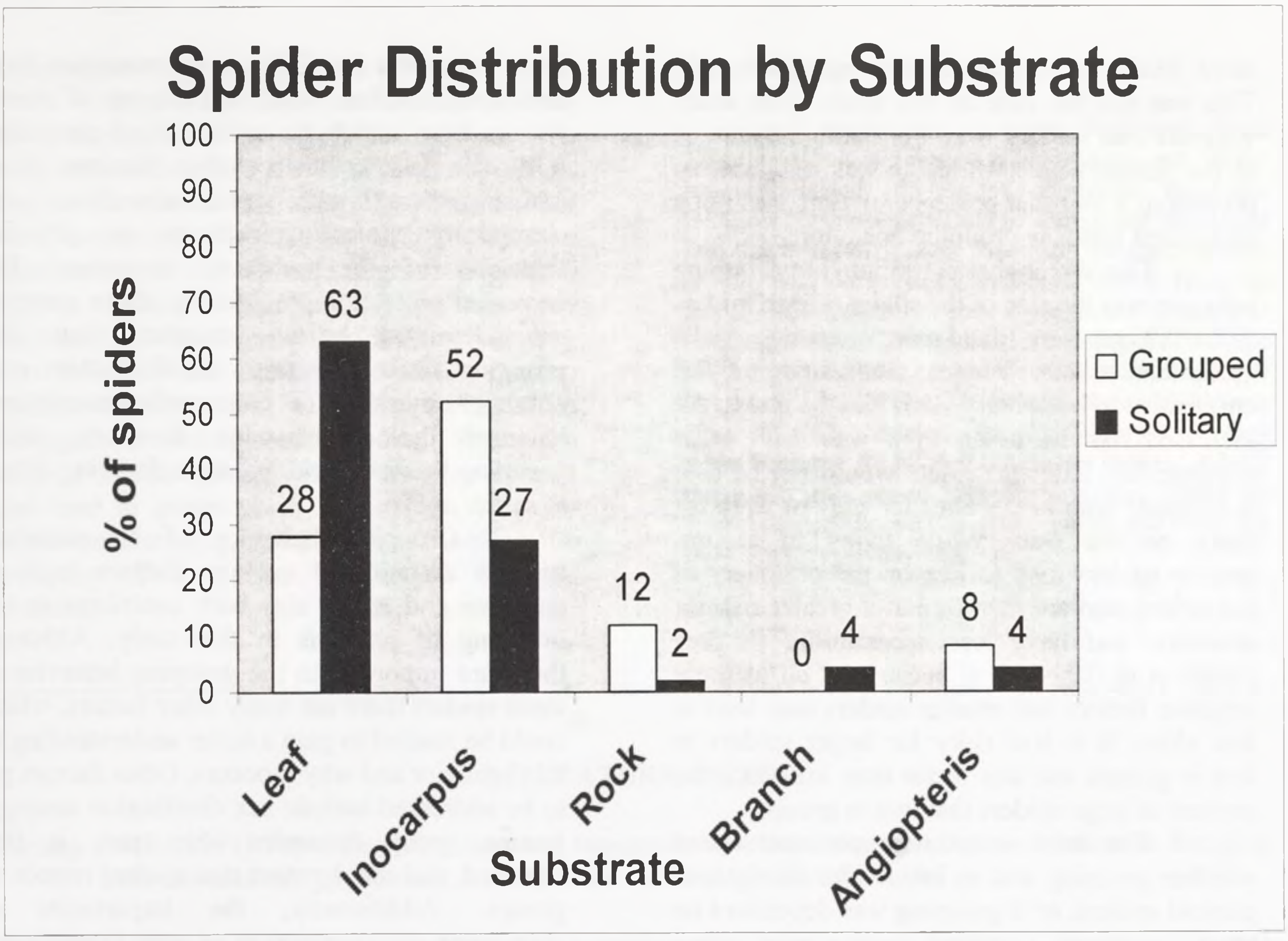

**Figure 3:** Distribution of grouped to solitary spiders by substrate after mark-recapture. Total percent of grouped spiders equals 100%. Total percent of solitary spiders equals 100%.

## Discussion

The habitat sampling observations as well as the mark-recapture suggests that habitat does not affect the distribution of grouped versus solitary spiders. Riparian and non-riparian areas as well as areas with *Inocarpus fragifer* all contained similar proportions of grouped and solitary spiders. Although higher prey availability may be expected in riparian zones (Barclay 1991, Holloway & Barclay 2000), there was no increase in grouping of *P. ancoralis* in riparian forests. Therefore, either prey availability was not higher in the riparian zones sampled in this study, or prey availability is not a factor in grouping behavior of *P. ancoralis*. There are several contradictory models that describe the affects of prey availability on the dispersal of spiders. The Gillespie and Caraco model (1987) suggests that individuals in a prey rich habitat optimize prey intake by moving frequently. Alternately, Vollrath (1985) found that tropical species *Nephila clavipes* L. moved more frequently when food availability was low (Ward & Lubin 1993). To determine how prey availability directly affects grouping behavior future study is needed to determine how *P. ancoralis* disperses in prey rich and prey poor habitats.

Possessing captured prey was not an important factor in the grouping of the species. Spiders with prey were common in both grouped and solitary situations. This suggests that both solitary and grouped spiders have an equal chance of capturing prey. One possible explanation for this is that grouping has little or nothing to with prey.

In some spiders maternal care ends with the construction and safe placement of the eggs sac. Other species, such as pholcids, which carry their egg sacs in their mouths, actively attend to the eggs (Li et al. 1999). In this study the presence of eggs did not have a significant effect on the grouping behavior of the spiders. This suggests that the spiders were not grouping in order to protect a female who was carrying eggs. A spider that is carrying her egg sac in her mouth is incapable of protecting herself without dropping her eggs. Therefore, spiders that joined the web of one that was carrying eggs would be at a lower risk of attack. Such spiders might be more likely to join an already occupied web. This was not the case in this study. This study suggests that spiders were not taking advantage of the female's web while she was incapable of protecting it and that presence of eggs was not a significant factor in grouping behavior.

One factor that contributed to grouping behavior was the size of the spider. Large spiders (0.8 – 0.9 cm) were found more often as part of a group than solitary, whereas small spiders (<0.5 cm) tended to be solitary. This may be due to the increased risk of living in a web with other conspecifics. Smaller spiders would not be able to compete with larger ones for prey or even for space on the web. While living in groups, smaller spiders tend to live on the periphery of the colony and are therefore at a greater risk for predation and have less accessibility to prey (Jakob et al. 1999). It is because of all of these negative factors that smaller spiders may tend to live alone. It is less risky for larger spiders to live in groups and this factor may influence the amount of large spiders that live in groups.

The mark-recapture experiment tested whether grouping was an innate characteristic of pholcid spiders, or if grouping was dependent on other conditions. Captured spiders were either originally solitary or grouped and were marked accordingly. The re-release of the spiders examined if spiders that were originally solitary remained solitary and if spiders that were originally grouped remained grouped. The results of this experiment showed that spider grouping was not an innate characteristic. If a spider was captured as a part of a group the spider was not significantly likely to be grouped again following the re-release.

One factor that did have a significant effect on the grouping of spiders was the substrate on which the spider was found. Grouped spiders were more likely to be found on *Inocarpus* trunks, rocks, and *Angiopteris* ferns, while solitary spiders were more common on leaves and branches. One explanation for this is that the structure of the substrates that the grouped spiders were found on was more complex than that of the solitary spiders. Substrate complexity increases the number of anchorage points appropriate for web architecture (Schuck-Paim & Alonso 2001). This would allow more spiders to successfully build webs in a certain area and increase the number of grouped spiders in areas where complexity is greater.

Alternatively, the tendency for greater grouping on particular substrates may be due to some substrates providing more protection from abiotic factors. Sun, wind, and rain are all abiotic factors from which the spiders need protection (Gillespie 1987). Other studies (Stratton et al. 1979) indicate that spider abundance and community structure increases as physical structure of the substrate increases. The increased protection provided by these complex substrates may be an important factor for grouping. Protection from abiotic factors may outweigh the risk of conspecific interactions amongst the spiders and therefore, make grouping less of a priority when deciding where to settle.

Grouping behavior of *P. ancoralis* remains difficult to explain. Factors such as substrate and spider size both contribute to the grouping of pholcids in this study. Although these are important to the grouping behavior of these spiders there are many other factors, which could be studied to gain a better understanding of this behavior and why it occurs. Other factors yet to be addressed include sex distribution amongst groups, group dynamics when prey is first captured, and the duration that spiders remain in groups. Additionally, the importance of abundance and distribution of prey in particular habitats can be further assessed to better understand grouping behavior. Studying these factors may give us a better understanding of what factors influence the grouping of *P. ancoralis* as well as other organisms that live in groups. It is important to understand the factors influencing grouping behavior in order to better understand the mechanisms involved in group living dynamics.

Learning more about the ecology of *P. ancoralis* and any other quite possibly invasive species contributes to island conservation. Alien species invasions severely impact island systems and could cause some native species to go extinct. Of the documented insect extinctions since 1600s, 10 have been continental while 51 have been on islands (Gillespie & Roderick 2002). It is necessary to understand the factors contributing to the ecological success of a species in order to better understand and prevent invasions from other potentially invasive species (Holway et al. 2002). Determining whether a species is native is difficult for scientists working on island systems because knowledge of island species is very sparse (Gillespie & Roderick 2002). Any ecological information on island species is useful because there is such a lack of information available for conservation biologists.

## Conclusion

There are several factors that may affect the grouping behavior of pholcid spiders. This study concluded that the size of the spider as well as the substrate on which the web was built were important factors in determining the distribution of solitary versus grouped pholcid spiders. Future studies of this behavior are important to determine other factors that may also influence the dispersion of these spiders. Studies such as this will give conservation biologists a better understanding about the ecology of this species as well as other species that tend to group. Such information is useful when considering questions such as the effect the grouping of a species has on its invasive qualities.

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# The Nature of Web Parasitism by *Argyrodes argentatus (*Araneae: Theridiidae*)* on *Cyrtophora moluccensis* (Araneae: Araneidae)

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In this field and laboratory study I investigated the mechanism of web kleptoparasitism by *Argyrodes argentatus* (Araneae: Theridiidae) on *Cyrtophora moluccensis* (Araneae, Araneidae) host webs by studying the factors that influence the kleptoparasite population. I examined natural population variations of *A. argentatus* that occur with (1) different host web sizes, (2) different host body sizes, (3) host behaviors and (4) whether the host was in an individual or colonial web. Results from field surveys indicate that a positive relationship exists between the average number of *A. argentatus* on a web and the size of host web. Field tests support that there is no relationship between average numbers of *A. argentatus* on a web and the host size, however, tests in the lab suggest that *A. argentatus* prefers *C. moluccensis* hosts less than 2.54 centimeters long. Significance was found between aggressive behaviors by the host *C. moluccensis* and host web size, as well as host body size. Host feeding behaviors and whether the hosts web was individual or in a colony also showed significance, however, results show there is no relationship between population variations of *A. argentatus* and *C. moluccensis* behaviors. The numbers of *A. argentatus* on colonial host webs as opposed to individual host webs showed significant differences. These results show that web kleptoparasitism by *A. argentatus* on *C. moluccensis* is influenced by host web size, host body size and whether the host is in an individual web or a colony, however it is not explained by any one of these factors. The interaction results from a variety of factors working together which produce the parasite distributions and population characteristics observed.

KEYWORDS: kleptoparasitism, *Argyrodes argentatus*, *Cyrtophora moluccensis*, symbiosis, orb web

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## Introduction

Many species are obligate users of resources gleaned from other species. Frigate birds rarely ever feed by themselves, but rather harass other sea birds into regurgitating their meals. Jackals forage primarily by following larger predators and stealing what bits of food they can muster. Likewise, several species of invertebrates survive by associations with other species. At least 19 spider families are reported to live exclusively in webs spun by other spiders (Tso and Severinghaus, 2000). Perhaps the most widely publicized of these web "guests" are the theridiid spiders of the genus *Argyrodes*.

Of the 224 recognized species of *Argyrodes*, all but one specializes on living off the webs of other spiders. (Gillespie, unp. 2002). This mode of existence is reflected in the highly responsive sensory organs and long walking legs that aid *Argyrodes* in maneuvering host webs efficiently and inconspicuously. (Foelix, 1996)

*Argyrodes* maintain either a positive or negative symbiotic relationship with their host. They can be mutualistic with the host by removing unwanted prey from the periphery of the web, thus reducing clutter and damage to the prey catching surface (Exline and Levy, 1961), but more commonly, they are detrimental to the host. This is accomplished by several means; the *Argyrodes* steals regular prey items from the host (Canglialosi, 1991), they remove silk from the web or egg cases of the host (Tso, Severinghaus and Liu 1998,), they force the host to relocate its web more often (Tso and Severinghaus, 2000), or lastly they prey upon the host or its offspring (Gillespie, unp. 2002).

On the island of Moorea, French Polynesia, one of the lesser-known species of *Argyrodes*, *Argyrodes argentatus* (O.P. Cambridge) lives in close association with the araneid *Cyrtophora moluccensis* (Doleschall). *Cyrtophora*, a member of the orb weaving family, constructs huge modified orb webs. *C. moluccensis* in particular, creates a large inverted bowl shaped web of finely woven mesh, surrounded by a tall tangle of extremely tensile associated threads that may or may not link the webs of other *C. moluccensis*' in massive colonies. In this relationship *A. argentatus* is said to kleptoparasitize its host *C. moluccensis*. (Koh, 2000). Kleptoparasitism is defined as the phenomena in which one partner in a symbiosis benefits from the other by stealing its food or other resources.

To investigate the nature of the kleptoparasitic association between *Cyrtophora* *moluccensis* and *Argyrodes argentatus* I examined the impact of factors influencing parasite population densities on host webs by field observations and surveys, as well as lab experiments. This paper addresses the nature of this association by examining the effects of host size, host web area, host behavior and whether the host web is unique or part of a colony on host web parasite load.

## Methods and Materials

### *Study Site and Spiders*

Surveys were conducted around the island of Moorea from 15 Sept 2002, until 25 Sept 2002 to assess the possibility of field sites. Four accessible areas with multiple webs were selected and mapped. These areas included the Belvedere Creek Marae Trail, the Belvedere Overlook Trail, the Belvedere Three Coconuts Trail and the Vaiare-PaoPao Trail. (*See fig. 1*)

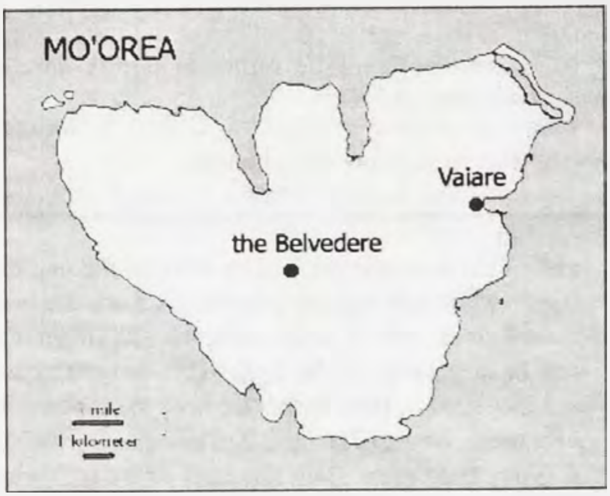

**Figure 1.** Map of Study Sites on Moorea, French Polynesia (17...30 W Lat., 149...50 S Long.).

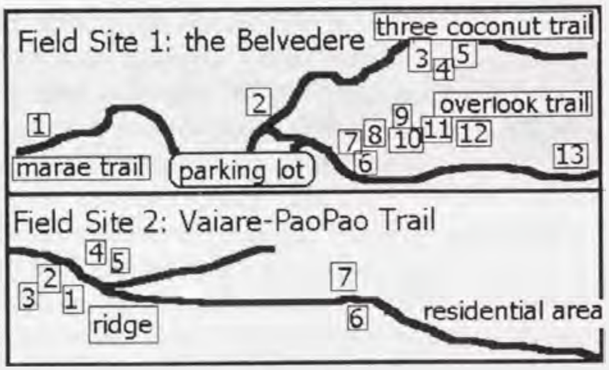

**Figure 2.** Maps of Field Sites 1 and 2 with location of all *C. moluccensis* web sites along each significant trail. UTM coordinates were: Belvedere: E 199,915.98 N 8,057,626.81, Vaiare: E 203,128.56 N 8,060,539.65.

Each trail was traversed multiple times and all *Cyrtophora moluccensis* webs found were marked with red flagging tape and a unique number. The sites were then surveyed 6 days per week beginning 27 Sept 2002 and ending 17 Nov 2002. (*See fig.2*)

The Belvedere sites consisted of closed riparian *Inocarpus* boundary habitat, and were monitored for about 7 weeks. The Vaiare-PaoPao sites were mostly tangled vine and scrub thickets but differed in percent cover in a gradient moving from the bottom of the ridge to the top. These sites were monitored for about 3 weeks. The amount of time spent at sites and the location of sites reflected the opportunity to visit.

For each web marked, the following data were collected; species of web builder, spider size, length of bowl, width of bowl, height of tangle, disposition of web and any behavioral observations.

### *Host Abundance and Characteristics*

#### *Species of web builder*

Species of web builder was determined by visual assessment. Host samples were collected and preserved in 95% ethanol, further identifications were made by students of University of California, Berkeley.

#### *Spider size*

Spider size was assessed by measuring to the nearest millimeter, the length of the spider in a resting position (see *fig.3*). Resting position was chosen because it gave a baseline measurement of the amount of space the spider occupied relative to the web.

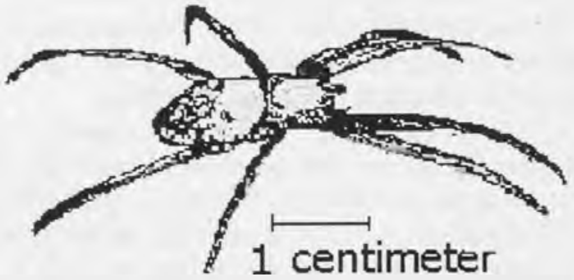

**Figure 3.** The adult *C. moluccensis* in resting position. These spiders occupy this position at the center of the bowl portion of their webs.

Measurements of carapace dimensions were not made because of uncertainty as to the spider's reproductive status. Female spider's abdomen size seemed to fluctuate with egg laying and web rebuilding events, so thus did not seem to be an honest measure of spider size when taken at any one time. Leg measurements were not made because of the possibility of damage to the spider and the web.

Spider size was categorized into Small, Medium and Large spiders. Small spiders were 2.54 millimeters or under, Medium spiders were between 50 millimeters and 2.54 millimeters while large spiders were over 50 millimeters long in resting position.

*Length of bowl, width of bowl*

Length of bowl and width of bowl were measured with an opaque ruler to the nearest millimeter. The bowl was characterized as the area of grid-like web that formed the primary prey catching surface. Area was estimated by multiplying measured length and width. (*see fig.4*)

*Height of tangle*

Height of tangle was measured with an opaque ruler to the nearest millimeter. Height of tangle was defined as the length of the upper to the lower limit of the total web. (see *fig.4*).

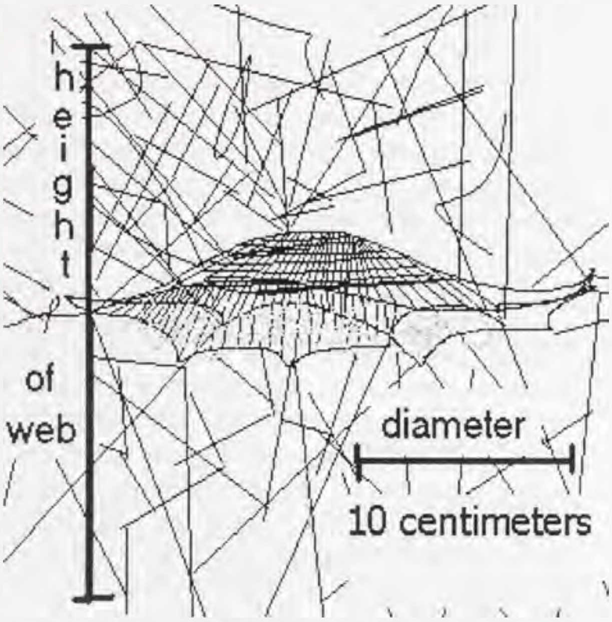

**Figure 4.** A *C. moluccensis* web showing both the measurement of height and diameter of the web. The bowl portion of the web is the central structure, while the tangle are all the other associated web threads.

*Disposition of web*

Disposition of web was defined as a whether or not the web was colonial or individual; colonial webs being defined as those which share associated threads with one or more additional webs.

*Behavioral Observations*

Behavioral observations were made every half hour spent at each web. After initial observations for a week, three integral behaviors were quantified; feeding, aggression and resting.

1. *Feeding behaviors* consisted of any actions associated with the capture or ingestion of prey.
2. *Aggressive behaviors* consisted of rush advances and web tweaks with the spider's third leg.
3. *Resting behavior*, the most common of all the behaviors, consisted of the spider being still in the middle of the web in a standardized position. (See *fig.3*)

Behaviors were noted by scanning observations in which each web was watched for a half hour. Within that period, each incidence of a behavior was noted. Behaviors were later normalized dividing the total behaviors observed by the total number of observations overall for each web.

*Parasite Abundance and Characteristics*

For each web a diagram was drawn and the presence of parasites mapped. Parasites were divided in categories A, P and S, signifying *Argyrodes argentatus*, *Pholcid pholcus* and an unknown species referred to as "Stealer", respectively. The density, location with respect to the top, bottom or side of the web, and any behavioral observations were recorded for every parasite in half hour intervals.

*Ideal Web Size/Host Distribution/Prey Amount*

Ideal web size was tested in a cage 2 meters tall by .75 of a meter wide. It consisted of three cement walls and enclosed by a large screen panel. Two large branches, three rocks, two water containers with plant leaves, a bar across the top of the cage and a table in the bottom half of the cage served as anchor points for webs. (See *fig. 5A*).

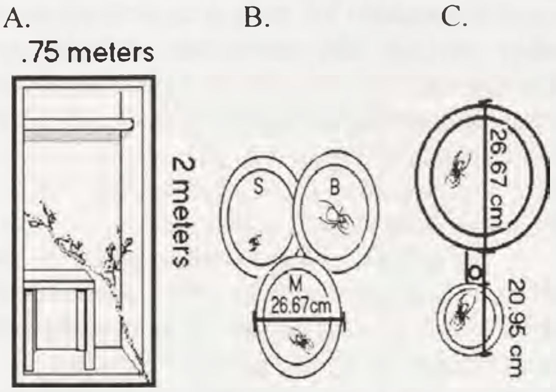

**Figure 5A,B and C.** 5A. shows the enclosure used to testing the ideal host web size. It is quite large so as not to constrain the spiders in web building. 5B. represents the container used to test if *A. argentatus* had any preference between hosts of different sizes. The S, M, and B on the containers denote what size host spiders were in each container. 5C. shows the container used to test if *A. argentatus* had any preference for web size. *A. argentatus* was inserted into the system by the little circle in the bridge in the middle of the two flowerpots.

These tests used 8 *Crytophora moluccensis* of sizes ranging from 6.35 millimeters to 63.5 millimeters (in resting position). Spiders were released into the cage and provisioned with live flies every 5 days. When spiders were unable to capture flies, flies were cooled in vials on ice for 90 seconds, and then placed on the spiders' web with tweezers. Spiders were allowed to acclimate to the cage for 4 weeks.

### *Ideal web size*

Ideal web size was tested by measuring all webs in the cage after four weeks. Measurements were made the same way as in the field.

### *Ideal host distribution*

Over the four-week period the movement of the spiders was recorded along with any associations between individuals.

### *Ideal prey amount*

Spiders were provisioned with as much as they would eat one day a week. Results of prey item accepted as well as the amount of prey eaten were recorded. Prey items were all added simultaneously or in intervals of thirty minutes.

### *Web Size Preference*

To test for web size preference, *A. argentatus* were presented with *C. moluccensis* webs of two different sizes. The percent of cases where the larger of the two webs was chosen over the smaller was analyzed for 15 two-hour trials. The test arena was constructed from two plastic flowerpots of differing size. The first flowerpot had a diameter of 26.67 centimeters, while the second had a diameter of 20.95 centimeters. The two flowerpots were connected by a 3.8-centimeter by 3.8-centimeter mesh and tin foil bridge with a hole punched in the top, the size of one mesh square. A 6.35-centimeter *C. moluccensis* was established in each pot. *A. argentatus* were introduced into the system by inverting a vial over the hole in the bridge. (See *fig.5C*)

### *Host Size Preference*

To test for host size preference, *A. argentatus* were presented with three equal area webs spun by a large, medium and small *C. moluccensis*. The webs were inhabited by a C. moluccensis 6.35 centimeters big, 2.54 centimeters big, and one abandoned web previously inhabited by a host 1.27 centimeters big, respectively. The percent of cases where one size host was chosen over the others was analyzed for 15 two-hour trials. The test arena was constructed from three flowerpots. The flowerpots were all of diameter 26.67 centimeters. Each flowerpot had a section 12.7 centimeters by 15.24 centimeters cut out of it with mesh in place over the cut out. The mesh is taped in place by duct tape, which was also used to secure the three flowerpots together. Screen netting was carpenter glued onto the tops of the flowerpots and small rectangles 2.54 centimeters by 1.9 centimeters were cut of the screen. Foam was placed in the cut outs to control entering and exiting the system. (See *fig. 5B*) A large, medium and small *C. moluccensis* was placed in each respective flowerpot and allowed to build webs and feed. The small host disappeared before trials began and the flowerpot was left abandoned. Observations in the wild showed that when webs with small hosts were abandoned it took up to 2 weeks for the parasite load to change at all, so the container was left as it was.

### *Web Disposition Preference*

To test if parasites preferred colonial to individual hosts, *C. moluccensis* spiders were placed in the same set up as the previous test.

Another medium sized *C. moluccensis* was introduced into the flowerpot containing the original medium spider, and allowed to spin a web. *A. argentatus* were then introduced to the system through the abandoned container and allowed to select a host web. Eight trials were run for 2 hours each.

*Statistics*

Data were entered, and graphs of relationships were generated from Excel spreadsheets. SYSTAT 7.0 was used for all linear regressions, ANOVAs and Chi squared tests.

**Results**

*Host Abundance and Characteristics*

*Spider size*

All *Cyrtophora* hosts surveyed were between .25 millimeters and 63.5 millimeters.

*Height of tangle/ Area of bowl*

Linear regression suggests that spider size was not significantly related to web area, however spider size did produce positive results when compared with the height of tangle (R squared = .275 P = .003). (*see Figure 6.*) Height of the tangle and the area of the bowl are significantly correlated (R squared = .990 P = .001). (*see Figure 7.*)

*Disposition of web*

15 webs in colonial associations and 15 individual webs were surveyed. There was a significant difference in the average number of *A. argentatus* per web between webs of different dispositions (R squared = .181, P = .019) according to a One Way ANOVA. On average, individual webs had 5 kleptoparasites, while colonial webs had 3. Colonial and individual webs differed in the amount of time the hosts spent in feeding behaviors (R squared = .129 P = .051), however they did not show significant differences in the amount of time hosts spent in aggressive behaviors, web area or the amount of egg cases present on web.

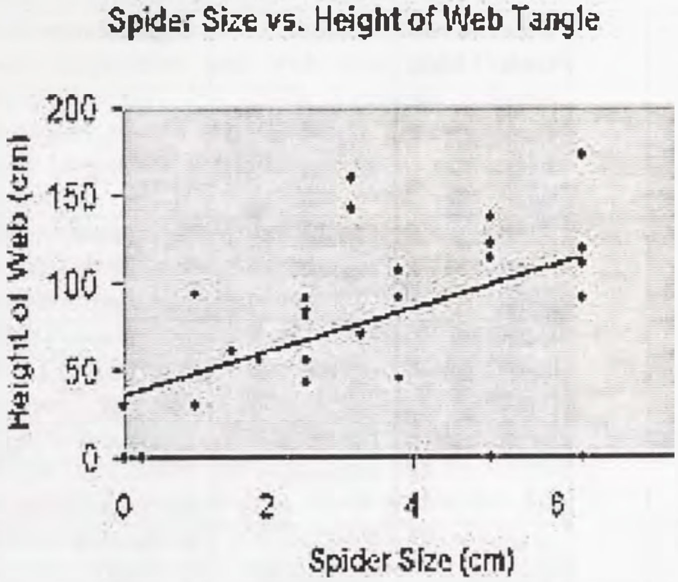

**Figure 6.** This graph shows the relationship between the height of the C. moluccensis web as measured in centimeters as compared to the size of the C. moluccensis inhabiting the web. Results show that this is a significant comparison:*(R squared = .275 P = .003).*

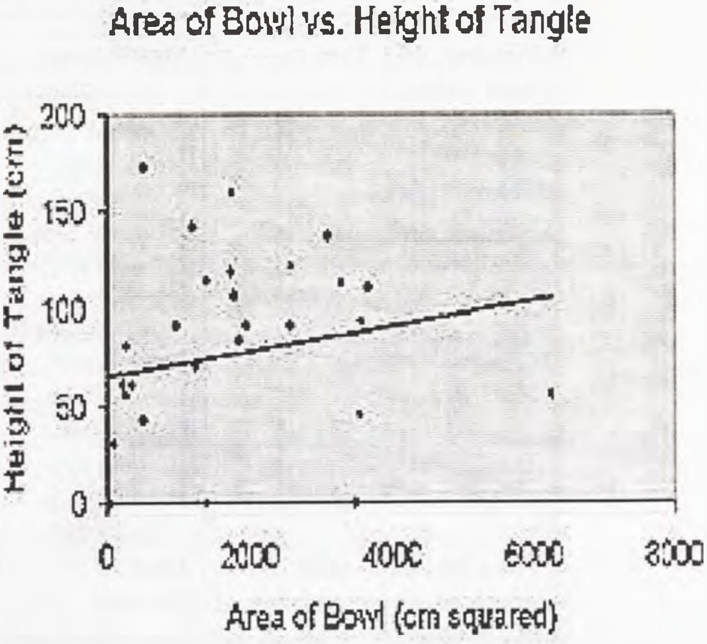

**Figure 7.** This graph shows the relationship between the area of the bowl portion of the C. moluccensis web and the height of the tangle portion. Results show that this is a significant comparison: *(R squared = .990 P = .001)*

*Behavioral Observations*

Values for the amount of time *C. moluccensis* spent in behaviors as measured in thirty-minute intervals were not normalized with an arcsine/square root equation. Even though the values were percents they already formed a normal distribution, and thus did not need to be transformed.

The percent of time the host spent in aggressive behaviors shows a significant relationship when compared with *C. moluccensis* web area. (See Table 1.) It also shows a significant difference when compared to general host sizes (small, medium, large)

The percent of time spent in aggressive behaviors as compared to a continuous scale of host size, the average number of *A. argentatus* per web, the amount of egg cases per web, and the disposition of the host web as colonial or individual were not significant.

The percent of time the host spent in feeding behaviors is not significant between individual and colonial webs. (See Table 2.) It shows no significance with general or continuous host size, web area, the average number of *A. argentatus* per web, or the number of egg cases present on a web.

*Ideal Web Size/Host Distribution/Prey Amount*

*Ideal web size*

*C. moluccensis* spiders over 5 cm had much larger webs in a lab setting as opposed to in the wild. Spiders between 2.5 cm and 5 cm had around the same area webs in both the wild and the lab. Spiders smaller than 2.5 cm had much smaller webs in a lab setting than in the wild.

*Ideal host distribution*

In the lab, all Large and Medium spiders relocated two times. A Medium and a Large *C. moluccensis* assorted together, as did a Small and a large host spider. The Small spider relocated its web 5 times.

*Ideal prey amount*

*C. moluccensis* in the wild caught an average of one prey item every 2 hours. In the lab, C. moluccensis spiders of any size would only accept one prey item a day. Extra prey items dumped on the web were either abandoned and discarded or ignored long enough to escape the web.

| **Percent of host time spent in aggressive behaviors vs..** | **Test** | **Significance** |
|---|---|---|
| Continuous Size of host | Linear regression | R- squared=. 120<br>P=. 071<br>Not significant |
| General Host size | ANOVA | R-squared = .214<br>P = .039<br>**Significant** |
| Web Area | Linear regression | R-squared =. 268<br>P=. 003<br>**Significant** |
| Average *A. argentatus* per Web | Linear regression | R-squared = .006<br>P=. 673<br>Not significant |
| Individual Web vs. Web in Colony | ANOVA | R-squared = .087<br>P = .1113<br>Not significant |

**Table 1.** This table shows the results from comparisons between the percent of time hosts spent in aggressive behaviors and several different factors. It lists the factors compared, the results, and the test used.

| **Percent of time host spent in feeding behaviors vs..** | **Test** | **Significance** |
|---|---|---|
| Continuous Size of Host | Linear regression | R-squared = .062<br>P =. 201<br>Not significant |
| General Host Size | ANOVA | R-squared = .043<br>P =. 553<br>Not significant |
| Web Area | Linear regression | R-squared = .032<br>P =. 332<br>Not significant |
| Average A. argentatus per Web | Linear regression | R-squared = .024<br>P =. 407<br>Not significant |
| Individual vs. Colonial web | ANOVA | R-squared = .129<br>P =. 051<br>**Somewhat significant** |

**Table2.** This table shows the results from comparisons between the percent of time hosts spent in feeding behaviors and several different factors. It lists the factors compared, the results, and the test used.

*Web Size Preference*

Results from the lab experiments did not support the idea that *A. argentatus* had any significant bias for web size. (Chi squared: Prob = .071) However, linear regression of results from the field finds significance between the average number of A. argentatus per web and the area of the web. (Linear regression; R squared=. 987 P=. 003).

*Host Size Preference*

Results from lab tests on *A. argentatus* preference for host size were very significant. (Chi squared; value 14.8, P = .001). Results from the field contested this however, finding for both a continuous scale of host size (linear regression; R squared =. 489, P = .126) and a general catalogue of Large, Medium and Small spiders (ANOVA; R squared = .146, P = .119) there was no real significance to the association.

*Web Disposition Preference*

Results from lab tests on web disposition preference by *A. argentatus* did not provide any significant relationship between the data. (Chi squared; Prob = .480). Likewise field data was found to be not significant between host web disposition and web area (ANOVA; R squared = .047, P = .248). Averaging web size, however gives a slightly larger a web area and web height for individual webs as opposed to colonial.

Web disposition was not found to be significant with respect to the number egg cases present on web (ANOVA; R squared = .058, P = .202). However, there was a significant difference in the general size of the hosts in individual as opposed to colonial webs. (ANOVA; R squared = .216, P = .010). Averaging the values obtained in the field, individual host webs had an average size of 2.55 centimeters. Hosts within a colony had an average size of 4.3 centimeters.

Likewise there was a significant difference in the amount of parasites per web between colonial and individual webs. (ANOVA: R-squared = .181 P = .019). Averaging the values from the field found that on colonial webs there was an average of 3 kleptoparasites per web at any given time, while for individual webs there was an average of 5 kleptoparasites.

## Discussion

In the field, *Cyrtophora* webs were observed to have an extremely high turnover rate. For spiders less than 4 centimeters in size, webs were abandoned approximately once every 2 and a half weeks. Webs were continually filled when abandoned if within a traversable distance from another *Cyrtophora* web, or if the original web contained multigenerational webs. It is not known to what distance immigration remains a profitable expenditure and at what point the immigrating host is far enough away that the benefits of immigrating to a new web are outweighed by the costs of moving. Generally succession proceeds with the largest spider within a given distance, although replacement only seems to occur if the spider is moving to a larger web. The replacement of hosts reflects a cost and benefits tradeoff between the size of the spider immigrating to the abandoned web, the size of the web to be abandoned if the spider moves, the size of the web to be gained, and the distance between the source of the immigrating host and the abandoned web.

Depending on one or more of these factors, the replacing spider on a web may be uncharacteristic of the original inhabitant. Webs spun by 4-centimeter spiders may be occupied by a 1-centimeter spider, depending on the composition of neighboring webs and the distance from them. Therefore, one would not expect a significant relationship between host size and web area. However, web height, area of bowl, and other architectural features do group significantly as a result of a single original web-builder.

At its creation, the web was a balance of host size, maximum prey catching surface area, and minimum energy expenditure. If in the field, webs had been manipulated such that all host webs were torn down, and hosts forced to rebuild, one would expect a greater degree of significance between the two variables. These conclusions agree with previous studies between *A. layuensis* and a similar orb weaving host spider, *Nephila plumipes*. (Grostal, Walter, 1999). This study found significant correlations between host body length and orb diameter, but only after the spiders had been forced to rebuild webs. Likewise work by Elgar (Elgar 1989) found positive relationships between the average numbers of *A. fissifrons* on the webs of *Psechrus* *argentatus*, a similar orb weaver spider, as compared to orb area and height of tangle.

In the lab setting, the ratio of spider size to average web area is different from that in the field. Small spiders tend to be associated with significantly larger webs in the field than in the lab. This could reflect the natural turnover rate in the wild, such that generally smaller spiders attempt to maximize their natural web building limits by becoming the most mobile and open to web replacement opportunities. However, some studies suggest that young orb weaving host spiders suffer the most heavy degree of silk consumption by parasites. Building a larger web in the presence of parasites could buffer the effects of silk loss, and provide a larger prey catching surface so that competition between the host and parasite for prey items wasn't limited to a small area. (Grostal , Walter 1998)

Medium spiders maintain about the same ratio of web area/ body size in both lab and field examples. This could be taken to signify that generally, medium body size honestly correlates with web area. The replacement rate of hosts may be the most stable at this phase. Spiders are able to establish a decent foraging area and control it most efficiently. Web succession certainly may happen, but at a lower frequency than smaller spiders. To accompany these hypotheses, medium spiders had the highest variance for web area values. They had very little variance in size, with no significant difference between all samples used in analysis.

Larger spiders have a much greater ratio of web area to body size in the lab as opposed to in the wild. This suggests that in the wild, larger spiders are constrained by some variable to keep their webs smaller.

The pattern of preferred web size on a scale from a small host to large follows roughly a normal distribution. This means that in the relationship of web area to host size there is an intermediate ideal point where the size of the host is best correlated to the size of the web. Ratios of web bowl area to spider size show that there is a peak of web area at a medium spider host size (1-2 inches) that maintains the most appropriate balance in between access to the most resources and the ability to control and manage these resources.

Web size and host size, are valid variables to consider in formulating alternate hypotheses, however several factors showed positive results when compared with the amount of time in a half hour interval, the host spider spent in aggressive or feeding behaviors. Aggressive behaviors coincided with host size and host area, meaning larger spiders, with a larger section of web to control and defend, are ultimately more aggressive. This supports the idea that kleptoparasites may be distributing on the basis of either web size or host size. This is assuming however, that a *C. moluccensis* is more aggressive when there are more kleptoparasites, however, this comparison was not significant, suggesting that there might be other reasons for the hosts to be aggressive. Interaction with conspecifics, defense from predation and web maintenance are some possible hypotheses as to why this pattern might be observed. Other studies have quantified the same aggressive behaviors with respect to the host but have not gone into analyzing them with different factors. (Severinghaus, Tso, Liu 2000). They do suggest however, that the behaviors are not very indicative because they find no real affect on host foraging by parasite presence.

Feeding behaviors seem to primarily relate to factors that were not tested in this study. The only significant results involving the time the host spent feeding was a slight difference between the hosts of individual webs as compared to colonial webs. Several studies have found a significant difference in prey species selected by both host and parasite, (Grostal, Walter 1998), (Severinghaus, Tso, Liu 2000) meaning the two species wouldn't complete over food. For the purposes of this study, however, observations support that they forage primarily on the same suite of prey items.

Web associations suggest several factors that could heavily influence the nature of the kleptoparasites relationship with the host. Because *C. moluccensis* is not obliged to be in a colony, differences between individual webs and colonial webs may help to distill what factors are important in determining the relationship between the two species. Results from fieldwork find that there is significance between the web disposition and host size, as well as average numbers of kleptoparasites per web. Colonial webs were found to have significantly larger *C. moluccensis* associated with them, as well as less kleptoparasites. Studies by Canglialosi (Canglialosi 1999), find the same results in studying another orb weaving spider, *Anelosimus eximus* in its association with *A. ululans*. Likewise, the trend of more kleptoparasites in individual webs as opposed to colonial is supported by studies using *Cyrtophora citricola* and *Argyrodes gibbosus*. (Leborgne, Cantarella, Pasquet 1998). So obviously there is some factor that spans species, and time sampled and area sampled in, to create this overall trend.

So why does this system allow for parasitism? Why are parasites in this location specific to only these types of webs? Allowing parasites on a web certainly must harm the direct fitness of the host by decreasing the amount of prey available, increasing the amount of energy spent in defense and lowering the foraging and survival opportunities of any offspring; however, it must also be to some advantage. If not a direct advantage, then at least in a indirect sense, that by allowing parasites to inhabit the web, hosts are able to spend less energy controlling the web and keeping it safe, and their relative fitness is not damaged by making it harder for immigrating small offspring to associate with a web. Smaller spiders are observed to be greater opportunists than other size spiders. With such a high mortality rate, it would seem evolutionarily savvy to try the most survival strategies possible and easy detachment from the original web associated with a small sized spider would provide the most opportunities to better the foraging surface area available while expending the least energy. The dispersal patterns of small *Cyrtophora* spiders should be similar to those of the parasitic *Argyrodes*. Essentially, smaller *C. moluccensis* spiders were observed to live as facultative parasites off the larger spiders.

In the wild, parasite load also makes roughly a normal distribution, much like the graph of the fitness of the host as compared to web area. Most parasites seem to occur in the ideal host state where there is the best balance between limiting factors. One might assume that this is because parasites are able to enjoy the largest surface are for prey capture combined with the most lenient control, but again, when theorizing why the middle state may be ideal for the host, the issue of control arose. Is the host benefiting by being able to enjoy the largest surface area possible to control, or are there different factors at work?

Results from this study support that the nature of kleptoparasitism depends on several factors, most significantly host web size, host body size, host web disposition and host behaviors to some extent. Although these results are all significant, none of them seem to concretely explain what factors create the relationship between the two species observed. Most likely, the kleptoparasite-host relationship between *A. argentatus* and *C. moluccensis* is based upon a combination of these factors working together. Other variables could also play a role in the complex mechanism that regulates the kleptoparasitic relationship between the host and parasite. Some other factors which would be interesting to study are parasite size distributions, sex ratios between both the host and parasite, age of the host and parasite, and the length of web association by the parasite.

Results from this study could be used to analyze the evolution of sociality in spiders. Although *C. moluccensis* would not be classified as a social invertebrate, it is a facultative colonial spider. Studying the effect of kleptoparasites could begin to shed light on the factors which influence the efficiency of social life on web building, offspring rearing, prey capture and defense.

This study could also be used as a springboard to do more concrete cost and benefit analysis of the effects of the host-kleptoparasite interaction. To truly understand the dynamics of the association between the species, and thus derive what makes the relationship function as it does, finite measures of energy tradeoffs inherent in the relationship would be important.

Likewise, further investigation of the factors which influence the segregation of *C. moluccensis* into individual webs or colonies, as well as more information on web succession and juvenile dispersal patterns would be of interest to expand upon the aforementioned ideas of offspring immigration creating opportunities for web kleptoparasites.

### *Conclusion*

Web kleptoparasitism is a fascinating phenomenon. Although the interaction is elegantly exemplified in spiders, the relationship occurs across taxa. This study helps to determine why this interaction is as it is. It explains some of the factors that promote such a unique form of symbiosis by analyzing the effects of host web size, host body size, host behaviors and host web disposition on parasite distribution and density, using *C. moluccensis* and *A. argentatus*. In studying the results of this paper, it becomes clearer how complex the interaction is, and how, although each one of these factors are significant, no one factor is wholly responsible for the interaction occurring.

This field and lab study provides a basic understanding of the factors that create kleptoparasitic associations. To truly understand the interaction, cost and benefit analysis would be helpful, and a better concept of *C. moluccensis* behavior, especially in a colonial setting.

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# The Distribution of Skinks (*Sauria, Scincidae*) Within the Habitats of Moorea, French Polynesia

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Abstract: The introduced skinks (Sauria, Scincidae) of Moorea, French Polynesia are unevenly distributed within the island's major habitats. In a four-week study, October 22 to November 18, 2002, a total of 369 skinks, including three species, (*Emoia cyanura, E. impar* and *Lipnia noctua)* and one melanistic morph of *Emoia*, were found to coexist in different proportions through coastal, residential, mountain forest and summit forest habitats. An overlap of species in each of the habitats suggests the speices are partitioning microhabitats within the vegetation zones. The mechanism behind the partitioning, whether it is due to abiotic preferences or species interactions, is not known.

Keyword: *Scincidae*, habitat distribution, resource partitioning, Oceania lizards, Moorea.

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## Introduction

When in sympatry, a niche overlap between morphologically and, therefore, ecologically similar species (Losos 1990) can result in interspecific competition for limiting resources (Gausse 1934). Competition results in decreased growth and reproductive rates, or increased death rate of all species involved (Bergon et al. 1990). In able to coexist without competition, species partition available resources (Fatuyma 1998). Habitat, food and time are the three most important dimensions that sympatric species partition in order to reduce competition (Pianka 1977). For example, Triclads and leeches partition live or damaged foods in British lakes (Seaby et al. 1996). Nocturnal and diurnal species of spiny mice in Israel divide foraging times to avoid competing (Jones et al. 2001). Although different taxonomic groups differ in priorities, most coexisting reptiles partition their habitat (Toft 1985).

Snakes are an exception to the generalization that reptiles segregate habitats to coexist (Toft 1985). In many ecosystems, snakes are the top predator of the food chain (Hairstone 1960). There are many specializations adapted to snake feeding behavior, especially ingesting large prey items, such as venom and constriction (Toft 1984). In this, snakes are highly dependent on their food type (Mushinsky et al. 1982). However, lizards are not specific on food type, and are described as being opportunistic feeders (James 1991). Therefore, lizards are not as likely to partition specific foods as they are the habitats where they can attain the most food. For lizards, habitat partitioning leads to a distribution of vegetation zones such as forests or grasslands, the microhabitats within the same vegetation zone such as the ground, tree trunk or rock out-cropping, or structural habitats like differing perch branch height and diameter of the branch (Schoener 1983) For example, in the semi-arid region of Northeastern Brazil, lizards are distributred by vegetation type (Vanzolini 1974). Likewise, near the blackwater tributary of the Orinco and Suromin rivers in Venezuala, different lizard speices occupy different structures of a tree (Ellinger et al. 2001).

However, unlike these extremely diverse continental areas, small islands typically have much fewer species than their adjacent mainland. According to the equilibrium theory of island biogeography, species number on islands decreases with a distance from the mainland and decrease in island size (MacArthur & Wilson 1967). For example, the tropical nation Vietnam hosts over 284 species of squamates, 112 species of lizards alone, nearly nine species per square kilometer (330,000 km$^2$ total area, Darevsky 1999). In contrast, the equatorial Solomon Islands just southeast of Vietnam host 76 squamate species (37,694 km$^2$ area, 0.002 species/km$^2$), and the Polynesian Island Samoa holds 15 squamates (3033 km2 area, 0.005 species/km$^2$, Bauer 1999). Yet, despite the relatively low species number, evidence of habitat segregation does exist. In Fiji and Roratonga, Cooks Islands, skinks species distribute forest and coastal areas (Zug 1991, Bruna and Case, 1996)

Moorea (17°30'S, 149°50'W) is a small (area 130 km$^2$) French Polynesian island near Tahiti (Map 1). The UC Berkeley Museum of Vertebrate Zoology contains specimens of three species of gecko and five species of skink from Moorea. No literature suggests the presence of any additional squamate species on the island. All species on record are introduced and common throughout Oceania. Skinks appear to thrive in Moorea, as they are easily seen scattering on the ground throughout the day. The high number of skink individuals may be a result of there being little known lizard predators. The only noted lizard predators on Moorea are cats (*felis domesticus)* Indian mynah (*Acridotheris tristis*) and dogs (*Canis domesticus*) (Bruna et. al. 1996). In contrast, islands like Hawaii and Fiji that have introduced snakes and mongoose, both renowned *squamate* predators (Zug 1991, McKnown 1996).

In this study, I test the likelihood that species compositions in each of the four major vegetation zone of Moorea differ by chance. A lack of difference between the species ratios of each habitat supports the null hypothesis that skinks are evenly distributed throughout the island. In this, either an absence of limiting resources allows the skinks to coexist without competition, or the skinks are partitioning other resource dimensions such as food or time. In contrast, significant results imply that the skinks are unevenly distributed throughout the habitats.

## Materials and Methods

Habitat

Based on preliminary observational study, Moorea was divided into four main vegetation zones: coastal, residential disturbed, inland forest and summit forest. The characteristics of each zone were designed in order to identify features that might be important explaining skink distribution. GPS coordinates of a position within each habitat are also given.

Coastal: The coastal habitat (17°31.261'S, 149°46.902'W) lies between the ocean water and the island's perimeter road. The land is flat, never rising more than one meter above sea level. Two main microhabitats form the coastal habitat, the rock shelf wall and the coconut/hibiscus grove. The man-made wall formation consists of five to six large, black igneous rocks piled out of the water. Where land has not been shaped by development, coconut groves remain. The vegetation of the grove consists of mostly coconut palms (*Cocos* spp), along with some small *Hibiscus* spp. and *Pandanus* spp. trees. The canopy reduces a minimal amount of sunlight. The consistency of sand beneath the trees varies, harder, more compact sands being further from the shore. Piles of coral rubble (on the intertidal line), fallen coconuts and coconut palm leaves are found on the ground throughout the habitat. Also, crabs burrow into the harder sands, although interactions between these crabs and skinks, if any, are not known.

Residential Disturbed: This zone (17°31.331'S, 149°47.308'W) covers the majority of land between the perimeter road and mountain forest. All native vegetation in this zone was cleared for development and agriculture (banana, *noni*, pineapple, mango, etc.). In areas of higher residential density (closer to the sea), most land is covered with grasses of varying height, scrub and bushes. *Hibiscus* and mango trees are also scattered throughout the area. Most agricultural land is just inland of main housing areas. The residential zone has minimal canopy cover, although dense grasses and bushes offer relief from sun.

Mountain Forest: The inland mountain, or *Inocarpus* forest rises from sea level inland of the residential zone to the summit. Large, tall, *Inocarpus* trees, easily identified by their heavily folded trunks, dominate the canopy allowing only splotches of sunlight to reach the ground. The ground is covered with fallen *Inocarpus* and *Hibiscus* leaves, and very little plant life.

Summit Forest: The summit (17°31.120'S, 149°48.085'W, 340 m high at lowest point) runs parallel to the coast, connecting the tall, steep mountain peaks. The vegetation of this zone is much more dense that that of the neighboring *Inocarpus* forest. On the summit, the large *Inocarpus* have not replaced the shorter, narrower *Pandanus* and native *Metrosideros.* Tall grasses, short ferns and dense bushes also cover the ground, making it difficult to maneuver anywhere but on the trail. Grounds are more exposed to sun than in the *Inocarpus* forests, and strong winds often blow over the summit ridge.

Experiments were held on the Vaiare coast and on the Vaiare side of the Vaiare-Pao Pao trail (Map 2). I chose this site based on the relative compactness of each of the four major vegetation zones (Map 3). The town Vaiare, the Island's largest ferry dock, lies on the east coast of Moorea, facing Tahiti. Directly inland of the coast lies the summit ridge. The Vaire-Pao Pao trail starts off the road, 100 meters south of the ferry. The trail runs through a residential section, up and over the mountain.

**Map 1: Moorea, French Polynesia,Oceania**

**Map 2: the Vaiare- Pao Pao trail on Moorea**

Data Acquisition

I acquired the data for four weeks, from October 22 to November 18. Every week, I visited each of the four habitats on different days. For one week, all data acquisition began at 8:00, while data acquisition began at 12:00 for another. For the other two weeks data acquisition began between 10:00 and 11:00. Each day I would walk through a habitat for four hours, a total of sixteen hours in each habitat. I divided the four hours evenly into active search, consisting of walking and turning over leaves, logs, coconuts, etc, and passive search, sitting quietly and observing. Lizards were spotted by the naked eye and identified on site by use of *A Field Guide to Reptiles and Amphibians of the Hawaiin Islands* (McKnown 1996) and specimen photographs from the UC Berkeley Museum of Vertebrate Zoology. Those lizards not identified on site were described and identified by additional sources (Ineich and Zug 1991, Bruna et al. 1996, Guillaume et. al 1994). Upon identification (or description), individuals were tallied as their respective species.

Data Analysis

The number of individuals of each species was recorded in a table. The Pearson Chi-square and Likelihood Chi-square analysis (SYSTAT) were used to test for a disassociation of species ratios within the different habitats.

## Results

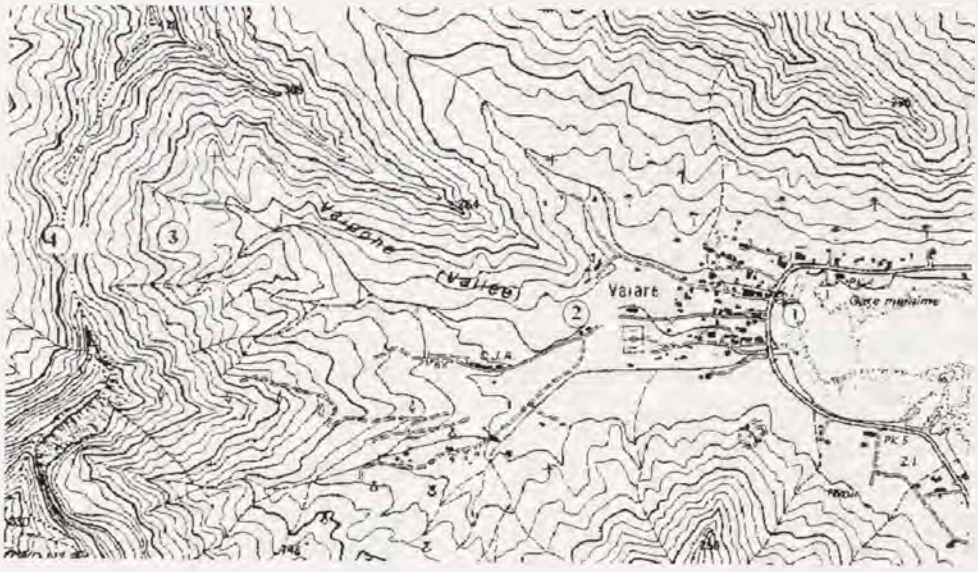
Three previously recorded species were identified throughout the research period. The striped morph of cryptic species *Emoia cyanura* and *Emoia impar* were found in all four habitats. The solid brown, "melanistic" morph of these *Emoia* species was seen everywhere except on the coast, and *Lipnia noctua* was found everywhere but on the summit (Table 1).

The mountain forest housed the largest skink population (154 total skinks, 9.625 skinks per hour); the summit held the lowest number of skinks (29 total, 1.825 skinks per hour). The coast (61 skinks, 3.8125 sk/hr.) and the residential (125 skinks, 7.8125 sk/hr.) areas each held intermediate amounts of skinks (Chart 1, Figure 1). Using the four morphs as four separate species, The Pearson Chi-square and Likelihood Chi-square analysis both resulted in a P-Value less than 0.001 in both cases (Chi-square value 53.4, 9df; Chi-square value=67.006, 9df).

## Discussion

Statistics support the hypothesis that skinks distribute themselves unevenly through the four major vegetation zones on Moorea. The ratio of species composition differs with high significantly (P-value<0.001) between each of the habitats. In this, each species must prefer certain vegetation zones to others.

The large amount of lizards found in the mountain forest and the residential area is probably due to a higher holding capacity in these zones compared to the coast and the summit forests. The mountain forests and residential area must therefore hold the most resources demanded for skink survival. Compared to the coast, the mountain forest and the residential area certain contain more microhabitat opportunities, such as heavy leaf litter and many types of trees and other vegetation. The summit forest could be too cold for most skinks do to constant winds, could have



**Map 3: The field sites on the Vaiare coast and Vaiare-Poa Poa trail. Each circled number is within the area of one of the four vegetation zones surveyed. 1=Coast, 2= Residential, 3= Mountain forest, 4=Summit forest.**

**Table 1: the number of individuals of each species found in each of the four habitats.**

TOTALS

Species By Habitat

| Species | Coastal | Residential | Mountain | Summit | Total |
|---|---|---|---|---|---|
| *Emoia cyanura* | 32 | 38 | 27 | 8 | 105 |
| *Emoia impar* | 27 | 49 | 56 | 17 | 149 |
| Melan. *Emoia* | 0 | 27 | 45 | 4 | 76 |
| *Lipnia noctua* | 2 | 11 | 26 | 0 | 39 |
| **Total** | 61 | 125 | 154 | 29 | 369 |

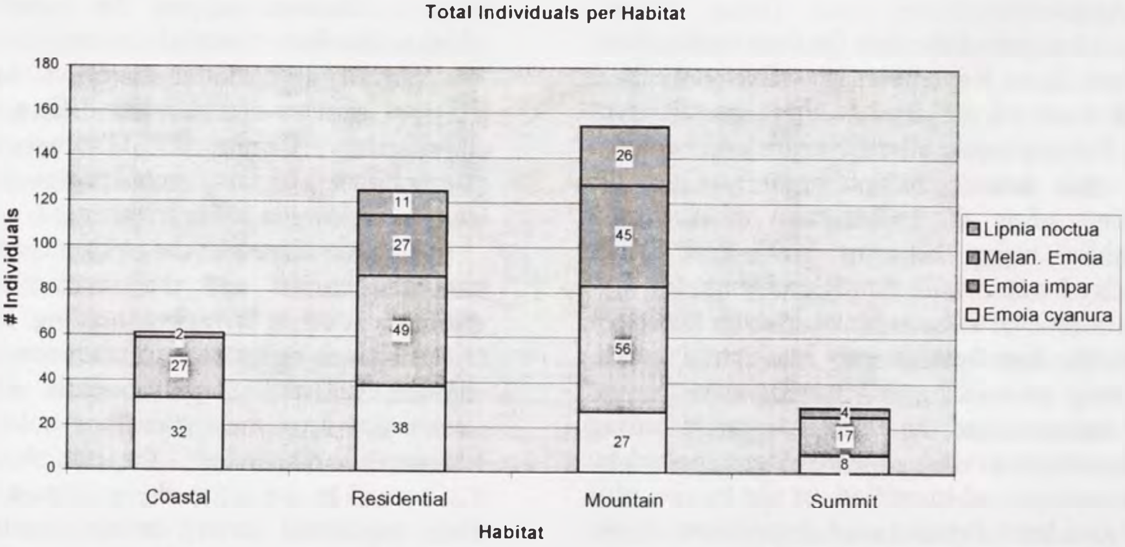


**Figure 1: The total of each skink species found in the four different habitats. Bar values display number of individuals of that species found.**

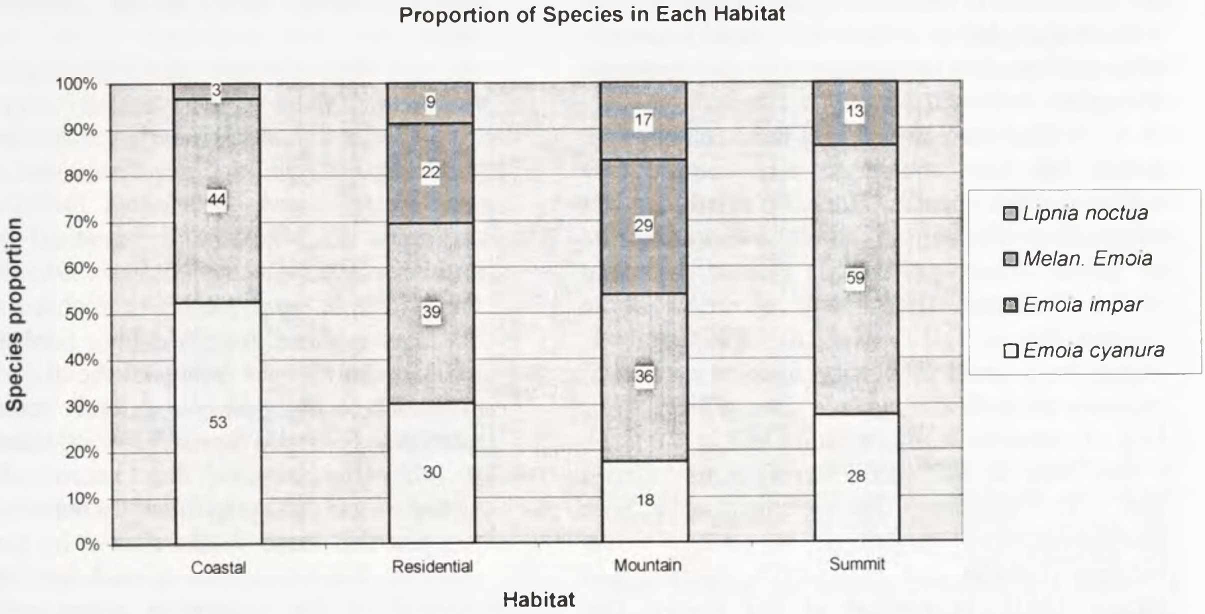


**Figure 2: The species composition of each vegetation zone. Bars represent the total skink assemblage for that habitat. Each section of the bar is the ratio that species represents in the assemblage. Bar values display ratio values of each species.**

less edible insects, or have less habitable vegetation than the other habitats.

Although populations of *E. cyanura* extend through all four habitats examined, the skink is most succesful in the coastal zone, suggesting a high preference for this habitat (Figure 2). The coast was also the only zone where the majority of skinks recorded were *E. cyanura*. These findings concur with results from similar research in Rarotonga, where the catch per unit area of *E. cyaunura* was significantly higher on the beach and disturbed habitats than in the forests (Bruna and Case, 1996). Although catch per unit area was not tested in this experiment, twice as many *E. cyanura* were tallied on the Moorean coast and residential (disturbed) area than in the high holding capacity forests (70:35 skinks, Table 1). Zug (1996) found *E. cyanura* to be the most abundant skink in the Fijian islands. Most of these *E. cyanura* were in sunny, disturbed habitats Zug (1996) suggests that this skink benefits the most from habitat change by man. Likewise, skinks are the only lizard found on Samoan shores and suburbs (Gill 1993). Although the reason is not known, *E. cyanura* certainly has a preference for the open, sun-exposed, coast and disturbed areas.

*E. impar*, or rather the "bronze" morph of *E. impar* (Bruna and Case 1996), distinguished from *E. cyanura* by its blue rather than greenish-brown tail (Ineich and Zug 1991, Guillaume et al 1994), also lives in all four Moorean habitats (Figure 2). Bruna, Case (1987) and Zug (1991) described *E. impar* as a primarily forest skink in Rarotonga and Fiji. Likewise, a higher number of *E. impar* was also found in the Moorean mountain forest (Table 1), however, this is probably because the forest simply has a higher holding capacity that any other habitats (Figure 1). Proportionally, *E. impar* is distributed nearly equally through all four habitats, although it does appear to be the predominant lizard in the summit. As the most abundant skink in the mountain forest, it is most likely that this lizard occupies the neighboring forest- especially since this summit offers some amount of the same resources, mainly varying amounts of shade and leaf litter. *E. impar* is the most abundant skink throughout Moorea, dominating all habitats except for the coast (Table 1). Bruna and Case (1996) suggest that either *E. cyanura* competitively excludes *E. impar* from the coast, or that *E. impar* is not able to tolerate coastal heat. In Samoa, Gill (1993) found *E. cyanura* and *E. impar* in the same forest microhabitats, but only *E. cyanura* on the coast. Once again, either *E. impar* is unlikely to live on the coast due to environmental factors such as intense sunlight, or a limit on coastal resources forces *E. cyanura* to compete with and dominate *E. impar*.

The taxonomy of the "melanistic" *Emoia* has been subject to much controversy within the last decade. Recently this lizard has morphologically and genetically been identified as an *E. impar* polymorph (Ineich and Zug 19991, Guillame et al. 1994). However, some suggest that not all "melanistic" *Emoia* are *E. impar*, but a small proportion of them may be *E. cyanura* as well (Bruna and Zug, 1996). For lack of certainty in the literature and in the field, I will refer to this lizard hereafter as "*Emoia* spp.". It is suggested that melanism serves as a thermoregulatory advantage in shady, forest habitats (Gibson and Falls 1979, Andren and Nilsen 1981). In support of this theory, this lizard was the only morph not seen in the sun-exposed coast Also, *Emoia* spp. was in highest proportion in the forest, where it nearly matched *E. impar* in numbers.

*Lipnia noctua* was the least abundant skink seen during fieldwork (Table1). However, the relatively low number of *L. noctua* may be a result of their less conspicuous behavior than other the skink species. Not much can be concluded be the skinks distribution within the habitats itself (Figure 1,2). However, some inferences can be made based on the microhabitats this skink was sighted in. Of all the Moorean skinks, *L. noctua* occupied the most diverse microhabitats Most *L. noctua* sat quietly and motionlessly within the folds of *Inocarpus* trees, sometimes as high as five meters high. In contrast, all three *Emoia* morphs scrambled loudly over dead leaves on the floor. In addition to *Inocarpus*, *L. noctua* was also found on horizontal rotting logs, especially in the residential area, and under dried coconuts on the beach. In Fiji, the abundance, distribution and habitat of this skink differ from island to island (Zug 1991). For the most part, the Fijian *L. nocuta* is a forest skink. However, *L. nocuta* lives on the forest floor on some islands, and high on trees on others. Due to it being both arboreal and terrestrial on New Guinea, Zweifel (1979) described *L. noctua* as a generalist, not highly adapted for any habitat. *L. noctua's* distribution and observations of the skinks relatively slow ground speed (compared to the other Moorean skinks) and climbing ability (compared to *Inocarpus* tree gecko, *Gehyra mutilata*) support this generalist theory. Due to the abundance of terrestrial skinks on Moorea, competition has likely forced *L.noctua* into the trees.

The absence of *Cryptoblepharus* spp. fom the field study further supports the hypothesis that the species are distributing the habitats. *Cryptoblepharus boutonni*, a species previously found on Moorea (MVZ), usually occupies dried coral and inter-tidal lava rock-outcroppings (Bruna et al 1996, Mcknown 1996). It is therefore believed that the species remains in these described inter tidal rock areas on Moorea and not in any of the major habitats. Therefore, *Cryptoblepharus* must remain in its preferred habitat where it faces little competition.

The results that ratios of species composition differ significantly between habitats suggest that these skinks coexist by partitioning habitat. The occurrence of each species through several of the vegetation zones, rather than clearly occupying only a specific zone, further implies that the species are dividing microhabitats present in more than one vegetation zone. For example, if one species prefers a specific amount of sun-exposure for thermoregulatory needs, then that species can live in any vegetation zone that offers microhabitats with the preferred sun-exposure, baring all other resource demands. It seems plausible, due to this study and past literature, that the three *Emoia* species partition the terrestrial environment due to sun exposure. Lizards have very narrow optimal temperature range (Huey 1982), and have been shown to partition microhabitats due to sun-exposure needs (Angart 2002). *E. caynura's* predominance in the coast and residential zones imply a preference for areas with more sun-exposure. In this, melanistic *Emoia* spp. would prefer minimum amount and *E. impar* would prefer an intermediate, omnipresent amount of sun-exposure. However, the thermoregulatory, much less any abtiotic demands of these species have not yet been measured and quantified. Therefore we do not know whether the species would independently distribute themselves to these habitats or not. Different limiting resources in different habitats could alter the species compositions. For example, *E. cyanura* could have a competitive advantage when a specific resource is limited in the coast. The same scenario could be giving *E. impar* the advantage on the summit.

Similar dilemmas exist for the generalist species (Zweifel 1979), the generalist

*L. noctua.* Based on this its microhabitats, it appears *L. noctua* can exist in any zones that offer habitable microhabitats away from the terrestrial, *Emoia* dominated, leaf littler. This slower moving species probably cannot compete with the swift and agile *Emoia*, and must live elsewhere. The *Inocarpus* trees, with their many folds and crevices, serve as the best, but not only, solution. However, competition may not be the mechanism behind *L. nocuta's* distribution. *L. noctua* may inhibit microhabitats that best suit its foraging strategy on this island. Other factors, such as reproductive needs, etc, may also be influencing the skinks habitat selection. Further investigation on the biotic and abiotic preferences of this and the other Moorean skinks must be made in order to properly assess the mechanisms of habitat selection.

## Conclusion

Despite the low diversity of skinks on Moorea, the ample resources, and the minimal threat of predation, these few species coexist by distributing themselves in the habitats. Future studies must go into the mechanism driving for this partitioning, whether it is preference or the result of species interaction. Insight on the reasons for habitat selection helps ensure the conservation of these and other island species throughout Oceania. Knowing the habitat preference of each species helps to predict the effects of introducing any of these lizards to other communities. Learning more on the competitive nature of these lizards will be especially helpful in conservation matters. If *E. Cyanura* does out-compete and exclude other lizards from the coast and disturbed areas, then clearing Pacific island land could result in the reduction, and elimination, of the islands herpetofauna diversity. These effects would be extremely detrimental on islands with native lizards like Fiji and Samoa. Increasing our knowledge is the only way to ensure conservation.

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# Size, Gender, and Coloration Effects on Territorial Behavior in the Amphidromous Goby *Sicyopterus pugnans*

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ABSTRACT. This study explored factors that confer territorial superiority in the amphidromous goby *Sicyopterus pugnans*. Heavier fish and males exhibit a higher frequency of aggressive behavior than lighter opponents and females, respectively. Physiological color changes convey information regarding the status of potential combatants. Experimental pairs of fish fought over a single shelter in freshwater tank. Records of passive and aggressive behaviors and time spent inside shelter were kept. Field observations in pools of the Opunohu River, Moorea, French Polynesia elicited distribution, coloration, and attack frequencies of *Sicyopterus taeniurus* and *S. pugnans*. While both species are algal grazers and do not spatially segregate, only male *S. pugnans* protects open territories. Female *S. pugnans* and large *S. taeniurus* individuals protect crevices and defend against aggressive attacks. In *S. pugnans*, presence of a horizontal stripe in females, an eye bar, and posterior vertical bars indicate aggressive dominance. A stripe above the mouth, loss of dominant coloration, and dorsal mottling indicate alarmed or submissive individuals. Coloration signals provide an energetically cheap and quick method of conveying information to potential opponents, thus preventing unnecessary injury and energy loss. Pools were located upstream from a weir that prevented the infiltration of *Anguilla marmorata, A. obscura, Kuhlia marginata,* and *Tilapia mossambica*. Low species diversity provided an environment absent of intense predation and interspecific competition. Increased population density and interactions between *S. pugnans* and *S. taeniurus* elucidate implications on distributional and behavioral theories of amphidromous fishes.

## Introduction

The continuous comparison of the risks and advantages of combat mediates the decision to initiate agonistic behavior. Aggression can protect an individual, its offspring, and the resources necessary to survive and reproduce (Archer 1988). Novel stimuli or major environmental change can elicit aggressive behavior in individuals (Archer 1988, Marler 1976). Groups commonly defend offspring from predators. Assemblages of blackheaded gulls guard their eggs by overwhelming oophagous crows (Kruuk 1964). Solitary common gobies defend their eggs against predatory eelpouts, despite possible risk of injury or death (Magnhagen and Vestergaard 1993).

Aside from preservation of self and offspring, many aggression studies examine the benefits of territoriality, defined as defense of a space (Maher and Lott 2000, Maher and Lott 1995). Ecosystems harbor biomass and energy necessary for survival and reproduction. Organisms that control the acquisition of resources garner more energy than non-territorial competitors. In addition to feeding territories, fishes defend lekking and breeding areas. In species that exhibit female mate choice, possession of a large breeding territory can attract fitter females. Females use the quality of a territory as a marker for the quality of the potential mate (Barlow 2000). Aggressively defending breeding territories against male competitors can help ensure a more fertile mate.

Success in agonistic encounters can also elevate the social status of an individual (Archer 1988). *Labroides dimidiatus* is a protogynous cleaner wrasse. The male maintains a harem and in the absence of the dominant male, the most dominant female undergoes a sex change and assumes control of the harem. The new male now reaps the fitness benefits of being a polygynous male (Barlow 2000). Dominant Harris sparrows accrue similar benefits by excluding weaker conspecifics from food supplies (Krebs and Davies 1987).

Aggressive and territorial behavior sustains not just benefits, but multiple costs. Fighting animals risk injury, energy and time expenditure, and death. Injury can shorten the lifespan of an animal or make it less desirable to potential mates (Broberg et al. 2000, Krebs and Davies 1987). Fights that do not cause injury, still incur costs. Gill and Wolf (1975) found that golden-winged sunbirds spend three times more energy defending a feeding territory than foraging for food. This energy loss is intensified because unlike feeding, fighting does not possess concurrent energy losses and gains. Additionally, fighting animals pay less attention to the environment, increasing the likelihood that predators will approach undetected. Carpenter and McMillen (1976) postulated a positive correlation between time spent fighting and predation risk in the iiwi, a Hawaiian honeycreeper.

Brown's (1964) theory of economic defensibility states that an organism weighs the costs and benefits of every single potential fight and decides whether or not the fight is worth the resources at stake. The presence of territoriality in fishes is based upon many environmental factors: population density, habitat topography, food quality, type, distribution, renewal rate, and predictability (Maher and Lott 2000). For example, if the location of a food source is unpredictable and sparse then territoriality of a specific location is unlikely to yield greater energy stores than non-territorial individuals that forage a large, unprotected space. Conversely, if food is exceedingly abundant than energy gains of a territorial individual are minimal compared to non-territorial individuals that can find food easily. If the energy gains are less than the energy necessary to defend a territory then aggressive defense of that space is not economical (Brown 1964).

Even in habitats that are economically defendable, aggressive defense does not always incur benefits. Individual opponents, as well as environments, must be continuously assessed to discern the most effective behavior. It is advantageous for a territorial fish to analyze its chances in a bout, thereby abdicating defeat if chances of injury are higher than winning (Suk and Choe 2001, Barlow 2000).

Fishes evaluate an opponent's markers of fighting ability at the beginning of a potential bout (Suk and Choe 2001). Direct markers such as size, gender, and prior residency convey information of an individuals fighting ability. Resource Holding Potential, or RHP, is the synthesis of all these markers and is a gauge of relative fighting ability (Archer 1988). Differences in RHP between opponents are inversely proportional to the duration and intensity of bouts (Olsson and Shine 2000).

Size is the most common determinant of relative RHP (Archer 1988). Size decides the outcome of competitive bouts among snail cichlids, painted greenlings, common gobies, Siamese fighting fish, and *Oreochromis mossambicus* (Brandtmann et al. 1999, DeMartini 1985, Suk and Choe 2001, Bronstein 1984, Turner and Huntingford 1986). In the Midas cichlid, weight differences as small as 2% can dictate the outcome of a fight (Barlow 2000).

Organisms that hold territories possess a fighting advantage over intruders, known as the prior residency effect (Krebs and Davies 1987). Resident lizards, *Niveoscincus microlepidotus*, beat intruder conspecifics in 72% of experimental bout, even when size differences were negligible (Olsson and Shine 2000). Two theories explain the advantage of holding a territory in combat. The territorial owner is more familiar with the immediate surroundings and can advantageously use local microtopography. Owners also have more incentive to stay and win a fight. Intruders have invested less energy in keeping territories; therefore they are more inclined to abdicate an escalating fight (Colgan 1983, Olsson and Shine 2000, Lozano and Lemon 1999, Rohwer 1982).

Gender may infer fighting advantages, either by differences in aggression level or physical differences in sexually dimorphic species. Magurran and Garcia (2000) note increased male aggression in polygynous species, while females may be more aggressive in monogamous or polyandrous species. However, these species primarily exhibit intrasexual aggression. Painted greenling, *Oxylebius pictus*, males principally fight other males during the breeding season, while females fight females chiefly during the nonbreeding season (DeMartini 1985). The Olympic mudminnow alters its aggression levels temporally, concentrating bouts in the breeding season (Hagen et al. 1972).

Coloration can be a badge of RHP, indirectly representing an individuals fighting ability. Physiological color change in fishes can be neurally or hormonally modulated. In stressful situations, hormones induce behavior concurrently with color change (Fujii 1993). Associations between social status and coloration give fish visual information regarding an opponent's history and ability. Honest coloration badges evolve in species that exhibit: interference competition, variation in ability, frequent confrontation, and minimal individual recognition abilities (Rohwer 1982). These species constantly require information on potential opponents, but are unable to efficiently gather it from size or memory. Dominant and submissive coloration can provide fish data regarding the social and aggressive status of a fish, thereby preventing unnecessary fighting and energy loss. Oscars, *Astronotus ocellatus*, defeated in fights display a coloration that inhibits aggression in subsequent opponents (Beeching 1995). *Chromidotilapia guentheri* announces its intentions by producing vertical patches when aggressive and horizontal stripes when escaping conflict (Barlow 2000).

Males that defend breeding territories are often highly sexually dimorphic in coloration. Females sexually select males with coloration indicative of a dominant and successful mate (Kodric-Brown 1998). Male coloration serves the dual purposes of informing rivals to stay away and females of relative fitness levels. Female threespine stickleback mate preference increases with the intensity of the male's coloration (Baube et al. 1995). The intensity of the male's color positively correlates with size and dominance over other males (Kodric-Brown 1996). By choosing red-bellied males, female sticklebacks maximize their own fitness by choosing the most successful mate (Baube et al. 1995).

Amphidromous gobies have been noted to exhibit color change, sexual dimorphism and territoriality (Marquet 1988, Askay 2000, Mauge et al. 1992). Territoriality is rare among freshwater fishes (Barlow 2000). Food sources in rivers and streams are unpredictable and dynamic, changing in location over short periods of time. A primary requirement for feeding territories is the existence of a stable energy source. Any riverine fish that defends one place will usually exhaust its food supply over time (Maher and Lott 2000). Algal feeders are exceptions to the rule. In an environment where the stratum is relatively static, algal beds can remain constant, immobile sources of energy (Ribbink et al. 1983). If algal beds are static, then the resource heterogeneity of a stream encourages territorial behavior. Concentration of predictable food sources will attract many individuals to the same feeding ground. The stronger individuals can exclude others from these feeding areas and gain access to considerably more resources than excluded competitors, thus making algal beds economically defendable (Maher and Lott 2000, Barlow 2000).

The amphidromous goby, *Sicyopterus pugnans*, inhabits high elevation streams on volcanic islands (Marquet 1988, Askay 2000). The family Gobiidae is characterized by blunt or slightly pointed snouts, elongate bodies, two separate dorsal fins and fused pectoral fins (Thresher 1984). *S. pugnans* employs its pectoral fins as a sucker, allowing it to climb rocky substrate into pools inaccessible by swimming. On Moorea, a series of agricultural dams and weirs block the upstream infiltration of many non-gobioid fishes (Concepcion and Nelson 1999). Physical obstacles block predators, such as *Anguilla marmorata*, creating negligible predator pressure on many upstream populations of *S. pugnans* (Resh et al. 1999, Smith 1993). The lack of predators and the fact that *S. pugnans* grazes algae makes it a prime candidate for territorial behavior (Maher and Lott 2000). Additionally, *S. pugnans* has only one primary algal competitor, *Sicyopterus taeniurus*. Overlapping living space and a lack of observed niche partitioning combined with the large percentage of the river's total biomass attributed to these species may drive them to compete over space, interspecifically and intraspecifically (Marquet 1988, Askay 2000, Marquet and Galzin 1992).

Little is known about the territorial behavior of amphidromous gobies. Fishes that live in species poor environments in high concentration provide a natural test of territorial theory with relatively few confounding variables. The purpose of this study is to experimentally and observationally examine territoriality in *S. pugnans*. Previous observations by Marquet (1988) and Askay (2000) evidence the existence of territorial behavior. This study proposes to test factors that confer advantages upon territorial individuals: size and gender. Coloration is also examined to discover if different coloration states correspond with social status and could act as markers of fighting ability and readiness. I hypothesize that large and male fishes will dominate, marking their victories with distinct coloration patterns. The null hypothesis states that no physical attribute confers a fighting advantage and territoriality is random among fishes.

## Methods and Materials

### *Collection and observation site locations*

This study observed and collected *Sicyopterus pugnans* and *S. taeniurus* from the central, perennial fork of the Opunohu River, Moorea, French Polynesia (Figure 1 and 2). Study and collection sites were located on 1.5km of the Opunohu River, upstream of a weir located at the French Polynesian Agricultural School. Hibiscus trees dominate the terrestrial cover and canopy over the river. The river bottom is composed of basaltic rocks covered in the algae *Schizothrix mexicana, Mougeotia genoflexa,* and *Hildenbrandia rivularis* (Shiley 1992). *Sicyopterus* grazes for diatoms found in the algae (Marquet 1988). Pools were .3m-1.5m deep, evenly interspersed and separated by shallow riffles. This study was conducted from September 23 through November 17, 2002.

*Species description and identification*

I identified *Sicyopterus pugnans* (Ogilvie-Grant, 1884) and *Sicyopterus taeniurus* (Günther, 1877) using species descriptions in Maugé et al. (1992). Sean Askay, Mike Moser, and Vince Resh supplied additional photographic and descriptive verification. *S. pugnans, S. taeniurus* and *Awaous ocellaris* were the only gobioid species present upstream of the weir.

*Experimental setup*

I collected *S. pugnans* individuals using nets in pools of depth greater than .4m. Shallower depths would have prevented the use of a mask and snorkel. Once fish were sighted, individuals were chased into crevices and forced into nets. Females were easier to catch than males, therefore comprising the majority of collected individuals. I placed all netted fishes into one-gallon plastic bags filled with stream water. Fish were transported to the Richard Gump Biological Research Station where all specimens were measured for weight and standard length. I defined standard length as the distance from the tip of the snout to the caudal peduncle. Sex and color form identification were conducted in the field, because individuals lost identifying colorations immediately after capture.

After measurements were taken, fish were replaced into plastic bags and submersed in experimental tanks. Fish were left in tanks overnight. Each 10-gallon tank was supplied a continuous flow of freshwater and a single 1.5cm^3 basaltic rock was placed in the shelter entrance (Figure 3).

Experimentation began the day after all subject fish were captured. Immediately prior to experimentation, coloration of each fish was recorded. I recorded presence or absence of four points of dark coloration: the eye bar, mouth stripe, horizontal stripe and posterior vertical bars. The eye bar extends from the eye to the mouth and is perpendicular to the lateral line. The mouth stripe extends along the upper lip of the mouth and continues as a horizontal stripe along the lateral line. Male *S. pugnans* lacks dark coloration along its lateral line. Four primary vertical bars may also be present posterior to the anterior dorsal fin.

Immediately after I recorded coloration, 2 experimental fish were simultaneously released into each tank. Fish were initially observed for 20 minutes and all social interactions and visits to the shelter were recorded. I tallied number of aggressive and passive encounters, number of visits to the shelter, time spent alone in the shelter, and time spent together in the shelter for each fish. A visit to the shelter was recorded if the caudal peduncle of the fish crossed the threshold of the shelter. Table 1 describes the criteria defining various aggressive and passive behaviors. Additional records and 20-minute observations were taken 3 hours after introduction and the following day. A total of three 20-minute observations were made for each pair of fish. After experimentation, 10 experimental fish were digitally photographed and all fish were released upstream from the any collection or observation site the following day.

**Figure 1.** Map of Moorea, French Polynesia. Inset is the Opunohu River. The Opunohu River flows into Opunohu Bay.

**Figure 2.** Map of the three primary forks of the Opunohu River. Study and collection sites are highlighted in purple and ranges for 1.5km upstream of the indicated weir.

*Natural Observations*

Observations were conducted in pools at least .4m deep and downstream from all previous collection sites. Shallower pools

**Aggressive Behavior:** Active interference with a rival.
**Passive Behavior:** Any behavior where one fish swims within 2cm of another without displaying stereotyped aggressive behaviors or inducing aggressive behavior in the recipient fish

| *Aggressive Behaviors* | **Description** |
|---|---|
| Lateral display | The body is held rigid with spine slightly arched. Dorsal, anal and caudal fins are fully extended, as well as the spiny rays of the anterior dorsal fin. Fish is usually perpendicular to the recipient fish. |
| Charging | Usually performed after lateral display, subject fish lunges 1-10cm at the recipient fish's flank. Behavior repeated until recipient fish is chased from territory. |
| Biting | If neither fish will abdicate and leave the shelter, one of both fish will bite the flank of the other fish. If both fish are biting, aggressors adopt a C-shaped position and circle around each other, biting the opponents flank until one fish abdicates. |
| *Passive Behavior* | **Description** |
| Polar resting | Subject fishes rest parallel to each other in opposite directions. Subject fish usually display this behavior immediately after introduction into the experimental tank. |
| Quivering | Rapid shaking of the head from side to side. |

**Table 1**. Experimental criteria for aggressive and passive behavior in social interactions of *Sicyopterus pugnans*. Archer (1988) defined aggressive behavior. Barlow (2000), Hagen et al. (1972) and personal observations defined specific, stereotyped passive and aggressive behaviors.

prevented visualization of social groups with mask and snorkel. I conducted observations at the fringe of pools for 20 minutes. A census of size of all defended territories, sex, color form, and species was taken for each pool. I applied Maher and Lotts's (1995) definition of territory as a space in an individual's home range where it is aggressive and usually dominant to invading individuals. The radius of a territory was the average distance territorial individuals chased intruders from a central location. The center of a territory was defined as the location territorial individuals most commonly resided and returned to after chasing intruders. Aggressive behavior alone did not constitute territoriality. Non-territorial aggressive behavior was defined as defense of bodily space, independent of individual's location. Presence of territorial and non-territorial behavior was tallied as well as the sex, color form and species of the aggressor and recipient fish.

*Statistical analyses*

Means and 95% confidence intervals were calculated estimating average pool composition and territory size. Paired sample t-tests examined differences between the occurrence of males, females, orange form, blue form, *S. pugnans*, and *S. taeniurus*. Descriptive analyses were made on all other natural observations.

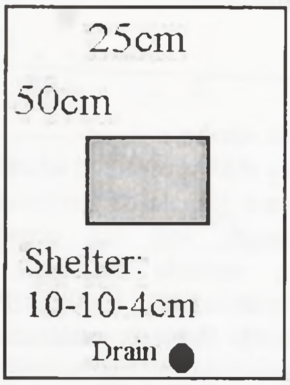

**Figure 3.** Diagram of experimental tank. Water flowed in and out of the tank at the drain. 2 fishes were placed in the tank and observed for 3 rounds of 20 minutes.

Correlation analysis was performed on standard length and weight in order to eliminate standard length as a confounding variable. Bivariate fit regression analysis was applied to independently compare relative attack frequency and time spent in the shelter with relative weight. A Kolmogorov-Smirnoff, K-S, one-sample test demonstrated if relative aggression was random regarding relative weight. Correlation analysis examined the effect of weight ratio on aggression. K-S two-sample tests analyzed the effect of weight on time spent in shelter.

| Relative Aggression | HIGHER | LOWER | HIGHER | LOWER | HIGHER | LOWER |
|---|---|---|---|---|---|---|
| df = 1 | Round 1 | | Round 2 | | Round 3 | |
| Vertical Bar | 4 | 4 | 11 | 6 | 8 | 4 |
| No Vertical Bar | 23 | 23 | 16 | 21 | 19 | 23 |
| | P = 1 | | P = 0.143 | | P = 0.190 | |
| Horizontal Stripe | 13 | 13 | 14 | 10 | 16 | 7 |
| No Horizontal Stripe | 9 | 9 | 8 | 12 | 6 | 15 |
| | P = 1 | | P = 0.226 | | P = 0.007 | |

**Table 2.** Presence or absence of vertical bars and horizontal stripe in paired combatants based upon relative aggression. Fishes possessing higher relative aggression levels attacked more frequently than their opponent. Chi-square tests calculated the significance of coloration differences between combatants.

K-S two-sample tests and distributions evaluated differences in attack frequency between the sexes and permanent color phase. A paired student's t-test analyzed aggression frequency by sex in male-female bouts. Chi-squared statistics tested for a difference in frequency of four coloration patterns (mouth stripe, eye bar, vertical bar, and horizontal stripe) exhibited in winners and losers. Excel 2000, JMPin 4.0.4, and Systat 7.0 performed all statistical calculations.

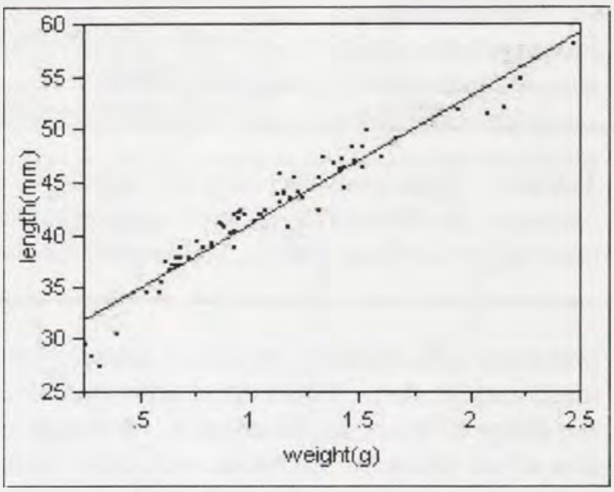

**Figure 4.** Bivariate regression of fish weight vs. length in experimental trials ($R^2$ = 0.93, $P < 0.0001$, N = 67).

## Results

### *Experimental Analysis*

This study examined whether mass, sex, and color were signals of territorial superiority. Standard length was not considered as a confounding variable because of its high correlation with mass ($R^2$ = 0.93, $P < 0.0001$, N = 67, Figure 4). Subjects initiated all aggressive behaviors inside the shelter or within 5cm of the threshold. In contests between two fish, the heavier fish displayed more relative aggression (Figure 5). Kolmogorov-Smirnoff, K-S, one sample analysis demonstrated the significance of the relative weight advantage (max diff. = 0.877, $P < 0.001$). Weight ratio did not correlate with total attack frequency ($R^2$ = 0.009, P = 0.390, N = 81, Figure 6). Heavier fish spent more time alone in the shelter than smaller fish, as shown by K-S two sample tests (max diff. = 0.296, P = 0.002). Both fish spent more time in the shelter together than the smaller fish spent alone in the shelter (max. diff = 0.272, P = 0.005). The difference bigger fish spent alone and with smaller fish in the shelter was insignificant (max. diff. = 0.074, P = 0.981).

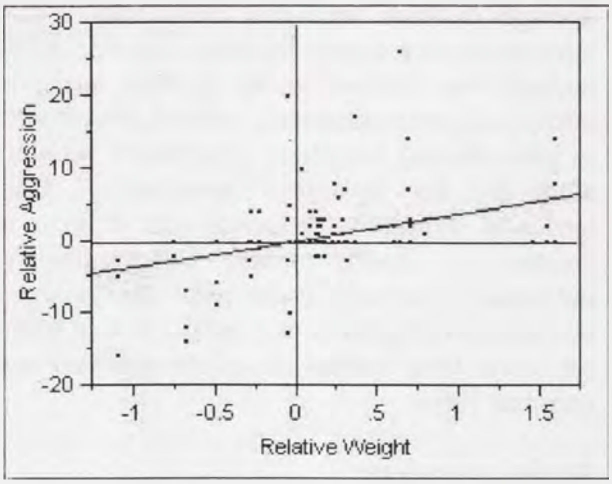

**Figure 5.** Bivariate regression of relative weight vs. relative aggression in experimentally paired fishes.

In female-male bouts, males were more aggressive than female opponents (max diff. = 0.667, P = 0.004). Males attacked 7.75 ± 4.12 (mean ± 95% C.I.) times per bout, while females attacked 0.75 ± 0.9 (mean ± 95% C.I.) times (t = 3.52, N = 12, P = 0.005). Regardless of the opponent's sex, males attacked their opponents in greater frequency (max. diff. = 0.475, P = 0.001).

The presence of horizontal stripes and vertical bars is a possible marker for the dominant fish in combat (Table 2). Amongst females, the presence of a horizontal stripe along the lateral line significantly indicated aggressive dominance (df = 1, P = 0.007). The permanent color phases, blue and orange, had no significant effect on aggression levels (max. diff. = 0.247, P = 0.443). Permanent color phases faded in captivity. Mottling was not recorded, because all experimental fishes exhibited constant mottling.

*Natural habitat dynamics*

Census of natural pools yielded the average frequency and 95% confidence intervals of *Sicyopterus* individuals. The average pool contained 10.4 ± 4.1% *S. pugnans* orange males, 6.1 ± 3.6% *S. pugnans* blue males, 30.0 ± 5.3% *S. pugnans* orange females, 22.5 ± 6.1% *S. pugnans* blue females, 21.0 ± 4.6% *S. taeniurus* males and 14.0 ± 4.1% *S. taeniurus* females. *S. taeniurus* and *S. pugnans* did not spatially segregate upstream of the weir; however, in a paired t-test, *S. pugnans* is more prevalent than *S. taeniurus* in each pool (t = 3.957, df = 22, P= 0.001). Pools contained more females than males, (t = 5.492, df = 22, P < 0.001) but there was no significant difference between the frequency of orange and blue color phases (t = 0.124, df = 22, P = 0.902). Higher elevations of all branches of the Opunohu River contained fewer adult males and more juveniles in both Sicyopterus species. Only *S. pugnans* males displayed territoriality in the open, each dominant male controlling an average of 0.44 ± .08m (Figure 7). Females only demonstrated territoriality over crevices. *S. taeniurus* and *S. pugnans* displayed territorial behavior over large open spaces downstream from the weir, but not upstream.

In 24 observed pools, four did not contain *S. pugnans* males. Site-specific aggression did not occur in these pools. The largest female *S. pugnans* in 3 of the 4 pools laterally displayed and attacked smaller conspecific females. In the 20 pools containing males, the largest male defended a site against all smaller fish, regardless of species. Males did not attack larger *S. pugnans* females and were only attacked by larger, disturbed *S. taeniurus* adults. *S. pugnans* males only conflicted with other territorial males when chasing intruders into adjacent territories. One blue male *S. pugnans* quivered with an equally sized blue female conspecific. After quivering the male chased a juvenile intruder and returned to its territory and chased the female away. While quivering, the bright blue male possessed a dark eye bar and four dark vertical bars ventral to the anterior dorsal fin. The recipient blue female lacked vertical bars and possessed a lateral line stripe. Fish coloration depended on the sex, color phase, and social situation. Mating, dominance, submissive, and alarm situations individually affected the presence or absence of the eye bar, mouth stripe, vertical bars, horizontal stripe, and mottling (Table 3, Figure 8). Permanent blue and orange coloration was brightest among territorial males. *S. taeniurus* did not display physiological color changes.

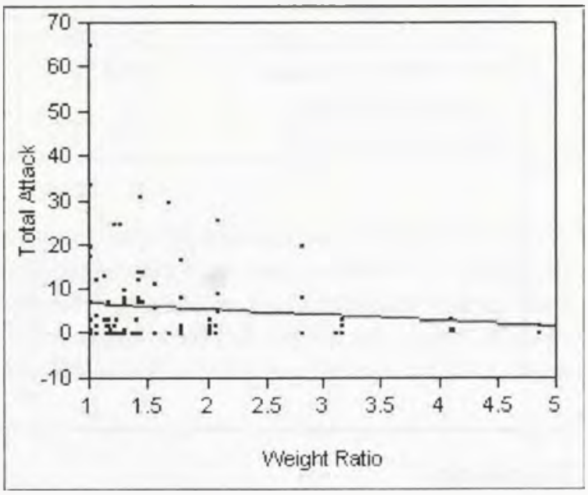

**Figure 6.** Bivariate regression of the weight ratio and number of total attacks displayed between combatants ($R^2 = 0.009$, $P = 0.390$, $N = 81$).

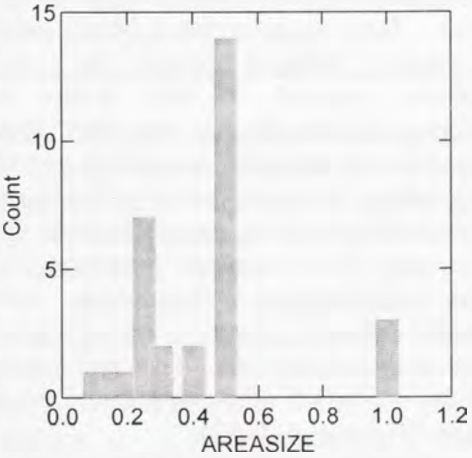

**Figure 7.** Histogram of male *S. pugnans* territory size ($m^2$) in the Opunohu River.

| Sex and Color Form | Presence (+) or Absence (-) of Coloration Pattern | | | | |
|---|---|---|---|---|---|
| **Dominant** | Mouth Stripe | Eye Bar | Vertical Bars | Horizontal Stripe | Dorsal Mottling |
| Yellow Phase Male | - | + | - | - | - |
| Yellow Phase Female | + | + | + | + | + |
| Blue Phase Male | - | + | + | - | - |
| Blue Phase Female | + | + | + | + | + |
| **Submissive** | | | | | |
| Yellow Phase Male | - | - | - | - | - |
| Yellow Phase Female | + | - | - | - | + |
| Blue Phase Male | - | - | - | - | - |
| Blue Phase Female | + | - | - | - | + |
| **Alarm/Stressed** | | | | | |
| Yellow Phase Male | + | - | - | - | + |
| Yellow Phase Female | + | - | - | + | + |
| Blue Phase Male | + | - | - | - | + |
| Blue Phase Female | + | - | - | + | + |
| **Mating Coloration** | | | | | |
| Yellow Phase Male | N/A | N/A | N/A | N/A | N/A |
| Yellow Phase Female | N/A | N/A | N/A | N/A | N/A |
| Blue Phase Male | - | + | + | - | - |
| Blue Phase Female | + | + | + | + | + |

**Table 3.** Presence or absence of specific coloration patterns depending on social situation in *Sicyopterus pugnans*. All observations were conducted experimentally at Gump Biological Reseach Station and in the field in the Opunohu River on Moorea, French Polynesia. The eye bar never disappears, but can fade from black to grey. Dominant fish have aggressively attacked submissive fish. Stressed fish have been chased and netted by researchers. Figure 8 displays the anatomical location of the coloration patterns.

## Discussion

### *Presence of territoriality*

Maher and Lott (1995) state the most commonly applied definition of territoriality is the aggressive defense of a space. Natural observations and experimentation show that *S. pugnans* females and males exhibit territorial behavior. Both sexes initiated lateral displays and chasing behavior inside the shelter. Individuals returned to the shelter after eliminating the intruder. In the field, females displayed similar behaviors in crevices and while feeding. Males defended 0.44m² of open space. Individuals returned to a central location within the territory after chasing intruders. The Olympic mudminnow, *Novumbra hubbsi*, repeatedly chases intruders from guarded vegetation clumps at least 28cm and return to patrol the vegetation after the intruder threat is removed (Hagen et al. 1972).

Environmental variables, such as canopy cover, water depth, silt, and flow rates, increase the likelihood that territories in the Opunohu River are economically defendable. Canopy cover negatively correlates with algal concentration and varies greatly within rivers, including the Opunohu (Askay 2000, Feldman 1996). Flow rates and depth affect the long-term stability of the substrate, creating benthic surfaces of varying stability. Stable sections of the stream that contain more abundant sources of energy promote territoriality (Maher and Lott 2000). Food must also be a limiting resource in order for feeding territories to be economically defendable (Carpenter and McMillen 1976, Munday et al. 2001). Algal beds growth rates correlate with canopy cover, which is highly variable in the Opunohu River (Smith 1993). Only small patches of the river grow at an optimal rate, possibly being a limiting resource.

The presence of a weir downstream from observed pools decreased the predator and competitor concentration. *Anguilla marmorata, A. obscura,* and *Tilapia mossambica* are all pisciverous and do not live above the weir, but inhabit pools immediately downstream (Resh et al. 1999, Thresher 1984, Trewavas 1982). The lack of predatory pressure allows the *Sicyopterus* species population upstream from the weir to reach higher density levels (Maher and Lott 2000). A large number of individuals living in an environment where food is a limiting resource receive energetic advantages by being territorial; however, the upper limit of population density for territoriality is not reached, because there is no evidence of excessive conflict over space in similarly sized individuals or in *S. taeniurus* (Krebs and Davies 1987, Maher and Lott 2000).

*Finding a territory*

*S. pugnans* and *S. taeniurus* are amphidromous fishes that migrate upstream while juveniles (McDowall 1988, Shen and Tzeng 2002). Male *S. pugnans* settles into territories as it migrates upstream. Lucas and Baras (2001) note a positive correlation between size and distance upriver in gobies. Observations of primarily juveniles, small females, and few males at higher elevations in the Opunohu River contradict this theory.

Algal thickness and availability decreases with elevation (Askay 2000). Due to the temporal variability in high elevation streams on volcanic islands, territory quality diminishes as elevation increases. A decrease in food quality provides creates less economically defendable territories (Maher and Lott 2000). Low amounts of food can attract fewer females, decreasing male territoriality (Maher and Lott 2000). If distance upstream negatively correlates with territory quality, then fish are more likely to adopt the first defendable territory that is discovered. Juvenile, due to small size, must swim to high elevations to find living space.

As juveniles become adults and grow, it becomes reproductively advantageous to migrate downstream to available territory. Once territories become economically defendable, adults will move downstream until it seizes the first opportunity to acquire a territory. Otherwise, juveniles remaining at high elevations will not be reproductively successful and fitness levels will drop (Figure 9).

*Mass effects*

Once individuals find a territory worth occupying, heavier individuals possess fighting advantages over lighter fishes. Both field and experimental observations elucidated a significant weight advantage (Figure 5). Heavier fish can generate more power and express higher metabolic rates than lighter fish (Archer 1988, Lahti 2002). Fishes with higher standard metabolic rates require more food and have greater incentive to defend feeding resources. *Rhinogobius brunneus* and *Oxylebius pictus* both exhibit a distinct weight advantage, which is the most common measure of RHP (Archer 1988, DeMartini 1985, Suk and Choe 2001).

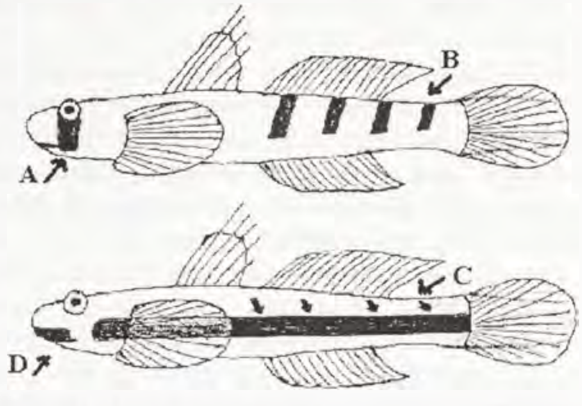

**Figure 8.** The four primary physiological coloration patterns in *S. pugnans*. "A" indicates the eye bar; "B" indicates vertical bars; "C" indicates the horizontal stripe (small arrows point to areas of increased color intensity); "D" indicates the mouth stripe.

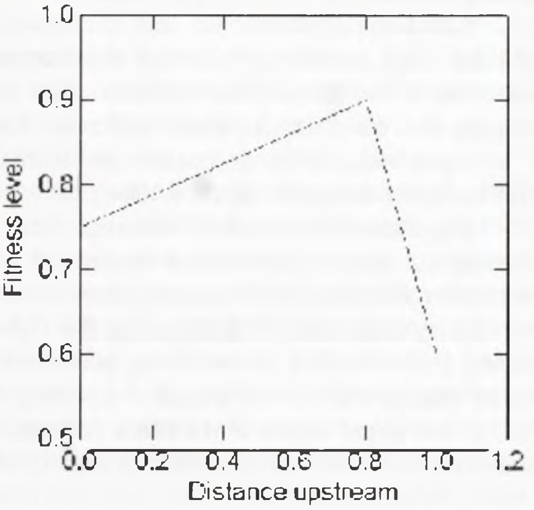

**Figure 9.** Graph of fitness levels against distance upstream. Individual fitness level can drop at high elevations due to poor habitat quality and lack of fit mates.

Fish conflicts begin with the least risky and energetic behaviors and proceed to increase the risk for energy loss and injury as fights progress. Therefore, highly mismatched opponents can end a fight quickly prior to injury or excessive energy loss. Opponents closely matched in weight fight longer and with more intensity than mismatched opponents (Barlow

2000, Krebs and Davies 1987, Suk and Choe 2001).

Experimental and observational results show no significant evidence that fight length and intensity is negatively correlated with weight ratio (Figure 6). In the field, invading adult males were not present. All relatively large, adult *S. pugnans* males possessed territorial space and no conflicts over territorial ownership were observed. It is likely that many territories are gained by occupying the territory of a recently deceased fish, eliminating the prior residency effect. Females failed to fight similarly sized females. Fights between similarly sized individuals might be more prevalent during the mating season, which occurs primarily in summer, after this study was conducted (DeMartini 1985, Kinzie III 1993). More observations over a greater scale of time and asymmetric experimental pairs might find fighting intensity to increase over time.

Additionally, fish had less incentive to fight for long periods of time in experimental tanks, due to the lack of food present. The tank lacked a food source and natural substrate. Lack of energetic incentive decreases an animal's drive to fight over space (Brown 1964).

Experimental results showed heavier fishes spend time in the shelter alone and with the smaller fish equally. This may have resulted from a larger, stressed fish deciding the risk of chasing the other fish, by attracting predators, is greater than territorial advantages. Leaving the smaller fish alone becomes the more economical behavior (Krebs and Davies 1987, Brown 1964).

### *Sexual effects*

Aside from weight advantages, maleness confers an advantage. Males were significantly more aggressive than females in bouts. Regardless of the opponent's sex, males displayed an overall increased level of aggression over females.

Sexual strategies, such as promiscuity and polygyny, increase male intrasexual aggression through sexual selection (Krebs and Davies 1987, Barlow 2000). Female fishes benefit by finding mates with the highest fitness possible. Fitness markers include size, coloration, and quality of territory. A male possessing a large territory with an abundant food supply exhibits its ability to fight off competitors effectively and can provide energy for offspring (Kodric-Brown 1998). Fighting intensity and motivation are important factors of RHP (Lozano and Lemon 1999).

It is unlikely that *S. pugnans* is monogamous or polyandrous, mating strategies that are not associated with strong sexual selection. Fighting intensity and motivation is usually symmetric between sexes in monogamous and polyandrous fishes. Any asymmetries usually favor increased female aggression (Magurran and Garcia 2000). This does not preclude intrasexual aggression among females. Female sand gobies and painted greenlings, *Oxylebius pictus*, compete over feeding territories (DeMartini 1985). Territoriality of shelters among female *S. pugnans* individuals is present and may stem from a limited number of adequate hiding places from potential predators. Female reproductive success hinges on the ability to defend shelters and energy sources necessary to breed successfully (Brandtmann et al. 1999)

### *Coloration Effects*

*S. pugnans* is sexually dimorphic; monomorphy is more prevalent in monogamous fishes, suggesting *S. pugnans* adopts a promiscuous or polygynous strategy (Barlow 2000). Rapid color changes and dimorphism is prevalent in polyandrous and promiscuous fish (DeMartini 1985). Factors indicative of sexual selection provide theoretical credence to sexual asymmetries in aggression (Krebs and Davies 1987).

Different social situations affect changes in dark coloration among *S. pugnans* individuals. Coloration in fishes has three primary purposes: crypsis and to convey information that allows individual recognition or discerns fitness and RHP (Krebs and Davies 1987).

Dorsal mottling in *S. pugnans* is a common stress response in fishes (Beeching 1995). Dorsal darkening and ventral lightening confuse a predator by blending in with the dark substrate or light sky, respectively (Fujii 1993). Individuals immediately gain a mottled pattern while being pursued and retain it throughout capture and experimentation. The darkening of the mouth stripe has a similar effect, because male usually possess bright white coloration above the mouth. The bright colors may signal predators, so darkening of the mouth stripe camouflages fishes facing predators.

The overall color loss in captured *S. pugnans* may be an additional effect of cryptic coloration. *Rhinogobius brunneus* changes its coloration based upon its background (Naitoh et al. 1985). Experimental *S. pugnans* subjects were placed in clear plastic tanks on a white table. Individuals dramatically lightened their permanent coloration and may be a result of stress and blending in with light backgrounds. Stressed fishes tend to lighten in overall color (Groneveld et al. 1995).

The most common coloration pattern found in all individuals is the eye bar. The eye bar hides the eye and makes the eye's location ambiguous to a predator or opponent (Fujii 1993). Dominant orange male *S. pugnans* individuals adopt eye bars as their only dark coloration.

The lack of a midline stripe and the darkening of vertical bars in dominant males may be a general signal of increased aggressiveness. Baerends et al. (1986) found the cichlid, *Chromidotilapia guentheri*, exhibited bars when successful in a fight and a stripe after defeat. Darkening and sharpening of coloration is common among fishes that recently won combat (Barlow 2000).

Male coloration may also play an important role in sexual selection among females. Female pupfish prefer darker males with more distinctive coloration (Kodric-Brown 1998). If sexual selection acts upon *S. pugnans*, dark coloration in dominant individuals would attract gravid females. Sexual selection would also warrant brighter permanent coloration in males, a feature present in male *S. pugnans* (Kodric –Brown 1996).

Changes in female coloration are muted relative those present in males, but may still function to prevent unnecessary intrasexual conflict and aid in cryptic coloration; A horizontal stripe in females *S. pugnans* may serve these two functions. Norepinephrine hormonally modulates coloration in melanophores and increases amounts of available energy (Wendelaar Bonga 1993). Short bursts of energy are beneficial in alarm situations (i.e. a quick escape from a predator) and dominant situations, allowing fish to chase away intruders quickly. Submissive fish can avoid territorial individuals, thereby eliminating the immediate need for quick energy.

Rohwer (1982) posits that generalized color patterns are not necessary in fishes that can individually recognize conspecifics. Coloration patterns can easily be bluffed and faked, while individual traits are honest. It is unlikely that *S. pugnans* can individually recognize conspecifics efficiently. A primary correlate to individual recognition is kin recognition. Amphidromous fishes have no preference for prenatal streams (Kinzie III 1993). Individuals are unlikely to encounter kin during their adult lives. Observations also failed to discern constant physical differences on the individual level. Learning chemical cues is possible in riverine fish and is common in salmon, but information transmission is slow and has a low resolution (Goodenough et al. 1993). Therefore, coloration in *S. pugnans* is most likely honest and accurately transmits information.

### *Sicyopterus interactions*

*S. Taeniurus* and *S. pugnans* share habitat and food resources (Marquet 1988). Munday et al. (2001) states that species fitting these criteria should compete for resources. The lack of prevalent interspecific confrontations suggests the two species live in resource equilibrium. *S. taeniurus* is a larger fish, but is less aggressive. Only relatively larger *S. taeniurus* adults attack *S. pugnans*. *S. taeniurus* attacks when approached and rarely initiated fights. Higher concentrations of *S. pugnans* suggest that heavier *S. taeniurus* individuals take the resources necessary for reproductive success. Smaller *S. taeniurus* and all *S. pugnans* individuals can aggressively compete for the remaining resources, unable to match the RHP of *S. taeniurus* adults. Further research can explore the reproductive habits and energy budgets of *S. taeniurus* adults in order to discover the reason they don't increase in population size and competitively eliminate *S. pugnans*. Large *S. taeniurus* adults held territories below the weir, suggesting that changes in resource and competitor distribution affect one species' behavior and not the other. Adult male *S. pugnans* held territories below the weir. Possible hypotheses include a possible population limit imposed by non-food resource limitations and the inability for larvae to migrate toward habitats with available resources due to randomness in stream recruitment (Maher and Lott 2000, Kinzie III 1993). Lack of physiological color changes may affect aggression by creating a relative vacuum of RHP information (Rohwer 1982).

### *Future Studies and Improvements*

Quantitative data on resource and fish distribution according to elevation and presence of competitors and predators needs to be examined in order to uncover possible correlations between behavior and environment. It may also serve to provide support hypotheses for the difference in territorial behavior in *S. taeniurus*. Tracking the movement of individual fish over time will give insights into the methods amphidromous fishes used to choose territories. Studies elucidating the reproductive strategies of *S. taeniurus* and *S. pugnans* can provide evolutionarily viable reasons explaining interspecies interactions. Future studies should conduct observations in a wider array of environments, discerning behavioral similarities and differences.

## Conclusion

This study observed *S. pugnans* in an environment relatively devoid of predator pressure and species diversity (Concepcion and Nelson 1999). The presence of weirs and dams throughout inhabited islands provides an opportunity to analyze the behavior of organisms that have not evolved behavioral and physiological adaptations to these recent environmental changes. *S. pugnans* guards economically defendable space and weight and gender can dictate the outcome of agonistic encounters. Color changes convey honest badges of fighting ability and experience. Experimental observations confirmed the role weight, gender, and color play. *S. pugnans* displays many features adapted to a more natural environment, such as mottling in response to predator presence. Conversely, this study examined an artificially modified and species poor habitat, which provided insights to social dynamics in habitats with relatively lower levels of interspecific competition. Possible increase in population density due to lack of predators and competitors elucidated a model for territory acquisition in amphidromous fishes.

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# The positive correlation between ecology and morphology in marine organisms: a study of chemical defenses

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ABSTRACT. The relationship between an organism's mode of life and its toxicity and antimicrobial activity was investigated in cnidarians, mollusks, and echinoderms over a five week period. It was hypothesized that organisms lacking physical means of defense and inhabiting exposed regions of the marine environment would have a higher concentration of anti-predatory toxins and antimicrobial compounds. Extracts from representative species of these three major invertebrate phyla provided significant comparisons of both toxicity and bioactivity. A brine shrimp cytotoxicity test showed toxicities ranging from highly toxic to non-toxic. Individuals lacking apparent physical defense methods showed a higher degree of toxicity than individuals possessing physical defense mechanisms. Microbial screens, using both bacteria and yeast, showed a wide range of bioactivity, ranging from strongly inhibitory to having no effect. Extracts from organisms without physical protection showed greater bioactivity. The measurements from these tests show a positive correlation with ecology and morphology. Slow-moving or sessile species that inhabit exposed environments and species lacking physical protection have relatively high concentrations of toxic compounds. Species that are well-defined morphologically show a lower degree of toxicity and bioactivity. The toxic and bioactive compounds found in these organisms may potentially have medical significance. Species may have adapted these antipredatory toxins as an alternative to morphological defense.

KEYWORDS: marine bioactivity, chemical defenses, marine toxicity

## Introduction

The longterm effects of predation are likely to have shaped the evolutionary trends in defense mechanisms among animals. Antipredator adaptations are frequently visible in the marine environment. The way an organism responds to predation depends on its morphology and behavior. Echinoderms, for example, may have developed morphologic structures such as hard exoskeletons and sharp spines in part to shield against predators. This protection enables these organisms to bottom-feed along exposed regions. Most species of mollusks have rigid shells of calcium carbonate offering shelter when threatened. Some Cephalopods and fish employ cryptic coloration or mimicry to evade predators. Cnidarians use poisonous darts (nematocysts) to fend off predators and paralyze prey. Many other animals including crabs and worms remain unattainable to predators by inhabiting concealed burrows or crevices (Allen & Steene 1994). To become unpalatable and even poisonous to predators, some organisms build up high concentrations of toxins (Alstyne & Paul 1988). These chemical defenses are the subject of this research.

Throughout the Society Islands, many marine organisms depend on toxic compounds as a primary mode of defense. Chemical defenses are anti-predatory chemicals that serve no physiological function for the organism (Pianka 1974). These defenses may include distasteful bodily fluids, repellent secretions, or toxic injections. Organisms acquire these toxins either by producing secondary metabolites themselves, or from dietary sources (Coll & Sammarco 1988). Secondary metabolites, or natural products, are compounds produced by the organisms but lack any metabolic use (Amsler, Mcclintock, & Baker 1999). Toxins acquired through dietary means are structurally modified and stored, allowing the consumer to use the poisonous chemicals for its own defense. Previous research has shown how this event takes place in *Glossodoris callida*, a species of nudibranch. *G. callida* was observed ingesting and storing the toxic metabolites from its prey *cacospongia*, a branching sponge. The nudibranch then concentrates these toxins into a mucus that covers its body for protection (Avila & Paul 1997). Developing toxicity, either through secondary metabolites or dietary means, requires an energetic cost. However, once obtained, toxicity limits the threat of predation and allows the organism to inhabit exposed environments where predation pressure is higher.

Predation is not the only agent selecting for defense mechanisms. Constant defense against bacteria and other microbes is essential for marine organisms. In a previous study, a screening for bioactive compounds was performed in sea cucumbers. Extracts from several species inhibited the growth of certain bacteria strains (Ridzwan et al. 1995). Organisms such as these sea cucumbers that lack the physical protection of an exoskeleton or safe recess may be more susceptible to microbial infection. Many animals also inhabit the marine benthos where bacterial concentrations are likely to be higher due to accumulation of detritus and waste. These apparently susceptible organisms may depend on high concentrations of antimicrobial compounds for survival (Peters 1998).

A large number of medicinal agents have been isolated from natural sources found in the marine environment (Cragg & Newman 2001). Studying the toxic and bioactive compounds in marine invertebrates may provide access to an unexploited reservoir of medicinal agents. These agents could serve as new sources of antibiotics, anticancer treatments, and other medically significant compounds.

The present study was conducted on the back reef of Cook's Bay, Moorea, in French Polynesia. It was designed to test the hypothesis that an organism's concentration of antimicrobial compounds and toxins are positively correlated with its mode of life. Soft bodied organisms inhabiting open regions where they are exposed to predation and microbial infection may possess higher degrees of toxicity and bioactivity. A positive correlation may suggest that chemical defenses evolved as an alternate to physical defenses. Alternatively, varying concentrations of toxic compounds could be distributed randomly among marine organisms. This could possibly be due to differing dietary requirements or environmental fluctuations. An example of this can be seen when a sudden rise in dinoflagelate concentration occurs (red tide). Mollusks exposed to this dinoflagelate bloom may accumulate high concentrations of toxins, becoming poisonous to vertebrates (Mackenzie et al. 2002). Representatives of three major invertebrate phyla, echinodermata, cnidaria, and molluska, were chosen to include species that appeared to be well defended and species that appeared susceptible either because of behavioral characteristics or the lack of mechanical defenses (table 1).

## Materials and methods

Using a mask and snorkel, a survey was conducted for two weeks at the back reef of Cook's Bay and Tamae Bay to find which phyla should be represented in this study. Phyla including organisms with apparent physical defense mechanisms and organisms appearing susceptible to predation and microbes were selected. Cook's Bay and Tamae Bay were selected because a variety of marine invertebrates were observed. The locations of the collecting sites are shown in figure 1. All species were found scattered throughout the back reef in less than two meters of water.

Representative species of Mollusks included nudibranchs: *Glossodoris atromarginata, Glossodoris cincta, Hexabranchus sanguineus,* and bivalves: *Limaria orientalis, Tridacna maxima,* and *Pinctada margaritifera.* The species of echinoderms consist of sea urchins: *Diadema savignyi, Echinothrix calamaris, Echinometra methaei,* sea stars: *Culcita novaeguineae, Amphiuro sp. 1,* and sea cucumbers; *Holothuria atra, Bohadschia argus,* and *Thelenota ananas.* Cnidarians were represented by sea anemones: *Stichodactyla haddoni, Hormathiidae amphianthus, Zoanthidea protopalythoa,* soft coral: *Ricordia yuma,* and hard corals: *Pocillopora verrucosa, Fungia costulata.* (Voucher specimens and references of the species used in this research can be found in the appendix.)

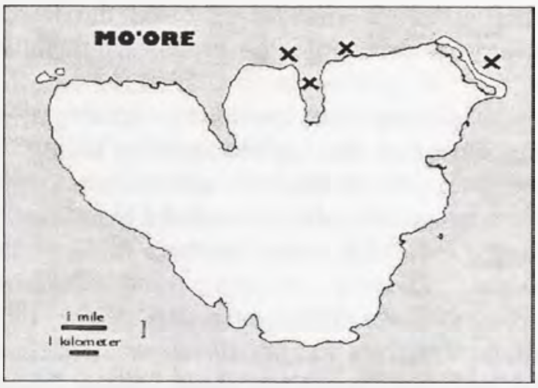
### *Extract preparation*

Sample organisms were collected and immediately placed in the freezer for six hours. Once the animal was dead, an emulsion of the entire organism was made using a mortar and pestal. If the sample was too rigid or dry to make an adequate emulsion, a very small amount of water was added (less than .25ml). Three individuals of each species were included in each emulsion, making one extraction per species. Five to eight millileters of each emulsion were stored at 0° C. Internal and external samples from *Thelenota ananas, Holothuria atra* and *Bohadschia argus* were tested individually due to unexpected toxicity results. Samples from the hard corals were acquired by first extracting tissue from the calcium carbonate skeleton. This tissue solution was then centrifuged at high speed for 5 minutes. An emulsion was made from the pellet that was created from the

**Table 1.** Table showing the primary mode of defense in each species and the predicted use of chemical defenses.

| Phyla: | Species | Apparent modes of defense | Prediction |
|---|---|---|---|
| **ECHINODERMATA** | *Diadema savignyi* | Spines and mobility | Low |
| | *Echinothrix calamaris* | Spines and mobility | Low |
| | *Echinometra methaei* | Spines and mobility | Low |
| | *Culcita novaeguineae* | Rigid skin and mobility | Low |
| | *Amphiuro sp. 1* | Rigid skin and mobility | Low |
| | *Holothuria atra* | Cuvierian tubules and bioactive compounds | High |
| | *Bohadschia argus* | Cuvierian tubules and bioactive compounds | High |
| | *Thelenota ananas* | Cuvierian tubules and bioactive compounds | High |
| **CNIDARIA** | *Stichodactyla haddoni* | Nematocysts and bioactive mucus | High |
| | *Hormathiidae amphianthus* | Nematocysts and bioactive mucus | High |
| | *Zoanthidea protopalythoa* | Nematocysts and bioactive mucus | High |
| | *Ricordia yuma* | Nematocysts and bioactive mucus | High |
| | *Pocillopora verrucosa* | Nematocysts, bioactive mucus and safe recess | Moderate |
| | *Fungia costulata* | Nematocysts, bioactive mucus and safe recess | Moderate |
| **MOLLUSKA** | *Glossodoris atromarginata* | Bioactive mucus | High |
| | *Glossodoris cincta* | Bioactive mucus | High |
| | *Hexabranchus sanguineus* | Bioactive mucus | High |
| | *Limaria orientalis* | Shell | Low |
| | *Tridacna maxima* | Shell | Low |
| | *Pinctada margaritifera* | Shell | Low |

**Figure 1:** Map of Moorea showing collecting sites.



centrifuge as well as the gelatinous substance on the surface. No ethanol was used to preserve any of the samples due to its antimicrobial characteristics (Lutz pers. com.). Extracts were used to test the toxicity and the antimicrobial activity of each species.

*Toxicity test*

For the toxicity tests, brine shrimp eggs (*Artemia salina*) were placed in aerated seawater for 48 hours (brine shrimp from Fischer company). Into separate vials, 25 brine shrimp were added using a micropipette. Additional seawater was added until the volume in each vial reached 5ml. Each vial was then labeled according to the species being tested. A 0.25mL sample of each echinoderm and mollusk species was then added to its appropriate vial. Due to a slightly higher toxin concentration, Cnidarians required only a 0.15 ml sample of each species to get comparable results. For a negative control, brine shrimp were only exposed to sea water. For a positive control, *Catharanthus roseus* (rosie periwinkle), a known cytotoxic plant, was used to verify the test (Farnsworth 1991). An emulsion was made of the periwinkle leaves in same manner as sample organisms being tested. For the mollusk tests and the echinoderm test, 0.25 ml of the Rosie periwinkle emulsion was added to a vial labeled positive control. For the cnidarian tests, only a 0.15ml sample of the periwinkle emulsion was used in the positive control. The purpose of this control was to see how many brine shrimp a known highly toxic organism could kill. After 24 hours, the number of dead brine shrimp was counted and recorded. Six replicate trials for each emulsion were conducted.

*Antimicrobial screen*

Antimicrobial tests were performed using yeast (*Saccharomyces cerevisiae*) and bacteria (*Lactobacillus bulgaricus*). The yeast was plated on Trypticase soy agar (3 parts pancreatic digest of casein, 1 part papaic digest of soybean, 1 part sodium chloride, and 3 parts agar). The bacteria was plated on standard agar (9 parts water, 1 part agar, .5 parts sugar). The plates was then incubated at 37° C for 24 hours until a thin layer of the respective microbe covered the entire agar surface. An incubator was built using 8" by 8" by 12" cardboard box with a 60 watt light bulb inside functioning as the heat source. A 0.15mL sample of each emulsion was dropped onto the microbe colonies and incubated again at 37° C for 24 hours. In the yeast tests, a topical fungicide (1% clotrimazole) was used as a positive control to show the affects of a lethal substance. In the bacteria tests, an antibiotic (bactroban mupirocin calcium 2%) was used as the positive control. In both tests, a negative control was run to allow the microbes to grow undisturbed. These controls are essential because they establish a range for comparing the qualitative results. The inhibition zone surrounding the spot was measured and recorded. These inhibition zones are regions on the agar surface where the microbes are killed by antimicrobial compounds (Lutz pers. com.). Four replicate trials for each emulsion were conducted using yeast. One test for each emulsion was conducted using bacteria because of time restraints.

*Statistical analysis*

The data from both he antimicrobial tests and the toxicity tests were statistically analyzed using an Anova Post-hoc Tukey Type test. The data were also used in a two-way anova analysis to see if chemical defenses are more dependent on an organism's ecology or phylogeny.

## Results

*Toxicity tests*

After 24 hours all 25 of the brine shrimp exposed to *Catharanthus roseus* were dead. Nearly all the brine shrimp exposed only to sea water survived the time period. In three of the initial trials, 1 to 2 dead brine shrimp were observed in the negative control. The number of dead brine shrimp mentioned is the average number dead after six trials. Among the echinoderms, external and internal extracts from *Thelenota ananas,* and internal extracts from *Bohadschia argus* had the highest concentrations of toxins. Extracts from these species killed 24 and 23 brine shrimp respectively. Internal extracts from *Holothuria Atra* also showed high toxicity killing 22 brine shrimp. External samples from *Holothuria Atra* and *Bohadschia argus* did not show significant toxicity, killing about 5 brine shrimp.

Among the sea urchins, *Echinometra methae* had the highest toxicity, killing 8 brine shrimp. *Diadema savignyi* and *Echinothrix calamaris* showed a slightly lower toxicity killing about 6 and 7 brine shrimp respectively. The Brittle star, *Amphiuro sp. 1*, killed about 9 brine shrimp. The cuhion star, *Culcita novaeguineae,* had the lowest rate of toxicity killing less than 5 brine shrimp (figure 2a).

Within the cnidarians, *Stichodactyla haddoni* had the highest toxicity killing 24 brine shrimp. *Zoanthidea protopalythoa* and the soft coral, *Ricordia yuma,* showed the next highest degree of toxicity killing 23 brine shrimp. The extracts from *Hormathiidae amphianthus* demonstrated sufficient toxicity to kill 20 brine shrimp. Extracts from *Pocillopora verrucosa* showed a lower toxicity killing nearly 5 brine shrimp. *Fungia costulata* showed the lowest degree of toxicity, killing 4 brine shrimp (figure 2b).

Among the mollusks, *Hexabranchus sanguineus* had the highest toxicity killing 24 brine shrimp. *Glossodoris atromarginata* and *Glossodoris cincta* also possessed a high toxicity killing nearly 23 brine shrimp. Two of the bivalves, *Limaria orientalis* and *Tridacna maxima,* killed 15 brine shrimp. The third bivalve, *Pinctada margaritifera,* was the least toxic representative of the phyla, killing 7 brine shrimp (figure 2c).

*Antimicrobial screen*

The samples were more lethal to the yeast than to the bacteria. Smaller and fewer inhibition zones were found surrounding the sample spot among the bacteria colonies. The extracts that killed the yeast were also deadly to the bacteria. The fungicide and the antibiotic used for positive controls killed all the yeast and bacteria respectively. The negative controls resulted in total microbe survival (figure 3).

Within Echinodermata, external and internal extracts from *Thlelnota ananas* showed the highest activity in the yeast tests, killing all the yeast within a 2mm zone surrounding the sample spot. In the bacteria test, the same extracts did not produce an inhibition zone around the sample, but no bacteria was living within the sample. The internal extract from *Bohadschia argus* also showed a high degree of antimicrobial activity in both tests, killing all bacteria and yeast within about a 1mm zone surrounding the extract. External extracts from this species had little affect on either microbe. The internal extract from *Holothuria atra* sample produced about a 1mm inhibtion zone within the yeast colony. The same extract killed all the bacteria within the spot, but it did not produce an inhibition zone. The external extract showed little microbial inhibition. *Amphiuro sp. 1* showed very little evidence of bioactivity in either antimicrobial test. Colonies of living yeast and bacteria were scattered throughout the sample. The *Echinothrix calamaris* sample occasionally killed a group of yeast cells, but many healthy colonies were found within the sample. This extract showed no evidence of antimicrobial activity in the bacteria screen. *Echinometra mathaei* showed very little evidence of antimicrobial activity. In both the yeast and bacteria tests healthy colonies were found within the sample. Similar to *Echinothrix calamaris*, an occasional group of dead yeast or bacteria was found within the sample. The *Culcita Novaeguineae* sample also showed a low degree of anitmicrobial activity in both tests. Scattered groups of dead yeast were found within the spot, but most of the yeast was unaffected by the sample. The majority of the bacteria was unaffected by this extract. *Diadema savignyi* showed no evidence of antimicrobial activity in either test (figure 3a).

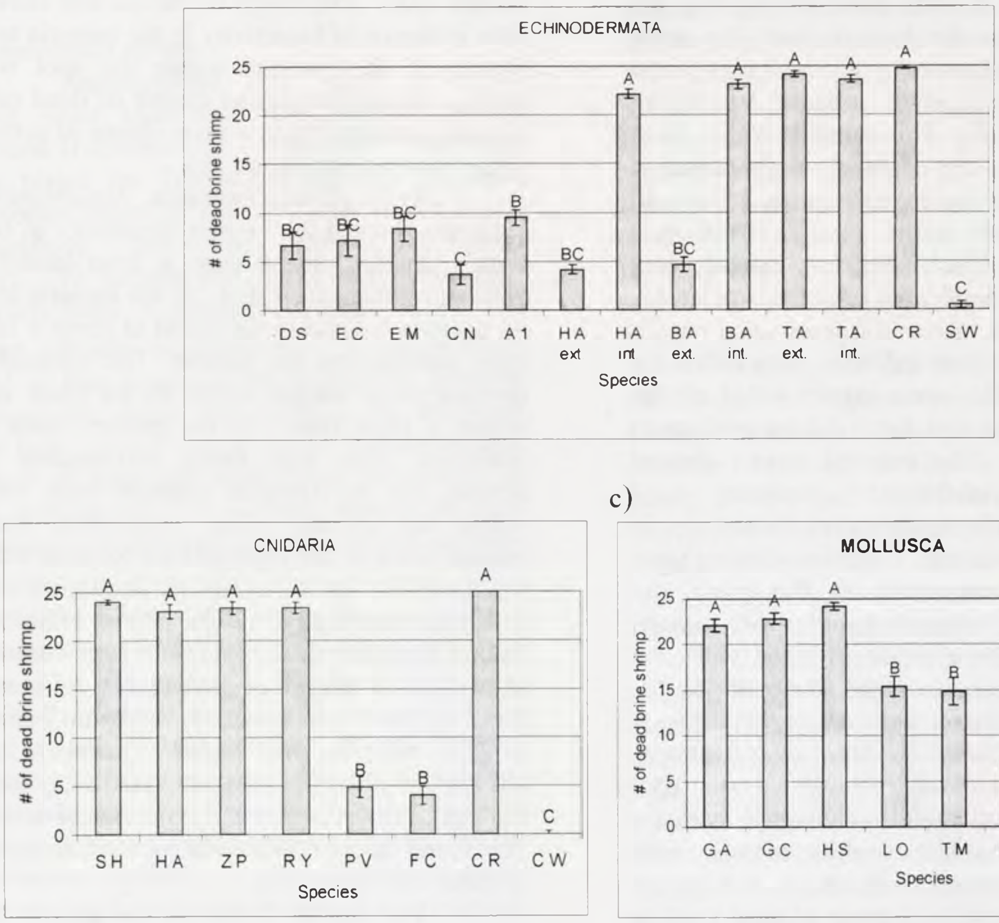
Among the cnidarians, *Stichodactyla haddoni* showed the highest activity in both the yeast and the bacteria screens. All yeast within about a 2mm zone was killed, and all bacteria within a 1mm zone were killed. Samples from *Zoanthidea protopalythoa*, *Hormathiidae amphianthus*, and *Ricordia yuma* showed high antimicrobial activity in the yeast screen, and relatively high activity in the bacteria screen. In all three cases no living colonies of yeast were found in a 1mm zone surrounding the spots. In the bacteria test, no bacteria colonies were found within the spots but no zones of inhibition were present. Both *Fungia costulata* and *Pocillopora verrucosa* showed little evidence of antimicrobial activity in the yeast screens. In each case, some yeast was killed but many healthy colonies were still found within the sample spots. *Pocillopra verrucosa* also showed little evidence of bioactivity in the bacteria tests. Nearly all the bacteria within the spot were living, with an occasional cluster of dead cells. *Fungia costulata* showed no evidence of activity in the bacteria test (figure 3b).

In the phylum molluska, *Hexabranchus sanguine* showed the highest bioactivity in both tests. On the yeast culture, a 2mm inhibition zone surrounded each spot. In the bacteria tests, no bacteria colonies were found in about a 1mm zone surrounding the sample. The *Glossidora atromarginata* sample killed all the yeast cells within a 1mm zone. In the bacteria tests, no inhibition zone was found surrounding the sample, but no bacterial colonies were living within the extract. The *Glossodoris cincta* extract killed all the yeast and the bacteria within the samples, but no zones of inhibition were found surrounding the sample spot in either test. Both *Limaria orientalis* and *Tridacna maxima* showed a low degree of bioactivity. As seen in the *Pocillopra verrucosa*, an occasional cluster of dead microbes was found. However, most colonies of yeast and bacteria were living within the spot. No evidence of antimicrobial activity was found in the *Pinctada margaritifera* sample in either test (figure 3c).

For figures 2 and 3, the generic and specific names of each organism are abbreviated. Among the echinoderms, D S, E C, E M, C N, A 1, H A, B A, and T A, refer to *Diadema savignyi*, *Echinothrix calamaris*, *Echinometra methaei*, *Culcita novaeguineae*, *Amphiuro sp. 1*, *Holothuria atra*, *Bohadschia argus*, and *Thelenota ananas* respectively. Among the cnidarians, S H, H A, Z P, A S, P V, F C refer to *Stichodactyla haddoni*, *Hormathiidae amphianthus*, *Zoanthidea protopalythoa*, *Alcyoniidae sinularia*, *Pocillopora verrucosa*, and *Fungia costulata* respectively.. Among the mollusks, G A, G C, H S, L O, T M, P M, refer to *Glossodoris atromarginata*, *Glossodoris cincta*, *Hexabranchus sanguineus*, *Limaria orientalis*, *Tridacna maxima*, and *Pinctada margaritifera* respectively. C R refers to Catharanthus Roseus, S W refers to sea water, CON refers to control, and NOT refers to nothing added

**Figure 2.** Results from the toxicity tests. The graphs show the number of dead brine shrimp found in each vial after 24 hours. Error bars show +/- 1 standard error. The letters above the bars indicate significantly different toxicity levels. Species with similar degrees of toxicity are labeled with the same letter.

a)



b) c)

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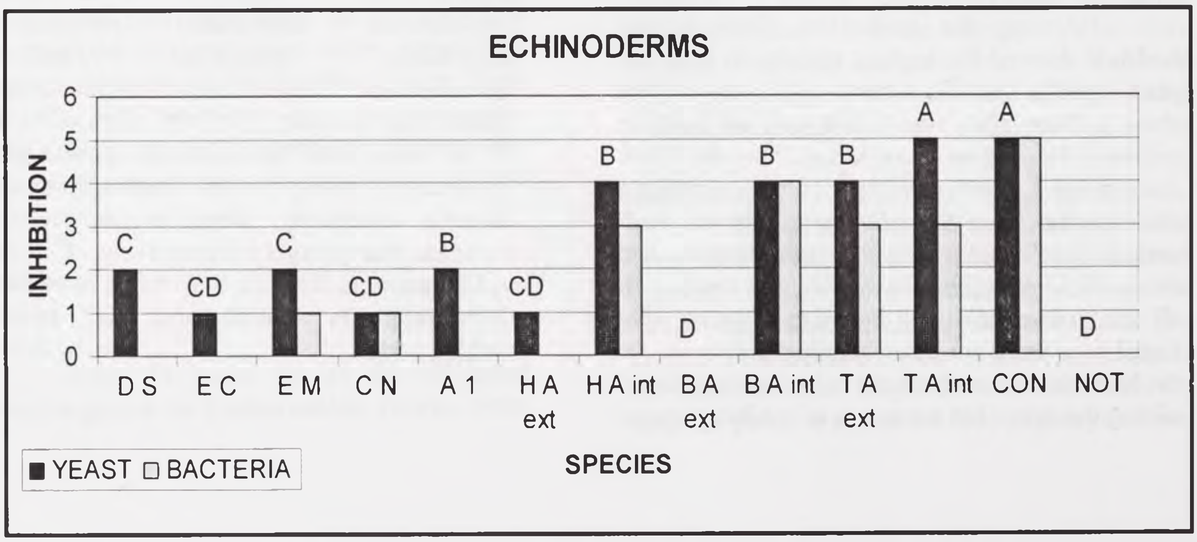
**Figure 3**: Qualitative results from the antimicrobial screens. The graphs show the degree of microbial inhibition of each extract. The letters above the bars indicate significantly different toxicity levels. Species with similar degrees of toxicity are labeled with the same letter. CON refers to fungicide in yeast tests and an antibiotic in bacteria tests. NOT refers to the negative control.

a)



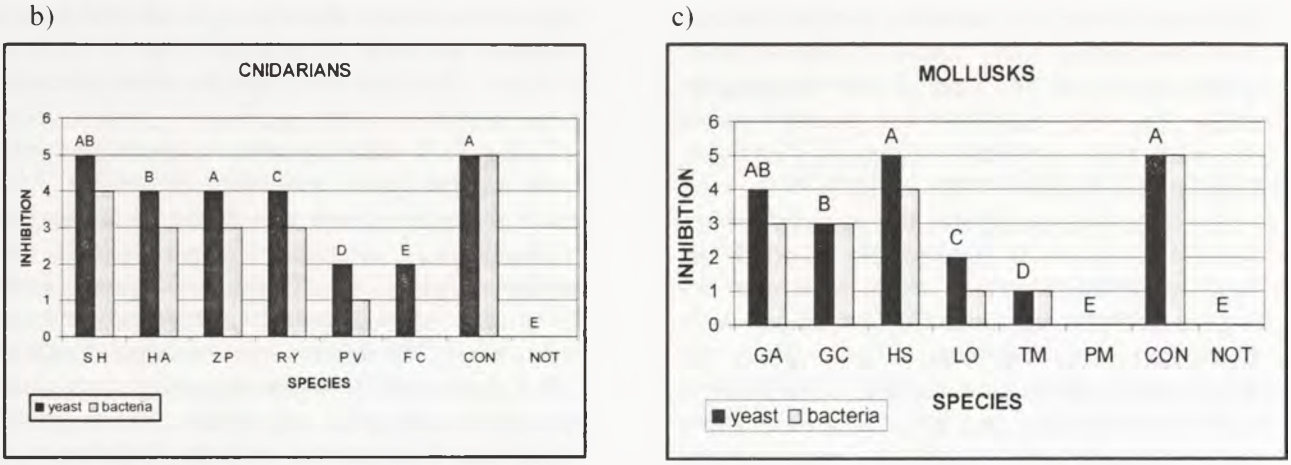
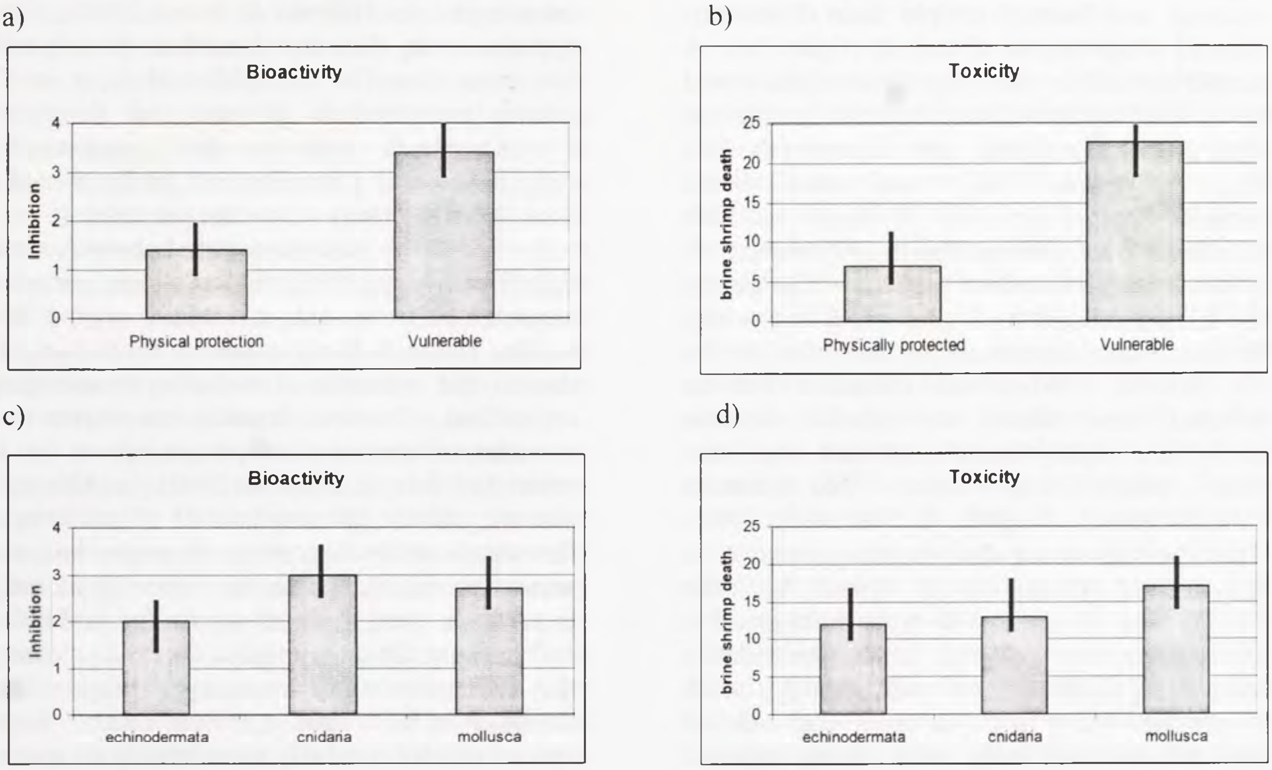


**Figure 4.** Figure 4 shows the results from a two-way Anova relating chemical defense to ecology and phylogeny. Graphs a and b compare the degree of bioactivity and toxicity between physically protected organisms and vulnerable organisms. Graphs c and d compare the degree of bioactivity and toxicity between phyla. Error bars represent +/- 1 standard error.



## Discussion

This study suggests a positive correlation between an animal's chemical defenses and its mode of life. Results showed that sea cucumbers, sea anemones, soft coral, and nudibranchs had the highest degree of both toxicity and antimicrobial activity within their respective phylum. These organisms also lack any apparent physical defense mechanism. One explanation for their dependence on chemical concentrations may be that these animals evolved toxic compounds rather than physical defenses. Toxic and bioactive compounds may have been selected for during the evolution of these organisms' ancestors, and descendants today rely on chemical compounds as an alternate to physical defenses (Amsler, McClintock, & Baker 2001).

As was predicted, the sea cucumbers, which appear more susceptible to predation, were significantly more toxic than the sea urchins and the sea stars (p: 0, f-ratio: 66.9; figure 2a). The sea urchins have spines, the brittle star is mobile and capable of shedding a limb when agitated, and the cushion star has a rigid epidermal layer. Because of these physical defenses, finding a minimal degree of toxicity in these organisms was expected.

Although the sea cucumbers showed the highest toxicity, the data suggested that different species concentrated toxic compounds in different regions of their body. Only internal extracts from *Holothuria atra* and *Bodadshcia argus* demonstrated toxicity. However, both the external and internal sample from *T. ananas* showed a high degree of toxicity (figure 2a). A simple method for verifying these results would be to feed epidermal and internal samples of each species to predatory fish. Based on the data from this research, fish should avoid internal samples from *H. atra* and *B. argus*, and both external and internal samples from *T. ananas*. A possible explanation for a higher toxicity among the internal samples of *H. atra* and *B. argus* may be due to their dependence on Cuvierian tubules for defense. When sea cucumber become irritated, these tubules are expelled, become sticky, and rapidly immobilize most organisms with which they come into contact (Vandenspiegel, Jangoux, & Flammang 2000). These tubules are the likely source of toxicity in the internal extracts (Delia, Hertel, & Wittle 1977). Both *H. atra* and *B. argus* were found to exude these toxic Cuvierian tubules immediately after being irritated. *T. ananas*, although much larger, was found to exude smaller amounts of the tubules, and only after being agitated intensely. One hypothesis is that as the ancestors of *T. ananas* evolved into a larger organism, exuding sufficient Cuvierian tubules for protection became too great an energetic cost. Instead, the sea cucumber concentrated more of its toxic compounds within its epidermal tissue. To test this hypothesis one would need to construct a phylogenetic diagram of these sea cucumbers to compare common ancestors. The hypothesis would gain support if both a gradual increase in epidermal toxicity and a decrease in Cuvierian tubule abundance is observed in *T. ananas'* ancestry.

The data show that the three species of sea cucumbers had a significantly higher degree of bioactivity than any other echinoderm used in this research (p: 0, f-ratio: 27.5; figure 3a). More antimicrobial activity was found in the internal extracts of *H. atra* and *B. argus* than in their epidermal samples. Similar results were found in a previous antibacterial screen of these sea cucumbers. In this experiment internal extracts of *H. atra* and *B. argus* showed greater gram-positive and gram-negative bacteria growth inhibition than external extracts (Ridzwan et. al. 1995). *T. ananas,* however, showed a relatively high degree of bioactivity in its external and internal tissues. This dissimilarity may be explained by a difference in hemolymph concentration that may exist in the external tissues of the three sea cucumbers. Hemolymph contains lysozimes, perforins and lectins that kill bacteria, parasites, and other harmful microorganisms (Glinski & Jarosz 2000). One hypothesis is that the bioactive hemolymph circulates closer to the epidermal layer in *T. ananas* compared to *H. atra* and *B. argus.* Future research could test this hypothesis by comparing the proximity of each animal's hemolymph pathway to its external surface.

All the cnidarians tested showed some degree of toxicity. Slightly less of the cnidarian extracts were needed to obtain comparable results. The most likely reason for this toxicity is due to the presence of nematocysts (stinging organelles). Previous research has shown that nematocysts contain a non-protein venom that is toxic to fish (Lindquist 2002). Although nematocysts are characteristic of all cnidarians, the organisms lacking physical protection, sea anemones and the soft coral, were significantly more toxic than the hard corals (p: 0, f-ratio: 108.1; figure 2b). A possible theory explaining this difference is that nematocysts may be less abundant in these species of hard corals. Some species of hard coral rely more heavily on mucus entanglement to capture prey, and have relatively few nematocysts (Golberg 2002). Sea anemones, which need sufficient toxicity to paralyze and capture larger prey, require more nematocysts (Karlsson & Ostman 1994).

Although soft corals feed on particulate prey like the hard corals, *Ricordia yuma* showed a higher degree of toxicity. Previous research has shown the intense degree of toxicity in several soft corals. Fish food treated with only 1% Dihidroflexibilide (a common secondary metabolite among soft corals) was strongly avoided by fish (Aceret et al. 2001). *A. sinularia* may depend on a greater degree of toxicity primarily as a defense mechanisms rather than a method for capturing prey.

All the cnidarians tested showed some degree of bioactivity in the microbial screens. However, the sea anemones and the soft corals showed a significantly higher bioactivity than the hard corals (p: 0, f-ratio: 39.7; figure 3b). Cnidarians possess an antimicrobial mucus that protects the exposed regions of the animals body. This mucus is too acidic for most microbes to successfully colonize. It also contains an enzyme (like a lysozyme) that can lyse bacteria that are capable of inhabiting acidic environments (Borneman & Lowrie 1998). One explanation for the soft coral and the sea anemones having higher bioactivity is that they have a greater amount of antimicrobial mucus. Sea anemones and soft corals are completely exposed to the environment and require bioactive mucus to surround their entire body. Hard corals have a physical barrier partially protecting them from microbial infection. The mucus is only required for protecting their exposed feeding tentacles. Future research could test this hypothesis by measuring the amount of mucus on the tentacles and on the bodies of hard corals, sea anemones, and soft corals. The results from my experiment suggest that hard corals will have more bioactive mucus surrounding their tentacles compared to their body. Soft corals and sea anemones will have uniformly high mucus coverage surrounding their tentacles and bodies.

Among the mollusks, nudibranchs were significantly more toxic than any of the bivalves (p: 0, f-ratio: 100.1; figure 2c). Unlike the other mollusks tested, nudibranchs depend primarily on high toxic compounds as their primary defense against predators. Most nudibranchs become toxic by sequestering toxic compounds from their prey (Karlsson & Ostman 1999). This can be seen in many species of mollusks that prey on cnidarians. These nudibranchs have developed immunity to the harmful effects of nematocysts and use them for their own defense (Karlsson & Ostman 1999). The data also show that two bivalves, *Tridacta maxima* and *Limaria orientalis,* showed a significantly higher degree of toxicity than *P. margaritifera* (p; 0, f-ratio: 29.9). In previous research an occurrence of various toxins was found in *T. maxima.* In this study 7 shelled mollusks were tested for the presence of toxic compounds. *T. maxima* was one of two species that showed significant toxicity (Kanno, Kotaki, & Yasumoto 1977). Because of its unique feeding habits, *T. maxima* depends on toxicity for survival. As *T. maxima* filter feeds, it separates its valves and allows some of its body to extend beyond the shell. The clam also employs photosynthesis as a method for feeding. In order to receive adequate sunlight, the animal must expose its flesh to the environment (Allen & Steene 1994). Like *T. maxima*, *L. orientalis* is often exposing itself to the environment. This animal has long fleshy appendages that extend out beyond the protection of its shell. Toxicity is needed to protect these appendages from hungry predators. Toxicity is also required to enable *L. orientalis'* unique style of movement. As it opens and closes its shell to move, *L. orientalis* exposes its entire body to predators. When agitated, both *T. maxima* and *L. orientalis* quickly retreat into their shells. This suggests that these animals rely on physical protection as their primary defense. Toxicity may only be needed for temporary protection while feeding or moving.

Extracts from a third bivalve, *Pinctada margaritifera,* showed very little evidence of toxicity. Perhaps this animal's mode of life does not require toxicity. Unlike *T. maxima,* this mollusk was never observed exposing any part of its body while (filter) feeding. The valves were often seen slightly open, but no flesh was ever observed extending outside the shell. Some species of mollusks rely only on their shell for protection. Depending on the type of predator common to its habitat, some bivalves can increase the thickness length of width of their shell to become less vulnerable (Smith & Jennings 2000). A likely explanation for the lack of toxicity is that *P. margaritifera* is dependent on this shell manipulation for defense. To test this idea, *P. margaritifera* juveniles could be exposed to waterborne cues of actively feeding predators (Smith & Jennings 2000). Once mature, the shells from these bivalves could then be compared to individuals not exposed to the cues. If differences are found among the clams, *P. margaritifera* is capable of changing its shell formation, and probably relies heavily on this form of defense.

The microbial screens showed that the nudibranchs possess a significantly higher degree of bioactivity than the bivalves (p: 0, f-ratio: 68.08; figure 3c). Like the cnidarians, most species of nudibranchs are covered by mucus that prevents microbial infection. The compounds making this mucus bioactive are diet-derived and differ depending on the nudibranch's prey (Avila & Paul 1997). The little microbial activity found in *T. maxima* and *L. orientalis* is probably due to the presence of macrophage-like phagocytes and antimicrobial peptides. These compounds are commonly found circulating within the animals' hemolymph to prevent infection (Roch 1997). Roch's research showed many bivalves having a high degree of bioactivity. This is inconsistent with the data from my study. In my research, only a slight case of antimicrobial was observed among the bivalves tested. A probable explanation for this discrepancy is that the bivalves used in Roch's research were naturally exposed to a greater abundance of infectious microorganisms. Future research could test this inconsistency by exposing the species of bivalves used in both experiments to the same quality of water. After a sufficient time period, if similar bioactivity is observed in all bivalves, a difference in water quality was the likely cause of our inconsistency.

Results from the two-way Anova showed that chemical defense is significantly dependent on ecological differences (p: 0, f-ratio: 29.8; figure 4). My research has shown that the phylum a species represents does not indicate the organism's degree of toxicity or bioactivity. Although cnidarians required a smaller amount of extract in the toxicity tests, the toxicity values from this phylum would not have been significantly higher from those of the other two phyla if the same amount of extract had been used for all three phyla. The presence or absence of physical protection is more important in determining toxicity. This may imply that chemical defenses have evolved independent of phylogeny, and are not the result of historical constraints. Future research could test this theory by isolating the toxic compounds found in these invertebrates. If chemical defenses have evolved independently, then the chemical make-up of these toxic compounds probably differ between phyla. Organisms from different phyla should possess different toxic compounds (Bakus 1981).

A possible explanation for the positive ecological correlation is that chemical defenses evolved repeatedly in taxa that lost physical defenses. To test this evolutionary hypothesis a phylogenetic analysis of relationships would need to be performed. A cladogram would need to be constructed showing how the taxa are related. The measurements of toxicity and bioactivity would then need to be mapped onto the cladogram to see if the more derived taxa are toxic. Mapping toxicity and bioactivity data onto a well resolved phylogenetic analysis is a promising area of research for investigating the evolution of chemical defenses.

The toxic and bioactive compounds in the organisms of this study may prove to have medical significance. The antimicrobial tests used in this research are similar to those performed by pharmaceutical companies that test for new sources of antibiotics (Cragg & Newman 2001). The significantly bioactive compounds found in the sea cucumbers, sea anemones, soft coral, and nudibranchs deserve attention because of their possible medical use. The brine shrimp toxicity test used in this research is also a preliminary test used in anticancer research (Lutz pers. comm.). The chemical compounds found in the highly toxic organisms of this study may be a promising topic for future anticancer research.

Future research on chemical defenses could be improved by reducing the variables that may have affected the results. In this study, the extracts from 3 individuals of each species were tested. Because adequate vials were limited, the extracts were stored in a single vial. Researchers should include more than 3 individuals from each species, and extracts should be stored in separate vials so that each individual can be tested independently. The method for extracting hard coral samples could also be improved. A sufficient amount of water may have been accidentally introduced to influence the results. More accurate results may be attained if the less water is added. In the toxicity tests, data could be made more accurate if the number of dead brine shrimp is counted at multiple time intervals. This will allow for a broader range of comparisons to be made between species. A major problem in this research was the inability to grow bacteria. Specific agar for cultivating bacteria should be used in future research so that the microbes can grow more easily.

## Conclusion

In this study, a variety of physical and chemical defense mechanisms were observed among a variety of marine invertebrates. The correlation between an organism's mode of life and its use of chemical defenses is likely due to ecological adaptations that occurred independently within each taxa.

**Acknowledgements**

I would like to thank Carol Hickman for all the help she gave me throughout my study. I would also like to thank George Roderick and Pete Oboyski for their help in statistics. Finally I would like to thank John Holl and Parissa Vassef for their continuous help with my tests.

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## Appendix

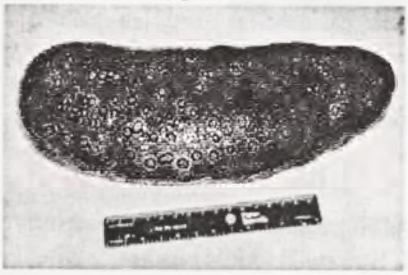
### Voucher photographs and references

## Echinoderms

*Thelenota ananas*

*Echinothrix calamaris*

*Bohadschia argus*



*Echinometra methaei*
See: Allen GR, Steene R (1994) Indo-Pacific coral reef field guide -

*Holothuria atra*

*Amphiuro sp. 1*
See: Allen GR, Steene R (1994) Indo-Pacific coral reef field guide.

*Diadema savignyi*

*Culcita novaeguineae*

## Cnidarians

*Pocillopora verrucosa*

*Fungia costulata*
See: Allen GR, Steene R (1994) Indo-Pacific coral reef field guide.

*Stichodactyla haddoni*

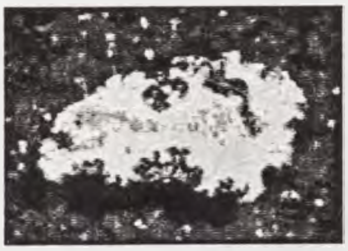
*Zoanthidea protopalythoa*
See: Allen GR, Steene R (1994) Indo-Pacific coral reef field guide

*Hormathiidae amphianthus*
See: Allen GR, Steene R (1994) Indo-Pacific coral reef field guide

*Ricordia yuma*
See: Allen GR, Steene R (1994) Indo-Pacific coral reef field guide

## Mollusks

*Glossodoris cincta*



*Gossodoris atromarginata*

*Hexabranchus sanguineus*
See: Allen GR, Steene R (1994) Indo-Pacific coral reef field guide.

*Tridacna maxima*

*Limaria orientalis*
See: Allen GR, Steene R (1994) Indo-Pacific coral reef field guide.

*Pinctada margaritifera*

Biol. Geomorph. Trop. Is. (2002) 11:95-104
UC Berkeley, IB 158 / ESPM 107

# Abundance and Response of *Carcharhinus melanopterus* to Feeding Stimuli in Mo'orea, French Polynesia

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ABSTRACT*:* The blacktip reef shark, *Carcharhinus melanopterus,* was observed in response to feeding stimuli in Mo'orea, French Polynesia. Natural abundance, abundance in response to feeding stimuli and abundance prior to tour boat arrival were observed at two established sites (1,2), and a third newly established site (3). The arrival time of *C. melanopterus* prior to food stimuli in the water was also observed in order to determine if the tour boat acted as an attractant. Interactions between time of day and shark abundance were compared with one another to determine if a significant interaction occurred at each site. Statistical analysis showed that time of day and site were significant indicators of abundance at established sites, but not at a newly established site. The data also indicate that at established sites a slight increase in abundance occurred prior to boat arrival, but it was not statistically significant. Statistical analysis of *C. melanopterus* arrival times at sites 1,2 and 3, and found that sites 1 and 2 were more similar than site 3, which did not have a response from *C. melanopterus* to food stimuli, time of day, or boat stimuli. Arrival prior to food placement in the water at sites 1 and 2 was observed and introduces the possibility that the sharks may be responding to the cue of boat presence, and not only food stimuli. The lack of response to stimuli at site 3 in contrast with the behaviors shown at sites 1 and 2 suggests that the natural behavior of *C. melanopterus* has been altered to an extent at established sites.

Keywords: *Carcharhinus melanopterus,* shark feeding, Mo'orea.

## Introduction

The allure and fascination of being within close contact of wild animals runs deep through the American culture, and those throughout the world. The want to be close to wildlife grows increasingly greater, as does the economic drive to create faucets allowing such exploitation of organisms. The economic success of bringing wildlife closer to the general public can be seen in the success of zoos, marine parks, and such recreational activities such as S.C.U.B.A Diving and snorkeling. S.C.U.B.A and snorkeling bring humans closer to the marine realm, which would otherwise be inaccessible. What once consisted of passively observing marine organisms now includes the existence of tour companies that bring humans closer to sharks, rays, and sea turtles by feeding the wild population at specific sites.

Shark feeding tours consist of either a S.C.U.B.A master, or tour guide for snorkels, taking tourists to an established location where they either hand feed sharks, or throw food into the water while the tourists watch from underwater.

In the 1960's the first shark-feeding site was established in the Bahamas (Decoudras, 11/6/02, perscom). Tourists were allowed to S.C.U.B.A with the sharks while one operator fed them. This form of tourism soon became a well known, and lucrative business which spread to other tropical locations. In the late 1970's, the success of shark feeding spread to French Polynesia and the first site was established on Bora Bora. It wasn't until the early 1980's that shark feeding was brought to Mo'orea and within the lagoon (Decoudras 11/6/02 pers.com.)

In Mo'orea shark feeding was first established primarily for S.C.U.B.A divers on the forereef, but it quickly became an attraction for snorkeling within the lagoon. Within the lagoon snorkelers were and are able to catch glimpses of a primarily *Carcharhinus melanopterus* population (Decoudras, 11/6/02, pers.com.).

While the popularity of shark feeding among tourists grew, the reverse began to occur for the native populations. Fisherman began complaining that they couldn't step into the water without having a swarm of blacktips harass them, while swimmers complained of the sharks no longer being timid when presented with a human (anonymous, 9/27/02, pers. com.).

In 1998, PGEM, or Plan de Gestion Espace Maritime, was proposed, which would no longer allow shark feeding within the lagoon and would regulate the feeding of sharks for S.C.U.B.A divers in the forereef. Although still in the process of becoming law, the Mayor of Moorea banned the practice within the lagoon over the past year.. Only three tour companies on the island still operate, and between those three companies, only two feeding sites exist. They are located on the north and northwestern portion of the island and are around the same age. The methods for feeding are similar for both sites, and the topography of the sites are similar as well. Tours cost between 2,000 cfp-55,000 cfp per trip, and can run up to two times per day. The total amount of time spent in the water with sharks is minimal and ranges from fifteen to approximately twenty-five minutes, depending on the company.

Feeding of *C. melanopterus* with such regularity raises a few questions that this study aims to answer:

1. Is the abundance of *C. melanopterus* at feeding sites altered throughout the day, or does it stays at a constant level?

2. Is the presence of the boat (and potentially it's motor), food stimuli in the water, or time of day, triggering the presence of *C. melanopterus*?

3.Does *C. melanopterus* have the same response at previously un-established sites?

**Figure 1.** Study Organism: *Carcharhinus melanopterus* as observed at Site 3. This individual was approximately 1.8m in length, and identified by the black coloration adorning the top of the dorsal fin, the black stripes lining the caudal, and pectoral fins. A longer black stripe runs vertically along the edge of the tailfin, which distinguishes it from the blacktip shark (Randall, et.al 1973).

## Materials and Methods

### *Study Organism*

*Carcharhinus melanopterus* was chosen for this study due to its abundance at the local feeding sites, as well as for its typically non-aggressive behavior towards humans.

*Carcharhinus melanopterus*, commonly known as the Blacktip Reef Shark, is found in tropical reef waters in the Pacific Ocean from Japan to French Polynesia, and westward to the Red Sea. It is one of twelve sharks species found in French Polynesia, and is one of the most abundant (Bagnis, R. et al. 1987).

They are typically found in lagoons, forereefs, and in waters that reach up to ten meters in depth. This species of shark frequents lagoon channels while low tide is present, and will then migrate into the reef flat as the tide level increases. Pups are found in the shallows of the intertidal zone where they can seek safety from other predators, such as more mature blacktips and lemon sharks. In natural settings they are most active near dusk and at night, but they are observable and present during daylight hours(Budker 1971).

*Carcharhinus melanopterus* is easily distinguishable by the black coloration that adorns the tip of its first dorsal fin, with a smaller band of white directly below. The second dorsal fin, lower caudal fins, and lower potion of the tailfin contain thin strips of black as well Randall et.al 1973) One of the smaller species of sharks, their size ranges from 33-52 cm (1320.5 inches) at birth, to 1.8 m (5.9 ft) at maturity. The snout is shorter and more rounded than other sharks found in French Polynesia. The dorsal portion of the body is light grey, with a slight gradient into a white abdominal area (Randall, et.al 1973).

### *Study Sites*

Sites 1 and 2 were chosen based on the pre-established locations of two tour groups. Site 3 was chosen based on similarity of topography and water depth to Sites 1 and 2, as well as accessibility by motorboat. (Appendices A,B, and C)

### *Site 1*

Site 1 was located at S 17°28.733, W 149°50.545, on the Northern side of the island of Mo'orea within the lagoon (Figure 2). The water depth was approximately 2.7-3.3 meters, depending on tide level and height of swells. The lagoon floor consisted of a fine-grain, sandy bottom with low-lying coral heads scattered throughout the vicinity. Water visibility was approximately 6.7meters but varied depending on the current strength and sunlight strength. A boating channel with the same depth was approximately 6.7 meters away to the south of the site, which incurred traffic both during feeding and during natural abundance observations. A deeper lagoon channel was located to the southeast of the site by approximately 100 meters, which fed water out of the lagoon and into the pass at Cook's Bay.

### *Site 2*

Site 2 was located on the Northwestern portion of the island near Fare Vai Moana, and within the lagoon at S 17°30.844, W 149°55.343. At this location the backreef receded from the reef flat, but did not decline into a deeper channel. The water depth was approximately 2.7 meters and had a floor substrate consisting of fine-grained sand and low lying coral heads. Visibility while in the water was approximately 6.7 meters (+/-) 1 meter depending on the amount of sunlight. The current was mainly calm and with little to no swells. A boating channel of the same water depth was approximately 6.7 meters to the southeast of the site, but did not acquire the same traffic as Site 1( Figure 2).

### *Site 3*

Site 3 was located to the East of the pass at Cook's Bay within the backreef and lagoon at S 17°28.735, W 149°49.123. It had a water depth of approximately 3.3 meters, varying depending on the size of the swells on the day of observation, with floor substrate composed of medium grained sand, and a higher density of corals, Sites 1 or 2. The swells at this site were medium to large in size, at times being approximately a meter. The current flowed in a Northeastern to Southwestern direction and was noticeably stronger than either of the two previous locations. To the west of the site, approximately 200 meters away was the water pass to Cook's Bay, and to the south was a deeper boating channel that received heavy traffic.

### *Establishment of a New Feeding Site*

The establishment of new feeding site required selecting an area of lagoon with similar substrate type, topography, and water depth to Site 1 and Site 2. A location was chosen on the Northern side of Mo'orea and to the east of the Cook's Bay pass in the back reef (Figure 2).

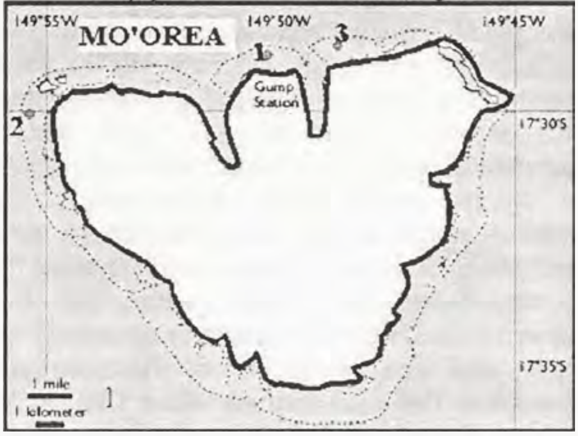

**Figure 2.** Map of Mo'orea, French Polynesia, showing approximate locations of study sites 1,2, and 3. Site 1: S 17°28.733, W 149°50.545; Site 2: S 17°30.844, W 149°55.343; Site 3: S 17°28.735, W 149°49.123.

At the newly established feeding site, an outboard motor boat possessing one Johnson 15 engine was used to travel to the site, which was located by the GPS tracking unit. A container was constructed out of a Brassiere Tahiti case (45x36x30cm), chicken wire, zip ties, and rope, in order to keep the food stimulus from floating away in the current (Appendix D ).

Upon arrival at the site, the boat was anchored; the bait box was deployed into the water, which contained strips of fish skin or internal organs, and approximately 0.5-1L of fish blood was poured into the water. The time of arrival, anchorage, deployment of bait container, and fish blood were recorded. My partner and I then entered the water and held onto the rope that anchored the boat. Observations, and shark counts, were taken for 10 minutes, at which point more blood was put into the water and the observational process continued for 10 more minutes. Scanning for sharks was performed from left to right, facing southeast, and then slowly rotating in a circle. In order to mimic the observations recorded from the tour groups approximately 20 more seconds per minute were spent looking in the southwest direction, which was the direction, the current flowed.

The experimental site was visited a total of thirteen times, at approximately 10:00 a.m. (+/-) ten minutes each day.

### *Determination of Baseline Natural Abundance*

In order to observe and quantify the magnitude that shark feeding has on the natural population, natural abundance observations were conducted at sites 1,2, and 3. Each site was visited at 4 randomly chosen times throughout the day in between the hours of 8-9am 11-1 p.m. and 2:30-5 p.m. Although randomly selected, observation times were adjusted to compensate for weather conditions and field partner accessibility. The sites were visited by using a 3.7m, two-person kayak. Upon arrival, the anchor to the kayak was dropped, the time recorded, and then my partner and I entered the water. Time points were taken every two minutes, and when a shark was observed, the time, size, and direction from which it came were recorded onto an underwater slate. After twenty minutes of observation my partner and I exited the water.

### *Determination of Abundance Prior to Boat Arrival*

The desired arrival time to Sites 1,2, and 3 was twenty minutes prior to the arrival of the feeding boat. A two-person kayak, 3.7m, in length was taken to each site, where it was anchored. Anchorage time, and time of entering into the water were recorded. Observations occurred for approximately twenty minutes or until the feeding boats began to arrive. At Site 1 we were able to stay in the water upon boat arrival due to being located approximately 7 meters to the south of the feeding site, which did not enable visibility of the feeding site, depending on where the boat anchored. However, at Site 2, upon seeing the feeding boat approach, my partner and I had to exit the water due to safety issues, and a desire voiced by the tour company for us to not be at the site. Observations at Site 3 were done twenty minutes prior to boat arrival as well, and we were not forced to vacate the water upon boat arrival.

### *Determination of Abundance During Feeding Times at Two Established Sites*

For Site 1 a tour boat approximately 7.4 meters, containing two Johnson V6 Ocean Runner engines was used to shuttle passengers to the site. The boat would anchor and a rope would be attached to a coral head approximately 10 meters away from the boat. The driver would enter into the water and call out for the presence of any sharks as soon as they were observed. Chunks of tuna, and other scraps of fish were thrown into the water and at which point the tourists were allowed to enter the water. The bait was thrown to the south of the boat and tourists, whom held onto the anchored rope for safety due to the strength of the current.

The time of arrival, anchoring, baiting, presence or absence of sharks, and time upon entering the water were recorded. Upon entering the water, an underwater slate, and a number 2 pencil were used to record time points and shark counts, while a Timex Indiglo watch was used for the actual time points. Shark counts were recorded in a non-interval fashion. When an accurate count of the sharks could be taken, +/- one shark, their numbers were tallied, along with the time. The amount of time spent in the water varied from 16-19 minutes, depending on when the driver instructed people to exit the water. A total of five visitations were made with this tour group.

Site 2 was visited a total of five times. Visitations occurred at approximately 10:00 a.m., and lasted for approximately 15-20 minutes. All observations were performed and recorded in the same method for Site 1. The tour boat at this location was slightly smaller than that at Site 1, with a length of approximately 6 meters. The boat used was powered by two Johnson V6 Ocean Runner engines.

### *Arrival Prior to Food Stimulus*

During each of the observations performed at Sites 1,2, and 3 the presence or absence of sharks prior to food stimuli being placed in the water was recorded.

### *Statistical Analysis*

An ANOVA test was ran on the data collected for natural abundance, abundance twenty minutes prior to boat arrival, and abundance during feeding time observations. The ANOVA was used to help determine if time of day, or site were significant indicators at any of the three sites for *C. melanopterus* abundance. . Further statistical analysis involving a post-hoc Tukey test was used to determine within sites, which times of day were significant indicators of shark abundance.

A Kruskal-Wallis test was used to determine whether the data obtained from the time it took for C. melanopterus to arrive after tour boat arrival was significant. Another Kruskal-Wallis test was then performed comparing only two sites at a time, and using a post-hoc Mann-Whitney test to determine which of the sites had significantly different arrival times. In order to compensate for the lack of arrival at Site 3, the time of arrival was inverted, which allowed a more normal distribution of data and data analysis

## Results

### *Baseline Natural Abundance, Abundance Prior to and During Feeding*

The average number of *C. melanopterus* observed during natural abundance, twenty minutes prior to feeding, and during feeding sessions are recorded and summarized in Table 1, and can be viewed in Figure 3. Observations taken during the feeding sessions regarding the presence or absence of *C. melanopterus* prior to food stimuli being administered are also located in Table 1.

### *Statistical Analysis*

The results from the ANOVA test, which compared whether the data collected for time of day and site were significant enough to predict abundance at either of the three locations, indicate that time of day and site were significant indicators of the abundance of sharks, with p values of less than 0.05 for each (Table 2). The interaction between time of day and site also proved to be a significant indicator of abundance, with a p value below 0.05. However, upon comparison of the three time categories within a site, with a Tukey test, the results suggest that for sites 1 and 2 the baseline natural abundance could not be differentiated from the twenty minutes prior to boat arrival Thus, only two significant abundances of *C. melanopterus* occurred throughout the day. Site 3 had no significant difference between the three time categories, with p values greater than 0.05 for each time category.

### *Response Time*

The Kruskal-Wallis test determined that the data obtained from measuring the time until first arrival of a *C. melanopterus* at sites 1, 2 and 3 during feeding observations proved to be significant ($p=<0.05$). Further analysis showed that all three sites had significantly different arrival times, p values were $<0.02$ for each, and that the site could predict the time of first arrival.

**Table 1**. Average number of *C. melanopterus* observed during three different time categories. BNA= Baseline Natural Abundance; AP= Abundance Prior to Boat Arrival, AD= Abundance During Feeding; and P?= Presence of *C. melanopterus* prior to food stimuli

| Site | BNA | AP | AD | P? |
|---|---|---|---|---|
| 1 | 0 | 4.5 | 11 | 4Y |
| 2 | 0.25 | 4.5 | 20 | 4Y |
| 3 | 0.25 | 0 | 0 | 13N |

## Discussion

### *Is the Abundance of C. melanopterus constant throughout the day?*

The abundance of *C. melanopterus* does not remain at a constant level throughout the day. An obvious alteration in abundance has been observed that occurs during feeding times. Surprisingly the slight increase in shark abundance twenty minutes prior to feeding, was not significant enough to determine that the time of day was cuing the sharks into the area. However, due to the inability of a count taken at the exact feeding site, it was difficult to observe the number of sharks present upon boat arrival. However, this discrepancy can be

### *Is the presence of boat, time of day, or food cuing the C. melanopterus?*

The exact cue eliciting response from *C. melanopterus* has yet to be determined. It was previously established that the sharks did not have a significant increase in abundance prior to boat arrival, which disregards time of day as a viable cue.

It is difficult, and presumptuous to separate time of day and food presence from one another due to the method of learning that could have been employed by *C. melanopterus*. In order for *C. melanopterus* to be responding to the presence of the boat, or time of day, associative learning would have had to occur at some point in the past. Associative learning involves the ability of an organism to equate one form of stimulus with the occurrence of another (Carew, 2000) A response to a boat as stimulus would only be triggered if its presence were associated with some form of reward. The food stimulus was found not to be the only cue for their response, due to the presence of *C. melanopterus* prior to the food stimulus being added to the water. Thus, it is probable that more than one cue was acting on the affected *C. melanopterus* population and from this data I interpreted that the sharks had previously associated the boats with a supply of food. However, other cues may have been factors as well such as individual sharks being attracted to the area due to sensing the presence of other feeding *C. melanopterus*, which should be investigated further.

(Parker 1999)

**Table 2.** Results of ANOVA comparing the significance of time of day and site as indicators of shark abundance. Sources with a P value less than 0.05 have significance.

| Source | Sum of Squares | dF | Mean-Square | F-Ratio | P |
|---|---|---|---|---|---|
| Time of Day | 948.219 | 2 | 474.109 | 98.675 | <0.05 |
| Site | 391.714 | 2 | 195.857 | 40.763 | <0.05 |
| TD*S | 652.614 | 4 | 163.153 | 33.957 | <0.05 |

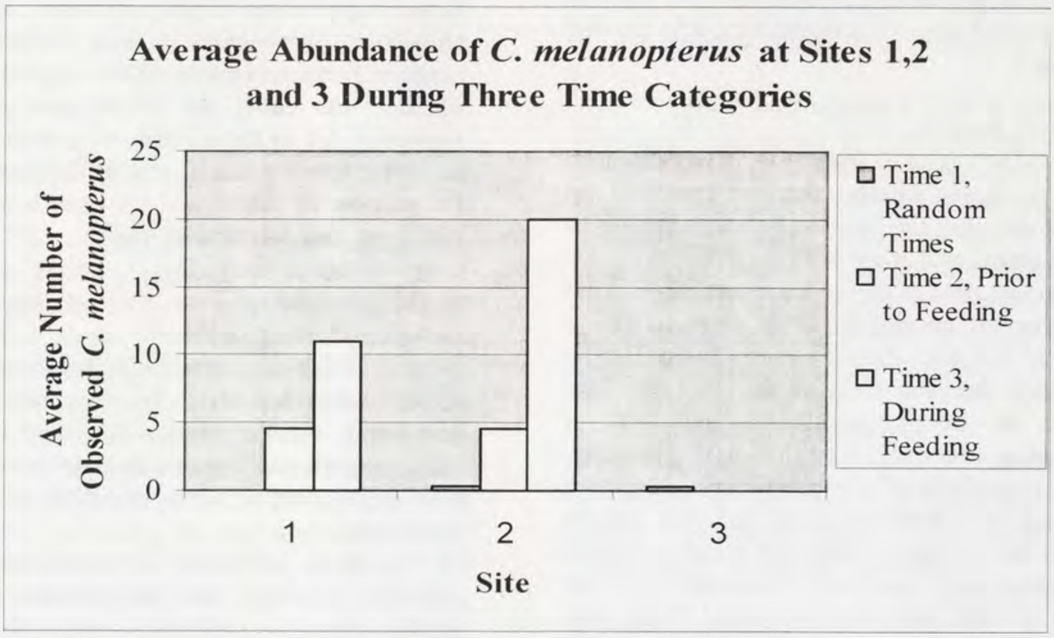

**Figure 3.** The average abundance of *C. melanopterus* observed at Sites 1, 2, and 3 for three different time categories. Time A is in reference to the four random times points taken for each site. Time B refers to the observations taken twenty minutes prior to boat arrival, and Time C refers to observations taken during feeding times with both the tour groups, and at the established site.

*Response at Newly Established Sites*

The lack of response by *C. melanopterus* to stimuli at Site 3, demonstrates that this species of shark did not have the same response at established sites, as they did at newly established sites. This finding was surprising due to the claim by local fisherman that *C. melanopterus* poses as a hazard while they are fishing, as well as a nuisance. This finding leads to idea that either the boldness of *C. melanopterus* has been exaggerated, or that the naive population at this location needs a greater amount of time to respond to the stimuli.

**Table 3.** Combination and comparison of sites 1-3. Combinations with p values less than 0.05 are considered significantly different from one another.

| Site Combination | Mann-Whitney U test | p value |
|---|---|---|
| 1,2 | <.01 | 0.009 |
| 1,3 | 64.999 | <.017 |
| 2,3 | 64.999 | <.017 |

A number of factors could have attributed to the lack of response at Site 3. Although chosen for its topography and location within the lagoon, Site 3 did have differences from the other two sites. The close proximity to the pass at Cook's Bay could limit the number of juvenile sharks present at this site. Their desire to be in the shallows of the backreef, and close to the algal crest for safety purposes may have kept them farther away from the bay pass, which ultimately acts as an entrance point for lemon sharks or other predators (Randall, Helfman, 1973).

The proximity to the reef crest may also have been a limiting factor in shark response. The location of Site 3 was closer to the boat channel and pass, which did not allow the same area of backreef to the southwest of the feeding site as Sites 1 or 2. The area located to the southwest of the feeding sites was important due to the direction of the incoming current. A greater area equates to the food stimuli being spread over a greater range of *C. melanopterus* habitat. At Site 3 the direction and strength of the current could have carried the scent of food stimuli quickly through the backreef and into the pass of the bay and deeper boating channel where a smaller population of *C. melanopterus* would be found, due to their lack of affinity for deep waters (Ellis, 1983).

### *Time of Arrival*

The results from the Kruskal-Wallis Statistical Analysis demonstrate that a difference between the average amounts of time it took for the first blacktip reef sharks to arrive after boat arrival occurred. The low p values obtained from the Kruskal-Wallis analysis demonstrates that the data points were significant and that a difference between the means of arrival times did occur. However, the Kruskal-Wallis analysis does not say between which two samples the difference occurred, and thus the Mann-Whitney test was used to find between which samples the mean was different.

Given the fact that both sites were approximately the same age, the question of why the difference in abundance and arrival time varied is raised. It is possible that the difference in shark abundance was greater at Site 2 due to its geographic location and topography. Site 2, was located on the northwestern side of the island where the reef crest curved around, causing a bowl shaped lagoon with a greater reef area than Site 1. There was also the lack of reef pass close to Site 2. The lack of reef pass would make the chances of a lemon shark, or other large predator, coming into the site area less likely, as well as the shallow depth of the water, which is undesirable habitat for lemon sharks (Bagnis et.al 1987). The lack of natural predator could explain the higher average number of sharks responding to site, and ultimately the shorter response time to boat stimuli.

### *Implications for PGEM*

Although an alteration of abundance was observed, other factors besides the tour boats must be taken into consideration for contributing to the response of *C. melanopterus* to presence of boats. Quite like the tour groups, fisherman often gut and deposit the internal organs of fish in the lagoon where they are fishing. The frequent deposit of food type items for *C. melanopterus* is a potential contributor to the increased boldness of the sharks' behavior.

The implication from my data that sharks are attracted to either the engine of the boat, or the physical presence of it, raises the question of how the sharks will react if they expect food, and are not given it. In a first-hand encounter described by Peter Benchley, the presence of humans in the water at an established feeding site caused an unnatural increase in shark abundance, which became progressively more frenzied and aggressive as no reward was given (Benchley 2002). The habituation of sharks to the presence of humans can potentially pose a threat to fisherman, swimmers, and S.C.U.B.A divers if they are at a feeding site and not offering food. Only once in my study were aggressive behaviors exhibited towards my field partner and myself, at which point we exited the water. The C. melanopterus in question was not exhibiting life threatening behavior, but exhibited common aggressive trademarks such as an arched back while swimming, swimming in a more angular fashion, and down-pointed pectoral fins (Randall et.al. 1973)

### *Future Studies*

For further examination into this topic more extensive studies should be preformed, which focus not only on what stimuli is eliciting responses, but also on population dynamics and structure. Tagging of the sharks at the feeding sites would allow individuals to be tracked and accounted for at each feeding time. Where the sharks are coming from, as well as if they visit a site more than once a day would offer information on their changing behaviors. The amount of food consumed is also a potential study. Sharks typically consume 3-14% of their body weight per week, and it would be helpful to know if the sharks consumed less of their natural prey, or the same amount with the added feeding stimuli (Budker, 1971). This information would give researchers and government officials a better understanding of how the shark population has been altered.

### *Conclusion*

The response of *C. melanopterus* to boat, food stimulus, and time of day differed between two established sites and one site in the process of becoming established. The quick response observed during feeding times indicates that populations of *C. melanopterus* are responding to stimuli other than food, like the physical presence of a boat, or the boat motor, which I was not able to differentiate between in this study. An obvious alteration of abundance occurred and should be examined in further detail.

### Acknowledgements

I would like to thank the Professors and GSI's for their help, support and wonderful ideas, Steve Carlson and Chris DiVittorio for their enthusiasm and help with my project while in the field, the Gump Research Station staff for their donations of fish parts, Cecile Gasper for her help with personal contacts on Mo'orea, Pierre Decoudras for offering information on the history of shark feeding and PGEM, Club Bali Hai and Mo'orea Camping for allowing me to accompany them during their feeding tours. Thank you especially to Tetohu Toanena, and Michele Roe for their patience and assistance during the shark feeding tours.

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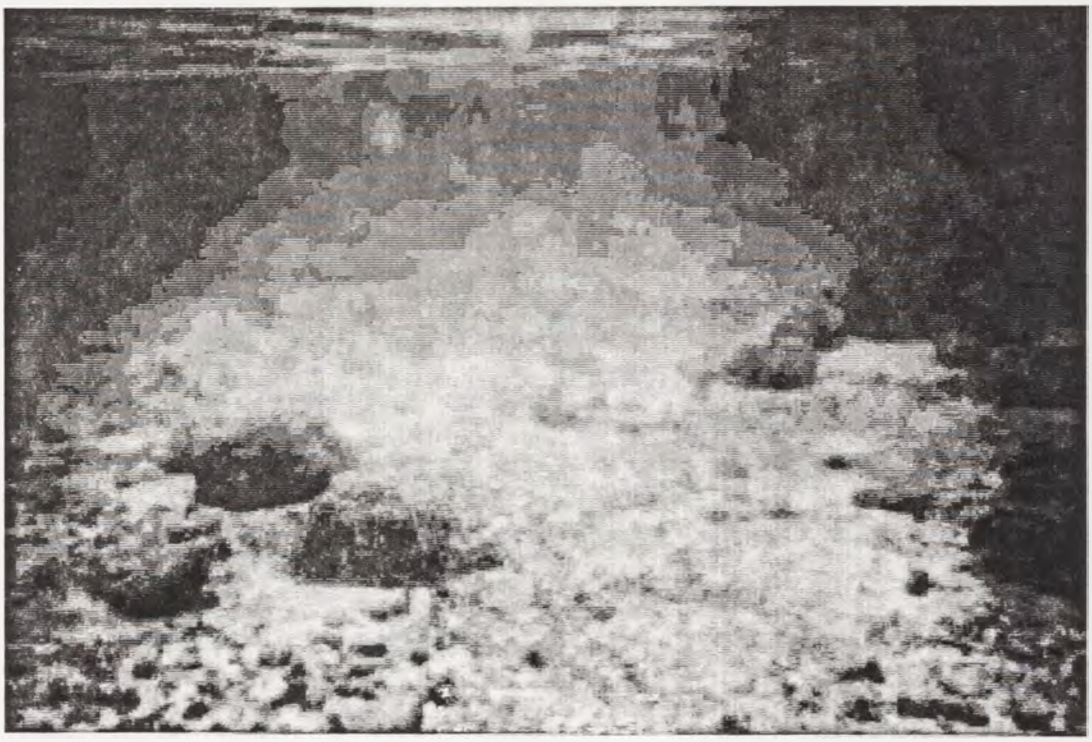
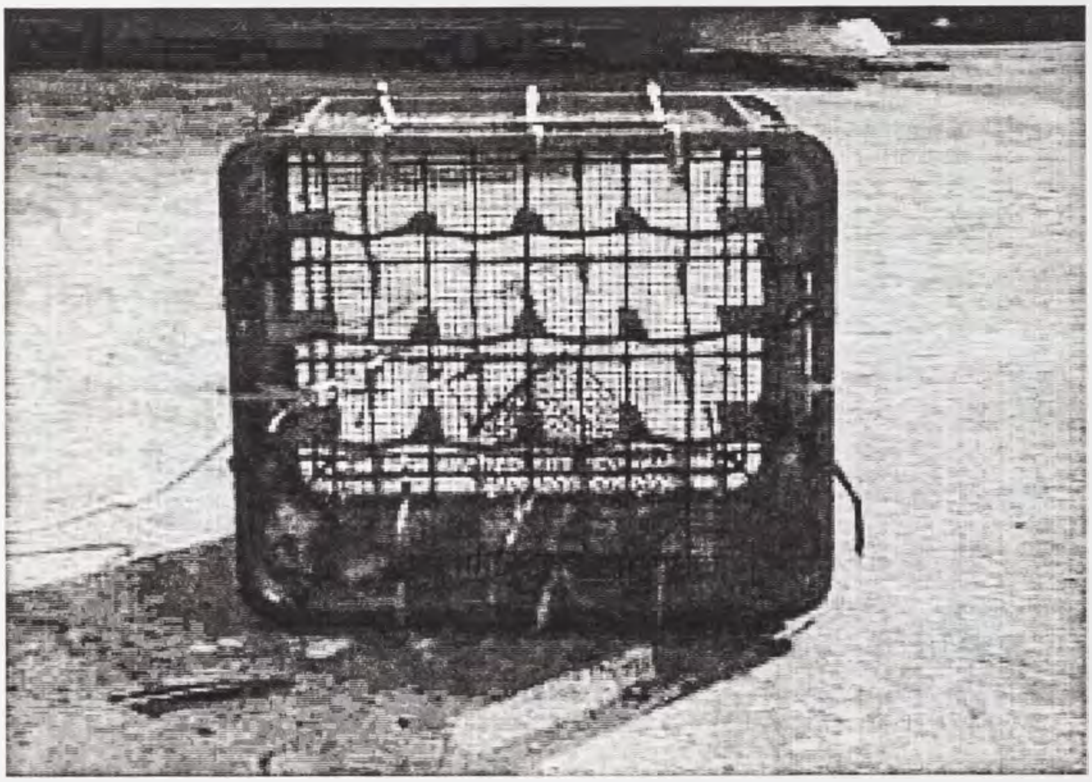
## APPENDIX

Appendix A. Topography and substrate at Site 1.

Appendix B. Topography and substrate at Site 2, with two blacktip reef sharks



**Appendix C**. Topography and substrate at Site 3



**Appendix D**. Food stimuli container. Dimensions (45x36x30cm)

Biol. Geomorph. Trop. Is. (2002) 11:105-118
UC Berkeley, IB 158 / ESPM 107

# The Effects of Sedimentation on *Porites* in Mo'orea, French Polynesia

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ABSTRACT. Sedimentation and its effect on coral bleaching of *Porites* were assessed for Cook's Bay, Mo'orea, French Polynesia. Sediment traps were placed in the field and collected weekly and at 24-hour intervals. Quadrats were used to survey *Porites* at each site and to record signs of bleaching. Dried sediment samples were analyzed for size using a micrometer and analyzed for composition using a petrograph. A lab experiment added sediment at different levels (250 mg/ $cm^2$, 500 mg/ $cm^2$, and 1000 mg/ $cm^2$) and different sediment sizes (83 microns and less than 83 microns) to coral heads of *Porites lutea*. A regression between mass of sediment (mg) for weeklong samples and percent of each quadrat bleached at each site gave an r-squared value of .004626 (p-value= .6533). A regression between mass of sediment (mg) for 24 hour-long traps and percent of the quadrat bleached at each site gave an r-squared value of .006706 (p-value= 0.7021). ANOVA tests found statistically significant differences among the controls (both 83 microns and less than 83 microns) and the treatment groups 250 mg/$cm^2$ and 500 mg/$cm^2$ (sediment size less than 83 microns). Particle size and composition does not differ at different sites throughout the bay (p-value= 1.000). Composition and sediment sizes are uniform within Cook's Bay. Sedimentation causes bleaching in *P. lutea* under laboratory conditions for smaller sediment sizes. The discrepancy between the results found in the field and those found in the lab exist because several other factors such as sunlight penetration, sea surface temperature, and competition vary at large spatial and temporal scales.

KEYWORDS. Mo'orea; French Polynesia; *Porites lutea*; Sedimentation; Bleaching

## Introduction

Over the last 25 years, coral bleaching events worldwide have increased in number and severity (Wilkinson 2000). Coral bleaching occurs when corals lose all or most of their pigmentation when their symbiotic zooxanthellae disperse. It can also occur when the chlorophyll concentrations in the coral's photosymbiotic zooxanthallae are reduced. This reduction in efficiency of utilizing photosynthetic energy occurs when the zooxanthellar photosystem is saturated by active radiation. If this excess excited energy is not decreased, the extra photo energy creates harmful free radicals that disrupt photosystems and cause bleaching of the coral colony (Mumby et al 2001).

Bleaching events have become more frequent in the last 20-30 years, and in the last 10 years in French Polynesia, four bleaching events have occurred (1991, 1994, 1998, and 1999) (Wilkinson 2000). Among these four bleaching events, 1998 caused the most widespread damage worldwide by affecting 42 countries. More specifically in French Polynesia, however, the 1991 bleaching event caused approximately 20% of the colonies in the outer slopes of many islands in the archipelago to die, while during the 1994 bleaching event most colonies recovered (mortality was not as high) (Salvat et al 2001; Wilkinson 2000). The 1999 bleaching event had much greater variability within each island and between different islands in French Polynesia.

The most widely excepted explanation for coral bleaching is that it is caused by exceptionally high sea surface temperatures acting in conjunction with ultra-violet radiation. This hypothesis is excepted because most massive bleaching events have occurred during the summer months, during calm sea conditions, and near the time when solar radiation should be at its highest (Mumby et al 2001). In April of 1994, the sea surface temperatures on Mo'orea (the second youngest island in the Society Archipelago and just Northwest of Tahiti) were 29.5°-30° C. This is approximately 1.5° C above the expected temperatures for that time of year and preceded the bleaching event by 2-3 weeks. This is consistent with previous data that bleaching events normally occur when thermal anomalies exist (Salvat et al 2001). All factors that could cause bleaching, however, such as sedimentation and salinity should be studied, because the causes of bleaching events are still unclear.

Sediment has been cited as one of the major destructive forces on reefs (Pearson 1981; Hubbard 1986: McManus 1988; Hodgson 1990; Rogers 1990; Babcock and Davies 1991). Sedimentation is an increase in the thickness of a sediment body caused by the addition of material from a plume or run-off from the land at a body of water's upper surface (Larcome and Woolfe 1999). High sediment loads to corals can have the affect of increasing respiration while in conjunction lowering photosynthetic rates of the endosymbiotic algae (zooxanthellae). Sedimentation can cause detrimental effects such as death of corals when they are buried by sediment, reduced growth by smothering and lack of light, inhibited coral planulae settlement, and modification of growth by producing corals that are more resistant to sedimentation. Many corals have the ability to shed sediments from their live tissues via secretion of mucus, movement of cilia, extension of polyps, and passive gravitational movement (Loya 1976). The energy expenditure, however, of removing these sediments decreases the coral's fitness by limiting resources available for other processes such as food capture, growth, tissue repair, and reproduction (Gleason 1998)

Colony death due to the persistent influx of sediment has been documented in several studies. For example, Marshall and Orr (1931) report that sedimentation accumulations of 1.6 g/cm$^2$ and 1.2 g/cm$^2$ over an 18 day period caused coral death to *Porites.* The results of sedimentation levels and their affects on *Porites* have varied substantially in different areas of the world and in the lab versus in the field. According to Hodgson (1990), there is a higher incidence of tissue damage in *Porites lobata* at 30 mg/cm$^2$/day in lab aquaria for 10 days by stirring "fine marine sediment" twice per day and allowing it to settle. In the Northern Great Barrier Reef, however, *Porites lobata* that was subject to calcium carbonate/quartz (63-250 microns in size) at influxes of 50, 200, and 400 mg/cm$^2$ for eight days *in situ* caused bleaching but not total colony mortality (Stafford-Smith 1992).

The purpose of this study was to determine if sedimentation contributes to the bleaching of *Porites*. Although *Porites* species seem to have a greater resilience to bleaching because of their slow metabolic and growth rates, the affects of bleaching on these massive scleractinian species of coral should also be explored. Because previous studies found that certain levels of sediment (mass per area) can cause total colony mortality, this study also determined, in the lab, what mass per area of sediment, while varying sediment sizes, causes bleaching and/or total colony mortality in *Porites lutea*. Sediment collected will be analyzed by size and composition to determine the natural distribution of sediment within Cook's Bay. Alternative hypotheses are 1) mass of sediment will be positively correlated with bleaching of coral colonies, 2) that sediment will cause bleaching, with the highest treatment causing total mortality or the largest extent of bleaching, and 3) that no bleaching will occur.

## Materials and Methods

### *Study Site*

Spot sampling was completed on coral reefs in Cook's Bay, and at the East back reef near the barrier reef in Mo'orea, French Polynesia. Mo'orea is a volcanic island , the second youngest island in the Society Archipelago, and it is located in the Eastern Pacific at 17° S and 149° W (see figure 1).

Steep volcanic mountains surround Cook's Bay on all sides, and there is a valley at the south end of the bay. Several agricultural fields are scattered within the valley and a river runs through it into the bay. During heavy rains (starting in November and ending approximately around April), a sediment plume develops that causes a huge influx of sediment particles into the bay. When this study was taking place (October and November of 2002), there was an extensive dry spell that lasted until the last week of the experiment (November 11-17).

Within Cook's Bay, the fringing reef consists of three zones: the reef flat, the reef crest, and the reef wall (Adjeroud and Salvat 1996).

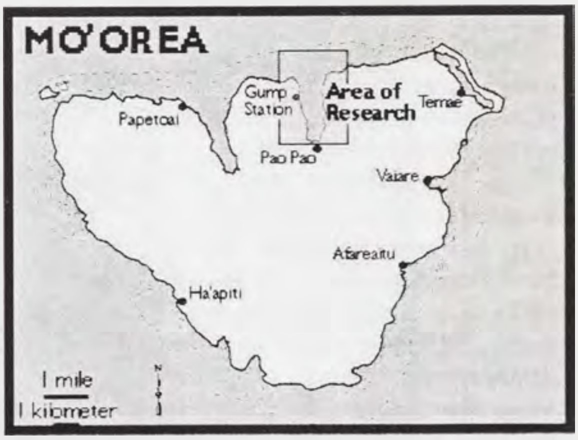

**Figure 1.** A map of Mo'orea, French Polynesia showing where the research for this study was completed.

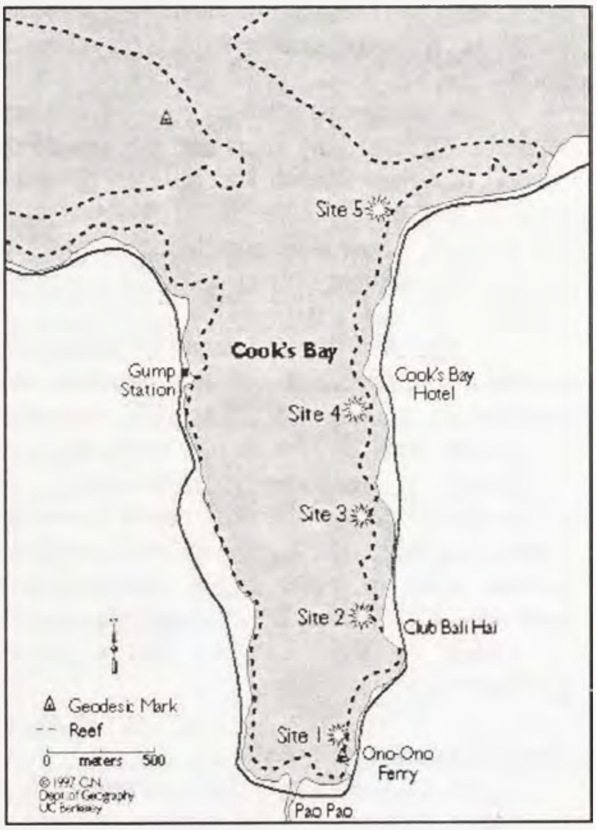

**Figure 2.** A map of the location of the five sites within the East side of Cook's Bay where this study was completed.

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*Field Experiment*

Sedimentation samples were completed in Cook's Bay starting near the entrance of the freshwater source out to the back reef (see figure 2). Twenty sediment traps were made and placed at five sites along the East side of Cook's Bay. The spot testing took place on the East side of Cook's Bay because the reef is present all along that side of the bay versus the West side of the bay, which has a steep drop-off where there is no coral. Four sediment traps were located at each spot within the bay and in front of colonies of *Porites*. Two of the sediment traps were left out for a week and then the sediment was collected. The other two traps were placed out for approximately 24 hours and collected twice a week.

The traps were made using PVC piping with a height: diameter ratio of 5. According to Hargrave and Burns (1979), this is the best method to collect sediment in a turbulent area without resuspension of sediments within the traps. The pipes were placed in plastic flowerpots and wet mixed cement was placed around them. The traps were placed in an air-conditioned room to be dried overnight. Each trap was numbered from 01-20 and then 10 traps were chosen at random to be either weeklong traps or 24 hour traps.

The 24-hour traps were collected twice a week for 5 1/2 weeks. The weeklong traps were collected every Wednesday for 5 weeks. For sediment collection, each trap was emptied into a plastic or glass bottle and then the lid was shut. At the dry lab, samples were allowed to settle and then the liquid was decanted. Samples were then filtered using 125 millimeter filter paper and allowed to dry in an air-conditioned room (approximately 1.5 days). They were then weighed and the results were recorded.

Dried samples were taken back to the California and were analyzed for size and composition. Ten weeklong samples (week 4 and week 5 at all five sites) were chosen to analyze. The size of sediment was measured using a micrometer and composition was determined using a petrographic microscope. Samples were disaggregated using de-ionized water and Quaternary O. They were then washed with de-ionized water, and a small aliquot was removed (by micropipette) and placed on a slide. One hundred particles were measured along a transect in the microscope.

At each site in Cook's Bay, 10 meter by 4 meter quadrats were surveyed for massive *Porites* (*Porites lutea*, *Porites lobata*, *and Porites austrialensis*) and *Porites rus*. The number of colonies within each quadrat was recorded as well as which colonies showed signs of bleaching (paling, white spots, and/or fluorescence).

Statistics included a regression between mass of sediment collected and percent of *Porites* colonies bleached out of the total number of *Porites* colonies present within each sampling quadrat at each site. An ANOVA test was also completed to determine if size distribution of particles was different at each site.

*Altered Sediment levels*

*Porites lutea* heads were collected from the West outer reef slope of Cook's Bay (see appendix B for voucher specimen). Corals were collected from this area because sedimentation due to either anthropogenic influences or erosion was limited. *P.lutea* was also used because the coral heads were smaller heads that could be removed easily from the ocean/reef floor. The coral heads were then returned to the ocean after the study was complete.

The corals were placed in tanks (3 replicates for each treatment) with bay water and allowed to equilibrate for several days to ensure that they did not die before the experiments began. The sediment used for the experiments was taken from the area where the freshwater stream fed into Cook's Bay (see Appendices). The sand was sieved to separate fine particulate sizes (83 microns and less than 83 microns).

Sediment was added at different levels (250 mg/ cm$^2$, 500 mg/ cm$^2$, and 1000 mg/ cm$^2$) to each treatment tank, and the corals sat for 7 days. Observations and temperatures were recorded approximately daily. All treatments were repeated twice for both sediment sizes.

Because it was not possible to measure the surface area of each coral head to any degree of accuracy, this study made the assumption that all sediment would fall either on the coral head or at the bottom of the tank. The area of each tank was multiplied by the treatment level (mass/cm$^2$) to determine how much sediment it would receive. In addition, sediment was mixed evenly to ensure that each coral within each treatment tank received the same mass of sediment per area. Another assumption was that the water flow would not remove sediment from the coral heads (i.e. any loss of sediment was due to the corals ability to remove sediment).

Before each treatment and following each experiment, digital photos were taken of each coral head. The extent of coral bleaching was assessed using Adobe Photoshop™. Red colors represented healthy tissues within the coral head while black colors represented bleached tissues. Histograms showing pixels in a range from black to red (unhealthy to healthy) were plotted for every coral before and after treatment. Means and standard deviations were recorded.

A Chi-squared test was first completed using visual observations of the presence of bleaching to determine if there was a difference between the controls and the treatments. ANOVA and Tukey's tests were then used to determine if there was a difference in the degree of bleaching among each treatment and the controls for all sediment sizes. An alpha level of .05 was used to determine statistically significant results.

## Results

### *Field Experiment*

A regression between mass of sediment (mg) for weeklong samples and percent of the quadrat bleached at each site gave an r-squared value of .004626 (p-value= .6533) (see figure 3 and table 1). There is no correlation between weeklong mass of sediment and bleaching in Cook's Bay.

A regression between mass of sediment (mg) for 24 hour-long traps and percent of the quadrat bleached at each site gave an r-squared value of .006706 (p-value= 0.7021) (see figure 4 and table 1). There is no correlation between the amount of sediment collected for 24 hours and bleaching in Cook's Bay.

The ANOVA test found no statistically significant differences in the particle size distribution among the five sites (p-value= 1.000) (see table 2). The ANOVA test found no statistically significant differences in composition among the five sites (p-value= 1.000) (see table 3). There was one significant p-value equal to 0.000 within composition at each site. All data was transformed from percent to arcsine so that the data had a normal distribution.

### *Altered Sediment Levels*

A Chi-squared test determined a significant difference in the number of colonies that bleached versus the control using the particle size less than 83 microns (p-value< .05). For the treatments using a sediment size of 83 microns, the Chi-squared test did not show a significant difference between the number of colonies that bleached versus the control (p-value>.05) (table 4).

An ANOVA and Tukey's test found statistically significant differences among the controls (both 83 microns and less than 83 microns) and the treatment groups 250 mg/cm$^2$ and 500 mg/cm$^2$ (with sediment size less than 83 microns) (see table 5 and figure 5). The treatment groups 250 mg/cm$^2$ and 500 mg/cm$^2$ had a larger mean difference in the number of healthy pixels as compared to controls and the other treatment groups.

## Discussion

### *Field Experiment*

The alternative hypothesis that mass of sediment explains the amount of bleaching in *Porites* colonies should be rejected due to the results found in this study. Other factors are responsible for bleaching within Cook's Bay (see table 1). Sea surface temperatures, intense UV radiation, and competition between corals and algae may contribute more substantially to bleaching at the five sites, but another study

| **ANOVA**<br>Data Sample | R-squared value | F-ratio | P-value | Degrees of Freedom |
|---|---|---|---|---|
| 24-hour long traps | 0.004626 | 0.2045 | 0.6533 | 45 |
| Weeklong traps | 0.006706 | 0.7021 | 0.4040 | 105 |

**Table 1.** Results from the ANOVA test to determine if mass of sediment (mg) explains percentage of *Porites* colonies bleached in Cook's Bay. R-squared values show that approximately 0% of the sediment collected for 24 hours and over the course of the week are responsible for the bleaching at five sites located in Cook's Bay.

| **ANOVA**<br>Source | Sum of Squares | Degrees of Freedom | Mean Square Error | F-ratio | P- value |
|---|---|---|---|---|---|
| Site | 0.028 | 4 | 0.007 | 0.081 | 0.988 |
| Particle | 0.590 | 4 | 0.148 | 1.710 | 0.178 |
| Size*Particle | 0.206 | 16 | 0.013 | 0.149 | 1.000 |
| Error | 2.244 | 26 | 0.086 | | |

**Table 2.** Results from the ANOVA test to determine if particle size of sediment is distributed differently at five sites located at the East side of Cook's Bay. There is no variation in particle size within or between each of the five sites located in Cook's Bay.

| **ANOVA**<br>Source | Sum of Squares | Degrees of Freedom | Mean Square Error | F-ratio | P- value |
|---|---|---|---|---|---|
| Site | 0.009 | 4 | 0.002 | 0.006 | 1.000 |
| Composition | 9.495 | 2 | 4.747 | 13.635 | 0.000 |
| Composition*Site | 0.063 | 8 | 0.008 | 0.023 | 1.000 |
| Error | 5.571 | 16 | 0.348 | | |

**Table 3.** Results from the ANOVA test to determine if composition of sediment is different at five sites located at the East side of Cook's Bay. There is no variation in composition within or between each of the five sites located in Cook's Bay.

would need to test these factors. Because this study was conducted in the field, the high degree of spatial and temporal variability confounded the direct results of sedimentation on species of *Porites*. According to Anthony (1999), testing the hypotheses of the affects of suspended particulate matter or sedimentation requires a higher degree of environmental control than can be obtained in the field. Sedimentation fluxes are often associated with several other factors such as freshwater runoff from the land (Sakai and Nishihira 1991), wave action (Larcombe et al 1995), reduced light (Dallmeyer et al. 1982; Te 1997), and high nutrient loads (Mitchell and Furnas 1997). In addition to these factors, boat wakes created by the Ono-Ono Ferry may have also contributed to the sediment fluxes seen at all five sites.

Another factor that may have contributed to bleaching of *Porites* in the field is changes in salinity due to the freshwater source (the river running through Cook's Bay). Although previous studies found that salinity changes were only from 34 ppt to 36 ppt in Cook's Bay, this may also affect the bleaching of corals in the bay (Lindsey 2001). Adjeroud and Salvat did a study of distribution of corals within Oponohu Bay (West of Cook's Bay), and found that on March 9, 1992 after three days of heavy rain, salinity was measured at 2 ppt.

$\chi^2$ **Goodness of Fit Test**

| Particle size | # Bleached (out of 6) | Sum | Degrees of Freedom | P-value |
|---|---|---|---|---|
| 83-250 | 4 | 3.4 | 3 | x> .05 |
| 83-500 | 6 | | | |
| 83-1000 | 4 | | | |
| 83-control | 1 | | | |
| less than 83-250 | 6 | 17.2 | 3 | x<.05 |
| less than 83-500 | 6 | | | |
| less than 83-1000 | 6 | | | |
| less than 83-control | 0 | | | |

**Table 4.** Results from the Chi-squared test to determine if *P. lutea* colonies bleached during the four treatments (250 mg/cm$^2$, 500 mg/cm$^2$, 1000 mg/cm$^2$, and the control) for both sediment sizes 83 microns and less than 83 microns. Only sediment sizes of less than 83 microns caused bleaching in *Porites lutea.*

| ANOVA<br>Source | Sum of Squares | Degrees of Freedom | Mean Square Error | F-Ratio | P-value |
|---|---|---|---|---|---|
| Treatment | 64389.527 | 7 | 9198.504 | 4.757 | 0.001 |
| Error | 77347.655 | 40 | 1933.691 | | |

**Table 5.** Results from the ANOVA test using data from Adobe Photoshop™ to determine if colonies of *P. lutea* bleached among the four treatments (250 mg/cm$^2$, 500 mg/cm$^2$, 1000 mg/cm$^2$, and the control) and different sediment sizes (83 microns and less than 83 microns). Bleaching did occur with Tukey's Tests showing that 250 mg/cm$^2$ and 500 mg/cm$^2$ for sediment size less than 83 microns caused bleaching in *Porites lutea.*

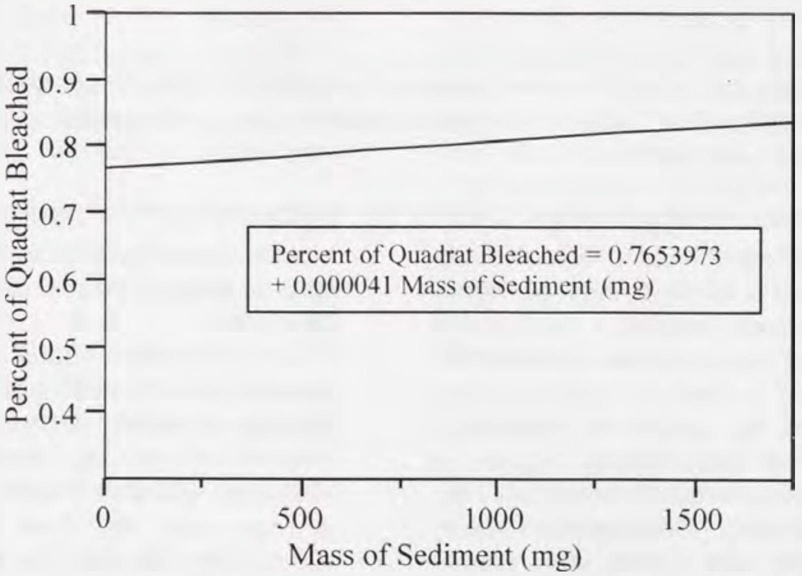

**Figure 3.** A regression of the percent of each quadrat bleached at five sites in the East side of Cook's Bay and the mass of sediment collected at each site over a period of a week. The percent of the quadrat bleached is not explained by the mass of sediment over a week present at five sites located in Cook's Bay.

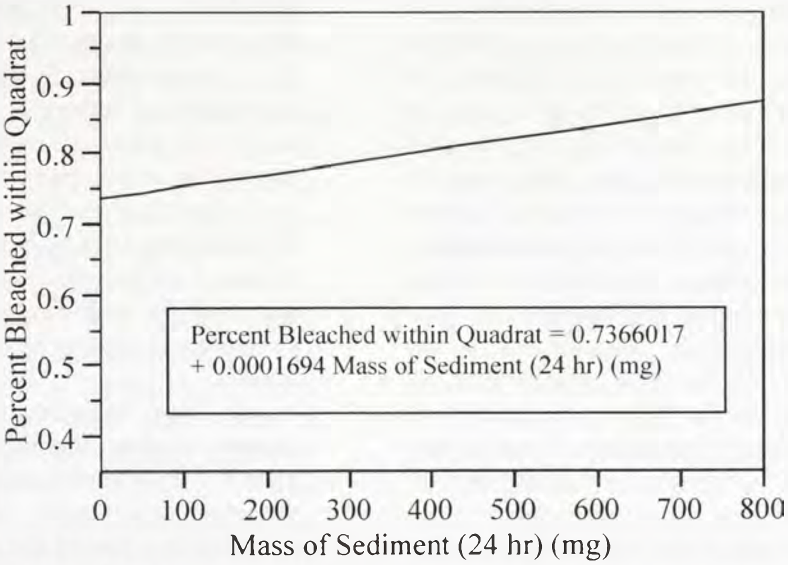

**Figure 4.** A regression of the percent of each quadrat bleached at five sites in the East side of Cook's Bay and the mass of sediment collected at each site over a period of 24 hours. The percent of the quadrat bleached is not explained by the mass of sediment over 24 hours present at five sites located in Cook's Bay.

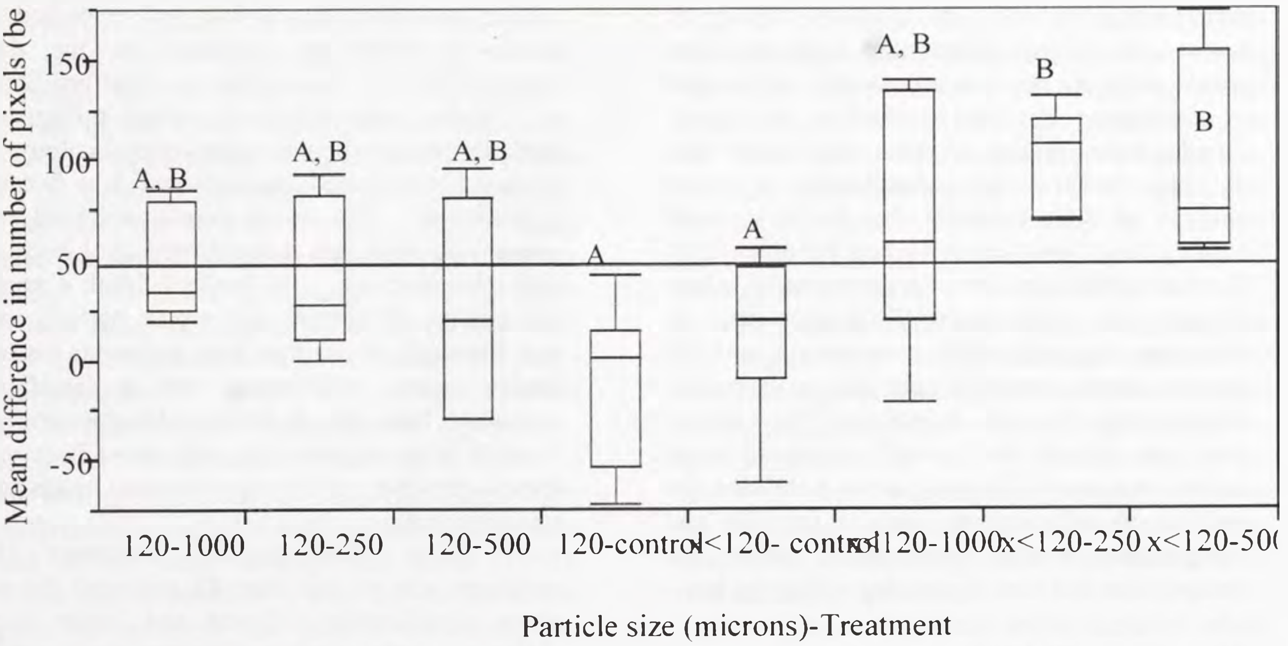

**Figure 5.** Quantiles and distributions of the mean difference in the number of pixels for the three treatments of sediment (250 mg/cm$^2$, 500 mg/cm$^2$, and 1000 mg/cm$^2$), controls, and sediment sizes. On the x-axis, treatments were labeled using particle size (120= 83 microns, less than 120= less than 83 microns)-treatment. Letters A and B stand for groups that are significantly different from each other.

They stated that usually land-end stations were the only areas affected by freshwater discharge, but that sometimes freshwater reached the middle of Oponohu Bay. Possibly, similar affects occur in Cook's bay, except that in Oponohu Bay, the reef infrastructure is not as continuous as it is in Cook's Bay.

In Cook's Bay, however, there are corals present close to the freshwater input into the bay. A consistent freshwater influx, such as in Oponohu Bay, would cause an absence of corals near the input of freshwater, but this does not occur in Cook's Bay. This is especially the case if salinity was reduced to only 2 ppt. This suggests that sedimentation and turbidity may play a more important role in causing bleaching and mortality in the area. Taking salinity measurements during the rainy season could test this hypothesis.

The composition of sediment within Cook's Bay and at the five sites sampled here are comparable (see table 2). No significant variation in the type of sediments found at the five sites existed as expected. A high level of carbonate occurs at each site because a continuous reef starts at the inlet of Cook's Bay and reaching all the way out to the barrier reef. Basalt is present because Mo'orea is a volcanic island and erosion plays an important role in the natural geology of the island.

The size distribution of particles at the five sites within Cook's Bay are not significantly different (see table 3). This is a surprising result, because one would expect that larger particles would settle out at a closer proximity to the inlet of freshwater (by the formation of flocs). Smaller particles should settle out further into the bay, because they would take a longer amount of time to settle due to their small settling velocities (Larcombe and Woolfe 1999). There are several reasons for these results. Only 10 samples were chosen (2 at each site) to determine size and particle composition, and this is not a large enough sample size to determine statistical significance. In addition, 100 particles may not reflect the overall composition of sediments contained at each site. Increasing the number of sediments to analyze for size and composition would reveal more about the composition and size of particles within the bay.

### *Altered Sediment Levels*

Statistically significant results show that sedimentation causes bleaching in *P. lutea* when exposed to sediment of size less than 83 microns (see table 4). This is in agreement with the alternative hypothesis that sedimentation causes bleaching in *Porites lutea* except only for small sediment sizes. For prolonged periods of time (in this case 7 days), bleaching results in tissues that are covered by sediment. In areas such as the back reef of the barrier reef on Mo'orea, the lagoon is regularly flushed by water flow created by waves crashing on the algal ridge. Sedimentation may not play as large a role in bleaching as compared to sea surface temperature or intense UV radiation. In Cook's Bay, inconsistent flushing mechanisms cause high levels of turbidity. This has severe effects on Poritid corals (Genus *Porites*) because they depend on water movement to cope with stress and cleaning (Marshall and Orr 1931; Bak and Elgershuizen 1976). In Cook's Bay, during rain events, a large sediment plume develops which increases the turbidity within the bay (personal observation) causing stress to corals within those areas.

For sediment that was sieved to 83 microns or less and then placed on the corals, after 1-2 days, dark agglutinations appeared near the edges of sediment. Mucous sheets were also recorded in a few of the corals, and in almost all treatments, by the end of the seventh day, there was no visible polyp extension. Although limited polyp extension was recorded for sediment sieved to 83 microns, by the end of seven days, polyp extension was still visible on some corals. This suggests that *P. lutea* has a harder time sloughing off finer sediments and the stress is higher as indicated by the dark agglutinations. According to Stafford-Smith (1992), sediment layers of several millimeters that had remained on an experimental colony for several days turned dark-colored and became agglutinated. The darker anoxic sediment was present on all corals that suffered total death or extensive damage. In Stafford-Smith's study, there is no differentiation between particle size, but this study shows that finer sediments cause a higher stress environment and a significant amount of bleaching as compared to the controls. Smaller sediment sizes not only cause *Porites* to bleach, but they also stress out coral heads to a greater extent.

The 1000 mg/cm$^2$ treatment (for sediment size of less than 83 microns) did not show significantly different and larger mean difference in the number of healthy pixels, which seems like a strange result. The greatest amount of sediment should cause the largest degree of bleaching because the layer of sediment would be thicker, but that was not the case. Several possible explanations for this discrepancy include a small sample size, the individual corals themselves, and the assumptions made at the beginning of the experiment.

The sample sizes for each treatment and particle size were only 6, which is normally not a large enough sample size to find a statistically significant result. Increasing the sample size would reveal a more statistically significant result. As for the individual coral colonies, their morphological and skeletal architecture strongly influenced their ability to sweep off sediment. Some of the corals chosen for treatment were bumpy while others had a larger percentage of flat or concave areas that could trap sediment within the polyps (Stafford-Smith 1992). Some corals were completely flat, but had no concave portions that would trap sediment, so most of it was swept off. This also contributed to varying results of bleaching within each experiment. As for the assumptions made at the beginning of the study, water movement may have been a considerable problem when recording ability of the coral to sweep off sediment. The inconsistent water flow of the tanks affected the degree to which sediment was swept off the live coral tissue.

No significant differences occurred between treatment levels within each experiment; therefore, the alternative hypothesis that the largest mass/area of sediment will cause the greatest extent of bleaching or mortality should be rejected. The thickness of sediment on the coral did not cause the bleaching, but the resistant layer of sediment covering the live tissues on the coral head caused the bleaching. The prolonged period of time that the sediment sat on the coral also played a role in bleaching. This does not support previous studies which have had varied results among different sediment loads. As stated previously, while Hodgson (1990) found that a level of 30 $mg/cm^2/day$ with fine marine sediment caused extensive bleaching and mortality in *P. lobata*, Stafford-Smith (1992) found that influxes from 50-400 $mg/cm^2$ for eight days *in situ* caused bleaching in *P. lobata* but no mortality. Stafford-Smith attributed these differences to variation between the same species and at the same locality and depth (1992). While this is possible, it may not be the actual mass of sediment that affects the coral, but whether sediment covers the corals to the degree that it cannot be removed. In addition, those studies looked at continuous sediment influxes whereas this study focused on one sediment load with a different species of *Porites*.

Sediment sizes within the field were much smaller than those used for the experiments. Although results from the lab show that small sediment sizes cause bleaching in corals and large sediment sizes do not cause bleaching, these results were not observed in the field. The sediment sizes were much smaller in the field, but these sediment particles were not resting on corals in as great an amount as they were in the lab. One reason for this is because this experiment was conducted during an extremely dry season. Rain only occurred during the last week of the experiment resulting in much larger amounts of sediment collected. If this experiment was done during the rainy season, sediment plumes would bring a much larger influx of sediment, and corals in the field might show signs of bleaching similar to those observed in the lab.

Although previous hypotheses and studies have focused on thermal anomalies and increased solar radiation as the major causes of bleaching, this study not only shows that bleaching can be caused by sedimentation, but that temperature increases may not cause bleaching. When coral heads were collected and placed in tanks for a week before the experiment began, temperatures were recorded for all tanks as well as while the experiment was occurring. All temperatures recorded were from 27° C to a maximum of 31° C. None of the corals in control treatments bleached at temperatures this high for two weeks. This is in contradiction with the normally accepted hypothesis that thermal anomalies are responsible for bleaching events (Salvat et al. 2001). During the bleaching event of 1994, in April, temperatures ranged from 29.5-30° C, which was about 1.5° C above that normally expected for that time of year and preceded the onset of bleaching by 2-3 weeks (Salvat et al. 2001). This experiment was not, however, conducted in the field, which may be the reason why corals did not bleach. A confounding variable (in addition to sea surface temperature) might also have contributed to the bleaching event of 1998. On an ocean-wide basis, this is not true, but in areas where flushing of sediment is reduced, sedimentation may contribute to bleaching more than increases in sea surface temperatures.

Of course, some errors occurred while completing this study. According to the results obtained from analyzing data using Adobe Photoshop™, not all treatments showed that the bleaching in the corals changed the overall number of healthy tissue pixels even though almost all corals were bleached. Several possible explanations include the small sample sizes used for each treatment, the lack of repetition of the study, and the way the photos were taken to analyze.

If the experiment had been repeated at least 10 times, the sample size would have increased to 30 coral heads per treatment and statistics would be more significant. This was not, however, feasible due to the time constraints and because this would have required the collection of over 200 coral heads. This would have been an excessive number of corals to take from the barrier reef, and it would have been hard to find so many small, healthy coral heads at the ocean floor.

As for the photographs taken from analysis on Adobe Photoshop™, the corals were hard to photograph due to their bumpy surfaces and the lack of light that penetrated the small concave crevices of the coral head. The bleaching changes were hard to capture in areas of the coral where these small crevices occurred. This definitely affected the histogram distributions of the mean number of pixels in photos before and after treatment. Adobe Photoshop™ is, however, an excellent tool to examine the degree of bleaching in a coral colony for experiments done in the lab. Instead of relying on simply personal observation, the histogram showing the number of pixels within the range from unhealthy tissues to healthy tissues is a great way to quantify extent of bleaching. Future studies should take this consistent method into consideration when analyzing extent of bleaching while also using cameras with a large number of pixels.

The tanks where the corals were being treated with altered sediment levels also had a few problems. While all tanks were subject to the same unfiltered water from Cook's Bay, this created a small disruption of the experiment. The water taken from Cook's Bay was also slightly turbid and contained small sized particulates and sediment. In the control tanks, these particles would sit on the corals for up to six days and in some cases caused a lightening of the live coral tissues. Particulate matter caused the change in color because it was only in areas where particulates would lie that any change in tissue color was found. This is why some of the control tanks had corals that bleached (see table 4).

Future studies should definitely examine turbidity and its affects on coral bleaching. Although percent of corals bleached did not correlate to mass of sediment, turbidity and bleaching may relate to each other. For areas such as Cook's Bay, it is possible that turbidity is a more crucial factor in determining the degree to which corals bleach in the bay due to the lack of sunlight penetration and these affects on zooxanthellae. In addition, this study would have substantially different results if it had been completed in the rainy season. Measurements such as salinity, sedimentation, and turbidity could all be completed. Also a more complete analysis of sediment sizes and compositions should be completed because it will contribute to a more complete understanding of sediment distribution within the bay.

Sedimentation in Cook's Bay should be monitored because of the extensive modification of shorelines and the input of sewage flushed directly into the lagoon. According to climate models, an increase in sea level of only 1 cm/yr over the next 30 years could destroy all existing shorelines. This would cause an increase in sediment loads to fringing reefs causing bleaching and total colony mortality (Wilkinson 2000). In addition, Bora Bora (another island in the Society Archipelago) is the only island with a major sewage treatment facility and most wastes are placed directly into reef lagoons (or in bays) at other islands and in Mo'orea (Wilkinson 2001).

Bleaching events should continue to be monitored because in the past two to three years, sea surface temperature anomalies rose above the long term 95% confidence interval limit for mean March temperatures for two months or longer. Also, the predicted future increase in strength and frequency of El Niño Southern Oscillation events in the pacific tropical environments will greatly impact the ability of reefs to recuperate. For example, in Rangiroa, French Polynesia, the worldwide 1998 bleaching event killed over 25% of *Porites* colonies and decreased coral cover of *Porites* by 40-80% in certain areas. Because many of these colonies were hundreds of years old, recovery will take over a 100 years for colonies back to their previous abundance. The only way complete rehabilitation would occur is if no future acute disturbances affected the reefs and if the dead corals were re-colonized rather than having new recruits build reef structures (Mumby et al. 2001).

## Conclusions

This study shows that the amount of sediment found within Cook's Bay does not correlate with the percentage of *Porites* bleached. Sedimentation causes bleaching in *P. lutea* under laboratory conditions for smaller sediment sizes. The discrepancy between these results exists because in the field several other factors such as sunlight penetration, sea surface temperature, and competition vary at large spatial and temporal scales. Composition and sediment sizes are consistently uniform within Cook's Bay, but further data should be analyzed to understand the sediment load to the bay. Although sea surface temperature anomalies seem to play a larger role in causing bleaching of corals within the field, the effects of sedimentation on corals cannot be ignored.

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**Appendix: A** GPS data for sites for study in the field

**Site 1**

GPS: 17° 30.270' S and 149° 49.150' W

This site is located perpendicular to Restaurant Honu Iti with the red roof and next to a white house with a green roof (Near the Ono Ono ferry). It is just north of the red buoy used for the ferry.

**Site 2**

GPS: 17° 30.021' S and 149° 49.082' W

This site is located perpendicular to Club Bali Hai. It is directly perpendicular to the 2nd bungalow to left of pointy triangle bungalow. There are also two red buoys and it is located at the red buoy that is farthest north.

**Site 3**

GPS: 17° 29.771' S and 149° 49.084' W

This site is located in the water perpendicular to the end of the row of coconut palms. There is one bent palm tree that is bent extensively into the water and traps were placed perpendicular to this point and north of it. Too far north is a house with a brown roof.

**Site 4**

GPS: 17° 29.521' S and 149° 49.092' W

This site is located before the floating dock (near house with bamboo roof). It is characterized by mostly *Porites rus* with very shallow, warm water.

**Site 5**

GPS: 17° 29.057' S and 149° .050' W

This site is located past the White Hotel that is no longer in business. Just North of the where the traps were located is a stout red buoy. Perpendicular to the site are two bungalows with bamboo roofs placed next to each other.

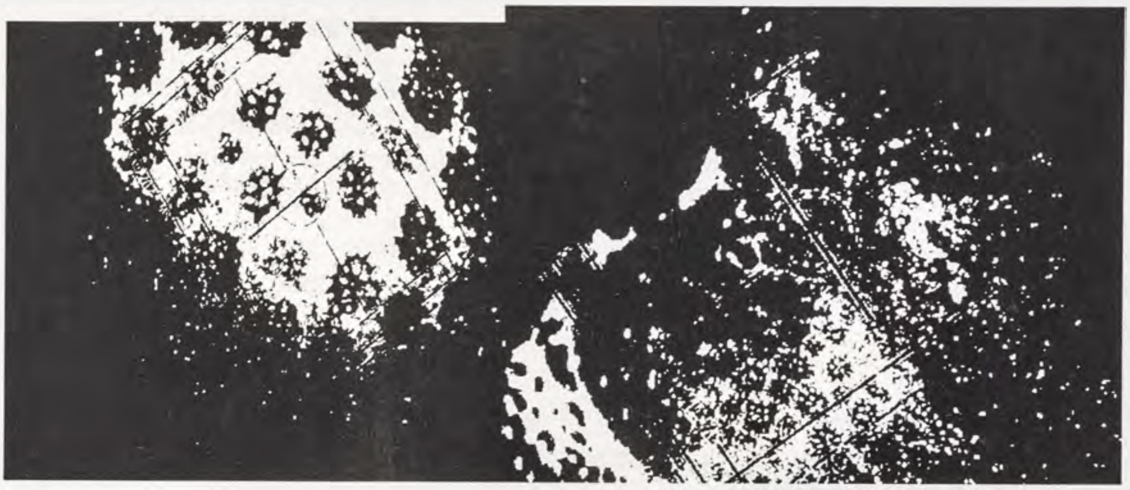
**Collection site**

West back reef (to the West of the channel leading into Cook's Bay)

GPS: 17° 28.648' S and 149° 50.097' W

*Porites lutea* was collected from sites with rubble, broken off branches of *Acropora* with algae and in between large colonies of *Porites* where there is sand and between large boulder corals on sandy bottom. Collection occurred within ~200 m of GPS values.

**Appendix: B** Voucher specimen of *P. lutea* magnified at 1x for the digital photo at the left, while the *P. lutea* on the right is magnified at 2x under a dissecting scope. Identified by Marissa Hirst using identification of corallite skeleton by Veron (1986).



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# The re-infection of bleached *Fungia sp.* corals using Zooxanthellae extracted from corals of various families and depths

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ABSTRACT. Corals belonging to the subgenus *Fungia* were experimentally bleached and experimentally re-infected with zooxanthellae extracted from corals of various families and depths, to investigate species and habitat specificity between symbiotic dinoflagellates (zooxanthellae) and bleached adult corals. This study is the first to experimentally re-infect bleached corals. Thirty-two *Fungia* corals were bleached using a combination of elevated water temperature and high intensity light. Bleached corals were divided into four treatment groups that were subjected to repeated inoculations of zooxanthellae extracted from donor corals of like and different depths and families. Statistical analysis of data, obtained using a novel method of quantification involving digital imaging, found significant differences in the rates of re-infection between treatment groups. Zooxanthellae extracted from corals of the same subgenus were better able to infect bleached corals than were zooxanthellae extracted from dissimilar genera. In addition, no difference was found between the re-infection rates of bleached corals treated with zooxanthellae extracted from different depths. Lastly a novel means of quantification involving the use of digital imaging and analysis of images in Adobe Photoshop™ was created in order to measure the rate of re-infection in bleached coral tissues.

KEYWORDS: Cnidarian, *Fungia*, scleractinian coral, zooxanthellae, symbiosis, re-infection, coral bleaching, Mo'orea

## Introduction

Algal-animal symbioses are well known for their important role in the ecology of shallow tropical seas. These symbioses are most commonly characterized by the association of a marine invertebrate with a coccoid, yellow-brown dinoflagellates known as zooxanthellae. The diversity of the animal component of these symbioses, which ranges from protozoa to mollusks, has long been recognized, however, the diversity of the algal component has only recently begun to be explored. Although the taxonomy of symbiotic dinoflagellates is incomplete, evidence indicates that symbionts comprise at least eight genera distributed across five or six classical orders in the Division Dinophyta (Iglesias-Prieto & Trench 1997, Trench 1997). Historically it was believed that there existed a one-to-one relationship between host and symbiont taxa (reviewed in Trench 1997). As dinoflagellate taxonomy has developed using morphological, biochemical, and genetic data obtained from laboratory cultured symbiotic dinoflagellates it has become increasingly clear that the one-to-one host to symbiont hypothesis must be abandoned for one in which symbioses are restricted to a "small and specific subset of the myriad combinations that theoretically might exist" (Toller et al. 2001, Trench 1997).

In order to understand the mechanisms structuring this 'small subset', numerous studies have taken place in which the importance of species specificity (Berner et al. 1993, Coffroth 2001, Davy et al 1997, Toller et al. 2001, Weis et al. 2001) and habitat specificity (Iglesias-Prieto & Trench 1997, Rowan & Knowlton 1995, Secord & Augustine 2000) has been explored. Studies focusing on questions regarding species specificity have found that the associations between invertebrate and zooxanthellae are essentially random with respect to phylogeny. These seemingly random associations often result in shocking findings in which hosts of distant evolutionary clades share identical symbionts (Hill & Wilcox 1998, Toller et al. 2001). While phylogeny has largely been unable to explain the structuring of the algal-invertebrate subsets, many studies of the influence of habitat have shown a strong correlation between depth (a proxy for light intensity) and the presence or absence of specific forms of symbionts (Iglesias-Prieto & Trench 1997, Rowan & Knowlton 1995, Secord & Augustine 2000). Although it is not clear evolutionarily why specific symbionts became associated with the subset of hosts they form symbioses with, it is clear that, within the context of a specific host, habitat plays an important role in deciding which symbiont taxon populates an individual host.

Perhaps due to the important role of habitat in the structuring of algal-invertebrate symbioses, small changes in water temperature (Brown & Ogden 1993, Glynn 1996), solar radiation (Gleason & Wellingon 1993, Shick et al. 1996), or both (Drollet et al. 1995) are capable of upsetting symbiosis, resulting in a phenomena called bleaching. When an invertebrate bleaches it expels its symbiotic algal partners, which are largely responsible for the invertebrate's pigmentation. It is from the noticeable loss of pigmentation that this phenomenon gets the name bleaching. The breakdown of the symbiosis has far more serious consequences than the loss of pigmentation. If the host is not able to re-establish a symbiosis with, either symbionts remaining in its tissues, or symbionts it may acquire from the water column, the host will die.

Due to difficulties with maintaining healthy colonies of scleractinian corals in the lab and quantifying zooxanthellae in scleractinian coral tissues, previous studies have centered on infection studies of aposymbiotic invertebrate larvae, re-infection studies of experimentally bleached invertebrate taxa excluding scleractinian corals, or field studies involving sampling and sequencing of natural systems. The Gump Field Station in Mo'orea, French Polynesia is uniquely situated on the beach overlooking a large healthy coral reef. The wet lab at the station draws water directly from the waters of the reef to provide a series of open-air tanks and aquaria with continually flowing fresh seawater. The use of flowing, unfiltered, fresh seawater allows researchers at the station to sustain animals in a laboratory setting for periods of time significantly longer than could be accomplished without such a setup. This study attempts to take advantage of both the Gump Field Station's unique wet lab and a novel method of quantification using digital imaging, to investigate the power of species specificity and habitat specificity in the algal-invertebrate symbiosis between corals of the subgenus *Fungia* and their un-described zooxanthellae endosymbionts. This will be the first study to experimentally re-infect bleached corals of any family.

## Materials and Methods

*Preparation of Bleached Corals:*

All collecting was performed in Mo'orea, French Polynesia and all experiments were conducted at the Gump Field Station in Mo'orea, French Polynesia. Bleached and partially bleached corals of the subgenus *Fungia* were collected from shallow depths (.5m to 2m) along the Vaipahu backreef (Fig. 1).

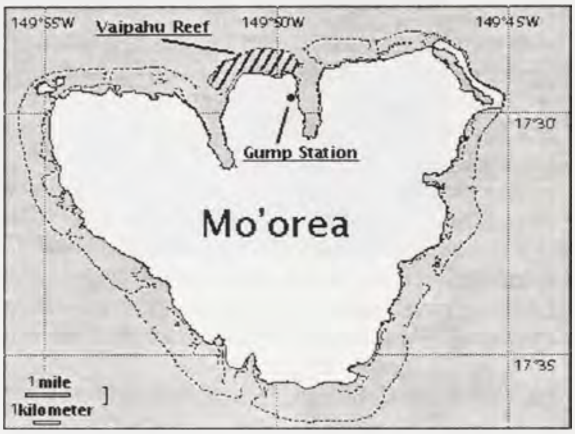

**Figure 1.** Map of Mo'orea, French Polynesia indicating the locations of the Vaipahu Reef and Gump Field Station. Specimens were collected from the Vaipahu backreef. Experiments were conducted at the Gump Field Station.

In order to complete the bleaching process, partially bleached corals were placed in small tubs of fresh seawater, and placed in the sun. Temperatures in the tubs were monitored, and water was changed to maintain a temperature between 33°C and 35°C. Corals were kept in direct sunlight 10cm below the water in an open-air fresh seawater flow through tank between bleaching exposures. During bleaching treatments corals were observed emitting pigmented mucus. Bleaching treatments were carried out for 3 days.

*Extraction of Zooxanthellae:*

Zooxanthellae were extracted by adapting the methods described by Weis et al. (2001). Freshly collected corals were stripped of tissue using a glassware scrub brush. Tissue was collected in a small amount of fresh seawater. Following brushing, tissue donors were kept in an open-air fresh seawater flow-through tank below a shade tarp to recover before being returned to the field. Tissue donors experienced 3.4% mortality. Tissue donors for each treatment were selected by surface area, in order to yield an approximately equal volume of zooxanthellae. The tissue-water homogenate was poured into eight 15ml falcon tubes and spun in a centrifuge at high speed for five minutes to pellet the zooxanthellae. The supernatant was poured off, and the pellets were re-suspended in 3ml of fresh seawater. The zooxanthellae extracts of the treatment groups consisted of a .4ml pellet of zooxanthellae suspended in 3ml of fresh seawater. Any excess zooxanthellae were removed from the falcon tube before the pellet was re-suspended. Zooxanthellae extracts were used within thirty minutes of preparation.

*Re-Infection of Bleached Corals:*

Following the bleaching treatments and two days prior to beginning the re-infection treatments corals were moved to shaded aquariums with fresh seawater flow through systems, where they would remain for the duration of the experiment. Thirty-two of the most thoroughly bleached individuals were selected by hand then randomly assigned to one of four treatment groups (Fig. 2).

| **Group** | **Treatment** |
|---|---|
| **Control** | No zooxanthellae |
| **Deep** | Zooxanthellae extracted from *Fungia sp.* found between 12m and 15m depth |
| ***Pocillopora*** | Zooxanthellae extracted from *Pocillopora sp.* found between .5m and 2m depth |
| **Shallow** | Zooxanthellae extracted from *Fungia sp.* found between .5m and 2m depth |

**Figure 2.** Chart describing treatments applied to each of four groups of experimental animals. The corals belonging to each group were all collected from shallow depths (.5m to 2m).

The control group was used to gauge the rate of re-colonization of the animal tissue by resident zooxanthellae. The control group also was used to control for zooxanthellae potentially acquired from the water column.

All bleached corals were collected in shallow water .5m to 2m in depth. Tissue donors for the Shallow and *Pocillopora* treatment groups were collected in water .5m to 2m in depth along the Vaipahu back reef. Tissue donors for the Deep treatment group were collected from depths between 12m and 15m.

Plankton was acquired using a light trap. Plankton trapped in the light trap was ground and strained. The resulting slurry was divided into 15ml falcon tubes and frozen for use during the duration of the experiment. Prior to re-infection treatment the plankton slurry would be thawed and brought to room temperature (~30°C).

Re-infection treatments were repeated a total of ten times every Monday, Wednesday, and Friday over the course of twenty-two days. Prior to each re-infection treatment each treatment group was moved from the aquaria to plastic tubs each containing 3600ml of fresh seawater. 15ml of plankton slurry was poured into each tub and stirred into solution in order to stimulate the corals to open their mouths to feed. The Deep, Shallow, and *Pocillopora* treatment groups then had zooxanthellae extract poured directly into the mouths of each coral, while the Control treatment group had plankton slurry poured directly into the mouth of each coral. The Control group was fed an additional 3ml of plankton slurry to compensate for any additional nutritional value the treatment groups may receive from digesting the material found in the zooxanthellae extract. The corals were then left to feed for the next 180 minutes before being returned to the aquaria.

During the first 30 minutes of this feeding process the cloud of zooxanthellae extract covering the mouth of each coral could be seen to move inside the mouth of the coral.

*Quantification of Pigmentation Change:*

Immediately prior to the re-infection treatment, on six of the ten treatment days, digital photographs were taken of each coral. In addition to the thirty-two bleached corals, a photograph of one of the tissue donors, prior to scrubbing, was taken to represent standard healthy pigmentation. Lighting and camera settings were kept consistent between photographs. The corals were photographed in a blue plastic tub under 3cm of fresh seawater. The blue tub served as a blue screen so that the coral could be separated from the background automatically by computer.

All images were processed in Adobe Photoshop™ 7.0. A Photoshop™ action was created to remove the coral from the background and process the image in such a manner that a histogram of the pigmentation could be created. This processing created an image in which the pigmentation from the standard healthy coral was used to perform a color range on the bleached corals so that all pigmented portions of the coral could be shaded red while all un-pigmented portions of the coral could be shaded black. The color range selected all colors exactly matching the standard one hundred percent and selects colors slightly different from the standard some percentage equivalent to the degree with which the color is different from the standard. In this manner un-pigmented portions of the coral are tinted black and scored with zeros while pigmented portions are scored between 1 and 255 depending on the intensity of pigmentation relative to the healthy pigmentation observed in the standard. The number of pixels counted in each bin 0 to 255 was displayed in a histogram in Photoshop™.

The medians of the histograms generated in Photoshop™ were used to describe the pigmentation of each of the corals on each day it was photographed. Analysis of variance (ANOVA) and repeated measures ANOVA tests were performed using Systat 10.2 software.

**Results:**

To compare re-infection success between treatment groups a repeated measures ANOVA was performed on the median pigmentation value recorded for each individual on each day. The treatments were found to be significantly different from one another (Fig. 3).

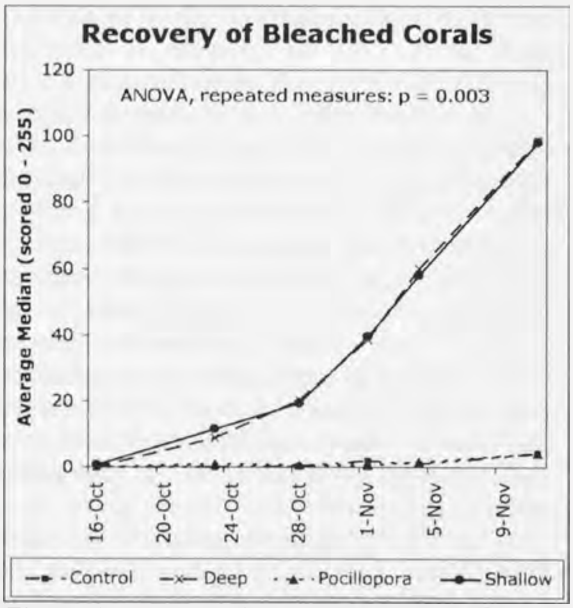

**Figure 3.** Average pigmentation median for each treatment group graphed against time, indicating the differential recovery rates of each treatment group. Treatment groups were found to be significantly different (p = 0.003).

The Deep and Shallow treatment groups exhibited a higher rate of recovery than the control group. The *Pocillopora* treatment group did not exhibit a higher rate of recovery than the control group. An ANOVA test was performed on pigmentation medians of corals prior to the experiment indicates that corals in each group started out with no statistically significant differences (p = 0.977).

Several changes occurred in corals that could not be quantified using the study's quantification method (Fig. 4).

| Corals Affected | Treatment Group | Observed Change |
|---|---|---|
| **BFC_37** | **Shallow** | **Gain of fluorescent green rim following first treatment and building intensity as treatments continued.** |
| BFC_02<br>BFC_08<br>BFC_11<br>BFC_13<br>**BFC_34** | *Pocillopora* | **Gain of dim pink pigmentation that grew in intensity as treatments continued.** |

**Figure 4.** Table detailing un-quantifiable changes in specific corals during the treatments. Corals listed in bold were most strongly effected by the described changes. All changes described only occurred during the treatment phase of the experiment.

The acquisition of a green rim in one of the corals of the Shallow treatment group was most unexpected. Green fluorescent rims observed, in the lab and the field, were present in many corals, but always remained present even following intense bleaching. No other corals lacking a green rim gained a green rim during the duration of the experiment.

Many members of the *Pocillopora* treatment group gained a pink pigmentation rather than the characteristic brown pigmentation found in *Fungia* corals (Fig 4). This pigmentation was similar to the pigmentation observed in *Pocillopora* corals found in the field. The pigmentation appeared suddenly after the first treatment, and very slowly grew in intensity in most of the corals. The pink *Fungia* corals never gained enough pink pigmentation to be quantified, even when changing the standard pigmentation to that of a healthy Pocillopora coral (i.e. median = 0).

All corals, regardless of treatment group, were observed to have noticeably more pigmentation each successive day during the treatment phase of the experiment. Though many corals had median pigmentation values of zero, small portions of pigmentation or dim pigmentation were present, indicating that low rates of re-colonization of aposymbiotic tissues were taking place. This slow rate of re-colonization of aposymbiotic tissues was only moderately picked up by the quantification method because of overshadowing by the high rate of re-infection in the deep and shallow treatment groups.

## Discussion:

This study is the first to examine the experimental re-infection of an adult scleractinian coral with zooxanthellae. Statistical analysis of data, obtained using a novel method of quantification involving digital imaging, found significant differences in the rates of re-infection between treatment groups. Zooxanthellae extracted from corals of the same subgenus were better able to infect bleached corals than were zooxanthellae extracted from dissimilar genera. In addition, no difference was found between the re-infection rates of bleached corals treated with zooxanthellae extracted from different depths.

The differential rates of re-infection between groups of bleached corals treated with homologous and heterologous zooxanthellae provides evidence for the existence of 'recognition and specificity systems between host and symbiont," two processes described by Trench (1993) as critical to cooperative biology necessary for the establishment of symbiosis. When an excess of viable symbionts were delivered directly into the mouths of subjects, that is, *Fungia* corals were best able to re-establish healthy symbiosis with zooxanthellae extracted from other *Fungia* corals, irregardless of the habitat from which the donor *Fungia* was taken (Fig 3). *Fungia* corals delivered zooxanthellae extracted from *Pocillopora* corals did not appear to be competent to re-establish symbiosis, as the recovering rates observed in these corals was not significantly different from bleached *Fungia* corals in the control group (Fig 3). The differential ability of *Fungia* corals to re-establish symbioses with homologous and heterologous zooxanthellae indicates that even following intense bleaching there remains a strong species specificity that is important in the recovery and long term survival of corals following a bleaching event. The apparent lack of any habitat specificity following a bleaching event can be interpreted either as a lack of habitat specificity, or as a flexibility of bleached corals to acquire any strain of zooxanthellae. While evidence for habitat structured diversity may be inconclusive, the strong species specificity evidenced by this study leads credence to the diversity of zooxanthellae and Trench's small sub-set hypothesis.

The finding of a strong species specificity of *Fungia sp.* corals following a bleaching event agrees with numerous studies investigating specificity of algal-invertebrate symbioses. Though this study is the first to experimentally re-infect adult corals, studies involving the re-infection of other adult taxa of invertebrates (Belda-Baillie et al. 2002, Berner et al. 1993, Davy et al. 1997, Fitt 1984, Hill & Wilcox 1998), the onset of symbiosis during larval or aposymbiotic juvenile life stages (Coffroth et al. 2001, Kinzie 1974, Schwarz et al. 1999, Schwarz et al. 2002, Weis et al. 2001), and the molecular sampling of un-manipulated recovering corals (Toller et al. 2001, Rowan & Powers 1991, Secord & Augustine 2000, Rowan & Knowlton 1995), have all reported on the power of species specificity in algal-invertebrate symbioses. Though it is hard to draw generalizations between these studies due to a wide range of quantification methods and varying scales of investigation, all cases reported that though novel infections are possible, homologous strains of zooxanthellae exhibit the strongest ability to form stable symbioses with their hosts. For example, studies by Weis and colleagues (Schwarz et al. 1999, Weis et al. 2001) on specificity during the onset of symbiosis in planula larvae of *Fungia scutaria* initially concluded that there was a low degree of species specificity in this symbiosis due to the ability of *F. scutaria* to form symbioses with zooxanthellae distantly related to the zooxanthellae naturally found in *F. scutaria*. These conclusions were challenged, however, when comparisons between the rates of re-infection of these novel symbioses was made with the rate of re-infection with homologous zooxanthellae. Weis and colleagues conclude that *F. scutaria* exhibit strong species specificity in their ability to establish symbioses with zooxanthellae (Weis et al. 2001). These conclusions tend to mirror those of this study, in which *Fungia* corals exhibit strong species specificity, but if we consider the un-quantifiable changes observed in the *Pocillopora* treatment group, rare associations with heterologous zooxanthellae may be possible. In studies on the recovery of naturally and experimentally bleached scleractinian corals *Montastraea annularis* and *M. faveolata* researchers found that while in most cases *Montastraea sp.* corals re-established symbioses with one of four strains of zooxanthellae known to naturally associate with these corals, in rare instances temporary associations with heterologous zooxanthellae were observed (Toller et al. 2001). Toller and colleagues hypothesize that the finding of rare zooxanthellae following a bleaching event may represent the existence of a succession regime in natural environments following a bleaching event (Toller et al. 2001). The similarity in the specificity of algal-invertebrate symbioses suggests that the phenomenon is not particular to any particular host taxon, but is instead a characteristic of all algal-invertebrate symbioses.

It is worthwhile here to discuss some of the experimental constraints of this study and how they relate to the interpretation of the data. Due to time constraints and concern for the health of the study organisms, bleached corals were not rendered completely aposymbiotic. Although a control group was used to control for changes in pigmentation that could be associated with the recovery of resident zooxanthellae, the effect of this resident zooxanthellae on heterologous zooxanthellae introduced during the treatments should not be ignored. Studies by Toller and colleagues (2001) suggest that even small populations of resident zooxanthellae may inhibit the infection of heterologous strains of zooxanthellae. This effect may be largely responsible for the observed species specificity of bleached *Fungia* corals. Although Toller (2001) suggests that intensely bleached corals, such as those used in this study, may overcome the inhibitory effects of the resident zooxanthellae, it would be best to construct an experimental design that would eliminate the possibly confounding effects of the resident zooxanthellae in future studies. Due to the large number of individual animals needed to conduct this study it was not possible to restrict the study to a single species of *Fungia*. Since no previous studies have focused on species specificity at the level of the genus or subgenus, it is difficult to know how using a mixed group of bleached *Fungia* corals and *Fungia* tissue donors could have affected the results. It does not appear that the use of different species within the subgenus *Fungia* negatively effected the data, however many of the un-quantifiable changes that occurred could be attributed to species specific responses, that should be more closely investigated in future studies. The use of cleaned zooxanthellae was not attempted due to the lack of appropriate equipment; however, zooxanthellae extracts contaminated by animal tissues have been shown to increase the success of infection (Weis et al. 2001). Despite the higher rate of infection that is associated with the use of contaminated zooxanthellae extracts, it is not clear whether the presence of foreign animal tissue could have strongly affected the differential re-infection results. The presence of a vacuole membrane surrounding individual zooxanthellae could have been a strong mechanism causing the failure or success of re-infection. In this example a host my not have been exhibiting a specificity for specific zooxanthellae, but instead was selecting for specific animal tissues. Past experimental evidence suggests that, though animal tissue could potentially be a confounding factor, there is also evidence that the increased rate of re-infection associated with the presence of animal tissue is a result of the role chemosensory cues in eliciting the feeding response in host animals that is necessary for the uptake of symbionts (Fitt 1984, Weis et al. 2001). Assuming this was the case in the current study, then any animal tissue contamination found in the zooxanthellae extracts had no effect on the acquisition of one strain of zooxanthellae over another. Further study regarding the role of chemosensory cues in the feeding response and subsequent uptake of symbionts needs to be conducted before a conclusive statement regarding the possible confounding effect contaminated extracts may have had on the differential re-infection of host animals. Despite any confounding factors introduced by the sources discussed above, the high statistical significance of the results speaks multitudes to the significance of species specificity in the structuring of algal-invertebrate symbioses.

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# Substrate Preference at Settlement and Metamorphosis in the Larvae of Two Sympatric Species of *Pullosquilla*, a Lysiosquilloid Stomatopod Crustacean from Moorea, French Polynesia

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ABSTRACT. Where an organism chooses to live plays an important role for the completion of its life cycle. The choice in adult habitat of marine invertebrates usually relies upon their larvae form. Many larvae are known to locate an ideal habitat using environmental cues. Some of these cues can be due to substrate preference. In this study, two species of stomatopod crustaceans of the genus *Pullosquilla* (*thomassini* AND *litoralis*) living sympatrically in reef waters around the island of Moorea, French Polynesia were used. Previous studies have shown that *P. thomassini* are in deeper, fine sand habitats of the lagoon while *P. litoralis* are in shallow course sand habitats of the lagoon. Larvae from each species were individually tested for preference to settle and metamorphose when given choice of two different sand types that represent the adult habitats. Adults were collected from the reef to see which species preferred which habitat. Results show the larvae of *P. thomassini* prefer fine sand only ($p = .002$) which correlated with zero adults collected from the coarse sand habitat. The larvae of *P. litoralis* showed no preference of sand to settle and metamorphose ($p = .371$), which correlated with the adults collected from the field. Adult *Pullosquilla* of both species were tested for sand preference and did not show a significant difference from the larval choice ($p > .05$) but could be biased due to low trial size ($n = 8$). These findings demonstrate that it is the larvae of *Pullosquilla* that are making habitat choice to settle and metamorphose.

KEYWORDS: stomatopod, *Pullosquilla*, crustacean, larva, settlement, metamorphosis, Moorea, French Polynesia.

## INTRODUCTION

Habitat choice is pivotal for the success of many organisms. For an organism to successfully complete its life cycle and propagate an ideal habitat is required. Many organisms will have a larval stage of the life cycle that controls where the adult habitat will be located. Adult bottom living organisms in the ocean are almost exclusively dependant on larval settlement for the choice of adult habitat. Invertebrate larvae from many entirely different phyla present uniformity in the order of events when choosing a habitat (Crisp 1974). Marine invertebrate pelagic larvae are able to exercise considerable control over their place of settlement (Crisp 1974). Settlement is a process beginning with the onset of a behavioral search for a suitable substratum and ending with metamorphosis and fixation (Crisp 1974 AND Rodriguez et al. 1993). A planktonic larva will search for clues as to what habitat is suitable for settlement and metamorphosis to continue its life cycle. These clues can fall under biotic and abiotic controls (Crisp 1974 AND Rodriguez et al. 1993). Some abiotic cues can be attributed to the type of substrate (Botero 1982). The particle size of a substrate has been shown to cue settlement and metamorphosis of polychaete larvae (Day & Wilson 1934, Wilson 1932, 1937). Many studies have found chemical cues for the recruitment of settlement and metamorphosis of planktonic larva (Crisp 1974, Morse 1993).

A clearer understanding of settlement behavior and substrate preference in planktonic crustacean larvae needs to be explored. The goal of this study is to explore habitat choice of two species of the lysiosquilloid stomatopod crustacean genus *Pullosquilla* (*thomassini* AND *litoralis*) living sympatrically in reef waters around the island of Moorea, French Polynesia. Initially the two species were described as living in shallow sandy beach habitats (Manning, 1978 & Michel AND Manning, 1971). In recent studies, *P. litoralis* adults have been shown to occupy the coarse sand habitat, which is shallow and closer to the barrier reef in the lagoon while *P. thomassini* adults occupy only the deep, fine sand habitats of the lagoon (Jutte 1997). Jutte et al (1998a) showed that the eye pigments of the adults were strikingly different in the two species and correlated with the depth of the adult habitat. In this study, the larvae of both species will be collected and given choice of two sand types that represent the two different habitats. In these experiments, substrate depth will be held constant, which will show if *Pullosquilla* larvae are using only substrate cues for their settlement and metamorphosis. The results of this study should determine if the larval forms of *P. litoralis* and *P. thomassini* are making the choice of habitat for the adult form to live. Some new insights into what cues the larvae are using to settle and metamorphose should also be gained. My hypotheses are that *P. thomassini* larvae will choose the fine sand significantly over the course sand and that *P. litoralis* larvae will choose the course sand significantly over the fine sand. The null for both hypotheses is that that neither species larvae will show preference to settle and metamorphose to either sand type.

## MATERIALS & METHODS

### *Study Animals*

The animals chosen for this project are two species of lysiosquilloid stomatopod crustaceans *Pullosquilla thomassini* and *Pullosquilla litoralis*, living sympatrically in reef waters around the island of Moorea, French Polynesia (Manning, 1978 & Michel AND Manning, 1971). The location where adults are frequently found and captured is Vaipahu reef, which is located northwest of Cook's Bay in Moorea (Figure 1). An adult (13-18mm total body length) *Pullosquilla* lives in monogamous pairs within U-shaped constructed burrows (Jutte, 1997, see Figure 2). *P. thomassini and P. litoralis* are easily distinguishable under magnification (Figure 3). In particular, the midventral surface of the telson of *P. thomassini* has numerous sharp spines that are not found on *P. litoralis*. The sand for these experiments was from the lagoon of Vaipahu reef. The coarse sand was taken closer to the barrier reef and the fine sand was taken where the reef begins to deepen near the channel. The free-swimming nearly transparent, late instar stage pelagic larvae (~12mm total body length) are abundantly found in the waters around Moorea. The two species of larvae cannot be distinguished from one another until after molting into post-larvae (personal observation see Figure 4). *Pullosquilla* larvae are readily identifiable from other stomatopod larvae because of their long rostral spine that has a pink tip (Figure 5). Upon capture, these larvae will readily molt into post-larvae.

### *Experimental Design*

Experimental buckets were designed to give an individual larva a choice of substrate in which to settle. Five buckets with a diameter of 55cm were filled to a height of 7.5cm with half coarse sand and half fine sand that was collected directly from Vaipahu reef (Figure 6). The two sand types were separated by a thin plastic divider and leveled off to the top of the divider to disallow any impediment of larval mobility. Water from Cook's Bay was placed into the buckets at ~15cm above the sand. Larvae were caught using a light trap (Porter, this volume), which was placed in the waters of Cook's Bay at night. These larvae were allowed to settle, molt and burrow overnight in the experimental buckets. The next day, the post-larvae were dug up to determine what species chose what sand type to settle upon and molt. The experimental buckets were rinsed with salt water from Cook's Bay after each trial.

To determine where the adults live in the natural habitat, collections were made in both coarse sand and in fine sand habitats of the Vaipahu reef system (see Figure 1). The depth of water ranged from .5m to 1.7m in the lagoon. Where the coarse sand habitat was .5m to 1m and the fine sand habitat was from 1m to 1.7m.Twenty-five adults from both habitats were randomly chosen, captured and brought into the laboratory for identification under magnification. These adults cannot be positively identified while in the field so the person capturing them cannot show any biased capturing techniques. Of these captured adults, some will be tested to see what habitat type they will choose to make a burrow. Two tanks (78cm x 41cm x 49.5cm) were filled to a height of 25cm with half coarse sand and half fine sand that was separated by a plastic divider. Each individual adult was placed into the middle of a tank and allowed to burrow. One day later, the adult animals were dug up and recorded. This was enough time to allow them to burrow in any part of the tank. The tanks were rinsed with bay water after each trial. The results of this experiment will be compared to those of the larvae choice experiment.

### *Data Recording*

*Pullosquilla* post-larvae that successfully settled in the sand of the experimental buckets were removed a day or two later and under magnification, were determined what species they were. The individual larvae were tallied with what habitat type they chose to settle upon and burrow. Twenty trials of both species were conducted over the coarse of seven weeks. Twenty-five adults from coarse sand habitat and twenty-five adults from fine sand habitat were captured and brought to the laboratory for species identification under magnification. Each species type and sex was tallied with the corresponding habitat from where it was captured. Eight adults of each species were successfully tried for habitat choice in the experimental tanks.

### *Data Analysis*

A chi-squared with goodness of fit statistical test using the program SYSTAT was used when comparing data in all data sets. This test was used to determine if the frequency of the distribution of the habitat choice in both the larvae substrate choice experiment and distribution of the adults collected directly from the field would reject or support my alternative hypotheses.

**FIGURE 1.** Moorea study site showing where adult *Pullosquilla* were collected from the sandy bottom of Vaipahu reef

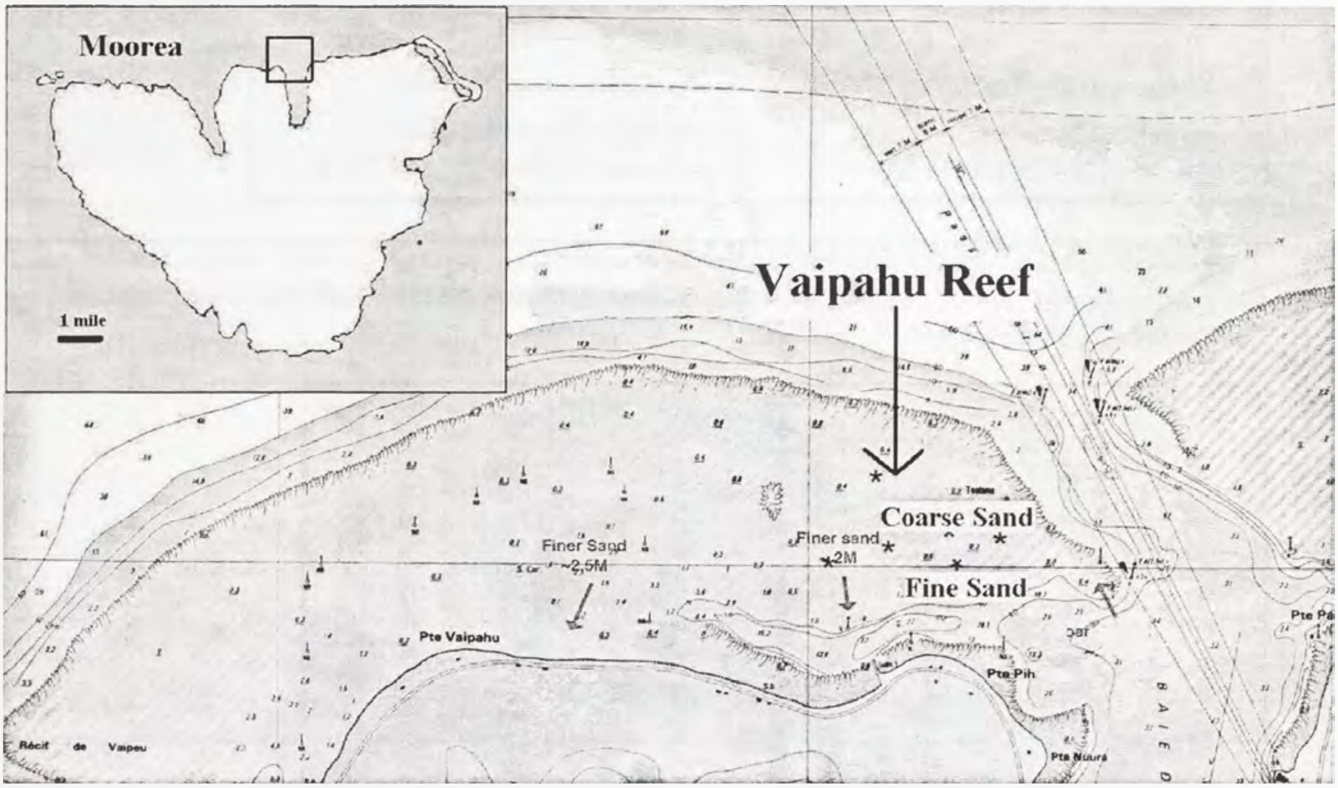


**FIGURE 2.** Side view of a typical burrow constructed by a male–female pair of *Pullosquilla* adults.

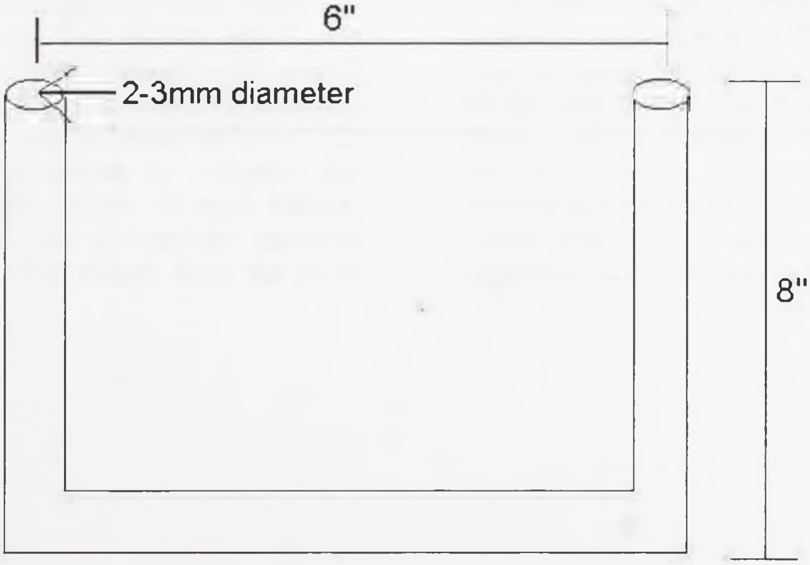
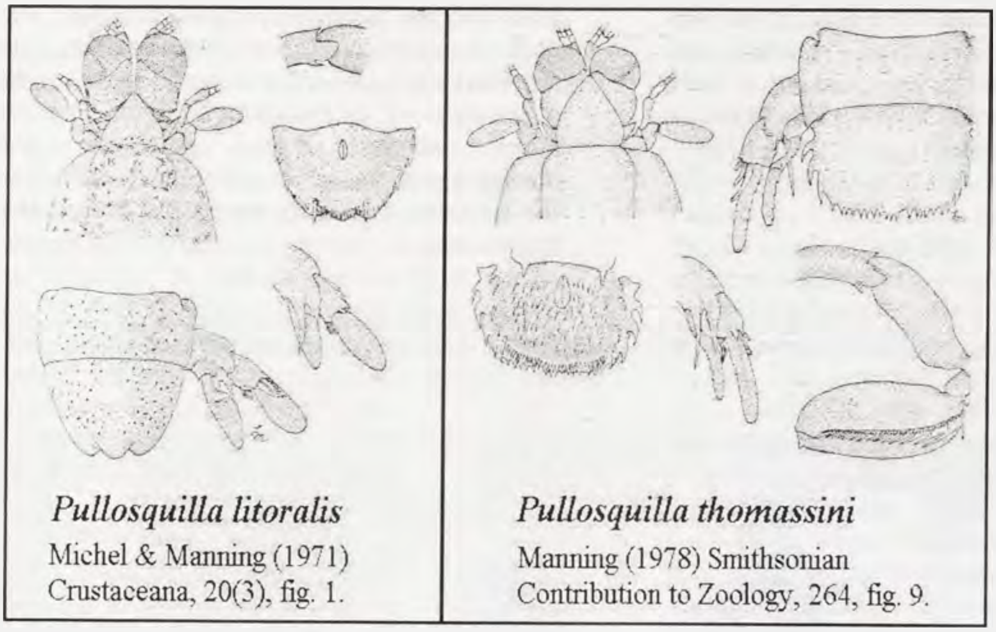


**FIGURE 3.** Distinguishing features of *P.thomassini* and *P. litoralis* (10x).



*Pullosquilla litoralis*
Michel & Manning (1971)
Crustaceana, 20(3), fig. 1.

*Pullosquilla thomassini*
Manning (1978) Smithsonian
Contribution to Zoology, 264, fig. 9.

**FIGURE 4.** Dorsal view of a late stage *Pullosquilla* larva. The larvae are 12mm from the tip of the rostral spine to the end of the telson.

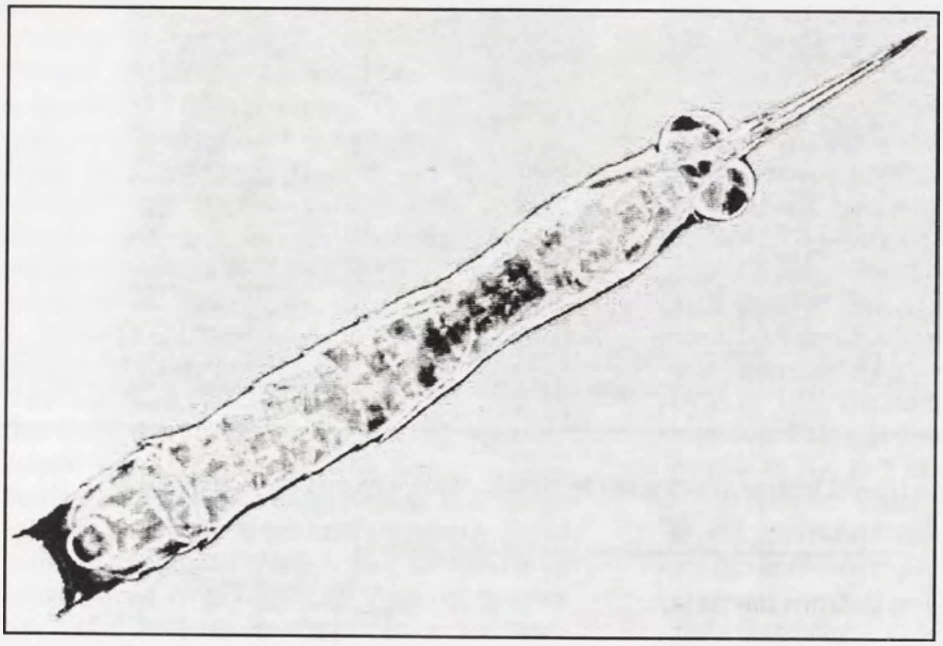


**FIGURE 5.** Ventral view of the head of a late stage *Pullosquilla* larva showing the characteristic long rostral spine with pink tip.

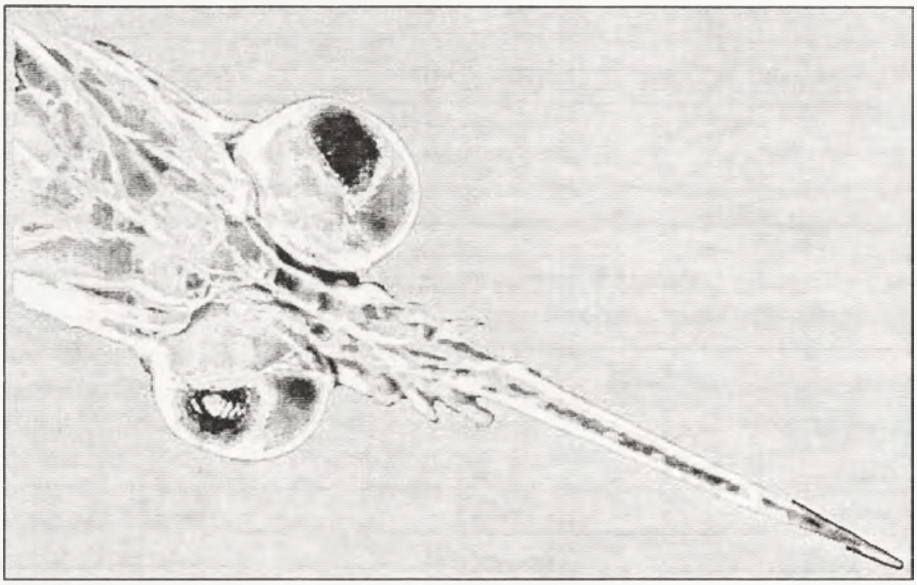
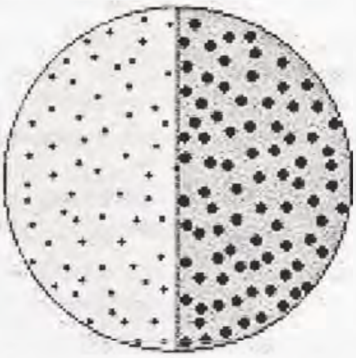


**FIGURE 6.** Diagram showing the design of an experimental bucket with a diameter of 55cm and filled with half coarse sand and half fine sand collected directly from Vaipahu reef.



## RESULTS

The results for the larval sand choice experiment are shown in TABLE 1. The calculated p-value for the distribution of species to each sand type is shown. Twenty individuals of each species were successfully tested. *Pullosquilla* adults that were captured from the field were tabulated and are shown in TABLE 2. A p-value was calculated to compare the distribution of adult species in each habitat. Twenty-five adults were successfully captured from each habitat. The results from the adult sand choice experiment are shown in TABLE 3. For each species, four adult males and four adult females were tried and observed. These results were compared with the larval sand choice experiment and shown in TABLE 4. A p-value was calculated to compare the distributions of larvae sand choice with those of the adult sand choice. Twenty individuals of each species were successfully tested. Observations of these trials showed that the adults would burrow where they would first come in contact with a substrate, regardless of what type of substrate it was.

**TABLE 1.** The number of *Pullosquilla* larvae that showed sand type choice during settlement and metamorphosis. The p-values compare the distribution larvae settling in the two sand types (n=20 for both species).

| Species | Fine Sand | Coarse Sand | P-Value |
|---|---|---|---|
| *P.thomassini* | 20 | 0 | .002 |
| *P.litoralis* | 12 | 8 | .371 |

**TABLE 2.** The number of adult *Pullosquilla* collected from the two sand habitats of Vaipahu reef. The calculated p-value is to compare the distribution of species collected from the two habitats.

| Species | Fine Sand | Coarse Sand | P-Value |
|---|---|---|---|
| *P. thomassini* | 14 | 0 | .0001 |
| *P. litoralis* | 11 | 25 | |
| Total Collected | 25 | 25 | |

**TABLE 3.** The results from the adult *Pullosquilla* sand choice experiment showing the number of adults that chose burrow in the particular sand type. (n=8 for both species).

| Species | Fine Sand | Coarse Sand |
|---|---|---|
| *P. thomassini* | 7 | 1 |
| *P. litoralis* | 7 | 1 |

**TABLE 4.** A comparison of the results from the adult and larval sand choice experiments. The p-values were calculated when comparing the distributions of the same species within the two experiments.

| Species | Fine Sand | Coarse Sand | P-Values |
|---|---|---|---|
| Larval *P. t*homassini | 20 | 0 | .107 |
| Adult *P. thomassini* | 7 | 1 | |
| Larval *P. litoralis* | 12 | 8 | |
| Adult *P. litoralis* | 7 | 1 | .159 |

## DISCUSSION

When only coarse sand was available, *P. thomassini* larvae would settle and metamorphose and could be found making burrows (early trials in this study). The larvae of this species chose only the fine sand (p =.002) when given substrate choice. Adult *P. thomassini* were only found in the fine sand habitat of the lagoon. It is clear to conclude that these animals only prefer to live in the fine sand habitat and that the larvae make this choice. The larvae of *P. litoralis* did not prefer to settle upon either sand type (p = .371). This experiment corresponded with the adult *P. litoralis* that were observed in both sand habitats on the reef. These findings disprove my hypothesis that the *P. litoralis* larvae would show preference to coarse sand during settlement and metamorphosis. The adult sand choice experiment yielded inconclusive results. There was no significant difference in substrate choice when comparing between adults and larvae of the same species. This is probably due to a small sample size (N = 8 for each species). Observations of these trials showed that the adults were not choosing the same substrate from where they were captured. The adults were dropped into the center of the tank and would immediately burrow wherever first contact with the substrate was made. This showed further evidence that the choice of habitat is not made by the adult forms of *Pullosquilla*. This interpretation is reasonable when the ecology and natural history of *Pullosquilla* stomatopods are taken into account. They are bottom living animals that make burrows into the substrate. Adult movement away from their burrows would be minimal and have high cost. Results from this experiment were able to demonstrate that the substrate provides the cue to settle and metamorphose for *P. thomassini* larvae. When given the choice between fine and coarse sand at a constant depth, all larvae chose the fine sand. This gives rise to new hypotheses about what possible cues the larvae show response to.

The first question, is the substrate medium (i.e. contour, texture, particle size) or organisms (i.e. conspecifics, prey foods, algal films) living in the substrate cueing response in the larvae? Chemical cues that derive from algal and/or bacterial films on the substrate have shown positive attraction responses from planktonic larvae in many studies (Brancato, 1982, Orlov 1996, Morse et al. 1984, Morse & Morse 1984). An alternative hypothesis is that the habitat has an inhibitory effect on recruitment and marine larvae would preferentially avoid this habitat. This avoidance of habitats has been shown in other planktonic larvae (Grosberg 1981, Maki et al. 1988). To test these hypotheses I propose future experiments that would be modeled after this study. Artificial substrates that matched those found in the natural habitats could potentially be paired with natural substrates in the same experimental design. *Pullosquilla* larvae could then decide what clues to respond to, the shape of the substrate or the organisms that live within the substrate.

Results from this study suggest the depth range of *Pullosquilla* larvae should be found. Adult *P. litoralis* are not known to inhabit deeper waters (37m) in the lagoons of Moorea (Jutte 1997, Caldwell personal communication). It would be interesting to find if *P. litoralis* larvae are found at the deeper depths. This could be accomplished following the protocol of using the light traps (Porter this edition).

## CONCLUSION

*P. thomassini* larvae have shown significant preference to settle and metamorphose upon the fine sand, which is shown to be their adult habitat. *P. thomassini* larvae have shown that the cue to settle and metamorphose is dependent on the substrate type and not on depth of the substrate. *P. litoralis* has shown no preference to live in either habitat type. The cue to cause settlement and metamorphosis for *P. litoralis* larvae could be dependent on depth of the substrate. This study found important information about the settlement behaviors of *Pullosquilla* stomatopods.

Future studies could lead to a better understanding of the processes working on the natural selection of these sympatric species. Studies of these could also lead to a better understanding of the systematics of stomatopods.

### Acknowledgments

I would like to thank the Richard B. Gump South Pacific Biological Station, Tony & Jacques You Sing for helping me construct my experimental buckets, Irma You Sing and Frank Murphy for having the station operating at all times, the French & Polynesian Governments for allowing me to conduct my project in Moorea, Professor Roy Caldwell for his expertise in the realm of stomatopod behavior & ecology and Stephanie Porter for use of her light traps and encouragement.

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UC Berkeley, IB 158 / ESPM 107

# A light trap survey of stomatopod larvae at Cook's Bay, Moorea

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ABSTRACT. Stomatopod crustaceans are important predators in tropical marine environments. Though the adults are benthic cavity-dwellers, their young are planktonic as larvae. A series of light trap transects at Cook's Bay in Moorea, French Polynesia taken over the course of one lunar cycle evaluated the spatial patterns of abundance and diversity of stomatopod larvae. A total of 813 stomatopod larvae were collected and identified with *Pullosquilla* sp. larvae being the most abundant taxa, composing over 70% of the total catch. In the back reef habitat, pullosquillids and diversity were highest to either side of the lagoonal channel. In the bay habitat, pullosquillid abundance and diversity were highest at the mouth of the bay, and lowest at the headwaters. No consistent vertical stratification was observed.

Keywords: light trap, plankton, recruitment, stomatopod, crustacean, larva

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**Introduction**

A reproductive strategy commonly employed by benthic marine organisms is to release large quantities of widely dispersive planktonic young. Maintenance of such benthic populations depends not only on factors such as predation and the availability of food and shelter, but at a very basic level, on the availability of recruits (young that settle out of the plankton into the adult population). Population dynamics of this type are counter to the popular conception of population equilibria, first developed for terrestrial birds and animals, where populations were mostly closed so young remained within their population of origin. Often marine invertebrates, like some plants, insects and coral reef fish have mostly open populations where many recruits originate in distant populations, so the local fecundity may not affect the recruitment of new individuals (Booth & Brosnan, 1995). In fact recent evidence suggests that many marine populations are regulated by recruitment and are held chronically below carrying capacity by low recruitment (Warner *et.al,*, 2000). Therefore, examining patterns of recruitment is crucial to understanding the dynamics, density and ranges of marine invertebrate populations.

Stomatopods, or mantis shrimps, are malacostracan crustaceans from the subclass Hoplocarida. While adult stomatopods lead a benthic lifestyle, often occupying a burrow or rock crevice, their young are planktonic. Many studies have described the sophisticated sensory and behavioral systems of adult stomatopods, especially the uses of their spearing or smashing raptorial appendages, their highly developed vision and their competitive interactions. Much less is known about the ecology of stomatopods, especially that of their pelagic larvae, despite the fact that the larvae are important planktonic predators and are present in tropical plankton communities world-wide (see Ahyong & Harling, 2000 for a review of species references).

Recent research on stomatopod larval dispersal and recruitment has focused on genetics. There is a strong positive correlation between the duration of larvae in the plankton and the actual distance the larvae disperse (Shanks, *et. al.*, 2001). Since the larvae of many stomatopod species persist in the plankton for over a month (Barber *et. al.*, 2002), it seems reasonable to assume that most stomatopods would have very high levels of gene flow and dispersal. However, a genetic analysis of *Haptosquilla pulchella*, a stomatopod distributed throughout the Indo-Pacific, shows that many populations may be self-seeding. While the larval dispersal potential of this stomatopod are very high, populations exhibit strong genetic mosaiacs between localities only 300 km apart (Barber *et. al.*, 2002). Therefore, much of the recruitment is limited to larvae from the local population rather than from larvae carried on ocean currents from distant populations.

While such genetic research can reveal much about the end effects of larval dispersal on population structures, it is important to understand the fine-scale process of larval dispersal. Researching the spatial distribution of late stomatopod larvae, pre-recruits, is important because it is a phase that links oceanic dispersal patterns to adult distributions, which are more well known.

While many models of plankton dispersal assume that currents distribute passive larvae, this is not always the case. Late fish larvae are often strong swimmers and appear to control their distribution in the plankton (Wilson & Meekan, 2001). For example reef fish larvae appear to select habitiats in which to settle—*Pomacentrus coelestis* will actively avoid lagoons in favor of other nearby habitats (Doherty *et. al.*, 1996). Recent research has focused on this late period in the larval development of marine species, since factors that affect the dispersal of larvae nearing their transition into benthic adults are an important determinant of replenishment patterns (Wilson & Meekan, 2001). The spatial patterns of pre-recruits is especially important for stomatopods as compared to larvae from mobile animals such as fish, since stomatopods undergo a complete change of lifestyle from a highly mobile planktonic larvae to a largely cavity-bound adult. Larvae must settle and metamorphose at a site that ensures good access to shelter, food and mates, making recruitment a crucial time period in the development of a stomatopod. There is likely to be strong selection for stomatopod larvae that can actively determine their location at the time of settlement.

In French Polynesia, the larval dispersal and recruitment patterns of reef fish are the topic of much ongoing research. The relatively open lagoons with deep passes and channels in many of the Society Islands allows for the direct export of eggs and larvae of lagoon-fish into the ocean (Leis *et. al.*, 1998), while the continuous wave action over the reef crest provides an entryway for pre-recruits in the surface waters returning to the reef flats to settle (Dufour, 1994). Very little is known about whether crustacean larvae exhibit a pattern of recruitment along the different reef habitats. Recruitment of strong swimming larvae like stomatopods has three major determinants: (1) the supply of larvae in the water, pre-recruitment, (2) choices the larvae make about where to settle and (3) the survival of the early settlers, pos-trecruitment (Keough & Downes, 1982; Eggleston & Armstrong, 1995). This study investigates the first part of this process by attempting to determine the spatial distribution of larval stomatopod abundance and diversity along a lagoon, bay and depth at Cook's Bay, Moorea.

## Methods and Materials

### *Ligth trap rationale*

Dufour & Galzin (1993) showed that the recruitment of fish larvae to the reefs of Moorea was a nocturnal event. Since a large pulse of larvae was detected as soon as light levels decreased, they hypothesized that larvae in the ocean waters adjacent to the reef were migrating at dark into the onshore surface currents and being carried over the reef crest by waves, thereby entering the reef system. This pattern may be a response to the reduced levels of predation by adult fish at dusk and dark (Bollens *et. al.*, 1992). Crustaceans may face similar pressures and it is likey that they too recruit to the reef at night. Many crustacean larvae are also difficult to sample during the day because they migrate to great depths, whereas at night they migrate to the surface. In fact, on coral reefs, concentrations of zooplankton are highest at night (Carleton *et. al.*, 2001). Since night time may be the time of greatest influx of new crustacean recruits as well as the time when recruits are most accessable, night time sampling may be better at detecting crustacean recruits than sampling during the day. Light traps are an increasingly popular method for sampling at night.

Also, crustacean larvae often have a patchy distribution in the water (*e.g.* Eggleston & Armstrong, 1995). Since light trap catches yield an average abundance of larvae over the time that they sample, they may more accurately reflect the average distribution of larvae at a site than a method such as plankton towing that generally samples for a relatively brief interval.

While early stomatopod larvae are weak swimmers and like other crustacean larvae, are probably subject to local ocean currents, late larvae are stronger swimmers and may have a greater control over their location so they will be able to enter the traps. Also, since late stomatopod larvae are strong swimmers, they may be underrepresented in plankton tows, since they may avoid the net, as many late fish, and possibly crustacean, larvae can (Choat *et. al.*, 1993; Eggleston & Armstrong, 1995). The only stages of stomatopod development known to be positively phototactic are the very early and late larval intars (Caldwell, pers. comm.), however it is unknown why.

### *Construction of light traps*

Before commencing the experiment three light trap prototypes were tested and their catch compared. The model most successful at catching stomatopod larvae was replicated and used for the experiment. Five replicate light traps were constructed following the basic format designed by Ginny Eckert (unpublished).

A translucent, roughly rectangular prismatic plastic 9.45 L water container (brand name, "Tahiti Premium Water") formed the body of the trap. Circular holes were cut into the four side panels of the container and funnels were sewn into the holes. Funnels were made of translucent plastic and were 65 mm in diameter at the larger end and had an 8 mm aperture at the narrow end (some of the narrow end was sawed off to enlarge the aperture to 8 mm). An electric drill with a small drill bit was used to make eight corresponding holes in each funnel and face of the plastic container. Fine gauge fishing line was then used to sew the parts together and the stitches were then covered with acetate-based silicon aquarium sealant to waterproof the attachments and to protect the stitches.

The collection cup, or catchment, at the bottom face of the trap was constructed from a cylindrical wide mouth plastic Nalgene-brand bottle. The body of the bottle was sawed in three: the solid bottom of the bottle was discarded and the remaining two pieces of plastic were glued together using PVC sealant, with a circle of 210 *u*m gauge plankton mesh between them. A large circular hole was cut into the lid of the bottle using a the cutting edge of a sharp drill bit. Similarly to the funnels, holes drilled into the lid and around a circular hole cut into the bottom face of the trap allowed the pieces to be sewn together and sealed with silicone. In this way only the lid of the catchment was attached to the trap so the body of the bottle could be freely detached. When removed from the water and held in an upright position, water draining from the trap carries plankton into the catchment (as depicted by the movement of the dots in Fig. 1)

Two 75 mg lead weights were cable tied inside the trap on diagonally opposite bottom corners of the trap using a drill to puncture the plastic. Two foam floats were attached to the outside of the top face of the trap, one on either side of the handle. The holes were sealed with silicone sealant. These weights and floats ensured each trap floated in an upright position, with the body of the trap completely submerged. The pre-existing handle of the water container was used to attach the trap to a nylon rope. The trap was lit using a minuature hand-held dive light cable tied to the interior wall. Dive lights were necessary since few other lights will function in deep water. Identical sets of Rayovac rechargeable NiMH batteries lit the traps for three hours before burning out (Fig. 1).

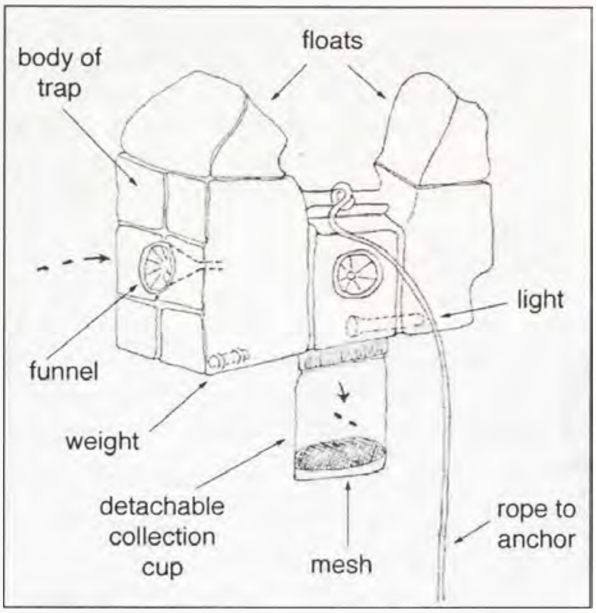

Fig. 1. Diagram of a light trap. Unless constrained, the trap floats at the surface with only the floats above the water. Light from the dive light reflects off the opaque body of the trap making the entire body of the trap glow. Small dots beside the funnel depict larvae about to enter the trap. When the trap is pulled out of the water, larvae are forced into the collection cup by the draining water, as depicted. Drawing courtesy of Carole Hickman, with text added.

### *Study site*

Field work was conducted at the University of California, Berkeley's Richard B. Gump South Pacific Biological Station in Moorea, French Polynesia from September to December of 2002. The field sites included waters up to 20 m deep on the east and west banks of Cook's Bay and the fringing and barrier reefs northwest of the bay. The dominant hydrodynamic feature of the lagoon environment on Moorea is the continuous influx of water as waves over the reef crest, and the outflow of water through the lagoon channel and out the passes in the barrier reef; the average residence time of water in this system is 6 hours (Dufour & Galzin, 1993). The dominant hydrodynamic feature of Cook's Bay may be a northward wind-driven surface current drawing in a deeper southward bottom flow. The Bay was very weakly influenced by a freshwater input since the study occurred during a severe extended drought (Fig. 2).

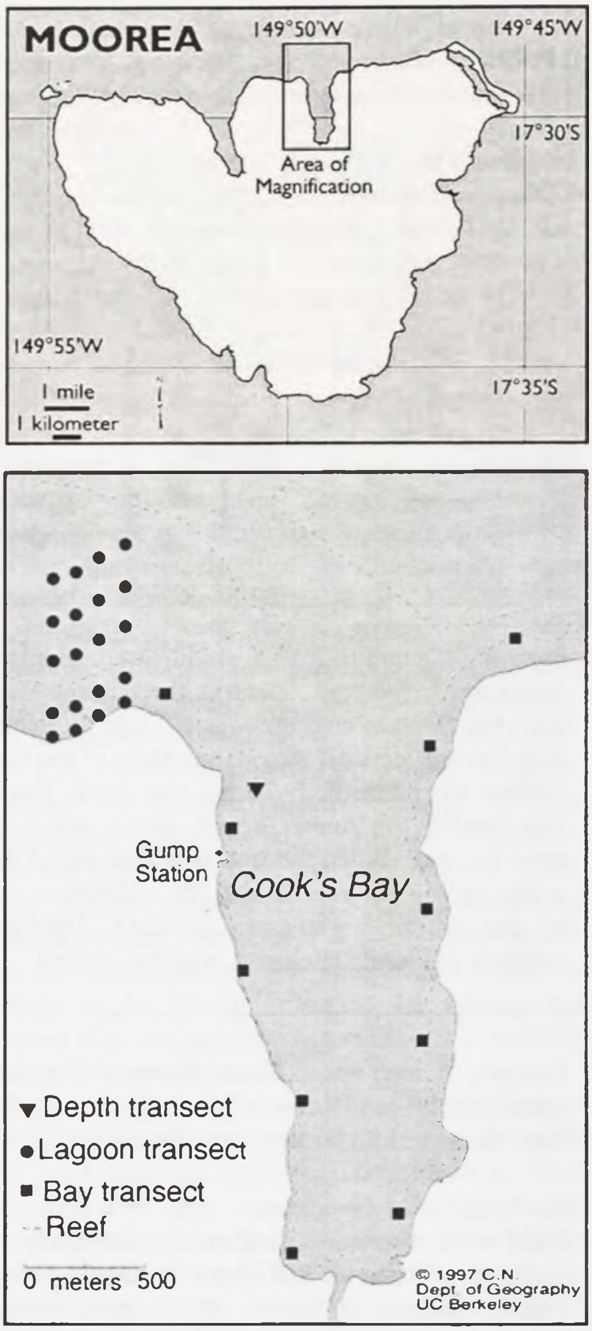

Figure 2: Maps depicting the study site. Above: the island of Moorea, located in the South Pacific. Below: map of the study sites at Cook's Bay.

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## *Methodolgy*

Traps were set and collected from a large two-peron kayak. Each trap in the backreef and bay transects was secured by tying it to a cinder block with a 5 m long rope which was tied off loosely so that the rope was 45° from vertical if a current was present. Due to the depth of the bay and the limitations of the kayak, traps could only be set in relatively shallow, nearshore water. Traps operated for 2.5 hours each night, starting at approximately 20:30 (+/- 1 hour). Each trap and catchment was labeled #1-5. In a light trap study on the Great Barrier Reef, Carleton *et. al.* (2001) found stomatopod larvae were most abundant (9 larvae/hour) from 24:00 – 01:00, however sampling during this time period was not logistically feasible. Carleton *et. al.* (2001) did detect stomatopods at all times through the night.

Once collected, catchments were removed and placed in seawater in water-tight, heavy-duty Ziploc plastic bags to keep the larvae alive. Some larvae were reared into juveniles in order to tentatively identify them according to Manning (1994) and the list of stomatopods known from Moorea (Caldwell, pers. comm.; Poupin, 1998). Some larval identifications were suggested by Caldwell, however most identifications were tentative since no stomatopod larvae from Moorea are described in the literature. Larvae that could not be identified were denoted by a distinctive feature; otherwise they were assigned an unique letter. Some larvae were described and diagrammed (Appendix. B). Voucher specimens of all stomatopod taxa (and bycatch species) have been placed in the Museum of Paleontology at the University of California, Berkeley.

### I. *Reef Gradient:*

Four transects of the reef immediately northwest of Cook's Bay were taken from the shoreline directly out to the reef crest (a north-south alignment) on 22 and 26 October, and 1 and 9 November, 2002. No samples could be taken at the reef crest since it is almost permanently at sea level. Similarly to many coral reef islands, waves breaking on the reef crest generated a constant flow of water over the barrier reef into the lagoon channel resulting in a constant current east and then north, out of the pass (Dufour & Galzin, 1993).

The first transect ended at 17° 29.173'S, 149°49.655' W (the most southeastern circle in Fig. 2). Each transect was taken 75 m west of the previous transect unless private property separted the transects, in which case the next closest location west on public land was chosen. Proximity to private property was avoided in this study because traps were easily visible and on one occasion a trap was stolen.

The distance from the shore to the shallowest part of the reef crest accessible by kayak was estimated to be 600 m. A waypoint was taken at the shoreline, then at the reef crest directly northward, where the first trap in the transect was laid. Successive traps were laid at 150 m increments back toward the shore, with the last trap being placed several meters from the actual shoreline. However, since a very deep boating cut through the transect, traps which would have otherwise been placed in the channel, were moved to either side of it. Traps at 0 and 150 m from the shore were on the fringing reef, while traps at 300, 450 and 600 m from the shore were on the lagoon side of the barrier reef.

The depth of water sampled by the traps varied from 0.5 – 2 m deep. Often the water contained coral heads rising from a sandy or solid substrate; traps were placed in the deepest water within a few meters of a site, generally in the water between any coral heads present.

### II. *Bay Gradient:*

Four transects of Cook's bay were conducted, two along the east (25 October, 7 November) and two along the west shoreline (21, 30 October 2002), from the southern end of the bay to the northern mouth of the bay. Since the bay is approximately 2km long, traps were set approximately every 500m along the bay, including both ends. Especially on the eastern shore, some transect points were very close to private property, so the next closest location not obviously inhabited was chosen. All traps floated at the surface and were set in nearshore water ranging in depth from 1-2 m deep.

### III. *Depth Gradient*

Four transects from 0 to 20 m deep were taken at a single location on the west bank of Cook's Bay just off the Gump Station (17°29.428'S, 149°49.557W) (20, 24, 28 October, 4 November 2002). Traps were tied to a nylon line at 0, 5, 10, 15 and 20 m deep. The entire line was sunk with a cinder block at exactly 20m, so that the top trap floated at the surface. The weights and floats attached to each trap ensured they remained in an upright position at depth and during removal from the water.

## Results

A total of 813 stomatopod larvae were collected and identified as one of 13 taxanomic categories. The four transects at each of the three study areas yielded a total of 60 light trap samples. The average catch rate was 5.98 larva/hour. The larvae detected in this study were for the most part either early, late or post larvae; almost no mid-stage larvae were caught (Table 1).

Pullosquillids were by far the most abundant taxa (over 70% of the total catch). Their large sample size allowed a meaningful analysis of their distribution along each gradient. However, since the abundance of pullosquillids in the water varied greatly, depending on the lunar cycle (Jutte, 1997), transect data from different nights were converted into a percentage of the total number caught that night. In this way, information about the distribution of pullosquillid abundance could be compared across the lunar cycle. Since proportional data of this type may not have a normal distribution, percentages were subjected to an arcsin transformation (Zar, 1974) before being tested by an ANOVA statistical test. Due to the high variability in the data and low sample size, few trends in the data were statistically significant to a $p < 0.05$.

Since taxa other than pullosquillids were rare in comparison, their presence was analyzed as part of two diversity indicies at each site. One index was simply the average number of taxa present at a site. The other was the Simpson's Diversity Index, combined information about the number of taxa present with the eveness of the taxa's distribution. The average results of these diversity indicies were analyzed by ANOVA statistical tests, however again high variability and low sample size resulted in few of the trends having statistical significance.

### *I. Reef Gradient*

A total of 178 individuals from 7 taxa were collected over the four sampling nights in the reef area. *Pullosquillia* sp. was the most abundant taxon and constituted 64% of the total catch. "Very early larvae" composed 22%, *A. multifasciata* 8% and the remaining 6% was composed of *Alima* sp., *Roulserenea* sp., *Lysiosquillina* sp., and post larva A. Larvae were caught at an average rate of 3.56 larvae per hour per trap.

Pullosquillid abundance was most often concentrated at 150 m and 300 m from the shore, on the fringing reef and barrier reef closest to the channel (Fig. 4). An ANOVA test detected a significant difference between the data sets ($p = 0.046$), however a Bonferroni post hoc test did not detect a significant difference in pairwise comparisons. The both diversity indicies suggested that diversity was highest at these locations as well.

Table 1. Composition of stomatopod larvae from the light trap collections. Data are pooled from all collections. Only restricted developmental classes of larvae were caught for each taxon; they are indicated. Occurrence indicates the number of individual light trap samples in which the taxon was present.

| Taxon | Larval stages | Total | Occurrences | Percent |
|---|---|---|---|---|
| *Pullosquila* sp. | late, postl | 586 | 48 | 72.1 |
| very early larvae | very early | 95 | 5 | 11.7 |
| *Roulserenea* sp. | post | 55 | 15 | 6.8 |
| *Acanthosquilla multifasciata* | mid, late, post | 33 | 15 | 4.1 |
| *Squilla* sp. | early | 17 | 4 | 2.1 |
| *Alima* sp. | late | 8 | 7 | 1.0 |
| *Lysiosquillina* sp. | late, post | 7 | 6 | 0.9 |
| "4-spine telson" | late, post | 3 | 2 | 0.4 |
| "fat arm" | post | 2 | 2 | 0.2 |
| larva A | post | 1 | 1 | 0.1 |
| larva B | post | 1 | 1 | 0.1 |
| larva C | post | 1 | 1 | 0.1 |
| larva D | post | 1 | 1 | 0.1 |
| no ID | post | 3 | 3 | 0.4 |
| **Total** | | 813 | 60 | 100 |

*II. Bay gradient*

A total of 263 individuals from 7 genera were collected over the four sampling nights in Cook's Bay. *Pullosquillia* sp. was by far the most abundant taxon and constituted 71% of the total catch. "Very early larva" composed 21%, and the remaining 8% was composed of *A. multifasciata*, *Roulserenea* sp., *Lysiosquillina* sp., early *Squilla* sp. and "fat arm". Larvae were caught at an average rate of 5.26 larvae per hour per trap. The data suggest that pullosquillid abundance and both diversity indicies were highest at the mouth of the bay. No statistically significant results were found, using an ANOVA.

*III. Depth gradient*

A total of 457 individuals from 11 genera were collected over the four sampling nights in Cook's Bay. *Pullosquillia* was the most abundant taxon and constituted 82% of the total catch. *Roulserenea* sp. composed 10%, and the remaining 8% was composed of early *Squilla* sp., *A. multifasciata*, *Alima* sp., "4-spine telson", *Lysiosquillina* sp, "fat arm", post larva B, post larva C and post larva D. Larvae were caught at an average rate of 9.14 larvae per hour per trap. The data suggested no vertical stratification pattern for pullosquillids. The number of taxa seemed highest at the surface and bottom traps.

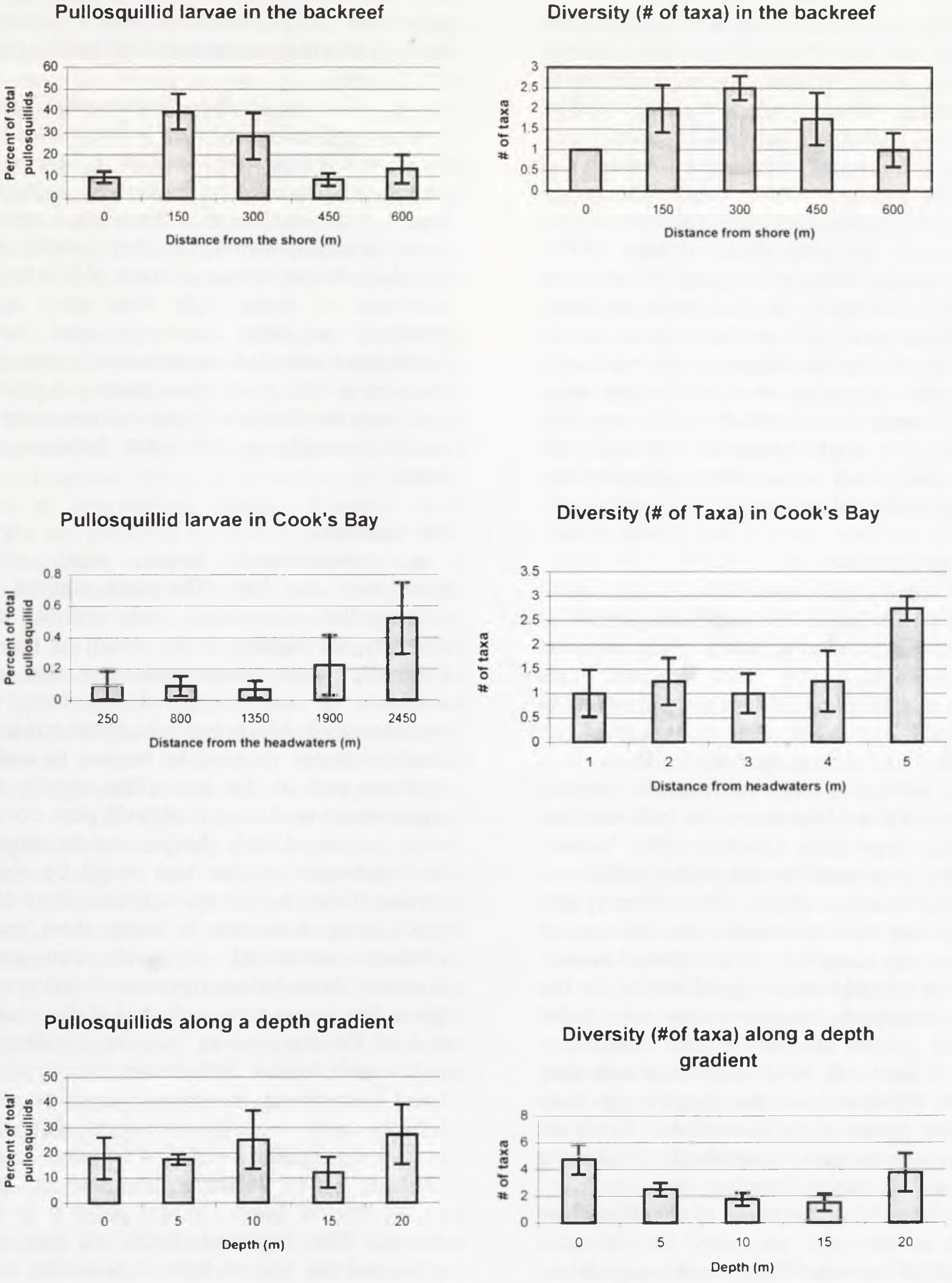
## Discussion

*Light trap performance*

The average catch rate of stomatopod larvae in this study, 5.98 larvae/hour, was comparable to the 3.57 larvae/hour of Carleton *et. al.* (2001) on the Great Barrier Reef using a trap design similar to that of Doherty (1987). The fact that the traps used in this study may be as or more efficient at trapping stomatopod larvae as those of Doherty (1987) is significant since the traps in this study were much smaller, lighter and were produced at a fraction of the cost (approximately $35 vs. $3000 per trap).

Since the volume of water sampled by the light traps is not quantifiable, light trap catch data are most valuable in a comparison at a single location over time or with other traps in very similar conditions. In the present study, current velocity may have influenced the data since light traps deployed in higher current waters would have sampled a greater volume of water than those in slower currents. However, it may be more difficult for larvae to swim to a trap in high current water than in slow, so it is difficult to predict how current speed would affect a catch (Carlton *et. al.*, 2001). Drifting traps may be useful in future studies of larvae that are less focused on specific locations (Meekan *et. Al.* 2001; Thorrold, 1992).

Light traps may have also sampled differential volumes of water due to differing proximities to deep water. Excluding the depth gradients, traps were always placed in water 2m deep or less. However at some locations near steep drop offs to deeper water, light may have been visible to some larvae in the deeper water. Therefore some traps may have sampled a greater volume of water than others due to their proximity to deeper water. Very few of the patterns of distribution in this study showed


Pullosquillid larvae in the backreef

Diversity (# of taxa) in the backreef

Pullosquillid larvae in Cook's Bay

Diversity (# of Taxa) in Cook's Bay

Pullosquillids along a depth gradient

Diversity (#of taxa) along a depth gradient

Fig. 3. Graphs depicting the average distribution of pullosquillid stomatopod larvae and the distribution of diversity (measured by the number of taxa) in the three study sites, the backreef, the Cook's Bay, and the depth gradient. Averages were determined by pooling data from each of the four transects at each site. Bars represent standard error

statistically significant results. It is likely that the variability in the data reflects the extreme variability of recruitment patterns common to many marine invertebrates (Keough, 1988). Stomatopod recruitment patterns to the reef may be influenced primarily by lunar periodicity and seasonality (Reaka, 1976). Many stomatopod species will synchronously breed and recruit at a specific lunar and tidal phases (Reaka, 1976). Since the present study investigates patterns over only one lunar cycle, it is difficult to judge whether the variability in the data is due to cyclical trends that would appear over a longer time period. Certainly in future studies lunar influence could be controlled for by sampling only during a single phase of the moon for several years and seasonality controlled by sampling during the same season for many years.

*Back Reef Habitat*

Stomatopod larvae were present at all areas of the backreef. Pullosquillids appear to be most abundant at 150 and 300 m from shore, the areas surrounding the boat channel. This suggests that pullosquillids are more abundant in the channel than in the other areas. Larval fish pre-recruits and early stages use the channels in back reef habitats as major pathways for entering the back reef to settle or to exit the back reef into the ocean, respectively (Dufour, 1994; Leis *et. al.*, 1998). It is possible that Pullosquillids use the channel in a similar way. Since diversity was also high near the boat channel, the data suggest other taxa may congregate at the channel as well. A channel might provide a good habitat for late larval stomatopods, because if they have a diel migration pattern similar to other crustaceans (Hobbs & Botsford, 1992), they may seek deep waters in which to spend the day. Though there is a strong current in the channel, late larvae are strong swimmers and could probably avoid being swept back out into the open ocean.

In addition, the banks of the channel are covered in the sand necessary for the adult pullosquillid burrows. These banks support the highest density of adult pullosquillids in the backreef, sometimes reaching densities of 25 individuals/m$^2$ (Caldwell, pers. comm..). Congregating in the channel would provide late pulloquillid larvae with easy access to high quality habitat. Also, *P. thomassini* will settle successfully in deep water (Jutte, 1997), and the channel would provide one of the few protected, sandy, deep water habitats on the reef. It would be interesting for a future study to determine if pre-recruit pullosquillids are able to sense the location of and to navigate to high quality habitat in the field.

One important cautionary note about the trends suggested by the data is that it is possible they are a sampling artifact reflecting the proximity to deep water of the 150 and 300 m traps. It is possible that these traps collected more stomatopods since they could have sampled a larger volume of water. While this is a weakness of using light traps over spatial gradients, no other commonly used method could have sampled simultaneously along the transects in this study since boats and plankton nets were not feasible in the shallow water and couldn't sample in the water between coral heads.

*Bay habitat*

Stomatopod larvae were present throughout the bay. The data suggest that pullosquillid abundance and overall larval diversity are highest at the mouth of the bay. Generally adult stomatopods are also more abundant at the mouth of the bay; most stomatopods in Moorea require either crevices in hard substrates or sand to burrow in and the southern end of the bay offers mostly silty, sediment-covered areas (Caldwell, pers. com.).

It is unlikely the pre-recruits caught at the headwaters of the bay would be able to survive if they settled there. Unless these larvae could enter a current to carry them over a kilometer northward, or could swim such a distance, these larvae represent failed recruits. Since few larvae were collected at the southern end of the bay, larvae may be avoiding this unfavorable habitat. Fish larvae on tropical reefs have been found to actively avoid poor fish habitats such as lagoons, so avoidance of unfavorable habitat would not be unprecedented (Wilson, 2001). However, this lack of larvae could also be due to current patterns or other factors. The logistical limits of this study prevented the use of light traps in the central waters of the bay and the pass out to the ocean. Sampling these deeper waters would make an interesting future study.

*Depth gradient*

Many crustacean planktonic larval assemblages undergo daily patterns of vertical stratification. For example, Dungeness crab (*Cancer magister)* late larvae may aggregate at depths of 25-160 m during the day, while congregating at the top meter of water during the night (*e.g.* Hobbs & Botsford, 1992; Jamieson & Phillips, 1993). Many taxa such as fish larvae are also most abundant over tropical reefs in the surface waters at night (Doherty & Carleton, 1997; Hendriks *et. al.*, 2001). These fish larvae may ascend or descend to exploit the vertical stratification of water currents to influence their dispersal and settlement patterns (Cowen *et. al.*, 2000). Vertical distribution will also affect the predators and food sources of the larvae (Jenkins *et.al.*, 1998).

However, no obvious trends in pullosquillid abundance were observable along the depth gradients—pullosquillids appeared to be somewhat homogeneously distributed in the water column. The data suggest that there were a greater number of taxa at the surface and bottom than at intermediate depths. Possibly these depths are preferred by stomatopods other than pullosquillids, however the low sample size of these taxa makes it difficult to draw conclusions. It is possible that some stomatopods may prefer certain depths at certain phases of the moon. If stomatopods are vertically orienting themselves by light intensity, the prescence or absence of moonlight might determine their preferred depth. However, a much longer study would be needed to investigate the effects of the lunar cycle.

While light traps have been used to sample fish larvae along a depth gradient, recent research suggests that it may be important to use light baffles so that light is only detectable at a specific depth of water, making the traps stratum-specific (Fisher & Bellwood, 2002). The present study assumes larvae will enter the nearest (brightest) trap. However, since light traps as deep as 10 m were visible from the surface of the water, they may have indescriminately attracted some larvae from a range of depths of water.

*Pullosquillids*

The fact that pullosquillid larvae were extremely abundant compared to other larvae can be attributed to several factors. Most importantly, pullosquillids are by far the most abundant stomatopods in the habitats studied (Caldwell, pers. com.). Being small stomatopods, (adults grow to under 2 cm), Pullosquillids devote much of their energetic resources to breeding, and monogamous pairs will produce successive clutches of 135-145 eggs. Larvae are planktonic for over a month, and have at least 6 instars. The high number of *Pullosquillid* larvae suggests the genus is not recruitment-limited.

In a previous study, Jutte (1997) used an underwater light and hand net to collect pullosquillids in Cook's Bay. She determined that only first and last stadia Pullosquillid larvae are positively phototactic and her catch consisted of only the late stage pullosquillids. Similarly in the present study, only late stage larvae were caught in the light traps (no early stage larvae were not identifiable except as stomatopods).

The trap design used in this study worked well for pullosquillids, however, some larger stomatopods were excluded because of the small funnel aperture size. For example, *Alima* sp. were large enough to have difficulty entering the trap. *Lysiosquillina* sp. were too large as larvae to enter the traps, only the smaller post larvae were collected (larvae and molts were occasionally found wedged beneath the floats of the trap). A future study on Moorean stomatopod larvae should use a funnel aperture diameter of close to 2 cm in order not to exclude large larvae. However, a larger aperture might require a larger trap in order not to greatly increase the chances of the larvae escaping.

## Conclusions

The light traps used in this study proved to be inexpensive and effective collection devices for stomatopod and other invertebrate larvae (Appendix C). Future studies could employ a far greater number of traps to increase the numbers of replicates, and could involve many questions, including those pertaining to a broad variety of invertebrates.

In the backreef and lagoon areas of this study, larvae were most common in the areas that provided the highest quality habitat for adults. Whether this is the result of larvae actively congregating at high quality habitats or is due to chance or other factors is unclear. Pre-recruitment distribution suggests that, at least in the case of pullosquillids, recruits do not generally carpet all available substrates, so post-recruitment factors do not entirely determine adult populations distributions. Pre-recruitment distribution seems to largely overlap with adult distribution. At the scale of this study, the results suggest that post-recruitment mortality is not likely to be a limiting factor in the range of adult distributions.

Pursuing further research into how the population dynamics of recruits compares to adult population dynamics in stomatopods over a long time scale would make a valuable contribution to the ongoing debate over the importance of recruitment as a limiting factor in the marine environment. Also, knowledge of the larval populations around Moorea will help inform the management of the marine reserves soon to be implemented in the area. For example, *Lysiosquillina maculata* adults have been locally fished to extinction on Moorea, however some larvae of the species continue to reach the island (Caldwell, pers. com.). By locating and protecting the source population of these larvae and ensuring a protected area on Moorea, it may be possible to re-establish an adult population on Moorea.

**Aknowledgements**

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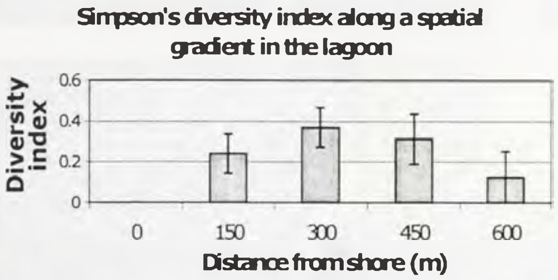
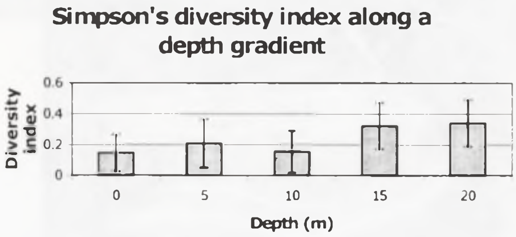
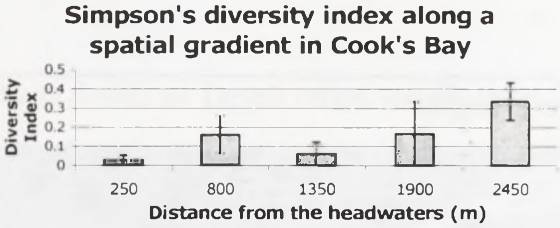
## APPENDICIES

**Appendix A.** Graphs of the Simpson's Diversity Index

Simpson's Diversity Index = 1 – sum(Pi^2), where Pn = the proportion of the total catch composed of a single taxa. A value of 0 indicates low diversity; a value approaching 1 indicates high diversity. (George Roderick, pers. com.)





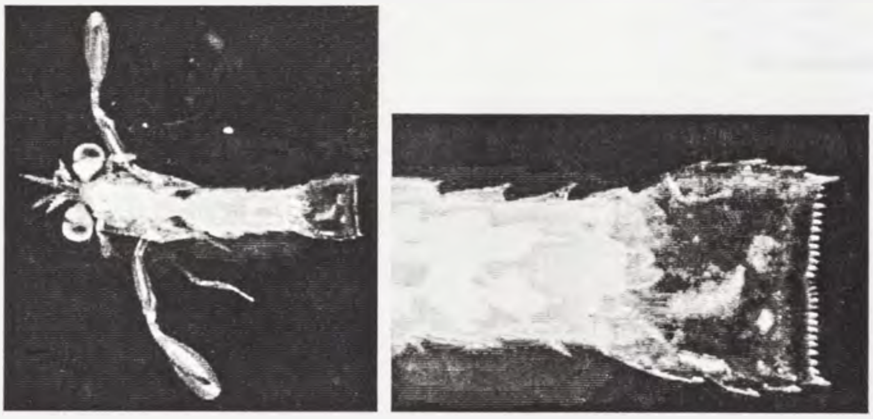


**Appendix B.**

1. Pictures of some of the stomatopod larvae at Cook's Bay

*Pullosquilla* sp. The two species present in Cook's Bay, *P. litoralis* and *P. thomassini* are indistinguishable as larvae and have similar morphologies as adults, though they occupy somewhat different habitats (Jutte, 1997). Larvae are easily identified by the characteristic pink tip on the rostral spine. See Wood (2002) for a further description.

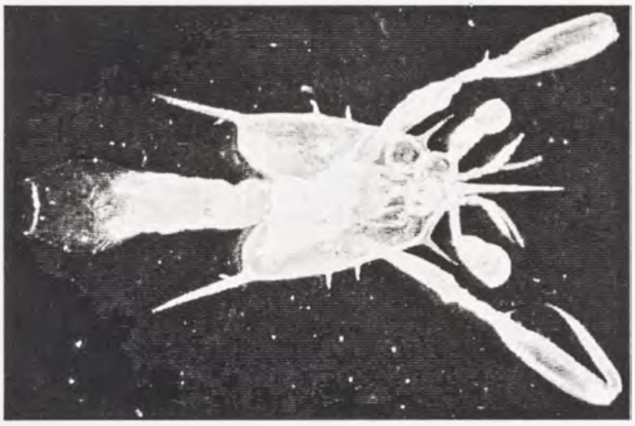
Very early larvae (no ID). Larvae were often only a few mm in length.



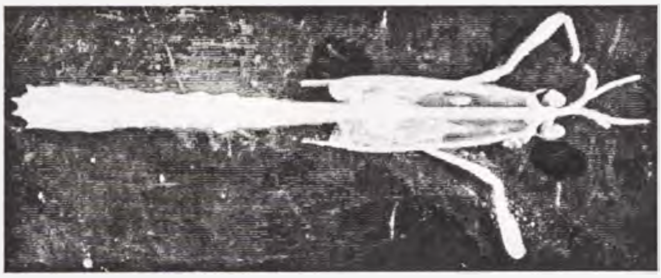
*Roulserenea* sp. Larvae were only caught in traps as post larvae.

*Acanthosquilla multifasciata.* Larvae had a characteristically large carapace with elongated rostral and carapace spines. Earlier instars had carapace spines that were longer than the body (shown in photo) and later instars had carapace spines shorter than the body.

*Early Squilla* sp. Length varied, usually about 5 mm long.



*Alima* sp. Very long (several cm), morphology is elongate and dorso-ventrally compressed, as if the larva was pressed in a book

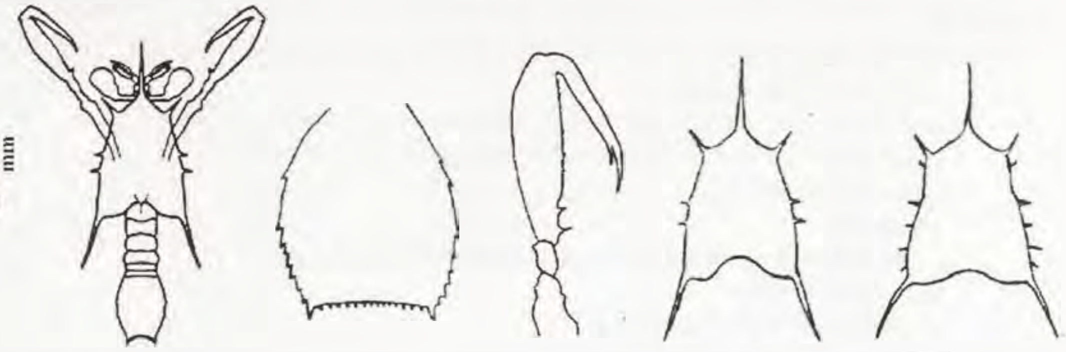


*Lysiosquillina* sp. Only the post larvae were caught, since the larvae were too large to fit in the funnel.

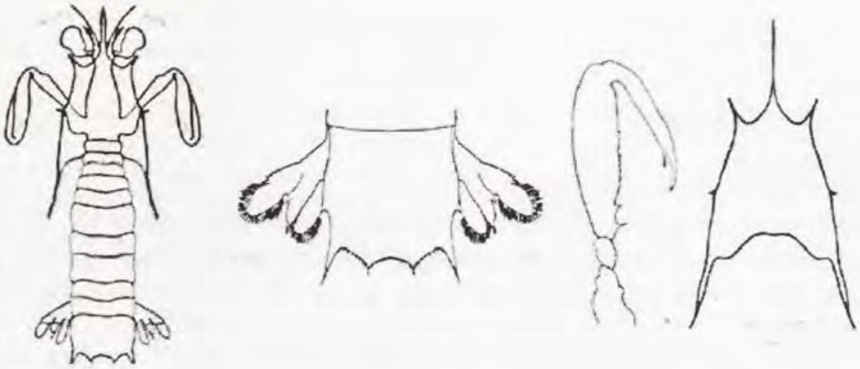
4-spine telson

II. Drawings of some of the stomatopod larvae at Cook's Bay

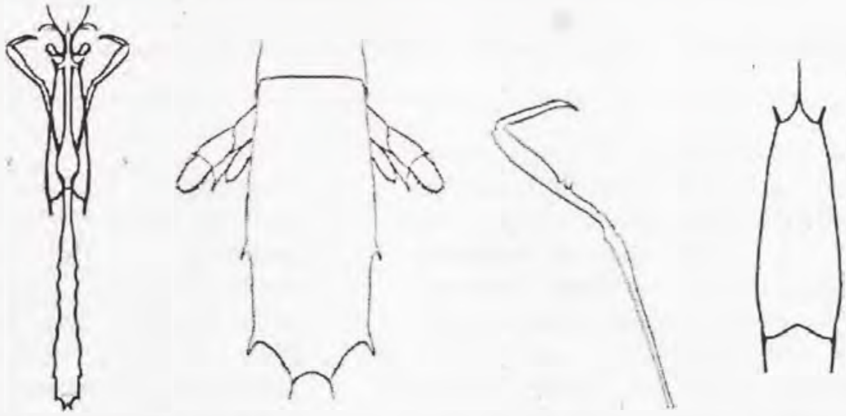
**searly squilla**
**4mm from base**
**of eyes to telson**



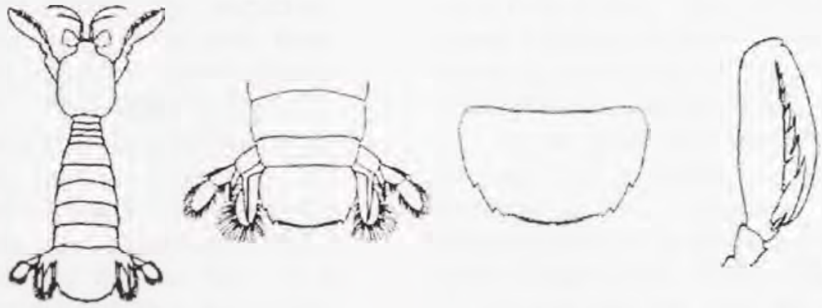
**4-spine telson**
**late larva**
**19 mm from base**
**of eye to telson**



**alima**
**late larva**
**sevearl cm**
**long**



**Lysiosquillina**
**sp. post larva**
**22 mm from**
**base of eye to**
**telson**



**Appendix C.** List of Other Organisms Collected by light traps

Abundant:

Mysid shrimp (bay)
Various copepod species
Diverse taxa of crab megalope (including anomura and branchyura)
Diverse taxa of crab zoea (including anomura and branchyura)
Unidentified worms
Ostracods
Scissurellid snails at a sexual stage (one night only)
Polycheate worms
Neritid gastropod larvae (lagoon)
Many species of unidentified fish larvae

Rare

Shrimp
Lobster late stage larva
Sea jellies
Assorted marine gastropod larvae
Octopus larvae (one taxa only mm's long, one taxa red and 2 cm long)
Planktonic forams (*Rosalina bulloides* in it's floating sexual stage)
One sea hare larva
One eel snake

Biol. Geomorph. Trop. Is. (2002) 11:153-162
UC Berkeley, IB 158 / ESPM 107

# Do stomatopods use visual observations to make decisions about evicting residents? A study on decision making in the stomatopod, *Gonodactylellus childi* (Manning 1971) in Moorea, French Polynesia

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ABSTRACT. Stomatopods have the most sophisticated peripheral visual system in the animal kingdom and have considerable learning and memory capabilities. Because stomatopods have a remarkable visual system and compete aggressively over cavities, this study tested whether stomatopods use information obtained by visual observation to make decisions about which residents to attempt to evict. *Gonodactylellus childi* were allowed to observe either two empty cavities, an empty cavity and a *G. childi* occupied cavity, or a cavity occupied by another *G. childi* and a cavity occupied by a less competitive resident, *Raoulserenea pygmaea*. I found that *G. childi* do not use visual observations to make decisions about evicting residents. In each group, the number of stomatopods that first approached and entered one cavity type was not significantly different from the number of stomatopods that first approached and entered the other cavity type. Additionally, the differences in ranks of the times of first approach and entry into either cavity did not differ significantly between the three groups. No behavioral differences were detected with respect to how stomatopods first approached and entered empty versus occupied cavities. These results suggest that 1. *G. childi* do not remember or learn the differences between the two types of cavities based on visual cues alone and/or 2. *G. childi* do not prefer which type of cavity to occupy. Further study on decision making behavior with other stomatopods is recommended to see whether observers adjust their agonistic behavior based on observed interaction.

KEYWORDS: stomatopod, *Gonodactylellus*, *Raoulserenea*, Moorea, behavior, learning, decision making, eviction

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## Introduction

Shelter is a vital resource for stomatopods. Shelter provides a safe environment in which these marine crustaceans can avoid predators, molt, mate, and brood eggs. Shelter also allows stomatopods to process prey (Caldwell 1979, Reaka 1980). For stomatopod species that dwell in pre-existing hollow cavities in coral rubble, the number of suitable living cavities probably is limited (Reaka 1980). Therefore, the acquisition of shelter becomes increasingly important. Stomatopods compete aggressively over these scarce cavities and are evicted frequently (Reaka 1980, Caldwell 1987). These fights over shelter are dangerous and potentially fatal because these interactions typically involve a stomatopod's powerful and deadly pair of raptorial appendages. Therefore, when stomatopods make decisions about when and how to fight for a given cavity, it is crucial that they know their opponent's fighting ability (Caldwell 1987).

Stomatopods can most directly obtain information about an opponent's fighting ability by engaging the enemy. But this approach involves considerable risk in species with powerful smashing-type raptorial appendages since a single blow from a resident can cause serious injury to intruders that probe into their cavities (Caldwell 1987). Another less costly way to assess an opponent's fighting ability is to determine an opponent's size. Size is an important variable in determining a fighting outcome and is usually correlated with fighting ability (Steger & Caldwell 1983). Caldwell and Dingle (1979) found that a 10% difference in body length between two *Gonodactylus viridis* was sufficient to influence the outcome of an open field contest. Thus, when making decisions about evicting residents, it is advantageous to know an occupying opponent's fighting ability without the dangers of confrontation.

Stomatopods have demonstrated the ability to learn and remember. The coral dwelling stomatopod, *G. falcatus* can recognize characteristics of its artificial burrow within five trials (Reaka 1980). Reaka (1980) observed that *G. falcatus* also can learn the characteristics of its local terrain. Smashers break open snail shells more efficiently over time (Caldwell & Dingle 1976). Spearers avoid cavities containing smasher residents (Caldwell 1988). Additionally, Caldwell (1985) demonstrated that *G. festae* can remember conspecifics that previously defeated them and conspecifics that were previously defeated. Caldwell (1992) also demonstrated that former *G. bredini* mates can recognize one another even after two weeks of separation. *G. bredini* is capable of long-term memory, and former mates can use the information learned earlier to mitigate aggressive interactions between each other. Finally, DeSalvo (2001) demonstrated that *G. childi* can learn to recognize the odor of *Raoulserenea pygmaea*.

In addition to their abilities to learn and remember, stomatopods have the most sophisticated peripheral visual system known in the animal kingdom. Their compound eyes rest on mobile stalks, which move independently of each other. Each eye is stereoscopic. Additionally, stomatopods exhibit polarization sensitivity, have color vision, and can see ultraviolet light. Stomatopods use their complex visual system for many things including scanning and navigating through their environment, communicating between conspecifics and other stomatopod species, tracking and capturing prey, and finding mates (Cronin et al. 1994, Marshall et al. 1996, Marshall et al. 1999, Chiao et al. 2000, Cronin & Marshall 2001).

When and why this unique visual system evolved in stomatopods is unknown. It may have evolved when stomatopods became increasingly predatory and developed their raptorial appendages (Cronin et al. 1994). A combination of the need for information to direct the strike, the need for information about dealing with dangerous competitors, and a relatively small brain capable of limited integrative abilities may explain why this visual system evolved. Speed during visual processing might also be a factor. With regard to these last two points, the eye and its peripheral integrative centers extract and process much of the information needed before it reaches the associative centers of the brain (Caldwell pers. com.).

Because stomatopods can learn and remember, have a remarkable visual system, and compete aggressively over cavities, this study tested whether stomatopods use information obtained by visual observation to make decisions about which residents to attempt to evict. To test for this ability, individuals of *Gonodactylellus childi* were allowed to observe cavities that were empty; occupied by another smasher resident, *G. childi*; or occupied by a spearer resident, *R. pygmaea*. It was hypothesized that if *G. childi* observed occupied and unoccupied cavities, they would first approach and enter cavities that were empty rather than cavities observed to be occupied. Also, we might expect that they would first approach and enter cavities that were occupied by the less aggressive and less dangerous *R. pygmaea* rather than those occupied by *G. childi*.

## Materials and Methods

### *Collection sites*

Stomatopods were collected from two sites in Cook's Bay on Moorea, French Polynesia (17°30' S, 149°50' W) (Figure 1). The majority of stomatopods were found on the back reef near the reef crest and west of the pass into Cook's Bay (17°29.407' S, 149°49.546' W). Other stomatopods were found in front of the Richard B. Gump South Pacific Biological Research Station. Suitable coral rubble, as defined by the presence of circular cavities, the absence of living corals, and a relatively light (low density) body, was collected from the sea floor at both sites. Rubble from the back reef was collected at low tide usually at 0600 hours or 1600 hours, in which sea level was less than half a meter deep. Rubble in front of the Gump Station was collected during daylight hours when sea level ranged from 0.5 - 1.5 meters.

**Figure 1.** Location of the two stomatopod collection sites denoted by large dots.

After rubble was collected, it was brought to shore. Stomatopods were extracted from the rubble by breaking it apart using a rock hammer. Rubble that was not smashed on the day it was collected was placed in continuous water-flow aquaria to be smashed the next day.

### *Maintenance*

Stomatopods were numbered, measured for body length, identified, and sexed under a dissecting microscope. All measurements were taken from the tips of the stomatopod's eyes to the tips of the submedian teeth of the telson. Under natural light regimes, stomatopods were housed in separate plastic containers (d=90 mm; h=94 mm) filled with 0.45 L of seawater. A piece of opaque PVC pipe (d= 20 mm, l = 20-35 mm) was added to each container to function as a cavity. Finally, a plastic lid covered each container to reduce evaporation.

Every three days during daylight hours, stomatopods were fed live food collected from rubble. Stomatopod smashers were given a snapping shrimp or small crab. Stomatopod spearers were given a snapping shrimp or small green fish. Seawater in the plastic containers was changed every two days. Seawater was also changed after a stomatopod fed completely or had molted recently. Recently molted stomatopods were given live snapping shrimp only, and were remeasured a week later.

### *Experimental apparatus*

The test arena consisted of a black plastic, rectangular container (l= 25 cm; w= 15.5 cm; h= 6.5 cm). Three metal 'partition holders' (l= 15 mm; w= 10 mm; h= 65 mm) were fixed on the sides of the arena and held a removable, T-shaped partition. The T-shaped partition was made of two pieces: a longer, clear glass side and a shorter, opaque plastic side. The partition divided the arena into three compartments.

Additionally, a clear, glass jar (l= 48 mm; d= 29 mm) was placed near the wall and at the center of the front compartment (Figure 2). A plastic lid with top cut out was fitted over the glass jar. Finally, a black, dissecting probe stick was inserted into the rim of the lid. The jar functioned to control for directional bias by the animal; upon making a choice, the animal was placed in the jar and released from the center of the arena (see protocol for details). The stick allowed the jar to be moved without the researcher's hand contacting the jar and disturbing the animal. Finally, clear plastic pieces were aligned along the top perimeter of the arena to prevent the animal from escaping during test trials. These plastic pieces stuck 35mm into the arena.

The arena was filled with 1.3 L of seawater. Fresh running seawater flowed continuously into the front corners of the arena and flowed out through holes (d= 0.42 cm) along the walls of the back of the arena. This was done to eliminate any chemicals released by the resident stomatopods, which could bias the experiment. Controlling for odor ensured that changes in the animal's behavior would be primarily due to what the animal had learned and remembered based on visual cues only.

### *Subjects*

A total of 78 stomatopods were used, 66 *G. childi* and 12 *R. pygmaea*. Stomatopod specialist Roy Caldwell of the University of California at Berkeley helped to identify stomatopods. Voucher specimens are held in the Museum of Paleontology at the University of California at Berkeley.

Individual *G. childi* (N=60) were designated as "observer" subjects. Observers were placed in the front compartment and allowed to observe the back compartments through the clear partition. Observer subjects were allowed to

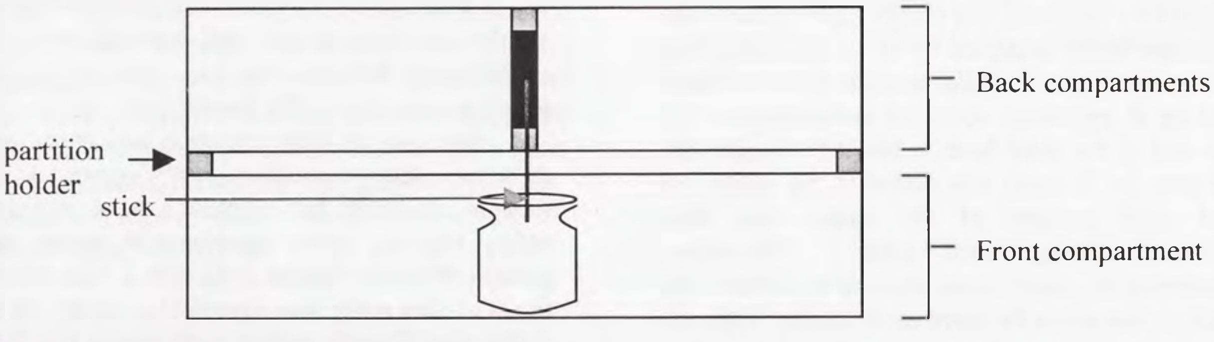

**Figure 2.** Top view of test arena.

observe only once, but were used multiple times as "resident" subjects.

Observers were placed into one of three experimental treatment groups. Group one (N=20) observed two empty cavities. Group two (N=20) observed one empty cavity and one cavity occupied by *G. childi*. Group three (N=20) observed one cavity occupied by *G. childi* and one cavity occupied by *R. pygmaea* (Table 1). Tests for group one were performed to look at the specific effects of observation on overall behavior. Tests for group two were performed to look at any possible avoidance of confrontation that the observer employs as a result of its observations. All observers were matched for sex and size (less than or equal to a 1 mm body length difference) between other *G. childi* and *R. pygmaea*, which were designated as "resident" subjects.

| Test group | Cavity 1 | Cavity 2 | Number of trials |
|---|---|---|---|
| Group 1 | Empty | Empty | 20 |
| Group 2 | Empty | *G. childi* | 20 |
| Group 3 | *G. childi* | *R. pygmaea* | 20 |

**Table 1**. Summary of the two types of cavities presented to each test group.

### *Experimental Protocol*

At the start and end of a test trial, the test arena, partitions, glass jar, jar lid, and stick were rinsed thoroughly first with freshwater followed by seawater. Depending on the treatment group, resident stomatopods were taken out of their home containers and placed into the back compartments. Then, the observer stomatopod was taken from its home container and placed into the front compartment.

For three hours, the observer was allowed to see the back of the arena. More specifically, group one observed two empty compartments, group two observed one empty compartment and a compartment occupied by *G. childi*, and group three observed a *G. childi* occupied compartment and an *R. pygmaea* occupied compartment. At the end of the third hour, a black PVC pipe (d= 20 mm; l= 26 mm) was added to the upper left and right corners of the arena with their entrances facing each other. Thereafter, observing *G. childi* were allowed to observe the back of the arena for three more hours. This six-hour observation phase took place at various times of the day for each animal, from 0600 hours to 1600 hours.

At the end of the observation phase, the observer was placed into the clear glass jar. The glass jar was then lifted up from its side and placed onto its bottom to prevent the observer from swimming out. Resident stomatopods, if any, were taken out of the back compartments. Then, the black PVC pipes were replaced with two new black PVC pipes. Next, the T-shaped partition was removed. Finally, opaque 'partition fillers' (l= 10 mm; w= 5 mm; h= 70mm) were inserted in the gaps of the metal partition holders to prevent observers from entering these potential artificial cavities.

After the last partition filler was inserted, the observer stayed in the jar for five minutes, where it eventually ceased swimming erratically. At the end of five minutes, the jar was tipped gently onto its side using the dissecting probe stick. Behavioral observations by the researcher began when the observing stomatopod exited the jar. Observations were made through a blind composed of cardboard. Recording the behavioral observations lasted for up to one hour or until the stomatopod entered a PVC pipe. When the stomatopod entered a PVC pipe, its behavior was observed further for 10 minutes more.

Observations that were recorded included: 1. first cavity the stomatopod approached; 2. first cavity it entered; 3. time taken to first approach a cavity; 4. time taken to first enter a cavity; 5. how it approached the cavity it first approached; and 6. how it entered the cavity it first entered.

## Results

The total number of stomatopods that first approached a particular cavity was 11 and 9, 11 and 9, and 10 and 10 for groups one, two and three, respectively (Table 2). The total number of stomatopods that first entered a particular cavity was 11 and 9, 12 and 8, and 9 and 11, for groups one, two and three, respectively (Table 3). A Pearson's Chi-square test showed that the number of first approach and entry did not differ significantly between the two types of cavities for all groups ($p > 0.05$; Tables 2-3).

The times of first approach and entry were examined between cavities within groups: 1. The median times of first approach to a particular cavity did not differ significantly within each group ($p > 0.05$; Figures 3-5), and 2. The median times of first entry into a particular cavity did not differ significantly within each group ($p > 0.05$; Figures 6-8).

| Test Group | Number of stomatopods that first approached Cavity 1 | Number of stomatopods that first approached Cavity 2 | $X^2$ value | p-value |
|---|---|---|---|---|
| **Group 1** | 11 | 9 | 0.2 | 0.655 |
| **Group 2** | 11 | 9 | 0.2 | 0.655 |
| **Group 3** | 10 | 10 | 0.0 | 1.000 |

**Table 2.** The total number of stomatopods that first approached each cavity. For all comparisons, degrees of freedom = 1. Alpha level = 0.05. See Table 1 for the types of cavities used in each group.

| Test Group | Number of stomatopods that first entered Cavity 1 | Number of stomatopods that first entered Cavity 2 | $X^2$ value | p-value |
|---|---|---|---|---|
| **Group 1** | 11 | 9 | 0.2 | 0.655 |
| **Group 2** | 12 | 8 | 0.8 | 0.371 |
| **Group 3** | 9 | 11 | 0.2 | 0.655 |

**Table 3.** The total number of stomatopods that first entered each cavity. For all comparisons, degrees of freedom = 1. Alpha level = 0.05. See Table 1 for the types of cavities used in each group.

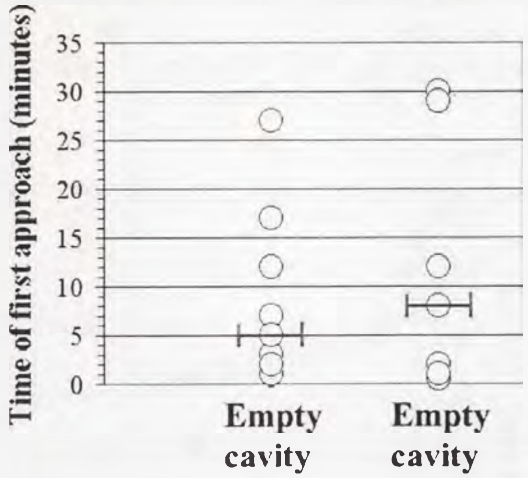

**Figure 3.** Group One's time data for individual times to first approach the empty cavity located in the left corner of the arena and the empty cavity located in the right corner of the arena. In the x-axis, the first empty cavity label represents the empty cavity located in the left corner of the arena. The second empty cavity label represents the empty cavity located in the right corner of the arena. Each point on the graph represents a trial. The "H-bar" indicates the median time of first approach to a particular cavity. 5 minutes was the median time for individuals to first approach the left empty cavity. 8 minutes was the median time of first approach to the right empty cavity. Mann-Whitney U value= 44.5. P-value= 0.703. Alpha level = 0.05.

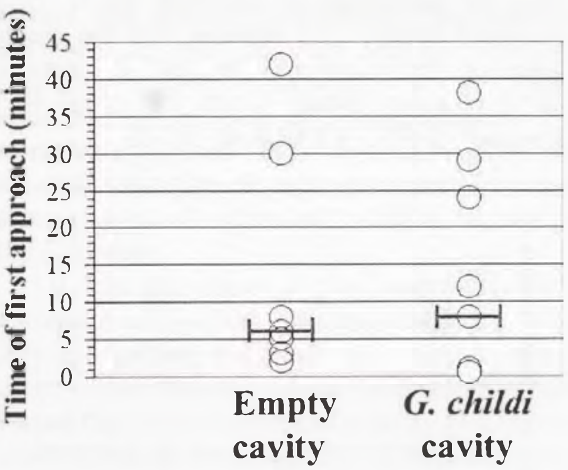

**Figure 4.** Group Two's time data for individual times to first approach the empty cavity and the *G. childi* occupied cavity. Each point on the graph represents a trial. The "H-bar" indicates the median time of first approach to a particular cavity. 6 minutes was the median time for individuals to first approach the empty cavity. 8 minutes was the median time of first approach to the *G. childi* occupied cavity. Mann-Whitney U value= 57.5. P-value= 0.542. Alpha level= 0.05.

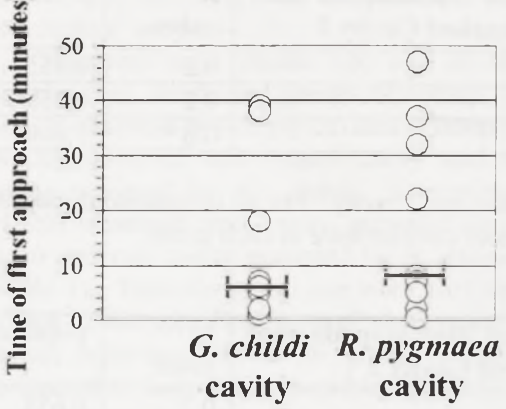

**Figure 5**. Group Three's time data for individual times to first approach the *G. childi* occupied cavity and the *R. pygmaea* cavity. Each point on the graph represents a trial. The "H-bar" indicates the median time of first approach to a particular cavity. 5.5 minutes was the median time for individuals to approach the *G. childi* occupied cavity. 7.5 minutes was the median time of first approach to the *R. pygmaea* occupied cavity. Mann-Whitney U value= 44.5. P-value= 0.677. Alpha level= 0.05.

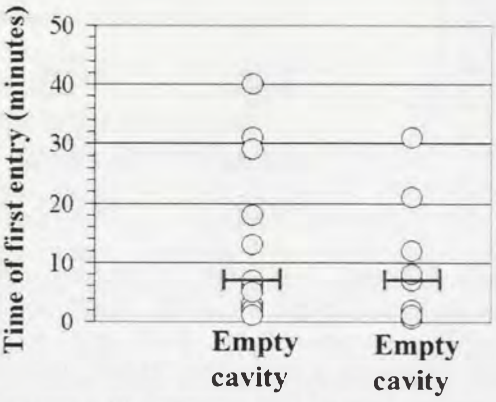

**Figure 6.** Group One's time data for individual times to first enter the empty cavity located in the left corner of the arena and the empty cavity located in the right corner of the arena. In the x-axis, the first empty cavity label represents the left empty cavity. The second empty cavity label represents the right empty cavity. Each point on the graph represents a trial. The "H-bar" indicates the median time of first entry into a particular cavity. 7 minutes was the median time for individuals to first enter the left empty cavity. 7 minutes was the median time of first entry into the right empty cavity. Mann-Whitney U value= 61.5. P-value= 0.361. Alpha level= 0.05.

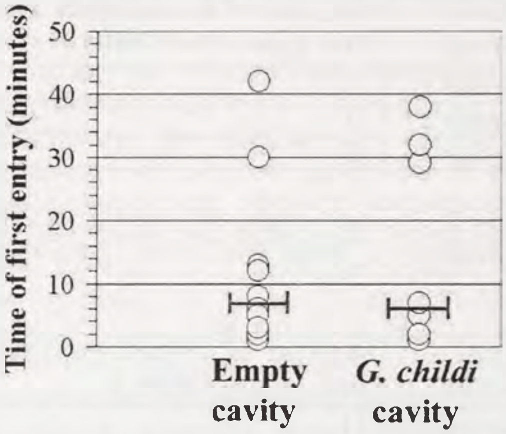

**Figure 7**. Group Two's time data for individual times to first enter the empty cavity and the *G. childi* occupied cavity. Each point on the graph represents a trial. The "H-bar" indicates the median time of first entry into a particular cavity. 7 minutes was the median time for individuals to first enter the empty cavity. 6 minutes was the median time of first entry into the *G. childi* occupied cavity. Mann-Whitney U value= 51.5. P-value= 0.786. Alpha level= 0.05.

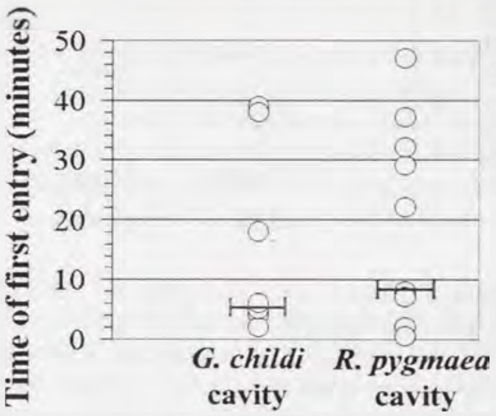

**Figure 8**. Group Three's time data for individual times to first enter the *G. childi* occupied cavity and the *R. pygmaea* occupied cavity. Each point on the graph represents a trial. The "H-bar" indicates the median time of first entry into a particular cavity. 5 minutes was the median time for individuals to first enter the *G. childi* occupied cavity. 8 minutes was the median time of first entry into the *R. pygmaea* occupied cavity. Mann-Whitney U value= 42.5. P-value= 0.594. Alpha level= 0.05.

The times of first approach and entry were examined between groups: 1. The differences in ranks of the times of first approach to either cavity did not differ significantly between the three groups (Kruskal-Wallis= 0.446, df= 2, p= 0.800), and 2. The differences in ranks of the times of first entry into either cavity did not differ significantly between the three groups (Kruskal-Wallis= 0.264, df= 2, p= 0.876). It was also shown with a Kolmogorov-Smirnov test that the distribution of times of first approach to either cavity did not differ significantly between groups (Figure 9). Group 1 and 2 had a p-value of 1.000. Group 2 and 3 had a p-value of 0.954. Group 1 and 3 had a p-value of 0.484. Also, the distribution of times of first entry into either cavity did not differ significantly between groups (Figure 10). Group 1 and 2 had a p-value of 1.000. Group 2 and 3 had a p-value of 1.000. Group 1 and 3 had a p-value of 0.749.

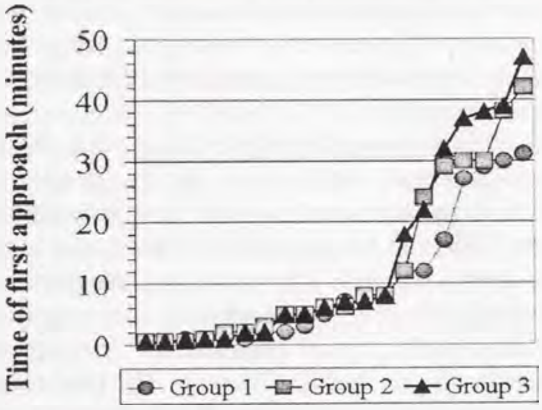

**Figure 9.** Distribution of times of first approach for each group. Each point on the graph represents a trial.

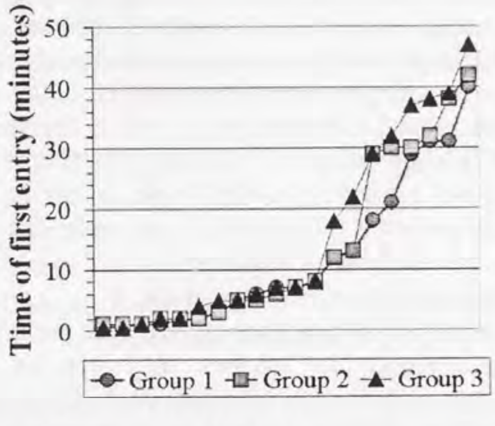

**Figure 10.** Distribution of times of first entry for each group. Each point on the graph represents a trial.

Behavioral data about how stomatopods approached and entered were observed as well. However, such data was not quantified because of time constraints to complete the project and initial unfamiliarity with the types of behaviors that stomatopods would exhibit. Behavioral differences in first approach and entry into a cavity could not be determined within and between each group because lots of variation existed. For example, some individuals from all the groups approached either cavity cautiously (i.e. while stomatopods approached a cavity, they paused at least once before reaching it). Some individuals completely passed the entrance of an occupied cavity, but approached cautiously toward an empty cavity. When individuals were beside the cavity entrance, it appeared that individuals first passed a cavity entrance as much as it would enter it.

It was observed that when a particular cavity was first approached, this same cavity was not always first entered. Additionally, stomatopods did not necessarily approach and enter cavities that they initially headed and moved toward upon exiting the glass jar. For example, if the stomatopods exited the jar and moved towards the right side of the front compartment, it did not always approach and enter the cavity located on the right side.

It was also observed that stomatopods did not consistently show the same behavior when they approached and entered a cavity a second time. For instance, some stomatopods first passed the cavity entrance of a cavity, which was observed as an empty cavity. Later, this same cavity was approached cautiously (paused repeatedly during its approach) until finally the stomatopod entered the cavity head on.

All observers approached and entered a cavity within one hour. Occasionally, some stomatopods waited for long periods of time (i.e. 35 minutes) before it would approach and enter either cavity. However, once the stomatopod approached a cavity, most behaved actively thereafter. Stomatopods approached and reentered both cavities during a trial and moved about all sides of the arena. Additionally, after the stomatopods visited each cavity, they were more than likely to stick to a wall corner or rest beside the glass jar rather than to remain sheltered in the artificial cavities. Rarely were stomatopods observed to stay in a cavity that it occupied.

**Discussion**

Results regarding the number of first approach and entry between the two cavities are discussed for each group. For group one, individuals randomly chose either the two empty cavities. Additionally, time spent to first approach and enter a particular cavity was not significantly different. More importantly, these results show that individuals had no bias for approaching and entering a cavity because it was on a particular side. The non-significantly different times show that stomatopods behaved similarly toward each cavity.

For group two, results show that when an empty cavity is available, stomatopods continue to first approach and enter a cavity that they observed to be occupied. Additionally, time spent to first approach and enter a particular cavity was not significantly different. Stomatopods were not more cautious or hesitant at first approaching and entering an occupied cavity as compared to an empty cavity. It is unclear why *G. childi* don't avoid occupied cavities. Such a decision to occupy an occupied cavity places the intruder at a greater risk to injury as compared to an empty cavity where he can avoid confrontation. However, such a decision is not necessarily non-adaptive and becomes especially important if stomatopods are looking for a mate.

Perhaps *G. childi* have no preference as to which type of cavity to occupy. The costs of a possible confrontation may not be too great. Another explanation is that *G. childi* do not use the information obtained by observing to make decisions about which type of cavity to occupy. *G. childi* may not have learned or remembered which cavity contained the other *G. childi* resident. Additionally, the observing stomatopod may not have learned to associate the cavity with the observed stomatopod as its resident; therefore, the observer treated this occupied cavity as if it was always empty.

For group three, observing stomatopods observed two occupied cavities. We might expect observers to confront a resident if it chose to evict a resident from its cavity. Of the two occupied cavities, it was predicted that observing *G. childi* would first approach and enter *R. pygmaea* occupied cavities more frequently than *G. childi* occupied cavities because the raptorial strike of a spearer inflicts less damage than a smasher's strike (Caldwell & Dingle 1976, Caldwell 1988). However, the type of cavity to first approach and enter was random. Additionally, time spent to first approach and enter a particular cavity was not significantly different.

One explanation is that *G. childi* have no preference as to which resident to evict. Observers may not have encountered an *R. pygmaea* in the field, and therefore was not able to assess the fighting ability of the *R. pygmaea* as compared to another *G. childi.* Although Caldwell and Manning (2000) observed both species co-occurring in the same areas and I found one instance in which *R. pygmaea* was with *G. childi* in the same corral rubble, field studies on population density and distribution for each species are necessary to conclude whether these species interact and compete with each other frequently.

Another explanation is that *G. childi* may not have learned or remembered which particular cavity contained the other *G. childi* or *R. pygmaea* resident. The observing stomatopod may not have learned to associate the cavity with the observed stomatopod as the resident; therefore he treated each cavity as if it were occupied by another stomatopod rather than being occupied by a stomatopod of the same or different species.

Results regarding the median time to first approach and enter either cavity between all three groups showed a trend that stomatopods who observed two occupied cavities take longer to first approach and enter as compared to stomatopods who observed two empty cavities. However, the times between all three groups were not significantly different. The time results for group two observers who observed one empty versus one occupied cavity are also supported by the time results between group one (who observed two empty cavities) and group three (who observed two occupied cavities). Thus, with respect to median times, group three approached and entered occupied cavities as slowly or as quickly as empty cavities. Results further support the conclusion that *G. childi* do not use visual observations to make decisions about evicting residents. This suggests that 1. *G. childi* do not prefer which type of cavity to occupy and/or 2. *G. childi* do not remember or learn the differences between the two types of cavities based on visual cues alone.

The use of visual cues to detect the presence of stomatopods in cavities may not be as important as the use of olfactory cues. Chemical recognition abilities evolved in stomatopods to avoid aggressive interactions between other stomatopods (Caldwell 1979). As with most stomatopods, *G. childi* relies heavily on chemical cues to identify individuals. After DeSalvo (2001) found that *G. childi* can recognize the odor of *R. pygmaea*, he demonstrated that *G. childi* can learn to recognize the odor of *R. pygmaea*. Experienced *G. childi* who previously interacted with *R. pygmaea* approached cavities containing the odor of *R. pygmaea* faster than naïve *G. childi* who did not previously interact with *R. pygmaea*.

Stomatopods are retiring animals and residents aren't usually visible to intruders (Caldwell 1987). In the context of the ecology of stomatopods, chemical cues would be most useful in detecting the presence of another stomatopod in a cavity. Preliminary experiments showed that *G. childi* intruders were able to quickly escape or coil away from a resident before the resident inflicted a blow. Probing into cavities does not seem too costly for *G. childi*, and learning to assess opponents from afar (i.e. using visual cues) may not be necessary. In fact, monitoring cavities may be too costly for stomatopods because it leaves observers exposed to predators.

The ability to learn or remember previously occupied cavities based on visual cues alone has also been tested in other stomatopods. Reaka (1980) found that after *G. falcatus* entered an artificial cavity that contained another *G. falcatus*, there was no significant difference between the time to enter a different but similar artificial cavity before and after the individual's "unpleasant" encounter. Because Reaka (1980) controlled for odor, she found that *G. falcatus* does not learn to avoid previously occupied cavities based on visual cues.

Although *G. childi* may have remarkable eyesight, they do not appear to learn aspects of cavities based on visual cues alone. Future studies might test whether other stomatopod species can use visual observations to make decisions about evicting residents. Stomatopods have the visual system to perform a visual learning paradigm, but do any exploit the full capabilities offered by their complex visual systems in making decisions?

Stomatopods make subtle distinctions based on size thus demonstrating that they have the cognitive abilities to process and use visual information to adjust their behavior in agonistic encounters. There are no other known invertebrate examples of observers adjusting their agonistic behavior based on observed interaction (Caldwell pers. com.). A more complete investigation of what limits and motivates *G. childi* and other stomatopods to make decisions about resident eviction should be explored further. The use of other cavity types might be included in the investigation of decision making behavior in cavity eviction. Possibilities include cavities previously occupied by other cavity competitors such as octopuses or cavities previously occupied by residents that successfully defended their cavities from intruders.

## Acknowledgements

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# Cleaning Behavior of Xanthid Crabs on the Scleractinian Corals *Pocillopora eydouxi* and *P. meandrina*

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ABSTRACT. A symbiotic relationship exists between crabs of the family *Xanthidae* and pocilloporid corals. On the island of Moorea, French Polynesia, changes in land use have led to increasing sedimentation in Pao Pao Bay. The mutualistic relationship between these crabs and their pocilloporid hosts elicits consideration for the role of crabs in maintaining coral vitality in areas of high sedimentation. The purpose of this study is to determine the function of *Trapezia* spp. crabs in keeping two species of *Pocillopora* (*Pocillopora eydouxi* and *P. meandrina*) clean and healthy. Fieldwork was conducted on the back reef of Moorea between the island's two major bays. Fifty corals were sampled to determine coral volume and crab species present. Twenty separate corals were removed from the back reef and placed in a controlled environment for testing. Sediment was added to each coral for 48 hours. Crabs were then removed and the experiment repeated. Difference in sediment cleared between corals with crabs and those without was highly significant ($p = .003$). Number of crabs in corals did not predict percent sediment removed ($R^2 = .012$). The mechanism of sediment removal remains yet to be determined. Three plausible explanations are: 1. Crabs play and active role in clearing sediment by physically removing sand grains. 2. Crabs play a passive role in clearing sediment as they move around and feed 3. Crabs consume sediment when feeding on coral mucus. Other factors must be taken into consideration including coral morphology, mucus shedding, and water movement. The symbiosis between *Pocillopora* and *Trapezia* spp. crabs may give it an advantage over other coral genera in areas of increasing sedimentation.

KEYWORDS: Coral, *Pocillopora*, *Trapezia*, symbiosis, mutualism

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## Introduction

Symbiosis is a phenomenon found often in the natural world. In its broadest sense it is a relationship where at least one of the species benefits from the other. It is often a brief occurrence but can exist between two species living in close association for long periods of time (Kimball 2002). Obligate symbiosis occurs when this association is necessary for the survival of at least one species. There are several types of symbioses but this paper focuses on the process of mutualism. In this symbiosis, both of the paired species benefit in some way from each other.

Branching corals of the genus *Pocillopora* are common in the South Pacific. They are a type of scleractinian coral defined by a hard, stony skeleton and secretion of calcium carbonate. These reef corals harbor a high diversity of macro-invertebrate communities (Patton 1974; 1976; Abele & Patton 1976; Coles 1980; Edwards & Emberton 1980; Black & Prince 1983).

Several obligate symbionts have been identified in previous studies including xanthid crabs (*Trapezia* and *Tetralia* species), a snapping shrimp (*Alpheus lottini*) and gobioid fishes of *Paragobiodon* spp. (Knudsen 1967; Patton 1974; Lassig 1977). On two different reefs in the eastern Pacific, Abele (1976) found that within the coral *Pocillopora damicornis*, 76 and 89 percent of the total fauna were decapod species and that 80 and 96 percent were decapod individuals. Xanthid crabs comprise a large proportion of the decapod crustaceans that live among the corals.

*Trapezia* is a genus in the family *Xanthidae*. They are brightly colored obligate associates of live pocilloporid corals. A mutualistic relationship between these crabs and their coral hosts has been documented in many studies. Crabs feed on mucus secreted from the coral and are sheltered from predation inside (Knudsen 1967; Patton 1974). *Trapezia* actively defend their host coral from attacks by the crown-of-thorns starfish, *Acanthaster Placi* (Pratchett 2001).

Species of *Trapezia* have pelagic larvae that must locate and settle on appropriate species of coral host (Knudsen 1967). Juveniles are first recognizable on their coral hosts at about 2mm carapace width. At this time they are usually found at or near the base of the host, living in tunnels or crevices. As sexual maturity approaches, the space available in these crevices becomes insufficient, and progressively more time is spent on the branches. At roughly 9-11 mm carapace width, heterosexual pairs are formed. Typically only one adult pair per species is found per host. This pair actively excludes all other adults of the same species. Interspecific agonistic encounters are apparently less severe since several host species of *Trapezia* may be found on a single large host (Preston 1973).

Sedimentation and eutrophication are thought to be the major cause of coral reef degradation worldwide (Ginsburg, 1993). The death of a coral host in turn displaces its associated symbionts. The highly mutualistic relationship between pocilloporid corals and *Trapezia* crabs elicits consideration for the role of crabs in maintaining coral vitality in areas of high sedimentation.

The island of Moorea in the Society Islands of French Polynesia has undergone changes in land use within the last several years. Conversion of natural forest vegetation to agriculture has increased soil erosion and subsequent transport of sediment to the ocean. This study takes place on the edges of Pao Pao Bay, an area of increasing sedimentation. The purpose of this research is to determine what role *Trapezia* spp. crabs play in keeping two species of pocilloporid corals (*Pocillopora Eydouxi* and *P. Meandrina*) clean and healthy.

The first part of this study looked at the natural history of *Trapezia* crabs and *Pocillopora* on the island of Moorea. Species types and abundance among coral was determined and compared to previous studies in other parts of the Pacific. These observations set up the basis for the experimental study by supporting the relationship between crabs and corals on the Moorean reefs.

The second part of this study tests the hypothesis that less sediment will remain on pocilloporid corals that contain crabs versus those without crabs after 48 hours of exposure. The study also aims to determine if *Trapezia* spp. exhibit differences in cleaning abilities and if corals hosting a greater number of individuals are cleaned more rapidly.

## Materials and Methods

### *Study Site*

All fieldwork and experimental analysis were conducted on the island of Moorea (17°30'S; 149°50'W) in the Society Islands of French Polynesia. Moorea is of volcanic origin with fringing and barrier reefs surrounding the island. All observations and coral samples were taken from the back reef between Pao Pao and Opunohu Bays (Figure 1). Water depth at the study sites varied between 1.5 and 6 meters.

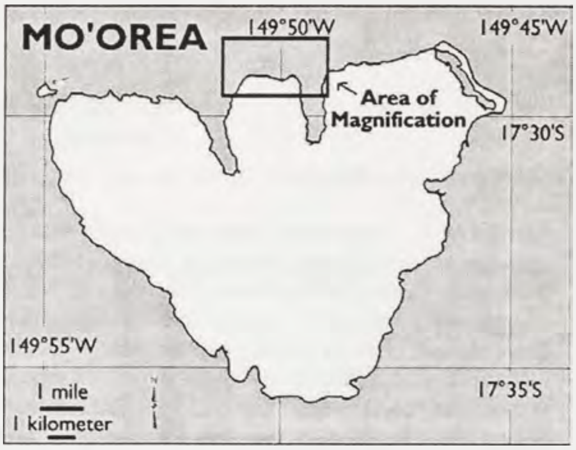


**Figure 1**. Map of Moorea with box around the area of study, the back reef between Pao Pao and Oponuhu Bays.

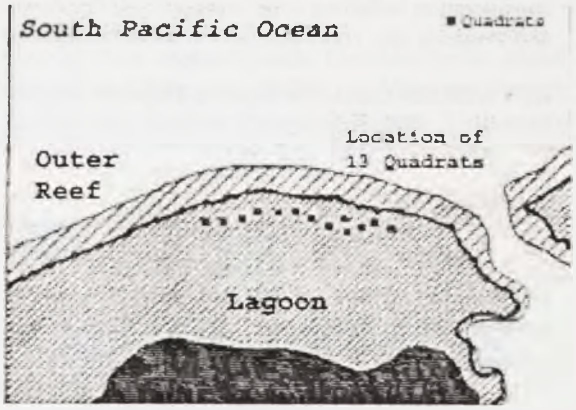


**Figure 2.** Area of magnification- Map of study site showing location of 13 quadrats along the reef crest.

Transect tape was used to measure out 5 meter x 5 meter quadrats. All quadrats were placed 10 to 50 meters inland from the reef crest in a parallel band that runs along the entire back reef (Figure 2). Corals were collected from the western portion of the back reef at depths of 1-4 meters and stored in a seawater tank at the Gump Biological Research station.

### *1. Natural history of Trapezia and Pocillopora*

A total of thirteen quadrats were laid out on the back reef during the months of October and November in the year 2002. An initial site in the middle of the reef was chosen (149°50'W, 17°28'S) for the first sample to be taken. Each sample after this was conducted within 200 meters in either direction. Quadrats were placed on areas of high *Pocillopora* density. Corals of the species *P. eydouxi* and *P. meandrina* were then examined for presence of large crabs, *Trapezia wardi* and *Trapezia flavomaculata* and small crabs, *Trapezia digitalis, Trapezia intermedia* and *Trapezia ferruginea*. Coral volume was estimated by multiplying the width, length and height of each coral. Unusual morphological features of corals as well as algal cover were also recorded.

All crabs collected from the corals were identified to species level. Photographs were taken of each species and compared to photos taken in previous studies (Preston 1973; Serene 1969). Male and female individuals were distinguished by looking at the obvious morphological differences on the underside of the crabs. (See appendix)

*2. Cleaning behavior of Trapezius crabs*

Twenty four corals of the species *P. eydouxi* and *P. meandrina* were collected from the back reef. These species were chosen for their similar morphological features. Other species of pocilloporid corals exhibit significant morphological differences which inhibit recruitment of large *Trapezia* spp. and may react differently to the effects of sedimentation. In addition, *P. eydouxi and P. meandrina* have relatively wide crevices which allow for easier identification and removal of crabs. An initial scan of the coral was made as soon as they entered the flow though tank. Coral volume, species types and numbers were recorded as well as presence of other organisms. Shrimp, fish and hermit crabs were then removed from the corals. Crabs measuring less than about 5 mm were not counted due to difficulty in detection and their ability to hide in very small crevices.

*Phase 1-Experimental:*

Sediment was collected from the patch reef off the Gump station and sifted with a 2mm sieve in order to remove rocks and other debris. 500 ml of sediment was placed in a 1000 ml graduated cylinder. Each coral was placed in a large bucket settled at the bottom of the tank. Sediment was poured onto the corals to cover all surfaces. The corals were then lifted out of the bucket and placed back into their respective positions in the tank. Sediment remaining within the bucket was recorded and subtracted from 500 ml to determine the amount of sediment on the coral. Cages were placed over corals with large *Trapezius* spp. in order to prevent coral switching.

Trials were run for 48 hours. After this period, observations were made on the placement and patterns of sediment displaced from the corals. The corals were then taken from the tank and placed in a dry bucket. A seawater hose was used to wash all sediment from the corals. The volume of this sediment was recorded and used to calculate the amount of sediment cleared in that 48 hour period.

*Phase 2-Controls:*

The experimental corals were examined for tissue death in order to determine if they were fit to run in a control test. Four corals had significant tissue death after the first phase of sedimentation. These corals were taken back to the reef and no control tests were run on them. A failure in the water flow for 2 days is believed to have caused this death. These were the only corals in the tank when the water flow failed. Of the twenty remaining corals only slight or no damage occurred and thus were deemed fit to be tested in the control experiment. All crabs larger than 5 mm were cleared from the corals using wooden skewers (Nearly all crabs were successfully removed alive and taken back to the reef). Sediment was placed and removed from the corals in the same fashion as the experimental tests.

In both the experimental and control tests, percent sediment cleared was recorded for each coral. A paired t-test was run to determine if a difference in sedimentation removal between the corals with crabs versus those without was occurring. A regression analysis was run to test for correlation between number of crabs in a coral host and percent sediment removed.

A second t-test was run to look for a difference in sediment removal between corals with large and small crabs versus corals occupied by only small crabs

## Results

*1. Natural History of Trapezia and Pocillopora.*

A total of fifty corals were examined between the thirteen quadrats on the back reef. The majority of corals examined contained at least one *Trapezia* species.

Of the corals sampled, 60 percent contained large crabs and 82 percent contained any sized species of crabs (Table 1). Coral volume ranged from 3000 cm$^3$ to 700,000 cm$^3$ with a mean volume of 137,000 cm$^3$. Average volume for corals with large *Trapezius* spp. (*T. wardi* and *T. flavomaculata*) was 192,000 cm$^3$

versus the average volume for corals containing only small species (*T. digitalis*, *T. ferruginea* and *T. intermedia*) of 50,000 cm³.

| Site | Percentage of Hosts Occupied |
|---|---|
| 1 | 60 |
| 2 | 100 |
| 3 | 100 |
| 4 | 100 |
| 5 | 75 |
| 6 | 80 |
| 7 | 100 |
| 8 | 100 |
| 9 | 66.6 |
| 10 | 33.3 |
| 11 | 60 |
| 12 | 100 |
| 13 | 100 |
| **Mean** | **82** |

**Table 1.** Fifty corals of the species *P. eydouxi* and *P. meandrina* were sampled for presence of crabs. 82 percent were occupied by some species of *Trapezia*.

*2. Cleaning Behavior of Trapezia*

Mean sedimentation removal for the control group was 57 percent versus 69 percent for the experimental group. The difference in sediment removal between the corals with *Trapezia* spp. versus those with out was highly significant (df = 19, p = .003 T-test, paired two sample for means). Sixteen out of twenty paired treatments showed higher sedimentation removal for the experimental group than for the control group (Figure 3).

A regression (Figure 4) of percent sediment cleared from corals on the number of Trapezia individuals per coral was not significant ($R^2 = .012$, $F = 0.210$, df. = 19, $p = .65$).

Sediment cleared in corals hosting large *Trapezia spp.* was not significantly higher or lower than corals containing only small species (df = 19 p = .17 T-test, two sample).

The placement of sediment beneath treated corals was different for control and experimental treatments. In the control group most of the sediment was scattered beneath the coral in no obvious pattern. In the experimental group sediment was both scattered and grouped into pyramid shaped mounds.

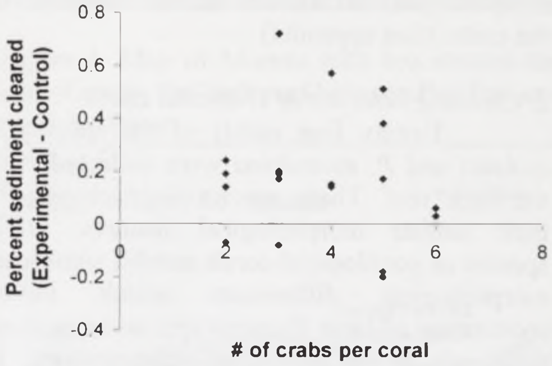
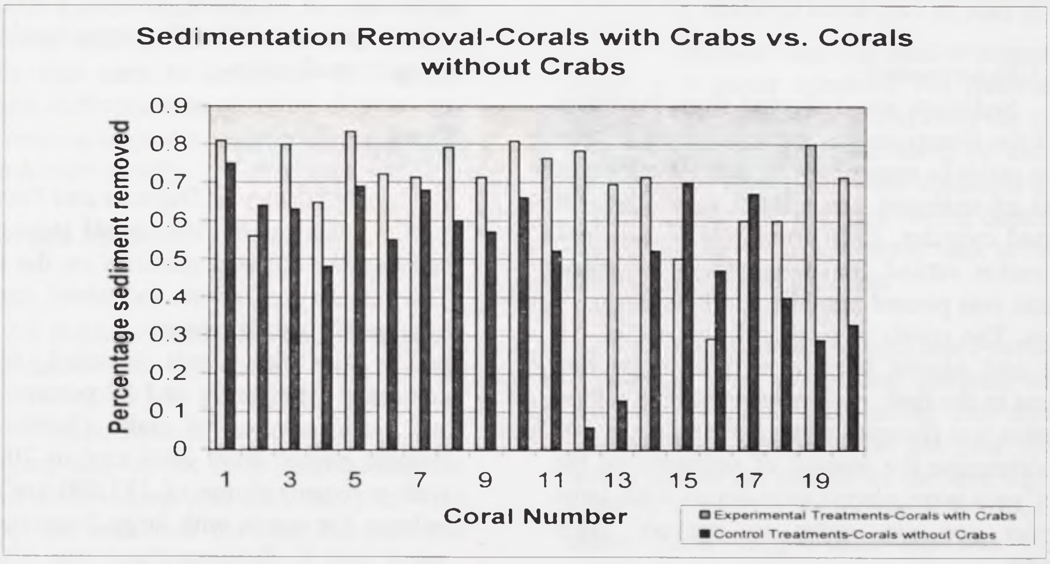


**Figure 4.** Regression of percent sediment removed from corals on the number of Trapezia individuals in corresponding corals was not significant (see text)

**Figure 3.** Comparison of percent sediment cleared between experimental and control treatments after 48 hours of exposure to sediment.



## Discussion

### *1. Natural History of Trapezia and Pocillopora*

Pocilloporid corals of Moorea are hosts to a number of xanthid crabs. A large majority of corals sampled contained crabs. Those that didn't were either very small, more than half dead or had such a large inter-branch space that it is likely the corals did not provide significant protection from predators. Preston (1973) sampled 11 sites with a total of 351 corals in Hawaii. 78 percent of corals sampled were occupied by at least one species of *Trapezia.* This is comparable to the slightly higher finding of this study at 82 percent.

Coral volume is an important factor in determining *Trapezia* association. Large crabs of the species *T. wardi* and *T. flavomaculata* were found on corals with larger volumes. This undoubtedly has to do with their inability to fit easily into the crevices of smaller corals. These species are not commonly found on smaller pocilloporid species such as *Pocillopora damicornis.* Both *T. wardi* and *T. flavomaculata* were not present among species found by Abele et al. (1985) on *P. damicornis.*

### *2. Cleaning behavior of Trapezia*

This study supported a relatively broad statement. That pocilloporid corals of the species *P. meandrina* and *P. eydouxi* containing *Trapezius* crabs have less sediment on them after 48 hours than those without. While it is reasonable to assume that *Trapezia* do promote coral cleanliness and vitality it remains yet to be determined the exact mechanism by which this occurs. There are three explanations that merit further consideration.

1. *Trapezia* spp. actively remove sediment from the coral: The organized mounds of sediment beneath the corals lend support to this argument. The crabs have the ability to remove sediment with their chelae. Coral polyps overwhelmed by sediment die and thus reduce overall mucus secretion by the coral. In time, enough sediment will kill corals and displace symbionts. By keeping the coral clean, both the food supply and the crab's home are preserved. Based on the mutualistic behavior documented in many other studies it is quite plausible that crabs play an active role in coral maintenance.
2. *Trapezia* spp. play a passive role by displacing sediment from the coral as they move around and feed: The crabs move throughout the branches frequently in order to feed and interact with other *Trapezia* individuals. It is very likely that some sediment, especially at the edges, is being displaced by this action. Knudsen (1967) described the feeding behavior of *Trapezia.* The crabs place their walking legs into and around the polyps and gather mucus by brushing dactyli back and forth on the coral surface. This brushing action may displace sediment.
3. *Trapezia* spp. consume sediment as they feed on coral mucus: Preston (1973) examined the stomach contents of *Trapezia* spp. and found mucus material along with sponge spicules and sand grains. A further study could examine the stomach contents of crabs before and after sediment addition to the coral in order to quantify the amount of sediment removed in this fashion

All of these hypotheses are easily testable in a controlled environment and provide a basis for future research. Twenty four hour observations with the use of surveillance technology would be needed in order to observe actual cleaning or passive removal of sediment. It is likely that all these explanations contribute to sediment clearing by crabs. Further tests are needed in order to determine the degree in which each part plays.

Although the experimental treatments had on average 12 percent more sediment cleared than the controls, a large amount of sediment was still cleared when crabs were not present. This leads to the conclusion that other forces were responsible for sediment clearing. Coral polyps have the ability to shed sediment through the use of mucus secretion (McClanahan and Obura 1977). Water movement throughout the tanks may have removed some of the looser sediment on the tips and edges of the coral. These two processes in conjunction with each other are responsible for the majority of sediment removal. In the open ocean, current and wave action contribute even more to the removal of sediment. In areas of low sedimentation and/or high wave action/strong currents, *Trapezia* probably have a small role in sedimentation removal. It is in areas of high sedimentation and low current/wave action where the role of these crabs becomes more important.

There was no significant difference in sediment cleared between corals with both large/small crabs and corals with only small crabs. It can be inferred then that smaller corals, (both small heads of *P. eydouxi* and *P. meandrina* as well as smaller species ie. *P. damicornis*), which cannot accommodate larger

*Trapezia* spp. have no disadvantage in areas of high sedimentation. A more detailed test (ANOVA) may be run in the future to test for differences in sediment removal between all species, not just large and small crabs.

Sedimentation removal is not affected by the number of occupants a host coral has. Inter and intra species interactions could be a factor. A single pair of one species may clean more efficiently than several crabs of different species. Preston (1973) found that crabs of different species showed antagonistic behavior towards one another. Interference competition usually results in some degree of spatial organization among the competing individuals (Miller 1967). These interactions among *Trapezia* spp. may reduce the efficiency of sediment removal.

Coral morphology must be considered when examining sediment clearing. Only two species of *Pocillopora* were used in order to eliminate confounding due to morphological differences. The conclusions of this study can only be applied to *Trapezia* within *P. eydouxi* and *P. meandrina.* It is possible that the relatively large inter-branch space found in these two species of coral is conducive to the elimination of sediment. A study examining the effects of xanthid crabs on multiple species of *Pocillopora* is needed in order to determine the importance of morphology on sediment clearing.

It must also be noted that corals collected were limited by size. The largest coral collected for experimental trials was 30,350 cm$^3$. This is quite a bit less than the average coral volume of species sampled in the natural environment (136,000 cm$^3$). Machinery would be required to remove and lift these larger corals out of the ocean. By choosing small corals the study may have been biased toward sedimentation removal. A very large coral may retain sediment for longer periods of time based on the higher number of crevices available for sediment to settle.

## Conclusion

This study opens up an opportunity for numerous areas of future research. The findings of this paper suggest that *Trapezia* do play some role in removing sediment from corals. The mechanism of that role and the degree of impact it will have on the coral reef ecosystem remains to be tested. Further studies might test the fitness of several genera of corals when exposed to sediment, some that are hosts to crabs and some that are not. This could provide important information on the possible advantages that *Pocillopora* has over other genera in areas of high sedimentation. As sediment input to Pao Pao Bay increases, the community structure of the coral reef may begin to change based on tolerance levels to sedimentation. The symbiosis between *Trapezia* and *Pocillopora* will likely play an important role in determining future coral composition of areas affected by increasing sedimentation.

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**Appendix**

Five crab species were extracted from twenty four corals of the species *P. eydouxi* and *P. meandrina*: 1. *Trapezia wardi* Serene, 2. *Trapezia ferruginea* Latrielle, 3. *Trapezia intermedia* Miers, 4. *Trapezia flavomaculata* Eydoux and Souleyet, 5. *Trapezia digitalis* Latrielle. Nomenclature follows Serene (1969)


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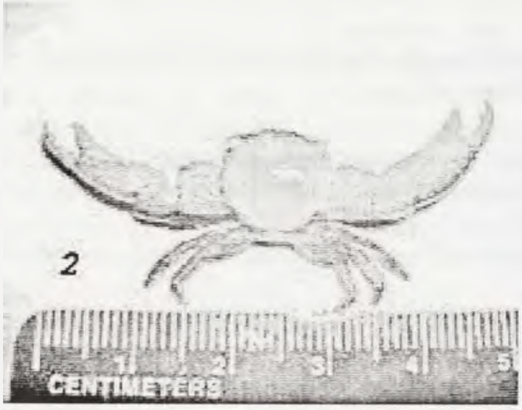
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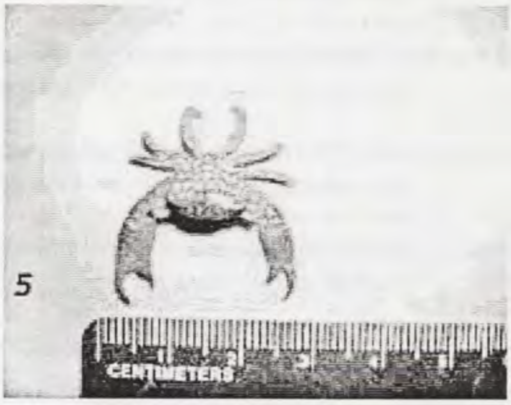
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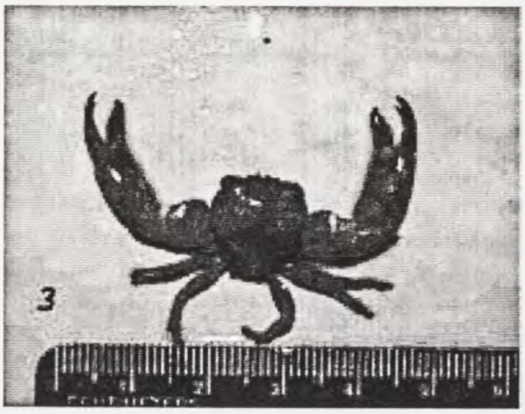
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# Shell selection and interspecific competition among intertidal hermit crab species on Moorea, French Polynesia

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ABSTRACT. The shell utilization patterns, shell selection preferences and interspecific competition of a tropical hermit crab assemblage (*Calcinus laevimanus, C. latens, Clibanarius humilis* and two *Cl.* species) were studied from the intertidal region of Moorea, French Polynesia. Hermit crab species, living gastropods and unoccupied shells were collected from three substrate types. Hermit crab species and their proportions varied in each substrate type. Pearson's Chi-squared test determined significance between crab species and the shells they utilized in each substrate type, indicating that the shell utilization pattern of a hermit crab species was comparable across all three substrate types. Significance between gastropod availability and shell utilization patterns varied with each hermit crab species and the substrate type they inhabited. Free access experiments were conducted giving crabs a choice of shells consisting of the families *Neritidae, Muricidae, Strombidae, Cypraeidae, Conidae* and *Mitridae*. Significance of free access experiments was found in larger individuals (> 0.2 g) of *C. laevimanus, C. latens* and *Cl.* species B, which all preferred *Muricid* shells. Significance was found in smaller individuals (<0.2g) of *C. laevimanus, Cl. humilis* and *Cl.* species A, which preferred *Neritidae, Muricidae* and *Muricidae*, respectively. Quality selection experiments involved crabs choosing between an intact shell and a damaged shell, with significance found in *C. laevimanus* and *Cl.humilis*, choosing intact shells. Interspecific competition experiments were conducted between species pairs (i.e. *C. laevimanus* vs. *C. latens)*, with two naked crabs competing over a single shell. Results show that weight and initial shell occupancy were of more significance than the actual species of the contestants.

Keywords: hermit crab, Moorea, shell selection, competition, *Calcinus, Clibanarius*.

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## Introduction

Competition due to limited resources can act as a selective agent on interacting populations. Sympatric species are thought to evolve a separation of resource utilization or habitat in order to maximize their survival in the environment. These ideas of niche separation, habitat and resource partitioning are basic principles of evolution that have been observed in many species assemblages (Arthur 1987).

Hermit crab communities provide a good study system for resource and habitat partitioning. They are crustaceans (Anomura: Diogenidae) that lack calcification of the abdominal exoskeleton and therefore, require an empty object to obtain protection from predators, desiccation and other physical stresses of the environment. Hermit crabs can acquire shells in four ways: from a dying or dead gastropod, from an unoccupied shell, or through interspecific or intraspecific competition with other hermit crabs (Brown 1993). Throughout the life of an individual hermit crab, several shell changes must occur. As a hermit crab grows, it must vacate its shell and locate one of more adequate size (Hazlett 1981). Otherwise, a tight fitting shell can significantly lower a crab's growth and fecundity rate, as well as make it more susceptible to predation (Angel 1999).

Because of the obligate crab-shell association and the potential for shells to be the limiting resource for hermit crabs, studies involving shell utilization patterns and competition among sympatric hermit crabs are highly warranted. Microhabitat partitioning can be observed among hermit crab communities, but overlapping spatial distributions are common. Gherardi and Nardone (1997) described microhabitat partitioning between two similar species of *Calcinus* found in the intertidal region of the Maldivian atolls. A third species, *Clibanarius humilis*, was found to occupy the same habitat as *C. laevimanus*, a much larger species. Their coexistence was marked by distinctly different shell utilization patterns due to their differences in size and morphology. Another study by Bertness (1981) described a three-species assemblage of Panama in which *Calcinus obscurus* was found in the middle to low intertidal region, *Clibanarius alidigitus* in the middle to high region and an undescribed *Pagarus* sp. occurring in the low intertidal area. This study showed that in areas where either *Clibanarius* or *Pagurus* coexisted with *Calcinus*, both former species were more likely to occupy shells of poorer quality than where they occurred alone. Addition of new shells to the area showed interference competition with *Calcinus* ousting *Clibanarius* from their shells. Both studies are significant because they give evidence to both microhabitat partitioning and competition among sympatric species. Additionally, many other studies have focused on whether or not microhabitat partitioning actively plays a role in diverging shell utilization patterns among hermit crabs. Some studies (Barnes & Grave 2000, Floeter 2000, Manjon-Cabeza 1999) have shown a significant separation of shell use among sympatric hermit crabs, while other studies (Abrams 1987ab, Leite 1998, Turra & Leite 2001) have described overlapping shell patterns.

While these studies concentrate on hermit crab use of particular shell species, it is also necessary to examine if species prefer particular shell characteristics. Wilbur (1990) concluded that *Pagarus longicarpus* chose shells based more on size, than shell species or quality. Individuals of the same species selectively chose intact shells over naturally drilled gastropod shells, both in the field and in laboratory manipulations (Pechenik & Lewis 2000). Botelho and Costa (2000) found that for the intertidal species *Clibanarius erythropus*, shell weight and internal volume were of more significance than the actual shell species. Examining how these factors (shell weight, internal volume, quality, etc.) influence hermit crab decisions adds yet another dimension to the complexity of any given hermit crab community.

The hermit crab community found on Moorea, French Polynesia, is extraordinarily diverse and studies focusing on Moorean hermit crabs are limited. Hahn (1997) studied shell use patterns of *Calcinus seurati* based on effects of water flow. He concluded that crabs inhabiting high flow environments are more likely to choose heavier shells than those from a still water site. Hatley (1997) examined shell preferences based on aperture size and found that *C. seurati* actively chose shells of larger aperture size (i.e. *Neritidae)*.

However, no studies have focused solely on the several species of hermit crabs that coexist in the intertidal region of Moorea. This assemblage consists of *Calcinus laevimanus* (Randall 1840), *C. latens* (Randall 1840), *Clibanarius humilis* (Dana 1851) and two other species of *Clibanarius*. The present study seeks to explore the shell utilization patterns and competitive interactions of this hermit crab community. 1) Do the five species of hermit crabs occupy the same substrate type? 2) Do they occupy similar shells, both in the field and when manipulated by laboratory experiments? 3) Do shell weight, volume, aperture size and quality influence shell preference? 4) Does interspecific competition exist among them and if so, is there a dominant species?

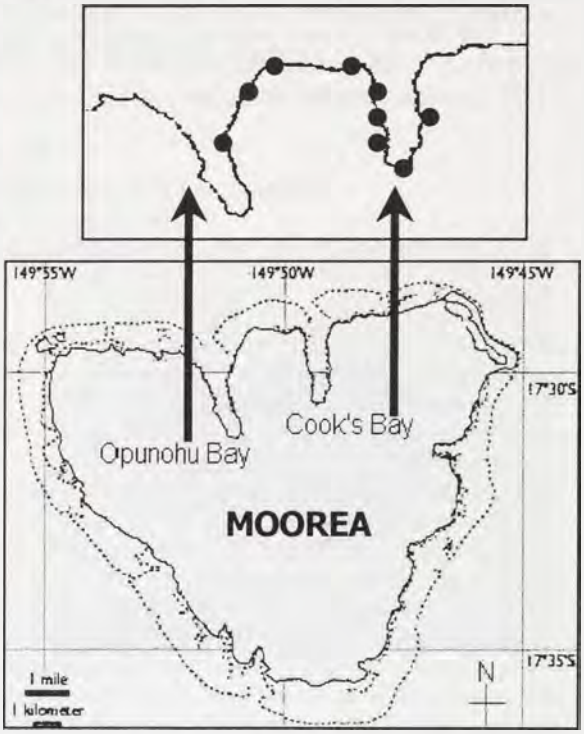


Figure 1: Map of Moorea (S149°50, W17°30) with sites located between Opunohu Bay and Cook's Bay. Maps courtesy of UC Berkeley Cartography Department.

## Materials and Methods

### *Site locations*

All field collections were done on Moorea, French Polynesia with collection sites located between the eastern bank of Opunohu Bay and the western bank of Cook's Bay. All laboratory experiments were conducted at the Richard B. Gump Research Station (Fig. 1).

### *Field Experiments*

#### *Natural Abundances of Hermit Crabs and Living Gastropods*

Sampling was conducted at all sites from October to November 2002. Sites were chosen

| Site # | Sub. | GPS Coordinates |
|---|---|---|
| 1 | 1 | S17°25.477, W121°36.387 |
| 2 | 1 | S17°29.122, W149°50.195 |
| 3 | 1 | S17°49.344, W149°50.912 |
| 4 | 2 | S17°25.911, W121°36.387 |
| 5 | 2 | S17°29.322, W149°49.589 |
| 6 | 2 | S17°29.531, W149°51.012 |
| 7 | 3 | S17°30.444, W149°49.458 |
| 8 | 3 | S17°29.167, W149°49.960 |
| 9 | 3 | S17°30.298, W149°51.012 |

Table 1: GPS coordinates of all sites, with corresponding substrate types.

based on their substrate composition. Three substrate types were defined. Substrate type 1 consisted of sand, coral rubble and rocks. Substrate type 2 was similar with approximately 50% of the coral and rocks covered with turf algae. Substrate type 3 was characterized by mud and all rocks and coral rubble were densely covered with algae.

Three sites (Tab. 1) from each substrate type were examined in which hermit crabs, living gastropods and non-occupied shells were collected within a 10m x 2m plot. Plots were situated so that ten meters ran parallel to the shoreline. Perpendicular to shore, one meter included the shoreline and the other was submerged in water. Water depth varied depending on tidal conditions during collection. All rocks and coral rubble were overturned in search of organisms. Ninety minute collections were conducted during high tide, while low tide collections were limited to sixty minutes.

*C. laevimanus*, *C. latens* and *Cl. humilis* were preliminarily identified by the Internet Database of Crustacea (Decapoda and Stomatopoda), mainly from the French Polynesian Islands (Poupin 2002) and confirmed by Joseph Poupin (Systemes d'Information Geographiques, Institut de Recherche de l'Ecole Navale, personal communication). Genus identification of *Clibanarius* species A and B were confirmed by Joseph Poupin and Patsy McLaughlin (Shannon Point Marine Center Western Washington University, personal communication) (Fig 2). Living gastropods and unoccupied shells were identified to family using an identification guide to the shells of Tahiti and French Polynesia (Salvat 1991).

Pearson's Chi-squared test was used to test significance between 1) substrate type and hermit crab populations, 2) hermit crab shell utilization patterns and substrate type and 3) shell availability and shell utilization patterns among the five species. For Pearson's Chi-squared tests, data was combined so that no more than one-fifth of the numerical entries were less than 5. Analysis of variance was used to determine the correlation among substrate type, hermit crab species and the shells they occupied. All statistical analysis was done using Systat 7.0.

### *Laboratory Experiments*

#### *Free Access Experiments*

Shell preferences for the five species of hermit crabs were investigated. All crabs collected previously were pooled together in a 32cm x 60cm aquarium. Constant water flow was maintained and crabs were fed detritus, algae and fruit. Crabs were chosen at random for experiments and were used only if they matched predetermined weight constraints. Small individuals did not exceed 0.2g while large individuals weighed greater than 0.2g. Crabs were removed from shells by either holding the apex of the shell to a 60W light bulb or by gently breaking the shell with a hammer. All damaged crabs were discarded. Naked crabs were dried and weighed to the nearest 0.01g.

Twenty small individuals from each of the five hermit crab species were used in the shell selection experiments. Because *Calcinus laevimanus*, *C. latens* and *Cl.* species B can grow to weights significantly greater than 0.2g, the same experiments were repeated for twenty larger individuals of these three species with ten sets of larger shells. A small crab individual was offered one of ten sets of six shells from families *Neritidae*, *Muricidae*, *Strombidae*, *Cypraeidae*, *Conidae* and *Mitridae*. Larger individuals were not offered *Mitridae*, since larger shells of this family were not common.

All shells used were of known weight, internal volume and aperture size. Shell weights were recorded to the nearest 0.01g after being dried for 12 hours. Internal volume was recorded by wrapping each shell in Parafilm and injecting water into the aperture using a syringe. The amount of water the shell could hold was determined by the difference between the final weight (shell, Parafilm and water) and the initial weight of the dry shell. The smallest aperture dimension was recorded to the nearest millimeter.

Individual crabs were placed in a 9cm x 13cm plastic tray with approximately 4cm of sea water and a set of shells. Crabs were given 24 hours to choose a shell. All sets of shells were

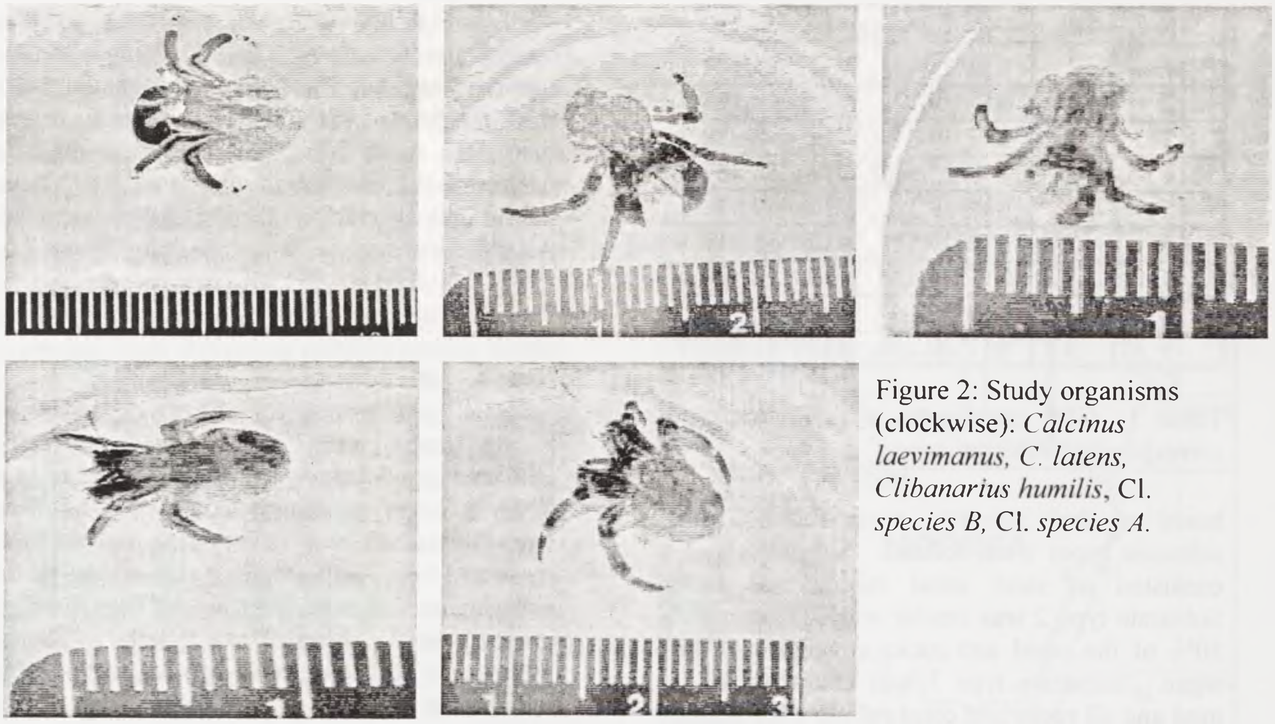


Figure 2: Study organisms (clockwise): *Calcinus laevimanus, C. latens, Clibanarius humilis,* Cl. *species B,* Cl. *species A.*

used for each hermit crab species and shell sets were again dried for 12 hours between experiments. To determine if each species was selectively choosing a particular species of shell, a Pearson's Chi-squared test was used. Spearman's Rank Correlation was used to find correlations among shells of the same family from the twenty shell sets, in terms of their weight, internal volume and aperture size. These parameters were then correlated with hermit crab weight using the same statistical method. Larger and smaller individuals were pooled for correlation data, so n=40 for *C. laevimanus, C. latens* and *Cl.* species B.

### *Quality Choice Experiments*

Ten sets of two shells were paired. Paired shells were of the same family, and of comparable weight and internal volume. Weight and volume were recorded using the same methods described above. Differences in weight and volume did not exceed 0.09g and 0.06ml, respectively. A 19/64" hole was drilled into one shell while its counterpart was left undamaged.

Twenty crabs from each of the five hermit crab species were used in the shell quality experiments so that each set of shells was used twice. Crabs from shell selection experiments were used in this portion of the study only if no damage occurred when removing the crab from its shell. New individuals were weighed to the nearest 0.01g. Crabs were given a set of shells that was similar to the weight of the shell the crab chose in the free access experiment described above, or by the weight of the shell occupied in the field.

Each crab was placed in a 100ml plastic cup with approximately 8cm of sea water and offered an undamaged and damaged shell. The shell occupied after a 24 hour period was recorded. To determine if each species was selectively choosing either a damaged or intact shell, a Pearson's Chi-squared test was used.

### *Interspecific Competition Experiments*

Ten competition experiments were conducted for each species pair (i.e. *C. laevimanus* vs. *C. latens, C. laevimanus* vs. *Cl. humilis, C. latens* vs. *C. humilis,* etc.). Ten additional experiments were conducted amongst larger sized crabs. Crabs from free access and quality choice experiments were used only if no damage occurred during removal from their shell. All new crabs were weighed to the nearest 0.01g.

Competition experiments included two crabs, an owner and an intruder. Weights were matched as close as possible, with the limitation that the weight of the smaller contestant must be greater than 75% of the weight of the larger contestant. For each pair of competing individuals, five runs were conducted in which Species A was the initial shell occupant and five runs in which Species A was the intruder. Shells occupied by the owner were chosen based either

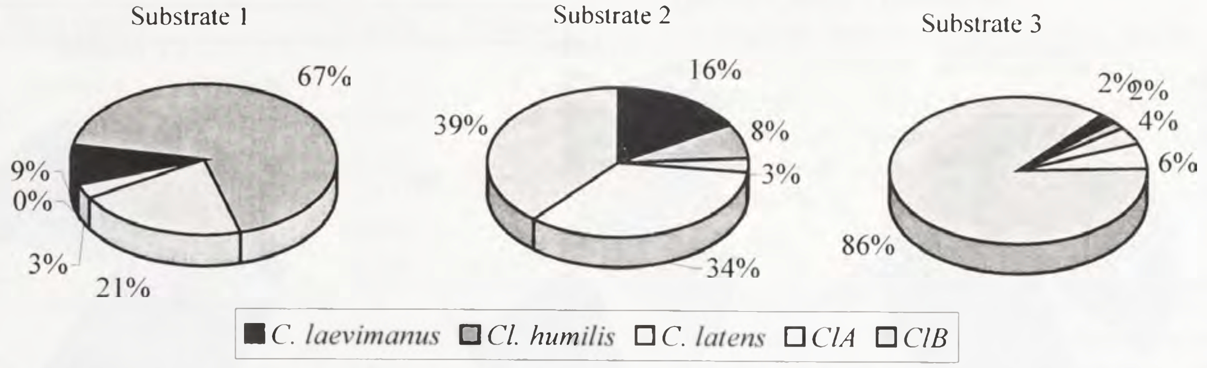


Figure 3: Proportions of hermit crabs found in each substrate type.

on the weight of the shell the crab chose in the free access experiment described above, or by the weight of the shell occupied in the field. Contestants were placed in a 100ml plastic cup with approximately 8cm of sea water. Observations were taken periodically during a 36 hour time frame and water was changed once during the experiment. The last occupant recorded at the end of 36 hours was considered the "winner."

Pearson's Chi-squared tests were used to determine if a particular hermit crab species was significantly more dominant in each species pairing. Data from species pairs involving large and small individuals were not combined. Additional Chi-squared tests were done to determine if first occupancy or weight discrepancy was of significance.

**Results**

Throughout this section, abbreviations for each species of hermit crab are as follows: *Calcinus laevimanus (Calae), C. latens (Calat), Clibanarius humilis (Clhum), Cl.* species A *(ClA), Cl.* species B *(ClB).*

*Field Experiments*

*Natural Abundances of Hermit Crabs and Living Gastropods*

For each substrate type, hermit crab populations were not of equal proportions (Fig. 3). Substrate type 1 was predominantly occupied by *Clhum* and *Calae,* while substrate type 2 showed a more heterogeneous composition. *ClB* was found to be the most common species in substrate type 3. Chi-squared results showed significance between hermit crab composition and substrate type ($r = 2358.277$, $df = 8$, $p < 0.05$).

ANOVA results show a correlation among hermit crab species, substrate and shells used ($df = 72$, mean-square $= 322.371$, F-ratio $= 1.919$, $p = 0$). Chi-squared tests further show significance between hermit crab species and what shells they occupied in all three substrate types (Sub. 1: $r = 544.413$, $df = 36$, $p < 0.05$, Sub. 2: $r = 46.986$, $df = 8$, $p < 0.05$, Sub. 3: $r = 40.592$, $df = 8$, $p < 0.05$).

Significance between shell availability (living gastropods and unoccupied shells) and the shells occupied by hermit crabs gave varied results for each species of hermit crab and the substrate they were found in (Tab. 2).

*Laboratory Experiments*

*Free Access Experiments*

With the exception of *Clhum*, Pearson's Chi-

| Crab sp. | Substrate | Value | df | p value |
|---|---|---|---|---|
| *Calae* | 1 | 32.013 | 9 | 0 |
| | 2 | 15.565 | 9 | 0.077 |
| | 3 | 8.751 | 9 | 0.461 |
| *Calat* | 1 | 268.42 | 6 | 0 |
| | 2 | 55.613 | 9 | 0 |
| | 3 | 20.897 | 9 | 0.013 |
| *Clhum* | 1 | 192.23 | 9 | 0 |
| | 2 | 14.534 | 9 | 0.105 |
| | 3 | 130.26 | 9 | 0 |
| *ClA* | 1 | 16.512 | 9 | 0.057 |
| | 2 | 170.4 | 5 | 0 |
| | 3 | 164.32 | 9 | 0 |
| *ClB* | 1 | 0 | 9 | 1 |
| | 2 | 76.836 | 9 | 0 |
| | 3 | 642.22 | 9 | 0 |

Table 2: Pearson's Chi-squared results showing varied significance ($p < 0.05$) between the shells used by hermit crabs and shell availability in each substrate type.

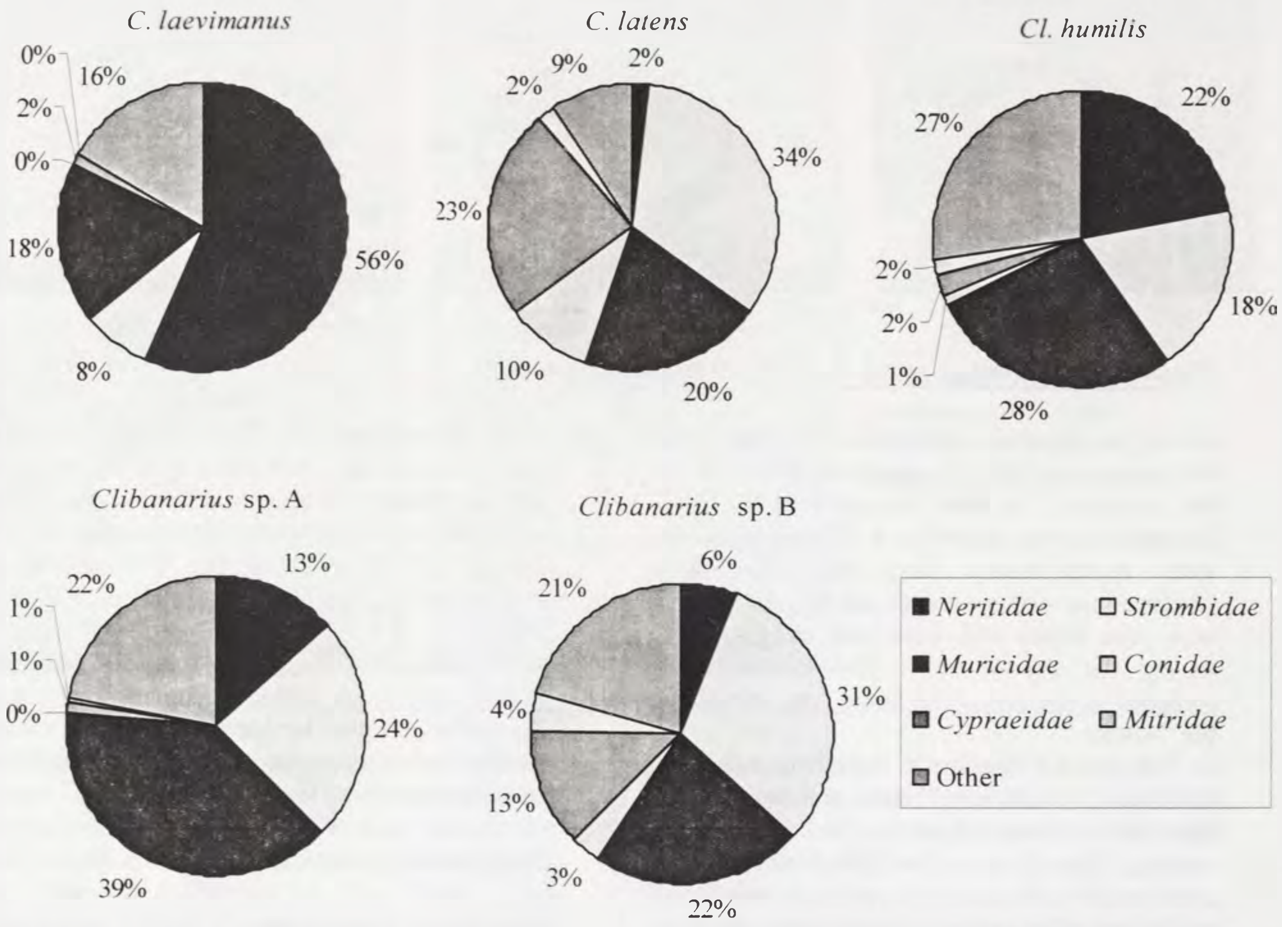


Figure 4: Shell utilization patterns of each species of hermit crab. Data pooled across substrate types.

| Hermit crab sp. | Value | df | p value |
|---|---|---|---|
| *C. laevimanus* | 7.2 | 1 | 0.07 |
| *C. latens* | 3.2 | 1 | 0.074 |
| *Cl. humilis* | 9.8 | 1 | 0.002 |
| *Cl.* species A | 5.4 | 1 | 0.02 |
| *Cl.* species B | 5 | 1 | 0.025 |

Table 3: (top) Pearson's Chi-squared results showing significance ($p < 0.05$) for free access experiments.

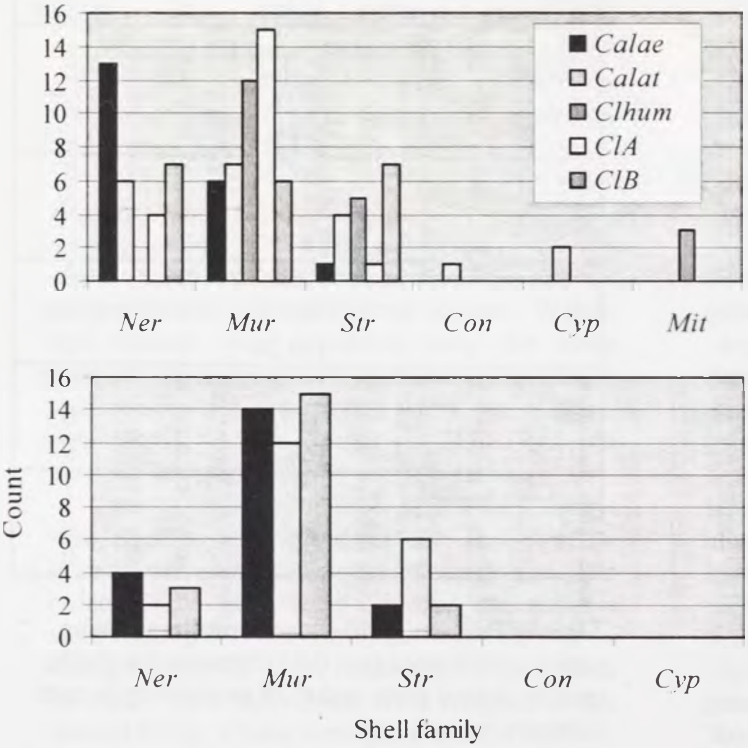


Figure 5: (left) Free access experiment results. Axes and legend are the same for both graphs. Shell families are abbreviated by the first three letters. Smaller individuals (top) for all five species of hermit crabs and larger individuals (bottom) for *Calcinus laevimanus, C. latens* and *Clibanarius* species B.

| Shell family | | Weight | Volume |
|---|---|---|---|
| *Neritidae* | Weight | 1 | |
| | Volume | 0.859 | 1 |
| | Aperture | 0.758 | 0.586 |
| *Muricidae* | Weight | 1 | |
| | Volume | 0.876 | 1 |
| | Aperture | 0.756 | 0.805 |
| *Conidae* | Weight | 1 | |
| | Volume | 0.64 | 1 |
| | Aperture | 0.874 | 0.616 |
| *Cypraeidae* | Weight | 1 | |
| | Volume | 0.684 | 1 |
| | Aperture | 0.816 | 0.843 |
| *Strombidae* | Weight | 1 | |
| | Volume | 0.814 | 1 |
| | Aperture | 0.79 | 0.813 |
| *Mitridae* | Weight | 1 | |
| | Volume | 0.867 | 1 |
| | Aperture | 0.215 | 0.326 |

Table 4: Spearman's Correlation data comparing shell weight, internal volume and aperture size for each shell family used in free access experiments.

squared test shows significance for hermit crab species and the shell they chose at the end of the 24 hour trial (Tab. 3). Differences in shell selection between large and small individuals of the same species were observed. Large individuals of *Calae, Calat* and *Cl*B all preferred *Muricidae* shells. Smaller individuals of *Calae* preferred *Neritidae,* while both *Calat* and *Cl*B preferences were more distributed across shell families (Fig. 5). Shell utilization patterns observed in the field for larger individuals were not similar to shell preferences observed in the laboratory. Patterns for smaller individuals, however, were similar.

For each of the twenty selection sets, shell weight and volume were significantly correlated for all families, except *Cypraeidae* and *Conidae* (Tab. 4). Aperture size was highly correlated with both weight and volume only in families *Muricidae, Cypraeidae* and *Strombidae.* Data from Spearman's Rank Correlation results (Tab. 5) show significant correlations between hermit crab weight and shell weight, volume and aperture size for all families, except *Clhum* and *Cl*A.

*Quality Choice Experiments*

Highest selectivity of quality shells was observed in *Clhum,* followed by *Calae, Cl*B, *Calat* and *Cl*A (Fig. 6). Chi-squared results. (Tab. 6) show significance for *Calae* and *Clhum* to selectively choose undamaged shells over damaged shells.

*Interspecific Competition Experiments*

Interspecific competition results (Tab. 7) show *Calae* to be the most dominant species, winning 81.67% of its fights (n = 60). Other results include *Calat* (53.33%, n = 60), *Clhum* (57.5%, n=40), *Cl*A (22.5%, n = 40) and *Cl*B (50%, n =60). Chi-squared results (Tab. 8) showed significance only for small individual species pairs of *Calae* vs. *Clhum* and *Calat* vs. *Cl*B and for the larger *Calae* vs. *Cl*B trials. Additional Chi-squared tests showed significance with which species occupied the shell first ($r = 6.923$, $df = 1$, $p < 0.05$) and with the weights of the contestants ($r = 12.308$, $df = 1$, $p<0.05$).

## Discussion

*Natural Abundances of Hermit Crabs and Living Gastropods*

Obvious differences in hermit crab composition were found in the three defined substrate types, giving evidence to habitat partitioning. Additional support for niche separation was provided by differences in shell utilization patterns found both in the field and the laboratory.

An obvious distinction of Substrate type 1 was the absence of *Cl.* species B. This can primarily be attributed to the lack of turf algae. As seen through observation in holding tanks, *Cl.* species B is an herbivore of this algae. Also, the algae covered environment could provide shelter and refuge, which was again observed in the field where the majority of *Cl.* species B individuals were found tangled in algal turf.

The interaction between the morphologically similar *C. laevimanus, C. latens* and *Cl.* species B varied within each substrate type. The coexistence of *C. laevimanus* and *C. latens* could be due to microhabitat partitioning. It was observed that *C. laevimanus* was more abundant in the high intertidal region, while *C. latens* was primarily found in the low intertidal region. This separation of habitat could significantly minimize competition and affect shell utilization patterns. In fact, it was found that *C. laevimanus* used shells of the *Neritidae* family over 50% of the time, and that *C. latens* used *Strombidae, Muricidae* and *Cypraeidae* in high proportions. These results support the idea of microhabitat partitioning since *Neritid* shells typically adhere to dry substrate, while *Strombid, Muricid and Cyprae* shells inhabit wet environments. Thus, shell sources are closer to the hermit crab species that use them. Further support of these shell utilization differences is provided with free access results for smaller individuals of the two species. However, results from free access experiments for larger individuals of these three species did not coincide with the shell utilization patterns seen in the field. Large individuals of *C. laevimanus* and *C. latens* were observed to typically use *Neritid* and *Conidae* shells, respectively. Shell preferences under laboratory experiments showed a preference for *Muricid* shells. A possible explanation for this difference is that *Muricid* shells of larger sizes were not very common in the field. Furthermore, Spearman's rank correlation shows a significant relationship between hermit crab weight and shell weight, volume and aperture size for the larger individuals. It can be concluded that these crab species choose shells based more on these parameters than the actual shell species.

In substrate type 2 and 3, however, the presence of *Cl.* species B can act on the populations of *C. laevimanus* and *C. latens*. In substrate type 2, *C. laevimanus* is still present in significant amounts, but the presence of *C. latens* decreased dramatically. This can be attributed to the overlap of spatial distribution of *C. latens* and *Cl.* species B, who were both observed to occupy the low intertidal region. Increased competition for shells and other resources, like space and food, can act as a selective agent. In fact, free access experiments found smaller individuals of *C. latens* and *Cl.* species B to prefer the same shells *(Neritidae, Muricidae* and *Strombidae)* in similar proportions. Competition experiment results show *Cl.* species B to be the more dominant species of the two, winning 7 of 20 fights. Thus, an overlap of resource use makes these two species vulnerable to competition, with *Cl.* species B being more successful.

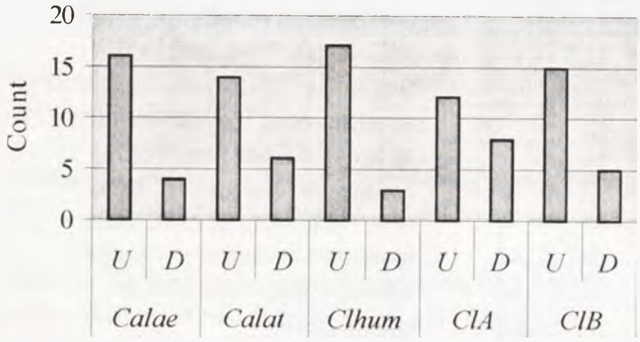
In substrate type 3, *Cl.* species B comprised the majority of the hermit crab population. With turf algae extremely abundant, a large population could be maintained due to the high amounts of food and shelter available. Other hermit crab populations in this kind of environment were highly limited. Even *C. laevimanus,* which was observed to live in a separate microhabitat, was limited. This could be due to the overwhelming growth of the turf algae, which could limit the food source of the herbivorous *C. laevimanus,* which was not observed to eat the turf algae. Moreover, population limitations of *Cl. humilis* and *Cl.* species A could be due to competition between juveniles of *Cl.* species B with these smaller species.

Another interaction of high importance was that of *Cl. humilis* and *Cl.* species A. Since these species are morphologically similar, reach comparable sizes and were found to have similar shell utilization patterns both in the field and in the lab, both species would benefit from habitat partitioning. In substrate type 1, *Cl. humilis* was found in abundant amounts with minimal amounts of *Cl.* species A. Substrate type 2 was reversed, with *Cl.* species A being the more prominent population. Factors that could account for a greater amount of *Cl.* species A in substrate type 2 could be the presence of turf algae as a food source and the presence of *Cl.* species B. *Cl.* species A could be a better competitor with *Cl.* species B than *Cl. humilis.* Although only ten trials were conducted of these species pairs, experimental results support this idea, with *Cl.* species A winning 5 of 10 fights and *Cl. humilis* winning 3 of 10.

Shell availability in each of these substrate types undoubtedly plays a significant role in the shell utilization patterns of the hermit crab assemblage. However, Pearson's Chi-squared results varied. No single species of hermit crab was seen to use available shells proportionally

| | Hermit crab species | | | | |
|---|---|---|---|---|---|
| | *Calae* | *Calat* | *Clhum* | *ClA* | *ClB* |
| Crab weight | 1 | 1 | 1 | 1 | 1 |
| Shell weight | 0.829 | 0.848 | -0.194 | 0.6 | 0.814 |
| volume | 0.798 | 0.812 | -0.161 | 0.647 | 0.812 |
| aperture | 0.684 | 0.809 | 0.038 | 0.643 | 0.797 |

Table 5: Spearman's Correlation results for each hermit crab species comparing crab weight, shell weight, volume and aperture size.



| Hermit crab sp. | Value | df | p value |
|---|---|---|---|
| *Calae* | 7.2 | 1 | 0.07 |
| *Calat* | 3.2 | 1 | 0.074 |
| *Clhum* | 9.8 | 1 | 0.002 |
| *Cl* A | 5.4 | 1 | 0.02 |
| *Cl* B | 5 | 1 | 0.025 |

Figure 6: (left) Results from quality choice experiments (U = undamaged, D = damaged).
Table 6: (right) Pearson's Chi-squared results showing significance ($p < 0.05$) between hermit crab species and preference for an intact shell.

across all substrate types. This indicates that, in some cases, hermit crabs were not choosing shells at random and selection was indeed occurring. However, the confounding factors associated with testing shell availability may alter results significantly. Because shells originate from other areas that were not taken into account, a true determination of present gastropods was not feasible. For instance, shells that are found in deeper waters were not included in the study, although a significant amount of shells used by hermit crabs come from these shell species washing ashore. Thus, environmental factors could bias results, specifically wave action. Areas of higher energy flow will undoubtedly bring more available shells to the intertidal region, whereas an environment with limited flow will not circulate shells as well.

### *Free Access and Quality Choice Experiments*

Free access and quality choice experiments gave evidence that each species of hermit crab is selective in the shell it chooses. Correlation results show that in order for a hermit crab to choose a particular shell, it must fit certain criteria for weight, volume, aperture size and quality. However, for *Cl.* species A and *Cl. humilis*, shell weight, volume and aperture size were not as highly correlated as compared to larger individuals. Results could have been skewed since only twenty trials were conducted for each, while statistical analysis for larger individuals was based on forty trials. Moreover, the negative correlation results for *Cl. humilis* can be due to the fact that data for *Mitridae* shells were included. Shell characteristics of the *Mitridae* family are difficult to accurately measure due to the shape and aperture size of the shell. Furthermore, data for quality choice experiments show that while the majority of the individuals chose undamaged shells, *Cl.* species A did not preferentially chose intact shells. This can be due to the fact that it was difficult to find larger sized individuals (> 0.12 g) of this hermit crab species. The shells comprising the quality choice sets may have been too large for their body weight. In fact, personal observation supports this in that individuals of *Cl.* species A actually used the drilled holes to protrude their antenna. Quality choice experiment results could also have been significantly altered if trials were conducted in a more natural environment, in which confounding factors, such as wave action and the presence of predators, were taken into account.

### *Interspecific Competition Experiments*

While competition over shells is occurring, Chi-squared results did not show significance for many of the species pairs. Because only ten trials were conducted per species pair, statistical analysis was difficult to use. Significance was found only when nine or more fights were won. Therefore, in order to achieve a more viable result, more trials should be conducted.

However, Chi-squared results did show significance between initial shell occupancy and the likelihood of winning the shell at the end of 36 hours. Initial occupancy of the shell can be advantageous since the occupant can hide within the shell to avoid the intruder. Also, weight plays a role in competition with the larger individual having an advantage. Hermit crabs can physically evict other hermit crabs out of shells (Hazlett 1981), which could potentially be

| | *Calae* | *Clhum* | *Calat* | *ClA* | *ClB* |
|---|---|---|---|---|---|
| *Calae* | | 0.0025 | 1 | 0.058 | 0.527 |
| *Clhum* | 10 | | 1 | 0.058 | 0.206 |
| *Calat* | 5 | 5 | | 0.0025 | 0.206 |
| *ClA* | 8 | 8 | 10 | | 1 |
| *ClB* | 6 | 3 | 3 | 5 | |

| | *Calae* | *Calat* | *ClB* |
|---|---|---|---|
| *Calae* | | 1 | 0.011 |
| *Calat* | 5 | | 0.527 |
| *ClB* | 9 | 4 | |

Table 7: Interspecific competition results for small individuals (top) and large individuals (bottom). Numbers in white cells indicate number of times a fight was won. "Winners" are species in top row. Numbers in grey cells indicate Chi-squared results showing significance for species pairs.

facilitated by a larger body size. Therefore, this study cannot conclude if one species is more dominant over one species, although it does give evidence that interspecific competition is dictated by a combination of factors, including, but not limited to, initial shell occupancy and contestant weight.

## Conclusions

The hermit crab assemblage found in the intertidal region of Moorea is dynamic in the shell utilization patterns of each crab species, as well as in the many competitive interactions that occur. Habitat partitioning is observed and this directly influences the shell types that are used. Therefore, it can be concluded that some degree of niche separation has occurred and competition is present in cases of sympatry and common resource needs. This dynamic interaction between a crab and its shell warrants future studies, which should include a larger area of study, bringing laboratory experiments into the field and examining intraspecific competition.

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# Ecological and ethnobotanical investigations of algae and the factors influencing their distribution on Mo'orea, French Polynesia

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**ABSTRACT.** Algae are a food source of prime importance throughout the Pacific, and though algae are currently consumed throughout at least two archipelagoes of French Polynesia, the extent of their use is minimal in the Society Islands. Practitioners of traditional Tahitian therapy *Ra'au Tahiti* revealed that eight marine species are or have once been used in the Austral Islands, one marine species is used in the Marquesas, while five marine species and two undetermined freshwater species are or have been used in the Society Islands. The purpose of this study is to evaluate the extent of the use of algae for food or medicine by interviewing Tahitian healers, as well as to survey and compare the distribution of algae in different environments and examine substrate composition in order to assess the impact of sedimentation deposited into the lagoon as a result of industrial agriculture. Transects were conducted at two different depths (and/or distance) from the shore at ten different sites evenly spaced around the island of Mo'orea. Percent algal cover, substrate composition, depth, temperature, salinity, turbidity, and phosphate and nitrate levels were measured. The ten sites were distributed into two site types; five sites were located at the mouth of an estuary and five were distant from any point source. The distribution of cumulative percent cover of algae and substrate types per transect were analyzed considering both factors of site type and depth. Variances in distributions of silt and of sand were significant across different site types and their distribution displayed a strong negative correlation. A regression showed a strong relationship between the cumulative percent cover of silt per transect and turbidity. Fossilized coral was the most suitable substrate for algal growth, as its distribution correlated with the distributions of many algal species or genera. Results and observations indicate that though the human impact on the lagoon is minimal, runoff from estuaries is detrimental to algal recruitment and growth.

**KEYWORDS:** edible algae, ethnobotany, survey, distribution, substrate, Mo'orea, French Polynesia

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## Introduction

Marine algae, as primary producers, are ecologically important and have been used economically as food and medicine for centuries. They have a fundamental role in the functioning and maintenance of the equilibria of all aquatic ecosystems and, by this token, of the entire biosphere (Perez 1992). Today, various species of marine algae not only constitute a food source of prime importance but also provide useful extracts used in numerous food, pharmaceutical, cosmetic, and industrial applications; these include agar, carrageenans (red algae *Gigartina* and *Chondrus crispus*; used in jellies, lotions, medicines), and alginates (made from the giant kelp *Macrocystis pyrifera* in the United States, from *Ascophyllum nodosum* and *Laminaria hyperborea* in Norway and Scotland; used as a polishing material, as a thickener, a stabilizer and suspending agent in foods, and a detoxifying agent – for instance, to remediate lead and strontium poisoning) (Guiry 2002). Payri report that at least six marine and one freshwater species of algae are currently consumed in the Marquesas archipelago of French Polynesia, where edible algae are easily collected on large, basaltic flats, while two species are consumed in the Austral Islands, seven species are used in the Fiji islands of Melanesia, and about thirty species in Hawaii (Claude Payri, pers. com.). The knowledge of seaweed as food has apparently been lost in most of French Polynesia, as theorized by Payri, de R. N'Yeurt, and Orempüller (2001). *Ulva lactuca* is an edible species eaten by ancient Tahitians, as recorded by Setchell (1926) as "Rimu miti" or 'salt seaweed', and remains in use in Hawaii and in the Marquesas. Exploring the ecology and the environmental factors possibly affecting the growth of the edible local species of algae could be useful in understanding why the use of algae is much less common in the Society Islands as it is in the Marquesas and the Austral archipelagoes. The consumption of marine products by islanders exemplifies the efficient use of available resources, which is particularly important on small islands with a growing population, limited arable land and a fragile ecosystem susceptible to disruptions detrimental to the production of food. The Japanese diet, which includes fifty-two species of algae, illustrates an adaptation to these particular sociological and geographical circumstances. The Chinese, who currently constitute the world's largest population, numbering more than 1.28 billion, as well as the world's greatest consumers of algae (perhaps not coincidentally), use seventy-four species (Payri, pers. com.).

The purpose of this study is to evaluate the extent of the use of marine algae for food or medicine in Polynesia, to examine the distribution of algae in different environments, and to assess the relations amongst substrate composition, turbidity, nutrient levels, the geomorphology of shallow and deep waters, and algal growth. The first part of this study consisted of meeting practitioners of the ancient tradition of Tahitian therapy, known as *Ra'au Tahiti* (abbreviated by many to *ra'au*), and questioning them concerning the use of algae for food or medicine, in order to find out the extent to which algae has been or is currently used. The practitioners of *ra'au* were chosen as informants because their knowledge of the use of plants and other natural products is far more comprehensive than that of most local inhabitants. It is worth noting that a severe ban was imposed throughout French Polynesia on the practice of *ra'au* by French legislature during most of the second half of the past century (Lombard, pers. com.), and it is likely that these regulations were more strictly enforced in the Society Islands, which became the established center of the French overseas department in 1880. The second part of the study will consist of a survey of the distribution of algae and substrate types in two different environments and two different depths chosen to estimate the impact of agriculture and industrial development on the lagoon, and thus on the growth of algae, and to examine the distribution differences between shallow or 'accessible' waters and deep, 'non-accessible' waters. The study ultimately seeks to elucidate whether or not algae constitute a part of the Polynesian diet, the extent to which it does, and why the use of algae is so scarce on Mo'orea and the rest of the Society Islands in comparison to other archipelagoes. Has this knowledge been lost due to Polynesia's colonization by France and the significant sociocultural as well as dietary changes that ensued? Is this loss a consequence of the severe ban on the practice of *Ra'au Tahiti* that lasted during fifty years and resulted in a switch of the predominant health care system from the ancient traditional therapy provided by local practitioners to Western medical practices provided by French doctors? Is algae not consumed in the Society Islands simply because the edible species are not widely enough distributed in shallow, accessible waters where they can be easily harvested? What environments are suitable to particular genera or species of algae? To what extent do modern lifestyles impact these shallow, accessible waters?

## Methods and Materials

### *Study sites*

The island of Mo'orea (17° 31' S 149° 50' W) is a volcanic island, located 11 km west of Tahiti, in the Society Archipelago of French Polynesia situated in the South Pacific Ocean. Data were collected during the months of October and November 2002. Ten sites were selected along the 59-kilometer perimeter of the island (Table 1, Figure 1). Each site was located more than five and less than seven kilometers away from the next, and was chosen so that five sites were located specifically at the mouth of an estuary of a minimum width of 3 meters, while five others were distant from any point source.

### *Transects*

Two 50-meter transects parallel to the shore were sampled at each site; the first transect was located in accessible or shallow waters, defined to have an average depth of less than one meter and/or a location of 25 m or less from the shore. The second transect was conducted in non-accessible or deep waters, defined to have an average depth of more than one meter and/or a location of 200 m or more from the shore. Samples were taken every 10 meters along each transect, yielding six readings per transect. A one square meter quadrat separated into one hundred squares (surface area of .01 $m^2$ each) was used to estimate the percent cover of algae, identified either by species or genus with the help of the book *Algae of French Polynesia* (Payri et al 2001), and to describe substrate composition by estimating the percent cover of various types of substrata, divided in five categories — live coral, fossilized coral, rock or rubble, sand, and muddy silt. The percent cover of seagrasses and cyanobacteria was recorded occasionally, when present. Thick algal turf, filamentous rhodophytes like the species *Spyridia filamentosa*, along with species rarely observed like the red Galaxauraceae species *Actinotrichia fragilis* and other species belonging to the *Galaxaura* genus, were classified as "Other." In addition, depth, temperature, and shoreline type were recorded at each reading. Two sets of water samples were collected from all ten sites near the shore, at about 30 cm below the surface, during low tide, and before the rainy season; the first was collected between 4:30 and 6:30 p.m. in late October, and the second was collected between 5:45 and 7:45 a.m. in early November. Levels of turbidity, salinity, phosphate and nitrate were measured for each set of water samples and averaged between the two sets to yield one final value for each parameter. Turbidity was measured in nephelometric turbidity units (NTU) using a digital turbidimeter. Salinity was measured

| SITE | COORDINATE |
|---|---|
| 1 EST | S 17° 30.433’<br>W 149° 49.312’ |
| 2 NON EST | S 17° 28.713’<br>W 149° 47.295’ |
| 3 NON EST | S 17° 30.659’<br>W 149° 46.140’ |
| 4 EST | S 17° 33.036’<br>W 149° 47.416’ |
| 5 EST | S 17° 35.175’<br>W 149° 48.447’ |
| 6 EST | S 17° 34.588’<br>W 149° 51.135’ |
| 7 NON EST | S 17° 32.782’<br>W 149° 53.400’ |
| 8 NON EST | S 17° 30.274’<br>W 149° 54.947’ |
| 9 EST | S 17° 29.517’<br>W 149° 52.318’ |
| 10 NON EST | S 17° 29.572’<br>W 149° 51.055’ |

**Table 1.** Site latitudes and longitudes

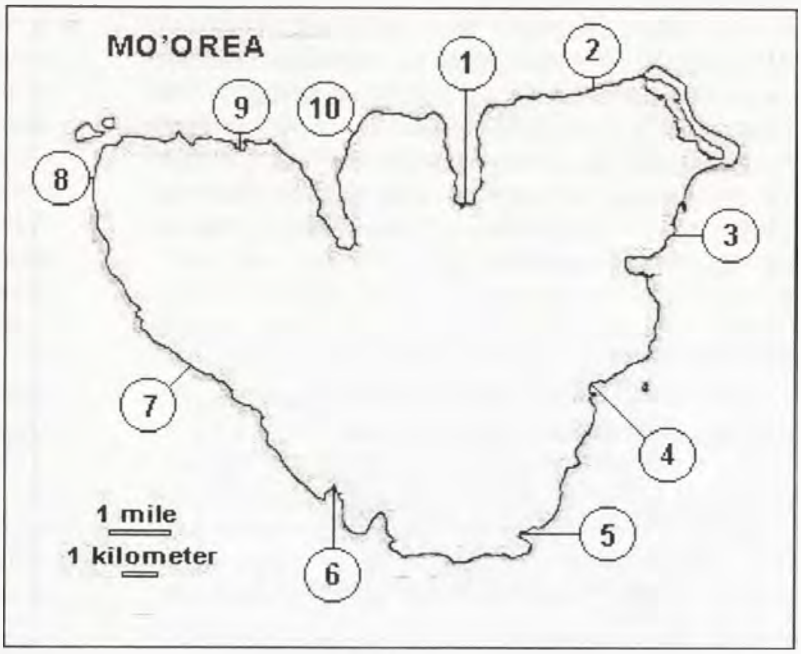

**Figure 1.** Map of the island of Mo’orea with locations of the ten study sites

with a refractometer in parts per thousand (‰), while levels of phosphate and nitrate were determined using Fischer Scientific, Inc. and LaMotte, Inc. saltwater calibrated chemical test kits.

### *Statistical Analyses*

Analysis of variance (2 way-ANOVA) tests were used to examine the distribution of macroalgal species present, as well as the distribution of various substrate categories, taking into account the two factors of depth and site type (proximity to a point source). Cumulative percent cover per transect was calculated for each genus or species and for each substrate type, yielding five readings for each of four site types (S EST, D EST, S NON EST, D NON EST). This analysis is meant to compare distribution between two different site types (estuary vs. non-estuary; source: EST), between two different depths (shallow/accessible vs. deep/non-accessible; source: DEPTH), and across both factors (source: EST*DEPTH).

Pearson correlations were drawn between the abundance of a particular algal genus or species and the abundance of a particular substrate, taking into account cumulative percent cover per transect for both factors, in order determine the substrate type that is the most suitable for any particular species. Correlations were also drawn among algal genera and species in order to establish which algae cohabit in similar environments, and among substrate categories. Finally, a regression was performed on the cumulative percent cover of each substrate type per site (1-10) and the average turbidity level (NTU) at that site in order to determine how sedimentation affects visibility and light penetration.

A cluster analysis was performed on the eighteen species or genera of algae to establish how species grouped according to distribution.

Kruskal-Wallis one-way analyses of variance and the Mann-Whitney U tests were used to establish whether variances of depth are significantly different across the four site types. Depth measurements (six per transect) recorded on all estuary sites (Sites 1, 4, 5, 6 and 9) were compared to depth measurements of all non-estuary sites (Sites 2, 3, 7, 8, and 10) in order to establish whether sediment carried by runoff from rivers into the ocean disturb shallow water environments by affecting the topography of their surface. Depth measurements recorded on all shallow/accessible transects of all estuary sites were also compared to those recorded on all shallow/accessible transects of all non-estuary sites. Finally, depth measurements recorded on all deep/non-accessible transects of all estuary sites were compared to those recorded on all deep/non-accessible transects of all non-estuary sites.

### *Ethnobotanical interviews*

Nine informants were consulted, and six, all of whom were practitioners of traditional Tahitian therapy (known as *Ra'au Tahiti*, or simply *ra'au*) were asked a set of five questions, as shown in Table 2, while being presented with either a fresh specimen of the species of study, or with pictures from the book *Algae of French Polynesia* (Payri, de R. N'Yeurt, and Orempüller, 2001).

1. Has this alga ever been used or consumed on Mo'orea and Tahiti?
2. Has this alga ever been used or consumed elsewhere in Polynesia?
3. If yes, where?
4. What was this alga used for, and how was it used?
5. Name and native origin of the informant

**Table 2.** Interview questions asked of known medicinal healers

## Results

### *Algal distribution*

Results of 2-way ANOVA tests for 9 species and 5 genera of algae, 1 genus of seagrass, cyanobacteria and undetermined algal turf (classified as *Other*) are tabulated in Appendix 1. Though many algal genera or species seemed to be preferentially distributed in certain site types and absent in others, only one species, *Turbinaria ornata*, was distributed significantly differently across both factors ($p < 0.05$), while another species, *Valonia aegagropila*, showed near significance in distribution variances ($p = 0.058$). The dendogram from the cluster analysis of algal distribution data is shown as Figure 2. *Ulva lactuca* was only found in shallow sites. *Enteromorpha entestinalis* and *Gracilaria spp* were found in both shallow and deep estuary sites, and only in shallow non-estuary sites. *C. racemosa* was found in both shallow and deep transects of estuary sites, while it was only found in deep transects of non-estuary sites. *Hypnea* spp was only found in non-estuary sites, at both depths. Boodlea *kaeneana*, *Halimeda spp*, and *Padina spp* were present at all site types. Distribution histograms showing the average percent cover of algae present in each site type at each depths are shown as Appendix 2a-d.

### *Substrate distribution*

Results of 2-way ANOVA tests for five categories of substrate are tabulated in Table 3. Variances in distribution are significant across different depths for live coral (Source: DEPTH; $p = 0.016$) and are near significant for fossilized coral (Source: DEPTH; $p = 0.054$). Both sand and muddy silt displayed significance in the variances of their distribution across different site types — silt being more abundant in estuary sites (Source: EST; $p = 0.000$) and sand being more abundant in non-estuary sites (Source: EST; $p = 0.000$). Though the 2-way ANOVA test results do not indicate the depth or site types at which a variable is more abundant, it is easy to observe abundance in this case by looking at the substrate distribution histograms (Appendices 3a-d).

| *Species / genus* | *Source of var.* | *F-ratio* | ***P-value*** |
|---|---|---|---|
| Live coral | EST | 1.911 | 0.186 |
| | DEPTH | 7.179 | **0.016** |
| | EST*DEPTH | 1.911 | 0.186 |
| Fossilized coral | EST | 1.141 | 0.301 |
| | DEPTH | 4.327 | **0.054** |
| | EST*DEPTH | 1.635 | 0.219 |
| Sand | EST | 80.599 | **0.000** |
| | DEPTH | 0.450 | 0.512 |
| | EST*DEPTH | 4.332 | 0.054 |
| Muddy silt | EST | 39.088 | **0.000** |
| | DEPTH | 0.097 | 0.759 |
| | EST*DEPTH | 0.097 | 0.759 |

**Table 3.** 2-way ANOVA results for substrate composition distribution across two factors, site type (source of variation: EST; estuary vs. non-estuary) and depth (source of variation: DEPTH; shallow/accessible vs. deep/non-accessible).

### *Correlations*

Pearson correlations performed among algal genera and species distributions, among distributions of substrate types, and between distributions of algal genera and species and of various substrate types were designated to be significant when $|r| > 0.500$.

Though according to observations many species or genera of algae seem to prefer a particular environment, at a particular depth or site type, only *Turbinaria ornata* showed significant variances in distribution across all factors (Source: EST; $p = 0.021$; Source: DEPTH; $p = 0.018$; Source: EST*DEPTH; $p = 0.021$). This species thrives in non-estuary, deep sites, probably due to its dependence on hard substratum like fossilized coral, which itself displayed near significance in the variances of its distribution across different depths (Source: DEPTH; $p = 0.054$), and with which *T. ornata* exhibited a strong positive correlation in distribution ($r = 0.703$). The distribution of *T. ornata* correlated with the distribution of live coral ($r = 0.581$), which itself also displayed significance in the variances of its distribution across different depths (Source: DEPTH; $p = 0.016$). *T. ornata* and *Valonia aegagropila* were strongly correlated in their distributions($r = 0.776$). *V. aegagropila* showed near significance in variances of distribution across all factors (Source: EST; $p = 0.058$; Source: DEPTH; $p = 0.058$; Source: EST*DEPTH; $p = 0.058$). The distribution of *Valonia aegagropila* also correlated with that of live coral ($r = 0.625$) and fossilized coral ($r = 0.618$).

The distribution of *Dictyota spp* significantly correlated with those of five different algal genera or species (*Valonia aegagropila*: $r = 0.783$; *Dictyosphaeria spp*: $r = 0.779$; *Turbinaria ornata*: $r = 0.776$; *Caulerpa racemosa*: $r = 0.729$; *Halimeda spp*: $r = 0.516$; *T. ornata*: $r = 0.507$) and with that of fossilized coral ($r = 0.505$). Fossilized coral seemed the substrate type on which algae most frequently grew: its distribution correlated with those of five algal genera and species (*T. ornata*, *Dictyota spp* and *V. aegagropila*, as mentioned above; *Dictyosphaeria spp* : $r = 0.610$; *C. racemosa* : $r = 0.522$; $r$-values for). The distributions of *U. lactuca* and *E. intestinalis* showed a very strong correlation ($r = 0.966$). The seagrass genus *Halophila* correlated negatively with all algal genera and species, for the exception of the category *Other* (which predominantly described algal turf).

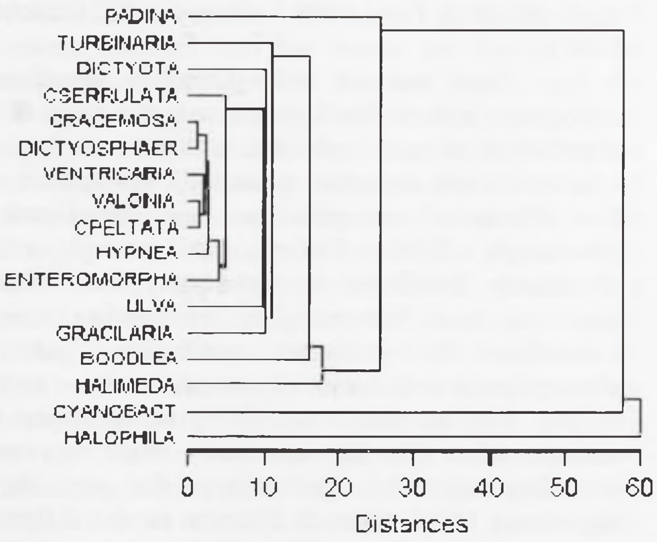

**Figure 2.** Cluster diagram of total algal distribution

The distributions of sand and of *Padina spp* showed a correlation ($r = 0.455$). Distribution of silt correlated negatively with those of all algal genera and species (range: $r = -0.149$ to $-0.372$) except for the seagrass genus *Halophila spp* ($r = 0.595$), *C. peltata* ($r = 0.177$), *Gracilaria spp* ($r = 0.305$), and *Other* ($r = 0.314$). Though *Halophila spp* (seagrass) predominantly grew in estuary sites and *Padina spp* seemed predominantly distributed in non-estuary sites, the variances in distribution between estuary and non-estuary sites were not significantly different though the $p$-values in both cases were low (Source of variation: EST; *Halophila spp*: $p = 0.101$; *Padina spp*: $p = 0.090$). This near-significance in distribution is probably due to the significantly greater distribution of silt in estuary sites, with which *Halophila spp* showed a correlation, and of sand in non-estuary sites, with which *Padina spp* showed a correlation ($r = 0.455$). Unsurprisingly, the distributions of silt and sand show a strong negative correlation ($r = -0.825$). There was a weak positive correlation between the distributions of live and fossilized coral ($r = 0.366$), and a weak negative correlation between the distributions of live coral and silt ($r = -0.349$).

Correlations between turbidity levels (NTU) and cumulative percent cover of all substrate types per site revealed a positive correlation between the distribution of silt and turbidity ($r = 0.727$) and a negative correlation between the distribution of sand and turbidity ($r = -0.675$). Regressions between turbidity measurements per site and the distribution of substrate types yielded one significant $P$ value of 0.046 when comparing silt distribution and turbidity, indicating a significant interaction between the two factors.

### *Depth variances*

Depth measurements recorded on all transects of all estuary sites (Sites 1, 4, 5, 6 and 9) were compared to depth measurements of all non-estuary sites (Sites 2, 3, 7, 8, and 10). According to results of analyses of variances for non-parametric data, variances of depth between the two samples were not significantly different ($p = 0.249$).

Depth measurements recorded on shallow/accessible transects of all estuary sites (S EST) were compared to depth measurements recorded on shallow/accessible transects of all non-estuary sites (S NON EST). Variances of depth between the two samples were not significantly different, though $p < 0.1$ ($p = 0.095$).

Depth measurements recorded on deep/non-accessible transects of all estuary sites (D EST) were compared to depth measurements of all non-estuary sites (D NON EST). Variances of depth between the two samples were not significantly different ($p$ = 0.200).

*Other factors*

Salinity levels (‰) are reported for each site in Appendix 4. Phosphate and nitrate levels were not taken into account because their concentrations were too low to be relevant, below detectable levels for all but one estuary site (Site 1 at the mouth of the Pao Pao estuary) where phosphate levels were slightly elevated.

*Ethnobotanical interviews*

A total of nine informants were consulted yet only six were interviewed, two of whom, namely Mme Frieder Lombard and Mama Lucie, are currently practicing *Ra'au Tahiti* professionally, and have for about 35 and more than 50 years, respectively. Mme Rita You Sing and Pasteur Heitarauri practice *ra'au* informally. Linda and Giselle were related to Mama Lucie, and all three agreed with each other considering that they have obtained their knowledge of *ra'au* from the same person — from Mama Lucie and Giselle's mother — so the results of their interviews are arranged in the same table. All informants, except for Linda, learned Tahitian therapy from their mother, as the knowledge and practice of *ra'au* is a matriarchal tradition passed orally through from one generation to the next, and tends to be the domain of members of the clergy as well. The results of interviews were compiled in Appendix 6.

## Discussion

*Ecological investigations*

According to observations made in the field, the distribution of reportedly edible species indicate a preference for shallow depths. *Enteromorpha intestinalis* seemed predominantly distributed in shallow waters, yet the variances in distribution between shallow/accessible and deep/non-accessible sites was not significantly different. By the same token, *Ulva lactuca* seemed to preferedly grow in shallow, non-estuary sites, and still the variances showed no significant differences. These species are two of the five reported comestible species that are or have once been used on Mo'orea and Tahiti, predominantly (yet not significantly) growing and cohabitating in shallow waters. This trend indicates the accuracy of the logical prediction that people are more likely to harvest and consume algae that grow in shallow, accessible waters. Three genera or species of edible algae were preferentially distributed in shallow waters according to their presence or absence across the four different type sites (S vs. D; EST vs. NON EST), namely *Ulva lactuca*, *Enteromorpha intestinalis*, and *Gracilaria spp*, a genus comprising the edible species *Gracilaria verrucosa*, widely cultivated and harvested industrially since 1949 (Perez 1992).. Surprisingly, the most popular edible algae of the South Pacific, *C. racemosa*, was found to grow predominantly (yet, again, not significantly) in deeper or less accessible waters, an observation which contradicts the mentioned prediction. Though these edible species seemed more frequently present in shallow waters according to observations, and according to frequency of presence or absence, the distribution of these species did not significantly vary across different depths due to their low general abundance. This low abundance points to possible variations in seasonality, a vital factor that is not taken into account in this study, and possibly the greatest source of possible bias.

The only species that showed significant variances in distribution across different depths and site types was *Turbinaria ornata*. The species *T. ornata* has elaborated numerous strategies to adapt to various environmental conditions, and the invasion of coral reefs by it and by other macroalgal species since the early eighties (Stiger & Payri 1999) coincides with recent massive coral bleaching phenomena which have become more severe and frequent in the past couple of decades (LaPointe 1989). Considering that the distribution of *T. ornata* is more closely correlated with the distribution of fossilized, bleached coral than it is with live coral, the greater frequency and severity of massive bleaching events might have resulted in providing more suitable substrate for algal recruitment (Payri 1987). The many significant correlations between the distribution of fossilized coral and those of various species or genera of algae reflect the crucial importance of anchoring surface availability in the spreading of algae (Stiger & Payri 1999; LaPointe 1989; Guilcher 1998).

Sand and silt both displayed significant differences in their distribution across site types. The distribution of sand correlated with that of *Padina spp*, which was described as an indicator species of the effects of eutrophication and development (Harbaugh, 2000). *Padina spp* seemed more abundantly distributed in non-estuary sites where sand was more common, an observation which contradicts the prediction made by *Padina*'s description as an indicator of eutrophication — as the estuary sites are much more likely to be subject to eutrophication than the non-estuary sites. However, the distribution of *Padina spp* did not show significant variances in distribution across different site types, so nothing can be said about its possible role as bioindicator of eutrophication. The lack of appropriate anchoring substrate in estuary sites might account for the sparse distribution of *Padina spp* and of most other species. The distribution of mudddy silt, which was significantly more abundant in estuary sites, correlated negatively with those of all algal genera and species except for *Gracilaria spp*, *C. peltata*, the seagrass genus *Halophila* which showed noticeable preference for this substrate type, and algal turfs, classified as *Other*. A regression between turbidity levels and the distribution of this silt furthermore points to a significant relationship between the two. This reveals that, though the runoff deposited from estuaries into the lagoon does not significantly alter algal growth across different site types, modern agricultural practices, mainly responsible for producing this runoff, do impact substrate distribution significantly; this not only affects algal and coral recruitment on the benthos but also light penetration through the water column. Silt is created by dredging and discharged by runoff, and usually remains in an area for long periods and is periodically resuspended during storms. Hard corals display an extreme susceptibility to sedimentation and the effects of elevated levels of turbidity caused by the dredging of estuaries (Dodge & Vaisnys 1977). Turbidity clogs the filter feeding mechanisms of coral polyps, causing the corals to lose energy due to the necessity of continually shedding a protective mucus layer (Lasker 1980). High turbidity which may cause severe damage to marine ecosystems by reducing the light available for photosynthesis necessary to the fixation of calcium carbonate and to the growth of algae, detrimentally affecting the entire food chain. Furthermore, this chronic stress makes corals more susceptible to disease and bleaching (Lasker 1980).

Although nutrient levels were predicted to be higher in estuary sites, phosphate and nitrate concentrations were too low to be significant. Runoff waters released into the ocean are known to be responsible for elevated nutrient levels, and the severe drought during which this study was conducted might account for the generally low levels of nutrients in estuary sites. Indeed, instead of flowing downstream, water was observed to be flowing into the estuary at four of five estuary sites; only at the Afareaitu estuary (Site 4) was there a trickle of water flowing into the ocean. It is worth noting that Site 4 was the site with the lowest salinity levels, with an average of 4‰. The nearest readings were 31‰ at the Papetoai estuary (Site 9) and 32‰ at the Ma'atea estuary (Site 5), while the two remaining estuary sites both had an average of 34‰ (Sites 1 & 6). Overall, the salinity of estuary sites averaged to 27.0 ± 11.6, while non-estuary sites averaged to 36.4 ± 0.4.

Variances of depth in shallow waters of estuary sites are not significantly different from variances of depth in shallow waters of non-estuary sites, though the *p*-value of is less than half of that comparing variances of depth in deep waters of estuary sites to deep waters of non-estuary sites. This *p*-value might have been lower with a greater number of replicates. By its nature, the muddy, silty sedimentation carried by runoff into the estuary is easily displaced, and thus likely to alter shallow water environments by affecting the topography of their surface. Furthermore, the presence of this kind of substrate is not very conducive to macroalgal growth; indeed, only the seagrass *Halophila* and some turf and filamentous rhodophytes (classified as '*Other*') seem to prosper and proliferate on silty substrate.

*Ethnobotanical uses*

Claude Payri, in a lecture on the biodiversity and uses of algae in French Polynesia, reported that two marine species of algae are used in the Austral Islands (*Caulerpa racemosa*, eaten throughout the world, often raw with coconut milk, and *Caulerpa bikinensis*, only found in the Austral and the Tuamotu archipelagoes) and six marine species (*Caulerpa racemosa*, *Ulva lactuca*, *Enteromorpha flexuosa*, *Cladophora patentiramea*, *Chnoospora minima*, and an undetermined marine species) and one freshwater species (undetermined as well) are used on the island of Ua Huka in the Marquesas archipelago (Payri, pers. com.). This present study reports the use of eleven species of algae in the Austral Islands and at least one in the Marquesas, as reported by three informants. Though only three persons confirmed the use of these species (four of which had not been previously recorded for human consumption), the results of this interview are worth describing due to the credibility of the main informant, Mama Lucie, who is evidently the most respected professional practitioner of *ra'au* on the island of Mo'orea and is reputed throughout Polynesia. Her services are requested on other islands and she often travels. Mama Lucie described five species in the *Caulerpa* genus, *Caulerpa racemosa*, *Caulerpa bikinensis*, *Caulerpa serrulata*, *Caulerpa urvilliana*, and *Caulerpa seuratii* — the latter three being first recorded for human uses — to be or have been consumed as food in the Austral Islands, as well as *Codium geppiorum*. The use of *Valonia aegagropila* was mentioned though not specified. Three of those eleven reported species, *Caulerpa racemosa*, *Enteromorpha intestinalis* and *Ulva lactuca*, were reported to be used for *ra'au*, though the purpose of their medicinal use was not explained. Coincidentally, these three species had previously been reported as comestible (Payri, N'Yeurt and Orempüller, 2001). Two species in the *Halimeda* genus, *H. distorta* and *Halimeda micronesia*, were reported to be used for decoration as hair ornaments. Out of these eleven species reported to be or have been used in the Austral Islands, only two were reported to be or have been used elsewhere; *Caulerpa racemosa* was reported to be used in the Marquesas, as reported by Claude Payri, and *Ulva lactuca* was reported to have once been used on Mo'orea and Tahiti, as reported by Setchell (1926).

Mme Frieder Lombard, who practices *ra'au* professionally as well, reported *Caulerpa racemosa* to be comestible, and called the alga "Rimu Ai Ai" (algae to chew or masticate). She also reported that *Hypnea pannosa*, previously reported as edible, was used in *ra'au* mixed with other sea products like coral and sea urchins to purge, to calm agitated babies and stabilize pregnant women's emotions. Mme Lombard also uses *Chnoospora minima* for *ra'au*, though its exact use was not specified. The species was first recorded for human consumption on the island of Ua Huka in the Marquesas by Payri and Conte (2002).

The use of *Ulva lactuca* was corroborated by two more informants. Rita You Sing described this species to have once been used for food on Mo'orea and Tahiti, eaten with coconut milk, while Pasteur Heitarauri said this alga is used in *ra'au* to treat goiter.

It is somewhat surprising that the consumption of algae has survived in the Austral Islands and the Marquesas archipelagoes while it has for the most part been forgotten or abandoned in the Society Islands, considering the strong evidence that Polynesians first arrived and settled in the Marquesas, from where they dispersed to the Hawaiian islands and other archipelagoes of French Polynesia. In theory, dietary habits and traditional medicinal recipes must have followed. Most informants mentioned the severe ban on the practice of traditional Tahitian therapy by French legislation, which was likely to have been more strictly enforced in the Society Islands; indeed, the two informants who were currently professional practitioners initially displayed obvious caution when giving information about the practice of *ra'au*. Although the socialized French medical system has become well established, local people, however, still do rely on the ancient traditional therapy; an average of 90% of people through the locales of Pao Pao and Temae still use *ra'au*, whereas 100% also use French medicine (Gaertner 1994). According to Mme Lombard, algae and other marine products were no longer used on these islands as frequently as they had once been due to modernization, industrialization and tourism, factors which all caused these marine organisms to retreat to greater depths or distances away from the shore. Healers are obviously unwilling to snorkel or SCUBA dive in order to gather these ingredients, and found ways to substitute them. Mme Lombard furthermore added that gathering medicinal plants had already become increasingly difficult due to socioecological changes and now required day long hikes into the mountains. This change in the practices of *ra'au* might also explain why healers who do use algae tend to use the two reported undetermined freshwater species that grow in pools and ponds of rivers rather than the marine species.

### *Future work*

A variety of subjects relating to this study could be analyzed in greater depth (no pun intended). Sites could be studied over a greater length of time to control the factor of seasonality, which is of great importance in algal growth and was not subject to control in the present study. More sites could be selected around the island to minimize bias relating to site distribution — for instance, alternating between estuary and non-estuary sites along the perimeter of the island. This factor is likely to be a source of bias in this study, considering that in the present study, three consecutive sites (Sites 4, 5 and 6) were all estuary sites, and that non-estuary algal and substrate distribution was not surveyed around the southern tip of the island. Additional transects could be conducted at greater depths and distances from the shore, in order to find out, for instance, how far the coverage of sedimentation deposited by estuaries extends into the lagoon. Other important factors — such as wave action, seasonality, and the topographical complexity of substrate surfaces — should be controlled in future studies. An interesting ethnobotanical investigation could consist of identifying and studying the ecology and uses of the undetermined freshwater algal species used by Tahitian healers.

## Conclusions

According to observations, the extensive amount of silt carried by the rivers and dumped into the lagoon at the mouths of estuaries seem strikingly disrupting to the environment when compared to the natural state of the substratum and water column. The geomorphology of shallow, accessible waters is of prime importance in defining the extent to which local people will or will not consume algae for food or medicine. The claims of one informant concerning the retreat of useful marine products to greater depths and distances from the shore might in this sense be relevant, considering that the sedimentation deposited on the substratum of the lagoon plays a role in minimizing settling surface availability. Furthermore, Polynesian society is rapidly changing, and though there has been a recent revival in the ancient tradition of *Ra'au Tahiti* and in the sense of pride felt by its practitioners and advocates, modernization has inevitably resulted in the loss of many traditions. Though little is known about algal consumption during the pre-European period in French Polynesia, it is not unreasonable to guess that the use of marine algae has in principle been lost in the Society Islands.

### Acknowledgements

I would like to sincerely thank all the Mo'orean who were helpful, hospitable, and kind, and who were willing to share the ancient and precious knowledge of *ra'au* with me. I would like to particularly thank Mama Lucie, her granddaughter Voehena, her sister Giselle, her daughter-in-law Linda, for all the mangoes, bananas, and pakaii and for welcoming me in their colorful homes, and to the exceptional Mme Lombard, to the You Sing family, to Papa Matarau, to Edouard Suhas for showing me his methodologies in isolating bioactive compounds from natural products, to Hinano Murphy who introduced me to the practices of *ra'au*, to my colleague John Holl, and to Claude Payri, who made this research possible.

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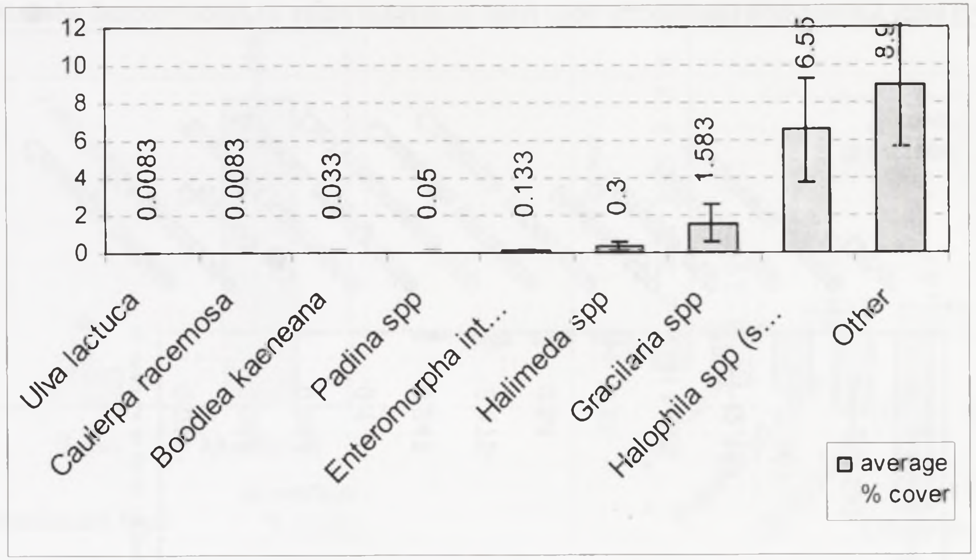
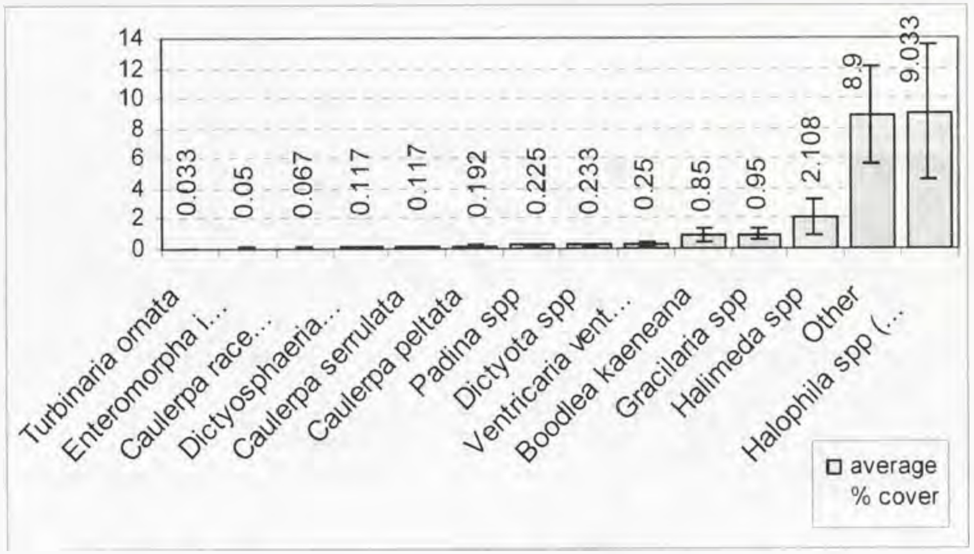
# APPENDIX

| *Species / genus* | *Source of var.* | *F-ratio* | ***P-value*** | Species / genus | Source of var. | F-ratio | **P-value** |
|---|---|---|---|---|---|---|---|
| *T. ornata* | EST | 6.605 | **0.021** | *Hypnea spp* | EST | 2.097 | 0.167 |
| | DEPTH | 6.892 | **0.018** | | DEPTH | 0.900 | 0.357 |
| | EST*DEPTH | 6.605 | **0.021** | | EST*DEPTH | 0.900 | 0.357 |
| *V. aegagropila* | EST | 4.184 | **0.058** | *Boodlea kaeneana* | EST | 0.559 | 0.466 |
| | DEPTH | 4.184 | **0.058** | | DEPTH | 0.490 | 0.494 |
| | EST*DEPTH | 4.184 | **0.058** | | EST*DEPTH | 1.647 | 0.218 |
| *Gracilaria spp* | EST | 2.759 | 0.116 | *Ulva lactuca* | EST | 1.528 | 0.234 |
| | DEPTH | 0.315 | 0.582 | | DEPTH | 1.550 | 0.231 |
| | EST*DEPTH | 0.107 | 0.748 | | EST*DEPTH | 1.528 | 0.234 |
| *Padina spp* | EST | 3.248 | 0.090 | *V. ventricosa* | EST | 0.585 | 0.455 |
| | DEPTH | 0.993 | 0.334 | | DEPTH | 1.514 | 0.236 |
| | EST*DEPTH | 1.208 | 0.288 | | EST*DEPTH | 0.585 | 0.455 |
| *Halophila spp* (seagrass) | EST | 3.035 | 0.101 | *C. peltata* | EST | 1.030 | 0.325 |
| | DEPTH | 0.077 | 0.785 | | DEPTH | 1.460 | 0.244 |
| | EST*DEPTH | 0.077 | 0.785 | | EST*DEPTH | 1.030 | 0.325 |
| *Dictyota spp* | EST | 3.029 | 0.101 | *C. racemosa* | EST | 0.735 | 0.404 |
| | DEPTH | 0.678 | 0.422 | | DEPTH | 1.205 | 0.288 |
| | EST*DEPTH | 0.299 | 0.592 | | EST*DEPTH | 0.787 | 0.388 |
| *C. serrulata* | EST | 2.757 | 0.116 | *Cyanobacteria* | EST | 1.000 | 0.332 |
| | DEPTH | 0.219 | 0.646 | | DEPTH | 1.000 | 0.332 |
| | EST*DEPTH | 0.525 | 0.479 | | EST*DEPTH | 1.000 | 0.332 |
| *E. intestinalis* | EST | 1.030 | 0.325 | *Halimeda spp* | EST | 0.868 | 0.365 |
| | DEPTH | 2.395 | 0.141 | | DEPTH | 0.011 | 0.916 |
| | EST*DEPTH | 1.476 | 0.242 | | EST*DEPTH | 0.749 | 0.400 |
| *Dictyosphaeria spp* | EST | 0.824 | 0.378 | *Other* (turf) | EST | 0.730 | 0.406 |
| | DEPTH | 2.245 | 0.153 | | DEPTH | 0.091 | 0.766 |
| | EST*DEPTH | 0.824 | 0.378 | | EST*DEPTH | 0.356 | 0.559 |

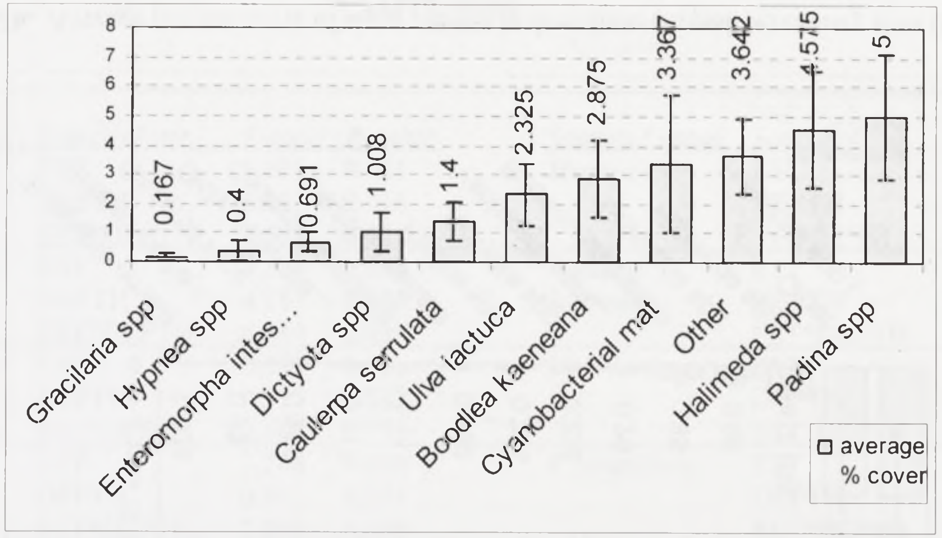
**Appendix 1.** 2-way ANOVA results for distribution of algal species and genera.



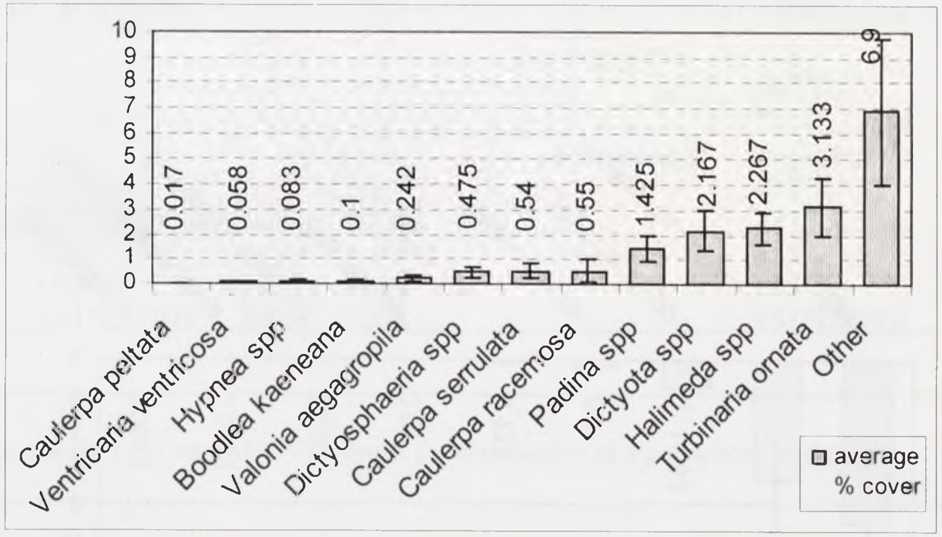
**Appendix 2a.** Average percent cover of algae present in shallow (accessible) estuary sites (S EST)



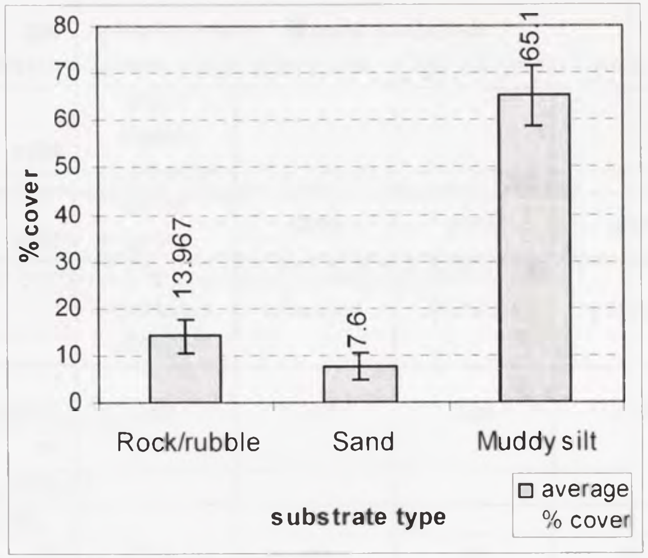
**Appendix 2b.** Average percent cover of algae present in deep (non-accessible) estuary sites (D EST)



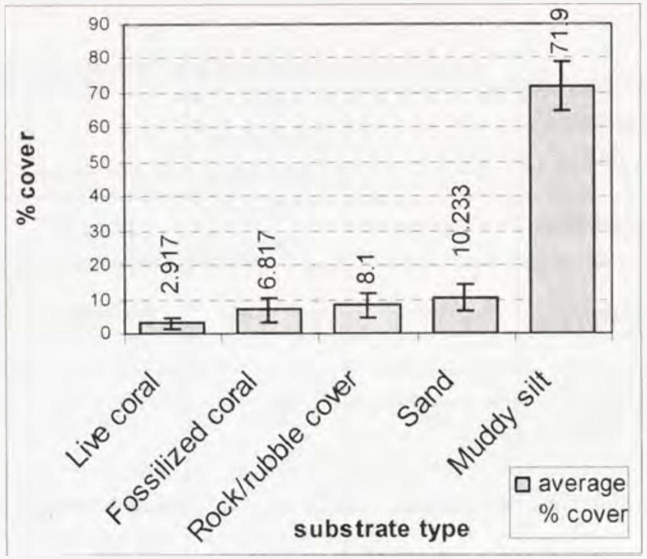
**Appendix 2c.** Average percent cover of algae present in shallow (accessible) non-estuary sites (S NON EST)



**Appendix 2d.** Average percent cover of algae present in deep (non-accessible) non-estuary sites (D NON EST)



**Appendix 3a.** Average substrate and sediment composition of shallow (accessible) estuary sites (S EST)



**Appendix 3b.** Average substrate and sediment composition of deep (non-accessible) estuary sites (D EST)

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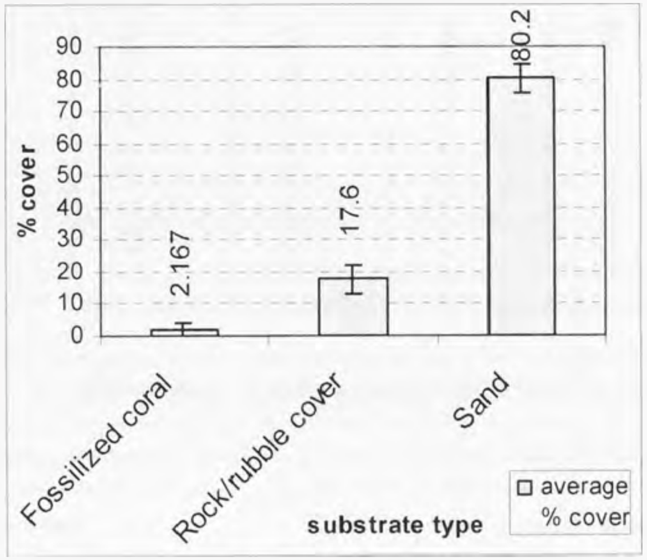
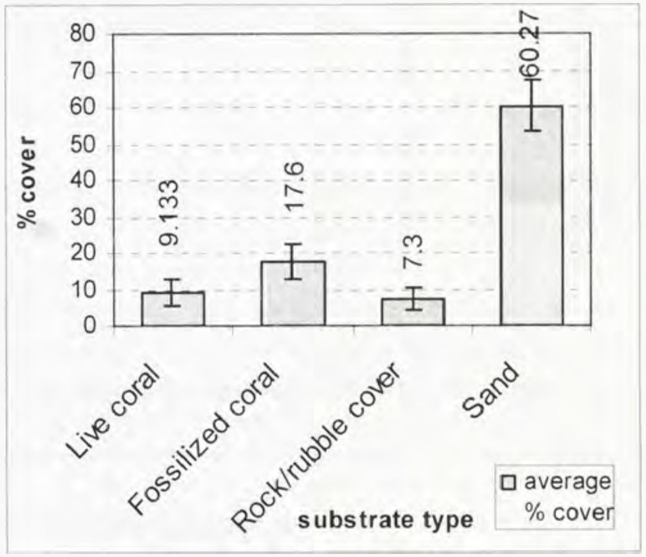


**Appendix 3c.** Average substrate and sediment composition of shallow (accessible) non-estuary sites (S NON EST)



**Appendix 3d.** Average substrate and sediment composition of deep (non-accessible) non-estuary (D NON EST)

| *Site #* | Site 1 | Site 2 | Site 3 | Site 4 | Site 5 | Site 6 | Site 7 | Site 8 | Site 9 | Site 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| *Salinity (‰)* | 34 | 36 | 37 | 4 | 32 | 34 | 36.5 | 36 | 31 | 36.5 |

**Appendix 4.** Average salinity levels at ten sites in parts per thousand

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**Appendix 5.** Compiled results from interviews with practicioners of traditional Tahitian therapy, *Ra'au Tahiti*

| | *Caulerpa racemosa* | *Caulerpa serrulata* | *Caulerpa urvilliana* | *Caulerpa bikinensis* | *Caulerpa seuratii* | *Codium geppiorum* | *Ulva lactuca* | *Enteromorpha flexuosa* | *Halimeda distorta*<br>*Halimeda micronesia* | *Valonia aegagropila* |
|---|---|---|---|---|---|---|---|---|---|---|
| Has this alga ever been used or consumed on Mo'orea and Tahiti? | no | no | no | no | no | no | yes | no | no | no |
| Has this alga ever been used or consumed elsewhere in Polynesia? | yes | yes | yes | yes | yes | yes | yes | yes | yes | yes |
| If yes, where? | Australs<br>Marquesas | Australs | Australs | Australs | Australs | Australs | Australs | Australs | Australs | – |
| What was this alga used for? | food<br>*ra'au* | food | food | food | food | food | food<br>*ra'au* | *ra'au* | decora-tion | food |
| How was this alga used? | eaten with lemon juice | – | – | – | – | – | eaten raw in poisson cru | – | hair ornament | – |
| Names and native origin of the informants | Mama Lucie, practioner of *Ra'au Tahiti* (since the 1950's), her sister Giselle, her daughter-in-law Linda<br>Austral islands, French Polynesia | | | | | | | | | |

| | *Ulva lactuca* | *Caulerpa racemosa* |
|---|---|---|
| Has this alga ever been used or consumed on Mo'orea and Tahiti? | yes | yes |
| Has this alga ever been used or consumed elsewhere in Polynesia? | – | – |
| If yes, where? | – | – |
| What was this alga used for? | *ra'au* | food |
| How was this alga used? | consumed to treat goiter | eaten with lime and coconut milk |
| Name and native origin of the informant | Pasteur Heitarauri. Mo'orea, French Polynesia | |

| | *Ulva lactuca* | *Enteromorpha flexuosa* |
|---|---|---|
| Has this alga ever been used or consumed on Mo'orea and Tahiti? | yes | yes |
| Has this alga ever been used or consumed elsewhere in Polynesia? | – | – |
| If yes, where? | – | – |
| What was this alga used for? | food | food |
| How was this alga used? | eaten raw with coconut milk | eaten with oil and vinegar |
| Name and native origin of the informant | Rita You Sing. Tahiti, Society Islands, French Polynesia | |

| | *Hypnea pannosa* | *Chnoospora minima* | *Caulerpa racemosa* | undetermined freshwater species n° *1* | undetermined freshwater species n° *2* |
|---|---|---|---|---|---|
| Has this alga ever been used or consumed on Mo'orea and Tahiti? | yes | yes | yes | yes | yes |
| Has this alga ever been used or consumed elsewhere in Polynesia? | – | – | yes | – | – |
| If yes, where? | – | – | – | – | – |
| What was this alga used for? | *ra'au* | *ra'au* | food | *ra'au* | *ra'au* |
| How was this alga used? | mixed with sea products like small crabs, sea urchins, coral, used for purging, to calm babies and stabilize pregnant women's emotions | – | eaten with lemon juice 'Rimu Ai Ai' [algae to masticate or chew] | mixed with flowers of the male papaya tree to kill intestinal worms; called 'Pipaetea,' described to be green, similar in shape to *Hypnea* but with rounded tips, grows like *Galaxaura* | mixed with 162 other plants in a cold bath to treat fractures, causing shivering, which settles the bones, and hardening of the skin, which works like a cast; therapy is called 'Ra'au Fativa,' and is said to involve the help of children; described to resemble algae belonging to the *Cladophoraceae* family |
| Name and native origin of the informant | Mme Frieder Lombard, practioner of *Ra'au Tahiti* (since the 1960's, publicly since 1994)<br>Tahiti, Society Islands, French Polynesia | | | | |

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# Spatial heterogeneity and species diversity of marine algae

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ABSTRACT. Understanding the effects of spatial heterogeneity in ecological communities is a central goal of community ecology. This study examines the relationship between small scale (1-10 cm) topographic heterogeneity and species richness among 57 marine algal species. Patterns of algal recruitment on artificial substrates (clay tiles) varying in their degree of spatial structure were compared with natural assemblages. A strong positive relationship between spatial heterogeneity and species richness was detected for recruitment tiles but not for natural assemblages. Algal species were further classified as "recalcitrant" or "labile" according to their possession of herbivore-resistant traits. A negative relationship was detected between spatial heterogeneity and degree of recalcitrance among algal species for experimental tiles but not for natural assemblages. This indicates that heterogeneity creates spatial refugia from herbivory for less protected species on short successional time scales but that this benefit disappears at longer time scales.

Monte Carlo simulation confirmed that the mechanism of the observed heterogeneity-richness relationship was the result of increased microhabitat diversity in more complex substrates. No relationship was detected in natural assemblages for several reasons. Body size scales in proportion to successional sere and age, and larger individuals may not perceive heterogeneity on the scale measured. Moreover, on a per-area basis, large bodied organisms are less species rich than small bodied ones. Thalus size of late successional species were typically one order of magnitude greater than the measured heterogeneity. Likewise, thalus size of early successional species were typically one order of magnitude less than the measured heterogeneity. Larger, more competitive late successional species eventually dominate the habitat, reducing local diversity. A preliminary synthesis is developed on the relationship between spatial heterogeneity, trophic structure, life-history variation, disturbance, and body size, in relation to relative abundance, diversity, and scale.

KEYWORDS: spatial heterogeneity, scale, diversity, algae, body size, community theory, herbivory, succession

## Introduction

Classical models of community dynamics predict there to be as many or fewer species than the number of limiting resources in an environment (Gause 1934, 1935, Lack 1954, Levins 1979, Tilman 1982). Using equilibrium solutions to Lotka-Volterra type competition equations, these models demonstrate appreciable success in predicting the outcome of two-species competition in closed, homogenous, laboratory systems (Tilman 1976). However, they fail to predict many other ecologically important phenomena, including the great diversity of species found in natural systems (Andrewartha & Birch 1954). This failure is largely due to these models' assumption of equilibrium conditions, spatial homogeneity, uniform life histories, and simple trophic structure (Hutchinson 1941, 1961, Tilman & Pacala 1993).

Relaxing any one of these simplifying assumptions invariably leads to an increase in the predicted number of coexisting species. Using diverse mathematical approaches, several models conclude that interspecific segregation (Skellam 1951, Horn & MacArthur 1972, Levin 1974) and intraspecific aggregation (Pacala & Silander 1985, Pacala 1986) could prevent the local extinction of competitively inferior species. Focusing on higher-order trophic interactions, Paine (1966) and Caswell (1978) among others argue that the introduction of a predator may promote coexistence of competing prey species by preventing competitive exclusion through a reduction in prey densities. Janzen (1970) and Connell (1971) hypothesize that increasing the number of trophic levels could account for high species richness through spatial and temporal variation in predation intensity. In these models, frequency-dependent predation reduces the densities of the superior competitors, promoting coexistence by allowing species to benefit from being rare.

Building on the early ideas of Hutchinson (1941, 1961), non-equilibrium models predicted coexistence though periodic reduction in the densities of all species (Connell 1978) and through fluctuations in certain environmental factors affecting the outcome of competition (Sale 1977, Huston 1979, Chesson 1986). Furthermore, life-history variation can promote coexistence in the form of a tradeoff between colonization ability and competitive ability, but only if the habitat is spatially subdivided (Levins & Culver 1971, Tilman 1994).

This rich body of theoretical mechanisms suggests new syntheses and experimental tests. Local diversity is, after all, likely best explained by a combination of these mechanisms coupled to regional processes operating over larger spatial and temporal scales (Levine & Rees 2002, Hubbell 2002, Levine 2000, Hanski & Gilpin 1997). Predictive failures may be reflective of inadequate syntheses. Lubchenko (1983, 1986) provides one of the best empirical investigations into the relative importance of these various diversity generating mechanisms. She demonstrates that substrate heterogeneity and seasonal variability in the intensity of physical disturbance and grazing explain widespread patterns of dominance and diversity among intertidal New England algal species. Substrate heterogeneity creates refugia from grazing which leads to the coexistence of ephemeral herbivore-vulnerable species and perennial herbivore-tolerant species. In the absence of either grazing or heterogeneity, competitive exclusion occurs. Additionally, the strength of this interaction is dependent on the seasonal intensity of disturbance in regulating predator numbers.

Despite recent interest in the biogeographic determinants of diversity (Roughgarden 1986, Hubbell 2002), local mechanisms still need to be studied (Ricklefs 1987). This is especially important after considering that many of the processes responsible for generating diversity among individuals on small scales (i.e. disturbance, heterogeneity, predation) also operate among populations on regional scales (i.e. glaciation, landscapes, disease epidemics) (Amarasekare 2000).

The goal of this study is to test the hypothesis that spatial heterogeneity promotes species coexistence by increasing microhabitat diversity. The generality of this mechanism will be compared across successional time scales, and the interactive effects of herbivory will be addressed. Spatial heterogeneity is defined as variation in a measured parameter between different samples in space (Kolasa & Rollo 1991). The type of spatial heterogeneity used is variation in small scale (1-10 cm) substrate topography.

## Methods

### *Study location*

This study was conducted in a lagoon on the island of Moorea, French Polynesia (S 17° 28.741, W 149° 49.884). Corals from the genera *Acropora*, *Porites*, and *Pocillopora* form large calcareous outcroppings that create most of the topographic heterogeneity present at the site. Corals are interspersed with coral-derived sand and debris. Dead intact corals provide the primary substrate for algal growth. Dominant macroalgae include *Turbinaria ornata*, *Sargassam* spp., *Halimeda* spp., and several species of calcareous encrusting algae. Herbivorous fish and crustaceans are highly abundant. Currents flow across the reef crest drawing water from the open ocean into the lagoon. Flow rate is highly variable depending on the size of the open ocean swell. Typical values are between one and 5 km/h (C. T. DiVittorio, personal observation).

### *Recruitment tiles*

In order to study patterns of algal recruitment, unglazed terra cotta tiles were placed in 2-3 meters of water durring October 2002. These tiles are ideal for recruitment studies because they closely resemble the texture of natural substrates and offer a uniform sampling area. The tiles measure 11.5 $cm^2$ and were secured to concrete blocks with metal rods that placed them 0.25 meters above the sea floor (Figure 1). Standard flat tiles were used to create four discrete levels of topographic heterogeneity with increasing microhabitat diversity. The first (least heterogeneous) level used standard, flat, up-facing tiles. For the second level, standard flat tiles were cut into 1 $cm^2$ x 11.5 cm long strips and attached to the flat surface of an intact tile at 1 cm intervals, creating parallel 1 $cm^2$ grooves. The third level consists of level two grooved tiles, with an additional standard flat tile attached down-facing on the bottom. The fourth (most heterogeneous) level used what was described for level three, with an additional up-facing standard flat tile attached 2 cm below the down-facing tile (Figure 1). An inventory of the microhabitat diversity present at each level is summarized in Figure 2. In order to control for sample area, multiple "units" as described above are used in the ratio 4: 2 : 1.3 : 1 for levels 1, 2, 3, and 4, respectively. Thus, each statistical replicate

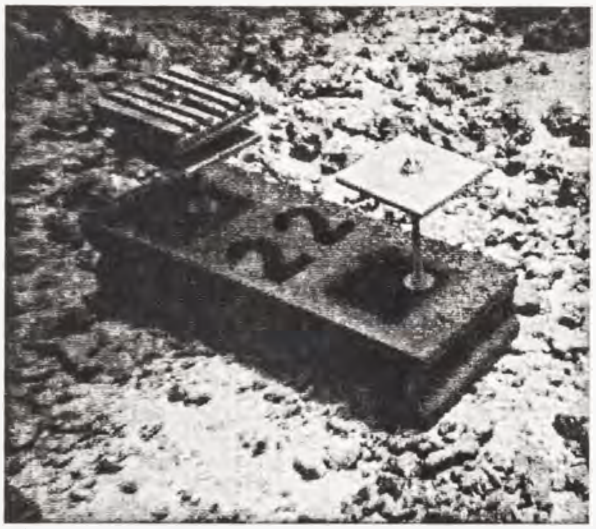

Figure 1: Photograph of the experimental apparatus at the beginning of the study. A level one complexity unit is shown on the right, and a level four complexity unit is shown on the left. The different tiles that compose the level four unit are visible. Tiles measure 11.5 $cm^2$. The sea floor is composed of mixed coral sand and debris.

consists of 4 level one units, 2 level two units, 1.3 level three units, and 1 level four unit. This method is superior to dividing the number of observed species by the sample area because it eliminates the confounding influence of nonlinearities in species-area relationships. Each treatment level was replicated five times and deployed in a randomized single-block design. After four weeks, replicates were collected sequentially and brought to the laboratory for analysis. Each tile was individually examined with a dissecting microscope using timed equal-effort sampling. Five minutes were spent on flat tiles and 10 minutes on grooved tiles (due to a doubling of surface area on grooved tiles). Presence of all algal species was recorded. Each new species was photographed on compound and dissecting microscopes at four magnifications for later identification. Data was analyzed using linear least squares regressions with level of heterogeneity as the independent variable and species richness as the dependent variable ($\alpha = 0.05$).

*Natural assemblages*

Substrate topographic heterogeneity and species diversity of 8 natural assemblages were directly measured at a nearby site. For each assemblage, topographic heterogeneity was measured using a one meter long chain with link length of 1.3 cm. An index of complexity was

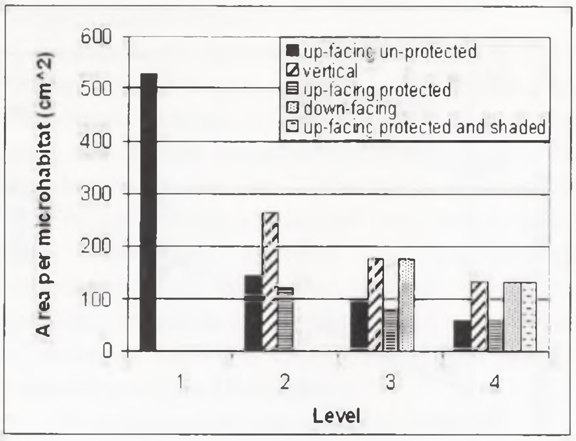

Figure 2: Microhabitat diversity for the four levels of experimental topographic complexity. Both the richness and evenness of microhabitats is increased at higher levels of complexity. Total microhabitat area is held constant for all levels.

calculated using the equation $S = 1 - ( a / x )$ where $x$ represents the actual straight length of the chain, and $a$ represents the linear distance traveled by the chain when made to conform to the contours of the substrate. Species richness was sampled by line-intercept sampling. Presence of all algal species intercepted by the chain was recorded. Data was analyzed using linear least squares regression with index of heterogeneity as the independent variable and species richness as the dependent variable ($\alpha = 0.05$).

*Heterogeneity and herbivory*

In order to gain insight into the mechanisms driving observed patterns in species richness, comparison was made between heterogeneity and the proportion of species exhibiting herbivore-resistant traits. "Recalcitrant" species were those that were either calcified, filamentous, or otherwise known to the author to exhibit herbivore-resistant physical or chemical traits. "Labile" species were those that exhibited mucilaginous, hollow, laminar, or single celled morphologies. Data was analyzed using linear least squares regression with level or index of heterogeneity as the independent variable, and proportion of species recalcitrant as the dependent variable ($\alpha = 0.05$).

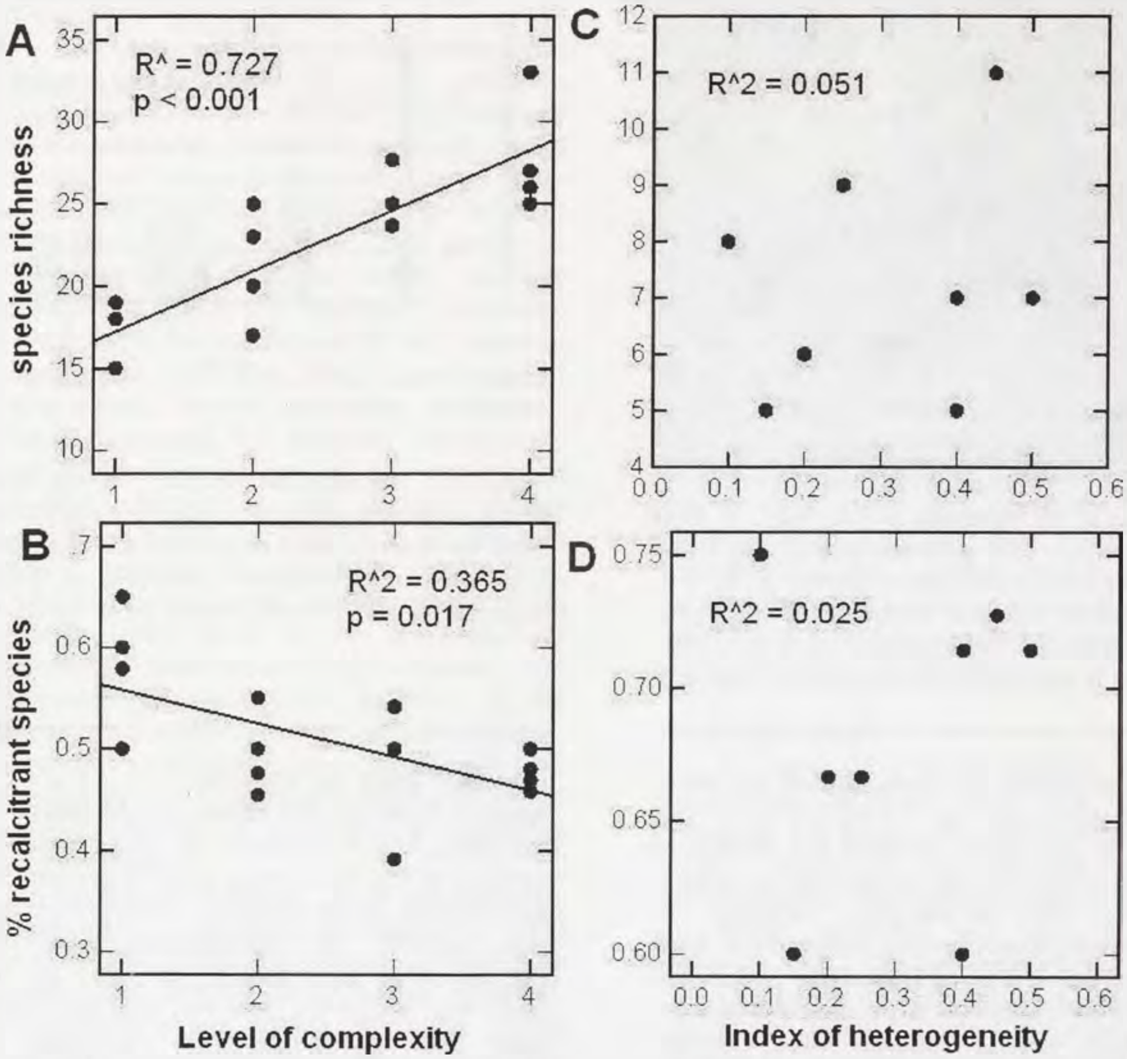

Figure 3: Strong relationships were detected on recruitment tiles for (a) species richness and spatial heterogeneity, and (b) proportion recalcitrant species and spatial heterogeneity. No relationship was found in natural assemblages for (c) species richness and heterogeneity, or for (d) proportion recalcitrant species and heterogeneity. All regressions are linear least squares with $\alpha = 0.05$. $n = 15$ and 8 for recruitment tiles and natural assemblages, respectively.

*Nonrandom subsets in species composition*

Heterogeneity is hypothesized to promote species richness by increasing the number of different microsites available for recruitment. Species present at one level of heterogeneity nonrandom subsets of the communities found on more heterogeneous tiles. Low heterogeneity tiles should share more species with high heterogeneity tiles than would be expected if community assembly were a random draw from a common species pool.

In order to determine whether nonrandom subsets exist, presence/absence data was pooled for all replicates in each level. This generated a 1 x $n$ matrix for each level of heterogeneity would also be present at the next highest level, in addition to a new suite of species occupying the new habitats. This model predicts that the community composition of low heterogeneity recruitment tiles should be where $n$ is the total number of species documented during study. Binary simple matching similarity coefficients were calculated for levels one and two, two and three, and three and four (Krebs 1999). A Monte Carlo numerical parameter estimation technique was used to generate a null distribution. Community composition data was then randomized and similarity coefficients recalculated. This process was iterated 1000 times. True similarity coefficients were tested against the simulated expectation using one-tailed 95% and 99% confidence intervals (alternate hypothesis: expectation < actual).

### *Statistical analysis*

All tests were performed with SYSTAT® version 7. Monte Carlo simulation was performed using the PopTools add-in for Microsoft Excel®.

## **Results**

### *Recruitment tiles*

53 distinct taxa were documented on recruitment tiles (Appendix A), representing 21 species of red algae, 9 species of green algae, 9 species of brown algae, 11 species of colonial cyanobacteria, and 2 species of diatom. Heterogeneity was strongly and positively related to species richness on experimental recruitment tiles ($R^2 = 0.727$, $p < 0.001$, $n = 15$) (Figure 3a). A moderately strong negative relationship was observed between heterogeneity and proportion of recalcitrant species ($R^2 = 0.365$, $p = 0.017$, $n = 15$) (Figure 3b). Frequent grazing by damselfish and surgeon fish on recruitment tiles was directly observed in the field. All microhabitats designated "up-facing unprotected" (Figure 2) exhibited scrape marks characteristic of grazing by fish. No evidence of grazing by fish were observed on any of the other microhabitat types. Greater than 90% of algae individuals observed measured less than 1 cm along their longest measurable axis with an average of 2-3 mm.

### *Natural assemblages*

11 taxa of algae were documented in the 8 natural assemblages sampled (Appendix A). *Turbinaria ornata, Halimeda* spp., *Sargassam* spp., and a pink coralline encrusting type were the dominant species. The total richness of natural assemblages sampled was much lower than the total richness of experimental recruitment tiles. However, the use of different sampling methods invalidates direct comparison of richness between experimental and natural assemblages (see Discussion).

No relationship was detected between topographic heterogeneity and species richness ($R^2 = 0.051$, $p = 0.590$, $n = 8$) (Figure 3c). No relationship was detected between topographic heterogeneity and proportion of recalcitrant species ($R^2 = 0.025$, $p = 0.710$, $n = 8$) (Figure 3d). No direct observations of grazing by fish were made, although fish can be seen grazing frequently on natural assemblages elsewhere on the reef. No evidence of grazing by fish was observed during sampling.

### *Nonrandom subsets*

The results of the Monte Carlo simulation is summarized in Table 1. Simulations detected highly nonrandom subsets for the pair-wise comparisons. The community composition of lower complexity level tiles share more species with higher level tiles than would be expected at random. All comparisons were significant at $\alpha = 0.05$.

Table 1: Results of Monte Carlo numerical simulations. All comparisons displayed greater similarity than would be expected based on random recruitment. Binary simple matching similarity coefficients were used. Simulated values based on 1000 iterations.

| comparison (levels) | actual coefficient | simulated coefficient | one-tailed CI | | significant at p = | |
|---|---|---|---|---|---|---|
| | | | 95% | 99% | 0.05 | 0.01 |
| 1 and 2 | 0.855 | 0.501 | 0.600 | 0.636 | yes | yes |
| 2 and 3 | 0.782 | 0.548 | 0.636 | 0.709 | yes | yes |
| 3 and 4 | 0.691 | 0.611 | 0.691 | 0.727 | yes | no |

## Discussion

### *Topographic heterogeneity and species richness*

Heterogeneity most likely affects species diversity through the creation of novel microhabitats for settlement, growth, and reproduction. Microhabitats differ in their amount and type of essential resources. Variation in resource availabilities leads to variation in interaction strengths and alters competitive hierarchies (Tilman 1982). Topographic variation additionally creates refugia, introducing spatial variation in predation intensity (Lubchenko 1986). Furthermore, topography influences physical processes of biological importance such as disturbance and fluid transport.

The expansion of the range of states assumed by these and other ecologically important parameters leads to the growth of what Whittaker (1975) termed an *n*-dimensional hypervolume, within which species are packed according to some limiting similarity in their requirements for existence (MacArthur & Levins 1967, MacArthur 1970). Within this framework, spatial heterogeneity has been postulated to be an important regulator of local diversity (Tilman & Pacala 1993). The results of this study strongly support this prediction (Figure 3). Furthermore, this relationship is thought to be generated by the direct effects of heterogeneity on microhabitat diversity (Table 1).

However, the generality of this relationship must be scale-explicit, both temporally and spatially. The question remains as to why the relationship was not observed in natural assemblages (Figure 6), and through what mechanism microhabitat diversity itself promotes species richness. As will be shown, successional time, organism body size, and the fractal nature of environmental heterogeneity must be considered (Hutchinson 1959, Morse et al. 1985, May 1986, Steinberg & Kareiva 1997, Stevenson 1997).

Before proceeding with any comparison of experimental and natural sites, some assumptions must be stated. First, no differences are assumed in settlement preferences between experimental and natural substrates. Second, rates of resource and propagule supply, environmental conditions and variability, and predation intensity are constant through time. While the former is debatable, the latter is surely a source of error. The question is whether this represents a significant source of bias so as to obscure the processes and patterns that operate in the system and that were or were not detected by the statistical analysis. A third source of error is also apparent, that sample sizes were different between experimental and natural assemblages ($n$ = 15 versus 8, respectively). This source of sampling error must be weighed against the strength of the detected correlations (or lack of correlations). I believe that the correlations (or lack thereof) are sufficiently robust so as to ignore the third source for the present.

These assumptions having been considered, some obvious differences exist between the communities on experimental versus natural substrates. First, recruitment communities can be considered early successional relative to the mature assemblages. Common features of early successional species include greater investment in rapid growth and reproduction, and less in resource acquisition (a correlate of competitive ability) and secondary defensive compounds. Moreover these tend to be small, have short lifetimes, and may exhibit higher degrees of specialization. By comparison, natural assemblages can be considered late successional. Common traits are relatively greater investment in defensive compounds and resource capture, and less in rapid growth and reproduction. Individuals are much larger at maturity, much longer lived, and may exhibit less habitat specificity. Based on these generalities from the common body of successional theory, five suggestions can be made about what explains the patterns observed in species richness.

First, the spatial refuge from herbivory afforded by increased microsite diversity would be beneficial to labile pioneer species but neutral to recalcitrant late successional species (Lubchenko 1983, 1986).

Second, pioneer species face high fitness costs from competition, which may select for specialization among these species (Helle 1985). Although there is little in the published literature studying this relationship, heterogeneity itself may also select for specialization in organisms whose body sizes allow that heterogeneity to be functionally important (see next paragraph) (Korona et al. 1994, Bell 1997, Rainey & Travisano 1998). Specialization often correlates with rarity (Jenkins & Hockey 2001, Harcourt et al. 2002, Jakobsson et al. 1998), and rare species comprise the majority of species richness of most communities (MacArthur 1957, 1960).

Third, body size scales up with age. If body size becomes too large, heterogeneity on the scale measured becomes transparent. This is especially true for marine algae, which utilize the substrate not as a source of heterogeneously distributed nutrients but simply as an attachment for the holdfast (Wilson 2000). Selection for specialization and the spatial herbivory refuge are eliminated as body size becomes too large.

Fourth, body size scales up with successional stage. This is a natural result of the fact that late successional organisms are older (Peters 1983).

Fifth, an important but little studied body of research on the distribution of organism body sizes in communities reveals that there are far more small bodied organisms than large bodied organisms, in terms of both individuals and species, both within and among trophic levels (Hutchinson and MacArthur 1955, Hutchinson 1961, Peters 1983, May 1986).

This framework is consistent with the findings of other published studies. In a recent study on patterns of grasshopper diversity in North America, Davidowitz and Rosenzwieg (1998) found that latitudinal gradients of grasshopper diversity are not related to the degree of local-scale heterogeneity. This finding agrees with the suggestion that the scale of a system's heterogeneity must be similar to the scale of organism body sizes. Small scale heterogeneity of prairies explains grasshopper diversity within prairies due to local processes such as increased opportunities for specialization and increased prey refugia. Small scale heterogeneity would not be expected to predict between prairie grasshopper diversity due to the scaling relationships discussed. Biogeographic determinants most likely supercede local processes at this scale of observation.

Buckling et al. (2000) demonstrated that within experimental microcosms, genomic diversity of the bacterium *Pseudomonas fluorescens* is enhanced by intermediate levels of disturbance (defined as an indiscriminant reduction in population density) but only in the presence of spatial structure. Homogenization of the microcosm environment by agitation lead to significant decreases in diversity for all levels of disturbance. Diversity was enhanced with intermediate levels of disturbance only when "spatial niches" were present. In this example, disturbance promotes spatial heterogeneity in the presence of disturbance, but disturbance does not itself create diversity. The design of the study makes this distinction tractable. In its purest form, the intermediate disturbance hypothesis has as an essential element, the heterogeneous distribution of disturbance in the habitat (Connell 1978, Sousa 1984). Disturbance itself, per se, does not generate or maintain diversity, except through a homogenous reduction in competitor densities (sensu Paine 1966). The spatial component is essential. This is consistent with the framework of this study, whereby disturbance operates indirectly to enhance diversity, through the promotion of spatial and temporal heterogeneity.

The findings of this study are also consistent with other small scale heterogeneity manipulation experiments with benthic invertebrates (Gilinsky 1984), plants (Vivian-Smith 1997), benthic microalgal films (Sommer 2000), and ammonia oxidizing soil bacteria (Webster et al. 2002).

**Summary**

The data collected suggests that spatial heterogeneity measured directly as the diversity of microhabitats, promotes species richness for those taxa whose body size and mobility permit functional perception of that heterogeneity. Functional heterogeneity can thus be defined as that which an organism experiences and may react to (Kolasa & Rollo 1991). Additionally, the mechanism of this relationship is the generation of unique suites of microhabitats to which different species' life-histories are better suited.

With regard to body size, some scaling properties need to be considered. Body size scales nonlinearly to the abundance and richness of taxa within those size classes (May 1978). Using information about the scaling relationships of body size, relative abundance, species richness, and environmental heterogeneity, it may be possible to predict the richness and relative abundances of species in early versus late succession. These are simple geometric relationships, but relationships that to my knowledge have not been explored in depth. There has been little work since Hutchinson wrote in his *Homage to Santa Rosalia*, "Irrespective of their position in a food chain, small size, by permitting animals to become specialized to the conditions offered by small diversified elements of the environmental mosaic, clearly makes possible a degree of diversity quite unknown among groups of larger organisms" (p. 155, Hutchinson 1959).

Further research is needed on the following topics. A dual empirical perspective is needed. One line of research needs to conduct more synthetic studies—multifactorial studies that examine the interactive effects of heterogeneity, disturbance, life-history variation, and all of the other proposed diversity generating processes. A second line of research should attempt to examine the effect of each of these processes operating without the confounding influence of spatial or temporal heterogeneity. Predation may not intrinsically promote diversity without spatial and temporal variation in its intensity. Likewise, disturbance may not intrinsically have any effect on diversity without the spatial and temporal heterogeneity it fosters.

Clearly, more work on body size and the fractal dimensions of heterogeneity needs to be performed (Morse et al. 1985, May 1986) and integrated with present thinking about species-area and species-abundance relationships. Geometrical relationships between these quantities can be linked formally to species diversity and relative abundance, and are worthy of future study.

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## APPENDIX A.

Taxa of algae documented during the study (Dick Moe, University of California Herbarium performed all identifications). Classification as recalcitrant or not is shown. All algae fully documented by digital photography at 10x, 40x, 100x and occasionally 1000x on compound microscopes, and up to 5x using dissecting microscopes.

| taxa | recalcitrant |
|---|---|
| **Red algae** | |
| Rhodomelaceae Polysiphonia? | yes |
| Rhodomelaceae Herposiphonia? | yes |
| Rhodomelaceae Herposiphonia? | yes |
| Polysiphonia | yes |
| Ceramiaceae | yes |
| *Ceramium* | *yes* |
| Herposiphonia? | yes |
| Ceramiaceae Ceramium | yes |
| Ceramiaceae | yes |
| Acrochaetiaceae Acrochaetium | yes |
| *Laurencia* | *no* |
| *Hypnea* | *no* |
| *Chondria* | *no* |
| Rhodymeniales Champiaceae | no |
| Dasyaceae Dasya | no |
| Callithamniella | no |
| Rhodomelaceae | no |
| *Laurencia* | *no* |
| *Laurencia* | *no* |
| Rhodomelaceae | no |
| unknown red | yes |
| **Brown algae** | |
| Sphacelariaceae? | yes |
| Ectocarpaceae Ectocarpus | yes |
| Ectocarpacaea? | Yes |
| Ectocarpaceae | Yes |
| *Sargassam* sp. | Yes |
| *Turbinaria ornata* | Yes |
| Ectocarpaceae | No |
| unknown brown 1 | yes |
| unknown brown 2 | yes |
| unknown brown 3 | no |
| unknown brown 4 | no |
| **Green algae** | |
| Ulvaceae | yes |
| Ulvaceae? | yes |
| Ulvaellaceae | yes |
| Ulvaellaceae | yes |
| *Enteromorpha* | *no* |
| *Cladophora* | *no* |
| *Halimeda* sp. | yes |
| unknown green 1 | no |
| unknown green 2 | no |
| unknown green 3 | yes |
| **Bluegreeen algae (cyanobacteria)** | |
| Oscillatoriaceae | yes |
| Oscillatoriaceae | yes |
| unknown bluegreen 1 | yes |
| unknown bluegreen 2 | yes |
| unknown bluegreen 3 | no |
| unknown bluegreen 4 | no |
| unknown bluegreen 5 | no |
| unknown bluegreen 6 | no |
| unknown bluegreen 7 | no |
| unknown bluegreen 8 | no |
| unknown bluegreen 9 | no |
| **Diatoms** | |
| unknown diatom 1 | yes |
| unknown diatom 2 | no |

## APPENDIX B.

Full digital photographic documentation of all species appearing in Appendix A are available by contacting the author (chris_td@uclink4.berkeley.edu, 4003 Valley Life Sciences Building, Department of Integrative Biology, University of California, Berkeley 94720).

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# Coral and Invertebrate Recruitment Patterns in Moorea, French Polynesia

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ABSTRACT. Coral reefs display a considerable spacio-temporal variability, which makes their species composition, age structure, reproduction and recovery from disturbance very difficult to predict. Coral reefs world-wide are becoming increasingly threatened by natural and anthropogenic impacts, and due to their enormous importance as a natural resource, increased knowledge of reefs' reproductive biology is necessary for proper management and restoration. The objectives of this study were to document patterns of coral and invertebrate recruitment to artificial substrata (unglazed ceramic tiles) in Moorea, French Polynesia and to determine if there is any effect of site, depth and/or grazing on recruitment. Four sites were tested, characterizing the two bays, back-reef and fore-reef habitats, as well as two depths at each site (15 & 40 ft.). At each site and depth there were three tiles covered by a wire mesh cage and three left uncaged to describe the herbivory effects on recruitment. A total of 9 coral recruits comprising two families, Pocilloporidae and Acroporidae, were found on 42 tiles in one month. No significant relationships between site, depth, and caged/uncaged were found for acropora recruits. For pocillopora recruits, a significant site effect was revealed ($p=0.029$), as well as a nearly significant caging effect ($p=0.055$). A significant effect of depth on pocillopora recruits was found, as well as a significant interaction between site and depth, revealed by a 2-way ANOVA ($p=0.029$). Invertebrate recruits were grouped into functional taxa- bivalves, tube-dwelling polychaetes, encrusting sponges and tunicates. Significant site effects were found for bivalves, porifera and bryozoans. Significant depth effects were found for bivalves, tunicates and polychaetes. Significant caging effects were found for bryozoans, tunicates and polychaetes.

Keywords: Acroporidae, Pocilloporidae, Bryozoa, depth, herbivory

## Introduction

Coral diversity and abundance is a striking feature of the reef environment and is the result of an incredibly complex web of individuals and interactions. Coral reefs species diversity rivals tropical rain forests (supporting 1-9 million species), and within its assemblages 32 of 33 phyla are represented (Reaka-Kudla 1996). Coral reefs are an enormously important resource for the countries privileged to have them as well as the entire planet. They provide coastal island communities protection from storms and coastline erosion, and an enormous supply of valuable fauna. Coral reefs account for only 0.2% of the world's oceans yet provide developing countries 25% of their annual fish catch (Sale 1999). In the context of world importance, coral reefs function as CO2 sinks, sequestering and processing CO2 from the atmosphere. A loss or weakening of this carbon sink will increase already inflated greenhouse gas levels and accelerate global warming (Garfield 2001).

In an effort to better understand the spatial and temporal abundances of corals, many studies have looked at recruitment. Recruitment is defined as the number of new individuals that settle and survive until the time of observation and may be an indication of patterns of larval arrival, availability and choice of suitable substrate or post-settlement mortality (Connell 1985). Factors that are known to direct coral settlement and/or recruitment include sedimentation (Hodgson 1990), biomass of the fouling community (Birkeland 1977), euthrophication (Tomascik 1991), solar irradiance in the visible and ultraviolet spectrum (Babcock & Mundy 1996, Kuffner, 2001), hydrographic conditions (Black et al. 1991) and grazing by fish and invertebrates (Gleason 1996).

Previous studies have looked at several aspects of coral recruitment. Nearly all studies are done using artificial plates as settlement substrata, since they provide a standardized and easily replicated measure of recruitment, which can be compared across regions and habitats (Harriot & Fisk 1987). Correlating the spatial and temporal variation of recruitment of corals to the adult community structure has proved exceedingly difficult. The time scale that directs adult coral community structure (years to centuries) varies greatly with that of recruiting corals and juveniles (days to years) (Edmunds

2000). This difference may explain why some studies have found a significant relationship between adult abundance and recruitment (Connell 1973, Johnson 1992), while others have not (Bak & Engel 1979, Hughes et al. 1999).

The objective of this study was to document the recruitment patterns of corals and other invertebrates on Moorea reefs, and to determine the effect of site, grazing and depth on recruitment. The differing hydrographic conditions of each site are hypothesized to influence significant differences in recruitment Grazing plays a large role in successful recruitment since newly settled recruits are extremely vulnerable, as they have not yet fully calcified. Grazing also helps to clear substrate of algae to provide space for recruiting corals and invertebrates

## Methods and Materials

*Study site*

This study was conducted on the island of Moorea (17°30' S; 149°50' W), which belongs to the Society Islands of French Polynesia. Moorea is a high volcanic island, triangular in shape with a circumference of 61 km. The island is edged by a fringing reef, up to 100m from shore, and a barrier reef 500-1000m from shore forming a narrow protected lagoon.

Recruitment patterns were examined at four sites on the northern side of the island. In order to characterize recruitment in the bays, one site was located within Opunohu Bay (or Papetoai Bay), and one site was located within Cook's Bay (or Pao Pao Bay). Opunohu Bay is located about 6km east from the northwest extremity of the island. It is 3500m long from barrier reef to lands end, and 600m wide on average. The bay opens to the ocean via Tareu Pass, which is approximately 250m wide. The study site was located along the western shore near Taiare Point. Cook's Bay is located 3000m to the east of Opunohu Bay. The back-reef site was located at Vaipahu reef near Opunohu Bay. The fore-reef site was located approximately 50m north of the barrier reef, near Tareau Pass.

*Recruitment*

Coral recruitment rates were determined using 10 x 10 x 1 cm unglazed ceramic tiles attached to concrete blocks. The tiles were biologically conditioned in running seawater for three days before attachment to the blocks, following recruitment studies by Harriot and Fisk (1987). Each block held two tiles, each 30 cm apart from the other, and held 30 cm above the block by metal bolts. Surrounding one tile on each block was a wire cage to prevent fish and invertebrate herbivory. The wire cage mesh was 5 mm. The other tile was left uncaged for comparison. The upper surface of each tile was smooth, but the bottom of each tile had several grooves running across from end to end. Since the two surfaces were different, no statistical analysis of top vs. bottom recruiting was done. Location of the recruits and other organisms was noted however. Organisms from the top and the bottom were simply pooled together, though there were very few organisms other than macro- and coralline algae on the top surfaces.

*Installation and removal of tiles*

Installation of tiles was begun 10/12/2002 and completed 10/14/2002. All blocks were brought down to the appropriate depth on SCUBA. Depth measurements were made with a dive computer. The blocks were placed in a suitable spot so that the tiles were oriented as flat as possible, each approximately 10 m apart.

The tiles in all sites were inspected about 14 days later to make sure the blocks were still positioned properly. During inspection I noticed a fair amount of filamentous algae growing on the wire cages. I carefully brushed this away, as it may have altered light availability to the tile, or hampered water flow through the cage. Also during inspection I found that two of the shallow fore-reef blocks were missing, and the third had been damaged by a large swell

Removal of the tiles was begun on 11/14/2002 and completed 11/17/2002. The tiles were removed in the same order as they were placed to standardize the amount of time underwater. The tiles were removed from the blocks underwater, placed in labeled plastic bags and transported to the laboratory. The tiles were kept in flow-through seawater tanks until they could be examined.

*Tile inspection and analysis*

The tiles were carefully examined under a dissecting microscope. Numbers of coral recruits, bryozoans, bivalves (oysters), porifera (sponges), ascidians (tunicates), and tubeworms were recorded. Photographs were taken through the microscope to aid in identification. Additional assistance of coral identification was given by Elodi. Mean numbers of recruits per tile were calculated and analyzed by 2-way ANOVA models to screen for interactions between site, depth and caging effects. Since the shallow fore-reef tiles were missing, no comparison between depth could be made for this site. The fore-reef data could not be included in the ANOVA models for depth.

## Results

Two families of coral recruits were found on combined tiles, Pocilloporidae and Acroporidae. Seven pocilloporids were found on tiles located in the fore-reef and back-reef sites. Two acroporids were found on tiles located in the back-reef and Opunohu Bay. The sizes of all coral recruits were the same (1.0mm). All coral recruits were found on the bottom surfaces of the tiles. For acroporidae, the results of a two factor ANOVA for the effects of site and caging showed no significant associations, although both recruits were found on uncaged tiles. There were also no significant effects of depth on the acropora recruits. The results of a two factor ANOVA for the effects of site on the pocillopora recruits revealed a significant effect (p=0.038), and a nearly significant effect of caging on pocillopora recruits. A significant effect of depth on pocillopora recruiting was found (p=0.029), as well as a significant interaction between site and depth (p=0.029).

Five taxa of invertebrates were found on the tiles. Bivalves (oysters) were found at all sites, but in greatest numbers in the bays and lower depths. ANOVA data yielded p-values of nearly zero for both site and depth effect. A strong interaction between site and depth was revealed. All were found on the underside of the tiles, and usually within grooves. There was no significant caging effect.

Five species of bryozoans were found on tiles, of which three could be identified to family with the help of a pacific reef invertebrate field guide. Individuals of all species were pooled together for analysis. All bryozoans were found on the undersides of the tiles, and nearly exclusively inside the bays (site: p=<0.001). The undersides of tiles within Opunohu Bay were completely carpeted with the curly species of Scrupocellaria. Bryozoans proffered the caged tiles (p=<0.001), yielding a significant interaction between site and caging (p=<0.001).

Tunicates recruited in lower numbers and were comprised of two or more species that could not be identified. They significantly recruited to deeper depths (p=0.029) and to caged tiles (p=0.039). The tunicates also recruited only to the undersides of the tiles.

Polychaetes recruited in the highest numbers on all tiles. They were only found on the bottom of the tiles. There were two species of tube-building polychaetes, one with a white shell and one with a brown shell. They could not be identified. The brown-shelled species was rare and only occurred in the back-reef and fore-reef sites. A significant effect of depth (p=0.032), and an interaction of site and depth were revealed (p=0.019). A significant effect of caging was also found (p=0.032).

Significant site effects were found for Porifera (p=0.009) as well as an interaction between site and caging (p=0.026).

## Discussion

Some significant trends were found for effects on recruiting corals. Pocillopora represented 7 of 9 recruits on all tiles. They significantly recruited to the fore-reef and back-reef tiles only, revealing a probable intolerance to increased sedimentation and algal cover. No statistically significant effects were found for the acropora recruits. They did not seem to show any depth or caging effect. With such low recruitment numbers it is difficult to draw any conclusions. A longer time underwater to more fully sample the recruitment would have been helpful.

The appearance of acropora recruits inside the bay is interesting due to the almost total lack of adult Acropora colonies in Opunohu Bay (Adjeroud, 1996). One possible explanation relates to the fact that acropora reproduces by spawning. Spawning corals release eggs and sperm in the water column, with larvae requiring several days to weeks to develop and settle (Babcock & Heyward 1986; Harrison & Wallace 1990). These larvae may disperse widely outside the natal reef by wind-driven or tidal currents (Willis & Oliver 1990). In contrast, brooding corals release fertilized larvae directly into the water, which are then ready to settle right away (Harrison & Wallace 1990). Acropora corals were spawning during the time of the study (Andrew Carroll, pers. comm.).

Bryozoan recruitment revealed several statistically significant trends. They nearly recruited exclusively to the bay sites, which reflects their feeding behavior. Bryozoans are colonial animals, resembling corals, that rely on cilia to beat in such a way as to generate a current of water toward their funnel-like mouths.

Food consists of small plankton or non-living organic particles. Bryozoans are known in some areas as "marine-fouling" organisms. They can form remarkably dense mats on ship hulls, pilings and other objects submerged in water. They tend to like bay or harbor environments, as they are exceptionally resistant to toxic waters. In such environments where local fish populations are reduced by marine pollution, they tend to spread quickly and form large populations. Bryozoans are extremely vulnerable to grazing by fish in natural benthic communities. In this study their numbers were severely reduced on uncaged tiles. In open water reef environments they are cryptic organisms, existing in coral crevices and on the underside of rocks and debris.

Significant site effect on bivalve recruits is immediately obvious. They recruited in high numbers inside the bays and very rarely in the fore-reef and back-reef. They also recruited more numerously at deeper depths inside the bays. In the deeper sites, the oysters are directly affected by grazing. Even still they are recruiting at higher levels in the deep site rather than the shallow site. In the shallow sites there are actually more successful recruits on the uncaged tiles. The caging effect must have more of and impact in the shallow depth, probably because of increased algal growth on the caged tiles at a shallow depth (more light=more algal growth) resulting in reduced water flow to the filter-feeding invertebrates.

Tunicate recruitment revealed some significant findings, despite its smaller numbers. Tunicates are very fragile benthic organisms that lead an extremely cryptic life. They prefer deeper depths and tight spots inside crevices and rubble. Only a few had recruited onto uncaged tiles, presumably because they were immediately grazed upon by local fish. Their vulnerability is reflected in their reproductive traits. As brooding ascidians, they release fully competent larvae into the water. Divers have recorded time from brooding to settlement and found an extremely abbreviated swimming period (measured in seconds), followed by immediate settlement and metamorphosis resulting in dispersal distances only a few meters (Stoner 1990). Since they are most vulnerable in their free-swimming larval state they have reduced the time necessary to complete their reproduction to a bare minimum.

The polychaetes recruitment patterns were similar to the tunicates in that they also were significantly affected by herbivory. In the bay sites uncaged recruitment numbers were much lower, and numerous broken shells were littered all over the tile. This trend was similar for depth as well. The ANOVA model shows an interaction between site and depth. That is to say that there was a greater difference between caged and uncaged recruitment in particular sites. In shallower depths, polychaetes were more heavily impacted by grazing than the deeper depths, but nevertheless there were more recruits at this depth. They seem to have decided (from an evolutionary standpoint) that a greater fecundity and population size will allow them to colonize an area with more food, even under more intense pressure from hungry predators.

A good interaction between effects is also seen in Porifera recruitment. There were more recruits in the back-reef and fore-reef site. When caging effect is looked at with site effect, it is clear that in Opunohu Bay and the back-reef site, there are more recruits on the uncaged tiles rather than the caged. In Cook's Bay and the fore-reef, uncaged tiles have lower numbers; their recruitment was directly effected by grazing predators. An explanation for this data is that the cages in Opunohu Bay and the back-reef must have been preventing grazers from eliminating an organism that competes with the Porifera for settling space. In these areas large amounts of macro-algae were growing on the tiles and inside the cages. Perhaps this algae, if not reduced to a natural abundance by grazers, affects the survival and recruitment of encrusting sponges by reducing water flow and thus food supply to the sponges, and/or altering physical and chemical settlement cues that settling sponges use to find suitable settling habitat.

A striking feature of the tiles from all sites was the lack of recruited corals and invertebrates on the upper surfaces of the tiles. In both bays and back-reef a thick layer of sediment covered the tops of the tiles (caged and uncaged), onto which a filamentous algal turf was loosely attached. Once the sediment and macro-algae was removed, only green and red filamentous algaes were attached to the surface.

The tops of tiles from the fore-reef were covered with several species of crustose coralline algae. They are heavily calcified by calcite crystals embedded in their cell walls, which provide them protection from grazing and wave resistance (Fabricus 2001). These algae were also found on the sides and bottoms of the tiles from the fore-reef and back-reef. Crustose algae are important to reef ecosystems for two reasons. First, they are a significant contributor to limestone formation and cementation of reef pavement (Barnes & Chalker 1990). Second, they are an important settling substratum for many types of reef benthos (Fabricus 2001). Their associated bacterial films are now known to induce settlement and metamorphosis of several reef organisms, most importantly scleractinian corals (Heyward and Negri 1999). Very few crustose algae were found on bay tiles, and those that were found were mostly recruited onto the undersides of the tiles.. A recent study has shown that crustose algae are negatively affected by sedimentation, and the effect intensifies in nutrient rich waters of bays where sticky mats of sediment form that are not easily removed by currents inside lagoons (Fabricus 2001). Fringing reefs inside lagoons are doubly affected by the lesser amounts of crustose algae. First, their ability too cement coral rubble together after die-off is decreased, reducing suitable settling substrate for several reef organisms. Also, since the algae is present in lesser amounts, there are fewer chemical cues to facilitate coral recruitment.

A significant trend in the recruitment data shows invertebrates' preference for bay sites. Bivalves, tube-building polychaetes and bryozoans all seem to prefer the bays. A possible explanation is the increased level of eutrophication. Both bays are subject to a constant input of agricultural run-off and other human waste. In Cook's Bay, a pineapple juice factory unloads a large amount of waste into the bay adjacent to the Gump Station. A common site (and smell) on the shores of the bays is the thick mat of rotting algae, mostly Turbinaria ornata, that release their nutrients upon decomposition. These inputs greatly increase the production of food for suspension feeders. A related study has found that rich upwelling currents on the Pacific coast near Panama lead to higher growth rates of benthic algae, and indirectly to increased growth of suspension-feeding invertebrates (Birkeland 1977). These assemblages can competitively exclude corals from recruiting, by taking up available space, and also potentially overgrow recruits and juvenile corals.

Coral reefs worldwide are becoming increasingly threatened by natural and anthropogenic impacts (Birkeland 1997). The 1997-1998 worldwide bleaching event had a likely unprecedented effect on coral assemblages (Shafer 1999), and Acanthaster planci (crown of thorns starfish) continues to devastate coral reefs in the Pacific region (Endean & Cameron 1990). Information on processes of natural reef recovery is becoming increasingly important to biologists who are trying to asses reef health and possible enhancement of reef recovery. The spacio-temporal recruitment of corals must be fully understood in order to understand how coral recovers from damage. A recent study outlines a potential use for accurate regional recruitment data (Kojis 2001). The study proposes that if local recruitment rates are high and the damage has not resulted in declined water quality or substrate conditions, the reef will most likely recover rapidly without intervention. Available resources could be allocated to managing the source of damage (if possible). In areas of lower recruitment rates, Carribean and the U.S. Virgin islands in particular, the slower recovery may warrant and expensive reef restoration program, especially on economically important reef ecosystems. Combining knowledge of the mechanisms of coral recruitment and reproduction with traditional reef management tactics of surveying adult abundance will lead to a better understanding and protection of one of our world's most important resources.

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HINANO
BLACKTIP REEF SHARK
hermit crab
NUDIBRANCHS
MOSQUITO
PALM TREES
STINGRAY
CORAL
Stomatopods
soldierfish
pholcid
tiare
gecko
MOOREA 2002