

Biology and Geology of Tropical Islands

Research Papers - Fall 2005



University of California, Berkeley
Richard B. Gump South Pacific Research Station
Moorea, La Polynésie française



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Biology and Geology of Tropical Islands

Moorea Research Papers

Volume 14

Fall 2005

Environmental Science, Policy and Management 107 and
Integrative Biology 158
University of California
Berkeley



The Moorea 2005 Class



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Front row: Sarah Daniels, Sarika Cullis-Suzuki, Alicia Bihler, Danielle Fuchs, Courtney Hart, Ian Sanchez, Carmen Yeung, Jennifer Brewer, Tanya Chapple

Not pictured: Brent Mishler, Scott Fay, Carol Hickman, Roy Caldwell, Rosemary Gillespie, Carole Hickman, George Roderick



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The Richard B. Gump South Pacific Research Station, Cook's Bay, Moorea
Photograph by Jere Lipps

The Moorea Class 2005

Introduction

“The Moorea Class”, or officially the *Biology and Geomorphology of Tropical Islands* (Environmental Science, Policy, and Management C107 and Integrative Biology C158), is a unique undergraduate experience. This semester-long class is based on the Island of Moorea, 18 kilometers from Tahiti in French Polynesia. Moorea is an ideal setting for a field science course: it's a small tropical paradise, the area of the city of San Francisco. Because of its isolation and history, Moorea is a microcosm of biological diversity. In this class, Berkeley undergraduates move from the lecture hall into the world of professional scientific investigation through the creation, design, and completion, of their own original research projects. Like any research expedition, the course includes planning, logistics, field reconnaissance, field research, and oral and written presentation of the final results. After three weeks at Berkeley preparing, equipping, and intense study, the class travels to Moorea, where the students encounter the diverse environments and research opportunities on Moorea and nearby Tahiti and Tetiaroa. Students explore the reefs, lagoons, shorelines, mangroves, and marsh swamps, rivers, tropical agricultural lands, and high elevation forests, to find their own special research interests. They encounter unique plants and animals, as well as rock formations, soils, and human activities spanning 1500 years. Like Captain James Cook and Charles Darwin, who explored these islands centuries before us, the students return with new

insights into scientific subjects, as well as themselves. During the last two weeks of the semester the students are back in Berkeley, where they finalize their work and deliver their final presentations at a public research symposium. Their scientific papers are published in this series of books, *Biology and Geomorphology of Tropical Islands*, which are distributed widely to libraries, researchers, and officials at Berkeley, on Moorea and Tahiti, as well as elsewhere. The books are now also available on-line.

Richard B. Gump, of San Francisco department store fame, donated land and facilities on Cook's Bay, Moorea to UC Berkeley in the mid-1980s for a research facility. Since then the Richard B. Gump South Pacific Research Station has benefited significantly from the support Gordon and Betty Moore family and Foundation, as well as numerous other generous individuals. With support of the National Science Foundation and other research foundations, the station is now a Long Term Ecological Research site and has supported dozens of multi-institutional research projects in both marine and terrestrial environments. The station also supports other undergraduate classes and graduate classes from around the world and has been active in local outreach and education through the Atitia Center. The students and instructors are grateful to all the far-sighted people who recognized the value of a research station on Moorea. More information about the Gump Station can be found at <http://moorea.berkeley.edu>.

Faculty members and Graduate Student Instructors (GSIs) with expertise in terrestrial biology, marine and freshwater biology, and geology, teach the course. Some give lectures at Berkeley while others provide instruction and assistance on Moorea. Three GSIs work with the class for the entire semester. "The Moorea Course" started in 1991 with Professors Roy Caldwell (Integrative Biology), David Stoddart (Geography), and Vince Resh (ESPM), teaching 13 students at the newly completed Gump Research Station. The course was a big success and has been offered every fall semester since. Demand for the course is always high and admission into the course is very competitive. As the living and research facilities expanded, the class grew larger, too: in 2005 the class included 21 students. Since its inception, over 200 students have conducted their own original research projects as a result of this course. Students have published their research in international scientific journals and most go on to graduate school or into other careers as professional scientists.

The 2005 Fall Class is no exception, with wonderfully talented students who developed a diverse set of original projects. This book presents the written research results produced by the class of Fall 2005.

Acknowledgments

The Moorea Course is complex. As such, we owe thanks to the students, faculty, Graduate Student Instructors, and our colleagues at Berkeley and on Moorea who made the 2005 class so successful. We all appreciate and remember fondly, the help, good times, camaraderie, and excitement, we shared together. Those of us in the field with the students also learned much from each of them, as they planned, designed, and explored, their own research.

At Berkeley, other faculty and staff presented lectures and workshops on a variety of topics, including Tom Carlson (Integrative Biology), Jim Hayward (UCB Diving Safety Officer), Norma Kobzina (Bioscience and Natural Resources Library), and Maggi Kelly (Geospatial Innovation Facility and ESPM). We especially thank Steve Owen (Integrative Biology) and MJ Carlson (ESPM), who keep the course going administratively. The staffs of the University and Jepson Herbaria, Museum of Paleontology, and Essig Museum of Entomology, assisted with workshops on producing scientific vouchers and curation. UC Berkeley's Visualization Center and Geospatial Innovation Facility provided additional training, assistance, and use of their resources.

On Moorea, we are grateful to many people, especially the leadership of the Gump Station, Executive Director Neil Davies, Associate Directors Hinano Teavai-Murphy and Frank Murphy, and wonderful staff, Valentine Brotherson, Irma You Sing, Tony You Sing, and Jacques You Sing. We thank also the local community on Moorea for advice, assistance and cultural experiences, particularly, Mari Mari Kellum. We also thank the le Centre de Recherches Insulaires et Observatoire de l'Environnement (CRIOBE), whose Directors, René Galzin and Serge Planes, and staff were generous with their time, as well as access to their library and collections. We are also grateful to Priscille Frogier and Jean-Yves Meyer of la Délégation à la Recherche, Gouvernement de Polynésie Française for their continuing interest in and support of this class. Our class relies on the assistance of experts at many other institutions worldwide for advice and information on various groups of organisms and scientific subjects. These experts are individually acknowledged in the student papers, and we thank them for aiding our students. Finally, we thank the generous donors who have helped to make the Gump Station one of the finest field laboratories in the tropics.

Without all this help of such various kinds, our students would not have had this unique opportunity to learn about the process of doing science, and to discover a unique environment, a new culture, and, especially, themselves. We have all been changed forever!

2005 Teaching Staff



Photo: Erica Spotswood

George Roderick Professor, ESPM, Lead Professor for 2005
James Bartolome, Professor, ESPM
Roy Caldwell, Professor, Integrative Biology
Rosemary Gillespie, Professor, ESPM
Carole Hickman, Professor, Integrative Biology
Brent Mishler, Professor, Integrative Biology
Scott Fay, GSI, Integrative Biology
Mathew Medeiros, GSI, ESPM
Michele Weber, GSI, Integrative Biology

Editor: Erica Spotswood, former GSI, ESPM

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THE ETHNOBOTANY AND BIOACTIVITY OF PTERIDOPHYTES MOOREA, FRENCH POLYNESIA

Nicole C. Baltrushes

Integrative Biology, University of California, Berkeley, California 94720 USA

Abstract. For millennia Humans have utilized plants that are rich in secondary compounds which have evolved presumably in response to herbivory. The plants that have these bioactive properties are important to traditional cultures and are a possible resource for the development of natural products for the rest of the world. Pteridophytes are a diverse and abundant group of plants whose secondary chemistry is still largely unknown. This study investigates the bioactivity of twelve pteridophyte species (*Microsorium grossum*, *Microsorium commutatum*, *Asplenium australasicum*, *Nephrolepis hirsutula*, *Davallia solida*, *Antrophyum plantagineum*, *Angiopteris evecta*, *Adiantum trapeziforme*, *Bolbitis lonchophora*, *Lygodium reticulatum*, *Lycopodiella cernua*, and *Dicranopteris linearis*) on Moorea, French Polynesia using bioassays for pesticidal potential, cytotoxicity and antifungal activity. Ethnobotanical information about the medicinal uses of these ferns was also collected and compared to the assay results. Nine of the twelve ferns were significantly more bioactive than the negative controls in at least one of the bioassays. The bioactivity of these ferns along with the insect damage results suggests that ferns may have evolved under similar herbivorous constraints as angiosperms, although further research is needed. Ethnobotanical information revealed variability in knowledge and use between healers, and the discourse between healers and scientists that was previously more tenuous is actively being pursued on both sides. The mutual advantages of cooperation will play a larger role in scientific investigation in the future.

Key words: *ethnopharmacology, secondary compound, bioassay, insecticide, cytotoxicity, antifungal*

INTRODUCTION

Humans are relatively recent players on the world stage, emerging in their present form only 160,000 years ago, compared to the first appearance of primitive vascular plants in the Silurian period, 420 Million years ago, and ferns in the Carboniferous period, 360 million years ago (Campbell and Reece 2005). Plants were not subject to human herbivory during most of their evolutionary history and thus the defenses that different plants have developed, i.e. toxic phytochemicals, prickly morphologies, or intricate life histories, are in response to other herbivores or exploiters. It is these defenses, however, that make many plants so useful to their recent human cohabitants.

Human subsistence has always been contingent on primary plant products such as starches and proteins. However, in addition to these essential nutritional compounds, secondary compounds produced by plants have also served an important role in human survival. The secondary compounds, which plants have presumably evolved as defenses against herbivory, or pollinator and seed disperser

attractants, are the sources of many human insecticides, medicines, oils, resins, tannins, waxes, and dyes among other products (Balandrin et. al 1985).

The knowledge of these compounds is evident in traditional herbal medicines and agriculture the world over. Many of the 2000 plant species with known insecticidal properties have been in use since before the Ancient Romans (Balandrin et. al 1985). The astounding biodiversity of plants, and the secondary compounds produced by these organisms are a virtual pharmacopoeia ready for human discovery. However, a scant 5-15 percent of the 250,000 to 750,000 species of higher plants have actually been tested for bioactivity (Kingham 2001). Furthermore, pteridophytes are a major plant group for which bioactivity data is not here reported.

Pteridophytes, ferns and fern allies, are ancient seedless vascular plants that can be found all over the world. It is the high abundance of ferns that is perhaps the first feature to catch a traveler's eye and mark one's entrance into the tropics. In fact, some 80% of the estimated 12,000-15,000 fern species occur in the tropics (Roos 1996). There are 73 species of ferns on Moorea alone, a

small island in French Polynesia (Murdock and Smith 2003).

However, is there any reason to expect that pteridophytes are bioactive? Paul Petard has identified 5 ferns as medicinally useful, which indicates possible bioactivity (1986). Also, differential herbivory can indicate possible bioactivity. It is a frequent observation that pteridophytes appear to be less herbivorized than other plants, however, recent research actually suggests that ferns are no less herbivorized than analogous herbaceous angiosperms (Hendrix 1983). The relative amount of herbivory can indicate whether pteridophytes use a general defense that makes them unpalatable, but not necessarily poisonous, or specific bioactive secondary compounds.

One striking pteridophyte lends support to the idea of the presence of bioactive compounds rather than general defense: bracken fern. Bracken fern, which can be found virtually anywhere except Antarctica, has been identified as a natural carcinogen to livestock, and ingestion of bracken fern can lead to bracken poisoning. The toxic agent is ptaquiloside which alkylates unbound DNA bases and cause genetic malfunctions and eventually cancer. Bracken fern ingestion has also been linked to cancer of the upper alimentary track in humans (Alonso-Amelot and Avendaño 2002). The presence of a distinct and potent toxin in this pteridophyte gives one reason to possibly expect bioactivity in other pteridophytes.

The diversity of secondary compounds in plants, and potentially in pteridophytes, has presumably evolved in response to, and been maintained by, insect herbivory. In a study of five generalist herbivorous insects, structurally related secondary compounds were found to produce different effects on different insects- some insects were affected while others were not (Macel et al. 2005). The targeting of certain insects by these plants suggests that these generalist insects could have played a part in the evolution of the compounds. The presence of bioactive secondary compounds in pteridophytes could provide insight into this lineage's place in an evolutionary context.

The purpose of this study was to assess the bioactivity of pteridophytes. Are there a significant amount of secondary compounds present in this group? A study of the insecticidal potential, cytotoxicity, and antifungal properties of ferns coupled with a measurement of the insect herbivory was carried out. Interviews with French Polynesian healers and elders illuminated the cultural context of these plants and their useful compounds. Also, the goal was to use science to encourage the practice of traditional medicine and

to expand local people's knowledge and utilization of their indigenous and introduced flora.

METHODS

Fern collection

Twelve species of ferns, representing varied morphologies and families, were collected from two sites in the Opunohu Valley- one in the rear of Mari Mari Kellum's property (06k 0197727 E, 8091220 W elevation 27m), and one at the Belvedere (06k 0199870 E, 8058417 W elevation 222m). Five ferns identified by Paul Petard (1986) as medicinally useful (*Microsorium grossum* (Langsd. & Fisch.), *Microsorium commutatum* (Blume), *Asplenium australasicum* (J. Sm.), *Nephrolepis hirsutula* (G. Forst.) and *Davallia solida* (G. Forst.)) were included purposefully. The other seven ferns (*Antrophyum plantagineum* (Cav.), *Angiopteris evecta* (G. Forst.), *Adiantum trapeziforme* L., *Bolbitis lonchophora* (Fée), *Lygodium reticulatum* Schkuhr, *Lycopodiella cernua* (L.), *Dicranopteris linearis* (Burm. f.)) were chosen for ease of collection and to represent different families. Ferns were identified using Andrew Murdock's Key to Ferns and Fern Allies from the UC Jepson Herbarium website, and nomenclature follows that used by Murdock and Smith (2003). Five individuals of each species were collected for extract preparation. In order to ensure that the specimens were distinct plant individuals 30 paces were taken between samples.

Insect damage

A second collection at each site was made in the same manner as for the extract preparation, and one frond was chosen randomly from each of five individuals from each species. Using a 1-centimeter square grid placed over the frond, the ratio of insect damage (chewed away, galls, or leaf mines) to unaffected area was calculated in order to assess the relative damage to different ferns caused by herbivores. Comparisons were made between the two sites and within the sites to determine whether insect damage was more dependent on location or on fern species.

Extract preparation

For each of 12 fern species 4 extracts were prepared: leaf matter in 90% ethanol, leaf matter in filtered water, roots and rhizomes in 90% ethanol, and roots and rhizomes in filtered water. Five grams of fresh plant matter were weighed and combined with 30 milliliters of water or alcohol in a blender. The mixtures were blended and the

water extracts were immediately filtered using filter paper. The alcohol extracts were blended, transferred to a vial, and refrigerated for three days. These extracts were shaken each day and then filtered in the same manner as the water extracts. (The difference in procedure for the extracts was implemented to avoid any spoiling that might result from the plant matter sitting in water for extended periods of time.) All extracts were refrigerated at 7° Celsius until use.

Insecticide assay

In order to assess the potential use of ferns as insecticides, five ferns representing five different families and exhibiting varied morphologies were chosen from the 12 species. Based on a protocol developed by Williams (1993), an insect pest was exposed to extract and then isolated on a food source. Glassy-winged sharpshooters, *Homalodisca coagulata*, (hereafter "GWSS") were used as the pest organism because of their recent invasion of Tahiti and French Polynesia. GWSS' are xylem-feeding insects that are native to the southeastern United States. They have posed a serious invasive threat to California vineyards and arrived in French Polynesia in 1999 (Wilder 2004).

Juvenile GWSS' were collected from plants on the UC Berkeley Gump Station and caged on a hibiscus tree until used. 10 GWSS' were placed into each vial and .1 ml of fern-leaf water extract was expelled into each vial. The vial was upended to expose each insect to the extract, and the insects were transferred quickly to a plastic bag with aeration holes and caged on a hibiscus hedge. The positive control was a commercial insecticide, active ingredient tetramethrin, and the negative control was water. These treatments were replicated 5 times each.

The utility as an insecticide was measured by counting the number of GWSS' dead in each treatment every 24 hours for five days. ANOVA and Tukey-Kramer HSD tests were used to analyze variance within and between treatments. Possible correlation between insecticide potential and insect damage of the different ferns was investigated.

Cytotoxicity assay

Brine shrimp, *Artemia salina*, were used to determine the cytotoxicity of each fern. Brine shrimp larvae are highly sensitive to compounds delivered to solution in very small amounts. Their common use as efficient and simple bioassay organisms began in 1956 (Michael 1956). The brine shrimp were hatched in seawater with aeration and a 60-watt light. Once hatched, 50 shrimp were

counted into each 15 ml vial and .1 ml of extract was added to each vial. The vials were filled to 10mL with seawater. In this study, the number of brine shrimp dead after 20 hours was recorded. Every extract was tested for cytotoxicity as well as a positive control, the extract of a known cytotoxic plant *Catharanthus roseus* L., and negative controls of filtered water and 90% ethanol. The test was replicated 6 times and average death rates were calculated for each extract. Using ANOVA, average death rates were compared to the controls for: (1) all extracts together (2) different plant part used (leaf or root) and (3) different solvent used.

Antifungal assay

The antifungal activity of *M. grossum*, *D. solida*, *D. linearis*, *L. cernua*, *A. evecta*, and *A. trapeziforme* was assayed using a culture of baking yeast, unicellular fungi. Baking yeast was chosen as an assay for its accessibility and similarities to fungi that might afflict humans. Agar was prepared with 100-g/L sugar content and 14g/L agar, and poured into labeled Petri dishes. Each Petri dish was inoculated with .5mL of a yeast and water mixture (2g/50mL).

An incubator was constructed from a cardboard box with two 60-watt desk lamps and holes cut in the box to attain a uniform temperature throughout. The petri dishes were incubated at 37degrees Celsius for either 6, 9, or 24 hours in order to measure both the inhibition of growth in expanding colonies and the death of existing colonies. After incubation, and the formation of an even lawn of fungi, filter paper discs, saturated with extract were placed onto the agar and the dishes were incubated for 48 hours. Water and alcohol were used as negative controls and solutions of a commercial antifungal, 1% econazole nitrate, in water or ethanol were used as positive controls. The diameter of the ring of inhibition/death, including the paper disc, was measured after this 48-hour period. For statistical comparisons, each treatment was reduced to presence or absence of change. These data were tested for significant differences between extracts and controls using ANOVA, and Tukey-Kramer HSD.

Ethnobotanical information

In order to assess the current use of ferns in Moorea, two healers were interviewed about their use of pteridophytes using a collection of pressed ferns presented to each. The healers interviewed, Mama Lucie Tavi and Rita You-Sing, are two of the few generalist healers in Moorea. Three elders, Papa Matarau, Papa Mape, and Papa

Mehai were collectively interviewed about their knowledge of pteridophyte ethnobotany using photos of the 12 different fern species. Tahitian name for the pteridophytes, medicinal use of each species, non-medicinal uses, and information about part of the plant used was solicited.

RESULTS

Insect Damage

Three fern species, *D. solida*, *N. hirsutula*, and *B. lonchophora*, were found at both the Belvedere and on the Kellum property. A t-test of insect damage on these ferns based on location found that the amount of insect damage at the two sites was not significantly different ($p \leq .29$, t -ratio=.56, $df=23$). Once the similarity of herbivory was established between sites, ferns that were not present in both sites could be compared confidently. An ANOVA analysis showed that insect damage on different fern species was significantly different ranging from an average of 0% to 15.8% ($p \leq .0002$, $df=11$, F -ratio=4.7). A Tukey-Kramer HSD test (see Figure 1) revealed that *D. linearis* (12.7%) and *N. hirsutula* (11.2%) both suffered a significantly higher percentage of insect damage compared to *D. solida* (2.3%) and *L. cernua* (0%). Insect damage was not dependent upon location but upon species. This suggests the possible presence of different secondary compound defenses between ferns.

Insecticide assay

The insecticidal potential of the ferns tested increased with time. A weak positive correlation between number of GWSS' dead and number of days of exposure was detected in a regression analysis ($R^2 = .15$, $df=1$, $p \leq .0001$). An ANOVA test showed that, while the difference between the commercial insecticide and water controls was statistically significant throughout the experiment the differences between controls and fern extracts varied with time ($p \leq .0001$, $df=6$, F -ratio=36.4). Using a Tukey Kramer HSD analysis of the first day, the insecticide was significantly more deleterious than the water control, with average GWSS dead (AGD hereafter) of 10 and .8 respectively (See Figure 2)

The *A. evecta* extract (AGD = 3.4) was significantly more lethal than the water control and all the other extracts, but significantly lower than the insecticide. The four other extracts tested were not significantly more potent than the water control on the first day (ANOVA test $p \leq .0001$, $df=6$, F -ratio=36.4).

In the following days the significant differences between *A. evecta* and the other extracts decreased until the fourth day when there was no significant difference between any of the extracts and the water control. However, significantly lower AGD compared to the insecticide was maintained by all treatments for all 5 days (ANOVA, $p \leq .0001$, $df=6$, F -ratio=8.0).

No significant correlation in a linear regression analysis between insect damage and

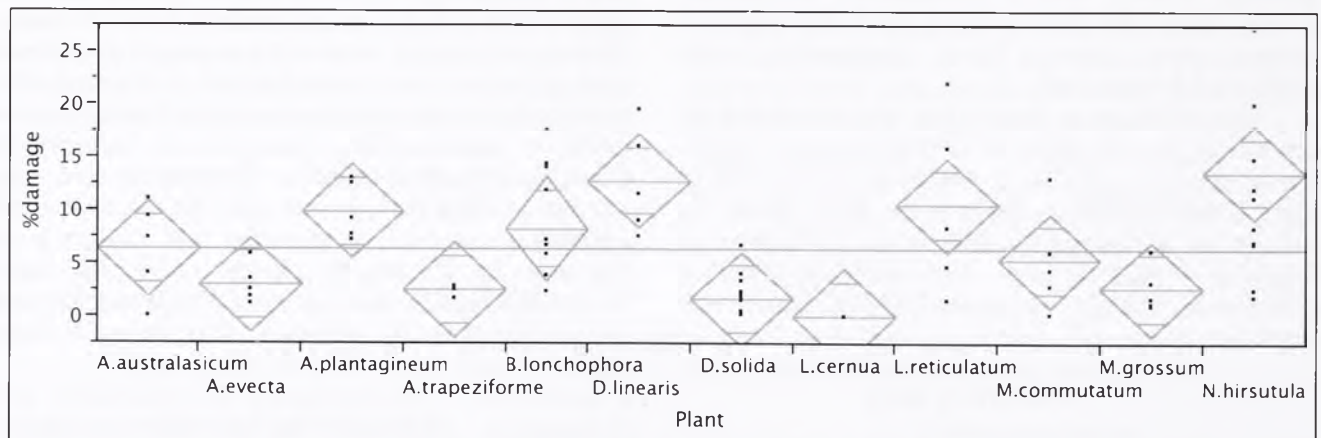


FIG 1. Variation in % insect damage on 12 pteridophytes on Moorea, showing the ANOVA and Tukey-Kramer analyses of the %insect damage on the different fern species. *D. linearis* and *N. hirsutula* are both significantly more damaged than *D. solida* and *L. cernua* ($p \leq .0002$, $df=11$, F -ratio=4.7)

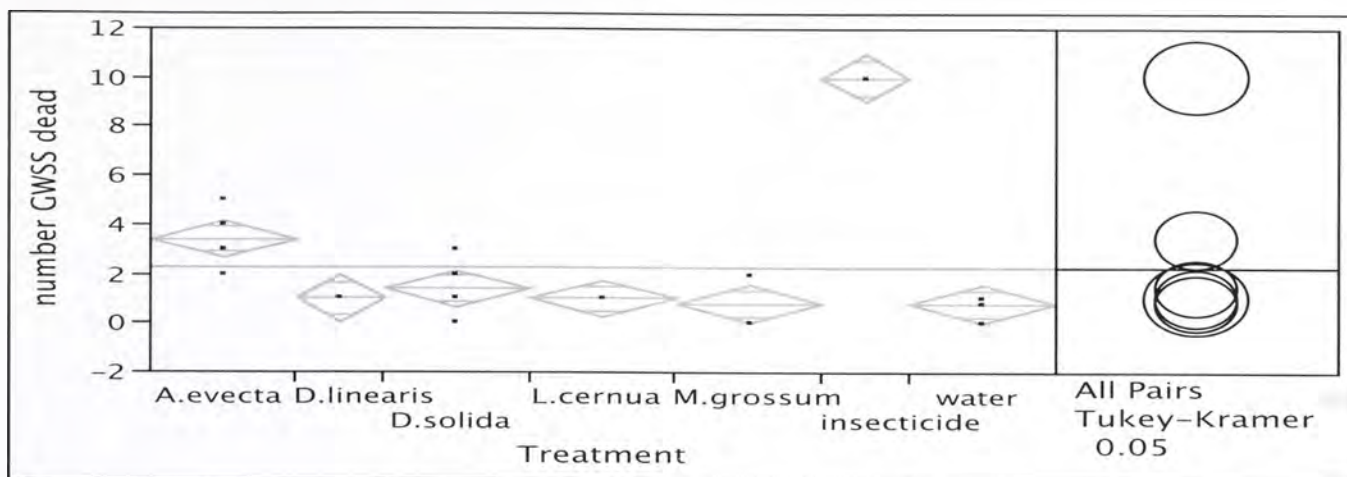


FIG 2. Insecticide results for Day #1 showing ANOVA and Tukey-Kramer analysis of variance. The insecticide, water and *A. evecta* treatments are significantly different ($p \leq .0001$, $df=6$, $F\text{-ratio}=36.4$).

insecticide potential was detected ($R^2=0$, $df=1$, $p \leq .98$).

Cytotoxicity assay

The *C. roseus* extract was significantly more cytotoxic than the negative controls and, as displayed in the Table 1, *A. plantagineum*, *M. commutatum*, and *A. australasicum* were all significantly more cytotoxic than the controls in a Tukey-Kramer analysis ($p \leq .0001$, $df=13$, $F\text{-ratio}=6.2$).

An ANOVA test comparing ethanol extracts to water extracts, shown in Figure 3, showed that

the ethanol extracts were significantly more cytotoxic than the water extracts ($p \leq .001$, $df=1$, $F\text{-ratio}=11$). Tukey-Kramer analysis of the ethanol extracts alone revealed that *A. australasicum*, *A. plantagineum*, *L. reticulatum*, *B. lonchophora*, and *M. grossum* were all more cytotoxic than the ethanol control ($p \leq .0001$, $df=13$, $F\text{-ratio}=4.76$). A similar comparison of water extracts alone showed that *M. commutatum*, *A. plantagineum*, and *L. cernua* were significantly more cytotoxic than the water control ($p \leq .0001$, $df=13$, $F\text{-ratio}=5.5$). Thus, the water and ethanol extracts were different in composition, either in type of compounds present or in concentrations.

A Tukey-Kramer test comparing root and leaf extracts revealed that the leaf extracts (Average death rate = 31.4) were significantly more cytotoxic than the controls (Average death rate = 14.0). The root extracts (Average death rate = 25.6) were not significantly more cytotoxic than the controls or significantly less cytotoxic than the leaf extracts ($p \leq .0012$, $df=2$, $F\text{-ratio}=6.7$). This indicates the different chemical composition of leaves and roots.

Average death rates and more comprehensive Tukey-Kramer HSD comparison data appear in Appendix A.

Antifungal assay

The average frequency of inhibitory change (FIC) for the commercial antifungal + controls (.64), and that for the negative controls (.14), though distinct were not significantly different in an ANOVA analysis ($p \leq .12$, $df=8$, $F\text{-ratio}=1.83$). However, in a Tukey-Kramer comparison the *M. grossum* 90% ethanol root extract was significantly more inhibitory (FIC=.79)

Treatment		Average death rate
<i>C.roseus</i>	A	49.33
<i>A.plantagineum</i>	A B C	40.00
<i>M.commutatum</i>	A B	38.92
<i>A.australasicum</i>	A B C D	36.42
<i>A.trapeziforme</i>	A B C D E	31.33
<i>M.grossum</i>	B C D E	30.08
<i>B.lonchophora</i>	B C D E	29.63
<i>L.cernua</i>	B C D E	29.38
<i>N.hirsutula</i>	B C D E	25.29
<i>D.solida</i>	B C D E	24.50
<i>L.reticulatum</i>	C D E	22.54
<i>D.linearis</i>	D E	18.83
<i>A.evecta</i>	D E	18.75
Water / alcohol	E	14.00

Table 1. Cytotoxicity results by species. Different solvent and plant part data are pooled in these results. Levels not connected by the same letter are significantly different ($p \leq .0001$ $\alpha=.05$).

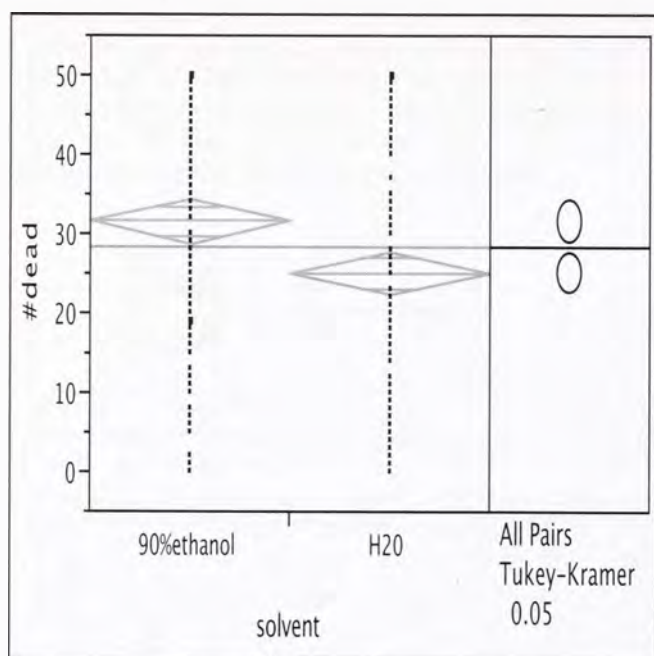


FIG 3. Cytotoxicity of pteridophyte extracts by solvent. 90% ethanol extracts are significantly more cytotoxic than water extracts (ANOVA, $p \leq .001$, $df=1$, $F\text{-ratio}=11$).

than the ethanol control, see Table 2 ($p \leq .037$, $df=7$, $F\text{-ratio}=2.4$).

Ethnobotanical information

While a similar format was used to assess knowledge of all people interviewed, their response styles varied. Rita You-Sing was very forthcoming with details of her use and preparation of the pteridophytes, while Mama Lucie was more reluctant to divulge her knowledge. Mama Lucie was only comfortable telling whether or not she used the plants and identifying the pteridophytes with Tahitian names if possible. The elders interviewed were all very forthcoming with the information they possessed. See Table 3 for Tahitian names and uses reported.

Mama Lucie reported that she used 8 of the 12 pteridophytes, while Rita You-Sing used only three, and the elders knew uses for six. See Appendices B and C for a partial transcription of the interviews with Mama Lucie and Rita You-Sing.

No one interviewed reported any antifungal, anti-cancer, or insecticide use of the pteridophytes.

DISCUSSION

The cytotoxicity bioassay using brine shrimp is a preliminary screening tool for anticancer compounds. The measured cytotoxic activity of

the pteridophytes in this study should be further analyzed using cultures of mammalian tumor cell lines, following T.D. Babu or Pomilio et al. protocols, to assess their potential use in medicine (1995, 1994). Until further research is conducted no conclusions can be made about possible use of pteridophytes in anti-cancer treatments.

The different activities based on solvent and plant part used suggest that the active compounds are concentrated in different parts of the ferns, and that the two solvents perhaps extracted different compounds.

The antifungal activity of the *M. grossum* root 90% ethanol extract was significantly higher than the negative controls, however further research is needed to assess its possible use in medicine. Further experiments could test the pteridophyte extracts' activity against more appropriate microbes such as *Candida albicans* or *Penicillium citrinum*. Although baking yeast and econazole nitrate were used by a previous student, John Holl (2002), and showed significantly antifungal results, these findings were not replicated in this study. The econazole nitrate had variable anti-fungal activity which suggests that it was not an appropriate anti-fungal for the baking yeast and that perhaps the yeast is not the best antifungal bioassay organism.

Insect damage is a large problem in agriculture, and commercial pesticides are costly and damaging to the environment. Plant toxins, which have been known to inhibit insect growth, reproduction and feeding, are a potential source of new pesticides. (Mansingh and Williams 1998; Grainge et. al. 1986; Williams and Mansingh 1996). The insecticidal activity of *A. evecta* against GWSS juveniles indicates the possibility of such a toxin in this fern. Experiments to assess *A. evecta*'s effect on insect reproduction, growth and behavior along

Treatment			FIC
<i>M. grossum</i>	A		0.79
Antifungal	A	B	0.64
<i>D. solida</i>	A	B	0.57
<i>D. linearis</i>	A	B	0.57
<i>L. cernua</i>	A	B	0.50
<i>A. evecta</i>	A	B	0.50
<i>A. trapeziforme</i>	A	B	0.43
-control		B	0.14

Table 2. Antifungal activity presence or absence results for 90%ethanol root extracts from 6 pteridophytes ($p \leq .037$, $df=7$, $F\text{-ratio}=2.4$). FIC=frequency of inhibitory change.

Scientific Name	Tahitian Name	Traditional medicinal use:
<i>M. grossum</i>	Metuapua -LYE	Heal broken bones, sinus headaches, wasp stings -Y* L E P
<i>M. commutatum</i>	Maire -LYE	-*LE
<i>A. australasicum</i>	Oaha -LYE	Heal headaches, colds -E *L P
<i>A. plantagineum</i>	Maire Oviri -Y	None known
<i>B. lonchophora</i>	None known	-*L
<i>A. trapeziforme</i>	None known	None known
<i>A. evecta</i>	Nahe -E	-P*E
<i>L. reticulatum</i>	None known	None known
<i>L. cernua</i>	Maiuu Tafai -LE	-Y *L
<i>N. hirsutula</i>	Maire Tahiti -L Amo'a -E	Heal surface lacerations -L E P
<i>D. solida</i>	Tiatia Mou'a -L Titi -Y E	Heal serious bodily injury, cleansing bath for newborns, -Y treat children's ailments -*L, and broken bones -E P
<i>D. linearis</i>	Nai-uu tafa'I -L Anuhe -Y	*L E

Table 3. Pteridophyte names and traditional uses as reported in interviews conducted with healers and elders on Moorea, French Polynesia, and from a reference ethnobotanical book by Paul Petard (1986). Notes: L= Mama Lucie, Y= Rita You-Sing, E= Elders(Papa Matarau, Papa Mape, Papa Mehai), P=Petard, *= plant is used but particulars were not given.

with an analysis of the compounds present in the pteridophyte could lead to the development of a natural, inexpensive, and eco-friendly insecticide.

The significant bioactivity of the ferns in these three bioassays suggests the presence of secondary compounds. Nine of the twelve ferns showed at least one significant bioactive result, and the significant differences in herbivory between ferns suggest variability in active compounds.

A phytochemical screening of pteridophytes in Western Ghats-South India revealed the presence of multiple compounds. All extracts possessed steroids, sugars, alkaloids, phenols, flavonoids, saponins, tannins, and amino acids, and for the 15 taxa analyzed ethanol extracted the most secondary compounds (Jesudass 2003). A similar screening of the pteridophytes collected in Moorea will be crucial in order to determine

whether their bioactivity is due to secondary chemistry.

The isolation of secondary compounds from ferns could add to the understanding of pteridophyte evolution. Recent molecular data suggests that ferns radiated in the Cretaceous period after the angiosperm-insect radiation (Schneider et al. 2004). Presence of insect specific defenses or abundant secondary compounds would indicate fern evolution under the same constraints as angiosperms and thus support this post-angiosperm radiation. However, a lack of secondary compounds and bioactivity would indicate, possibly, an earlier evolutionary radiation free of insect pressure. Far more research in phytochemistry and insect herbivory, however, will be needed to make hypotheses about the evolutionary history of pteridophytes.

Evolutionary phylogenies may explain similar characteristics of organisms. Such a phylogeny of pteridophytes breaks the group into four divisions. Eleven of the twelve ferns studied are from the polypodiophyta division, and *L. cernua* is from the Lycopodiophyta division. The Lycopodiophytes are a more ancient lineage than the polypodiophytes, and would thus be expected to have fewer secondary compounds (Campbell and Reece 2005). Indeed this study showed that *L. cernua* only showed significant bioactivity in its leaf extract. ANOVA analysis, however, showed no significant difference in cytotoxicity between the lycopod and polypod divisions ($p \leq .83$, $df=1$, $F\text{-ratio}=.05$). There was also no correlation between relatedness and bioactivity within the polypodiophytes. Ferns that were more closely related did not behave more similarly, as might be predicted. For example, *A. trapeziforme* and *A. plantagineum* are in the same order however *A. trapeziforme* was cytotoxic in only one comparison compared to *A. plantagineum* which was cytotoxic in three comparisons. For *M. grossum* and *M. commutatum*, which are in the same genus there was no great similarity in their activity.

Along with providing insight into the phytochemistry and evolution of pteridophytes, the results of this study are important in a cultural context. The pteridophytes most used by traditional Tahitian healers, *D. solida* and *M. grossum*, showed almost opposite bioactivity results. *D. solida* did not show any significant bioactivity, while *M. grossum* was both cytotoxic and the only antifungal agent. *A. plantagineum*, which was not identified as useful by any sources, was significantly cytotoxic in all comparisons. The bioassays performed perhaps were not appropriate to isolate the compounds in all the pteridophytes. But it is this difference between traditional knowledge and bioactivity results

which begs the opportunity for the exchange of knowledge: further ethnobotanical investigation could indicate which assays to attempt and these assays might reveal other useful compounds in previously unused plant resources.

Paul Petard's definitive work, *Plantes Utiles de Polynésie* (1986), published nearly 10 years ago, was the first comprehensive record of Polynesian Ethnobotany. It is an important tool not only for scientists interested in bioactivity, but for Polynesians. Both healers interviewed referred to this text during the interviews. However, the use of pteridophytes for traditional medicine found in this study differs from Petard's account. Four ferns, whose use was not recorded by Petard, were identified as useful in this study. Mama Lucie used 8 of the twelve ferns and explained that she experiments making new medicines with new plants, thus expanding her secret library of treatments (pers. com. 2005). This experimentation by isolated healers explains the discrepancy between literature and this study. An updated ethnobotanical text could help to spread useful medicinal knowledge between healers.

The barriers that insulate and isolate healers from each other and from the western world can prevent the active exchange of information. However, these constructs are actively being broken down by French Polynesians and this study contributes to a new age for integrative ethnobotany and traditional medicine in French Polynesia. In concert with French Polynesian NGOs and federations, scientific study of traditional treatments can help to legitimize cultural practice and help people make use of their botanical resources. This discourse between scientists and traditional healers was encouraged in a meeting of healers in Pao Pao, Moorea. Papa Matarau entreated the audience, "There is a strength coming up from the ground. Don't you want to see it sprout once again?" (pers. com. 2005). The scientific community can play a mutually beneficial role in this discourse, gathering information to guide the search for new medicines, as well as nurturing this renaissance in polynesian culture.

ACKNOWLEDGEMENTS

This project could not have been possible without the help of Valentine Brotherson. I would also like to thank all the professors and Graduate Student Instructors of this course for giving me their insight, guidance, and logistical support through all the foibles. I would also like to thank my benevolent benefactors Susan and Joe Baltrushes.

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APPENDIX A. Cytotoxicity Results

Treatment	Average death rate			
<i>C.roseus</i>	A			49.33
<i>A.plantagineum</i>	A	B	C	40.50
<i>A.australasicum</i>	A	B	C D	38.33
<i>L.reticulatum</i>	A	B		37.92
<i>B.lonchophora</i>	A	B	C	37.42
<i>M.grossum</i>	A	B	C	36.75
<i>M.commutatum</i>	A	B	C D	36.42
<i>A.trapeziforme</i>	A	B	C D	35.75
<i>N.hirsutula</i>	A	B	C D	30.83
<i>D.solida</i>	A	B	C D	27.42
<i>L.cernua</i>		B	C D	22.50
<i>D.linearis</i>		B	C D	21.67
<i>A.evecta</i>			C D	18.83
none			D	13.83

Table 3. Cytotoxicity of 90% ethanol extracts of 12 pteridophytes in a Tukey-Kramer HSD analysis. Levels not connected by the same letter are significantly different. ($p \leq .0001$, $df=13$, F-ratio=4.76)

Treatment	Average Death Rate			
<i>C.roseus</i>	A			49.33
<i>M.commutatum</i>	A	B		44.17
<i>D.solida</i>	A	B	C	35.17
<i>N.hirsutula</i>	A	B	C D	31.17
<i>A.trapeziforme</i>		B	C D	26.58
<i>L.cernua</i>			C D	23.92
<i>M.grossum</i>			C D	23.42
<i>B.lonchophora</i>			C D	22.42
<i>L.reticulatum</i>			C D	21.42
None			D	14.00
<i>D.linearis</i>			D	13.75
<i>A.evecta</i>			D	13.50

Table 5. Cytotoxicity of root extracts of 12 pteridophytes in a Tukey-Kramer HSD analysis. Levels not connected by the same letter are significantly different ($p \leq .0001$, $df=11$, F-ratio=7.8). Extracts were not made from *A.plantagineum* or *A.australasicum* root matter because their roots are difficult to collect and isolate.

Treatment	Average Death Rate			
<i>C.roseus</i>	A			49.33
<i>M.commutatum</i>	A	B		41.42
<i>A.plantagineum</i>	A	B	C	39.50
<i>L.cernua</i>	A	B	C	36.25
<i>A.australasicum</i>	A	B	C D	30.67
<i>A.trapeziforme</i>	A	B	C D	26.92
<i>M.grossum</i>		B	C D	23.42
<i>B.lonchophora</i>		B	C D	21.83
<i>D.solida</i>		B	C D	21.58
<i>N.hirsutula</i>			C D	19.75
<i>A.evecta</i>			C D	18.67
<i>D.linearis</i>			C D	16.00
None			C D	14.17
<i>L.reticulatum</i>			D	7.17

Table 4. Cytotoxicity of water extracts of 12 pteridophytes in a Tukey-Kramer HSD analysis. Levels not connected by the same letter are significantly different ($p \leq .001$, $df=1$, F-ratio=11).

Treatment	Average Death Rate			
<i>C.roseus</i>	A			49.33
<i>A.plantagineum</i>	A	B		40.00
<i>B.lonchophora</i>	A	B	C	36.83
<i>M.grossum</i>	A	B	C	36.75
<i>A.australasicum</i>	A	B	C	36.42
<i>A.trapeziforme</i>	A	B	C	36.08
<i>L.cernua</i>	A	B	C	34.83
<i>M.commutatum</i>	A	B	C	33.67
<i>A.evecta</i>		B	C D	24.00
<i>D.linearis</i>		B	C D	23.92
<i>L.reticulatum</i>		B	C D	23.67
<i>N.hirsutula</i>			C D	19.42
None			D	14.00
<i>D.solida</i>			D	13.83

Table 6. Cytotoxicity of leaf extracts of 12 pteridophytes in a Tukey-Kramer HSD analysis. Levels not connected by the same letter are significantly different ($p \leq .0001$, $df=13$, F-ratio=6.7).

APPENDIX B
Mama Lucie Interview, Moorea, French Polynesia October 2005

Scientific Name	Given Name	Used?
A. plantaginuem	None	None
A. australasicum	Oaha	Yes
A. evecata	-----	-----
A. trapeziforme	None	No
B. lonchophora	None	Yes
D. solida	Tiatia Mou'a	Yes*
D. linearis	Naiuu tafa'I	Yes
L. cernua	Maiuu Tafai	Yes
L. reticulatum	None	None
M. commutatum	Maire	Yes, leaves and roots
M. grossum	Metuapuaa	Yes, fronds without sori only
N. hirsutula	Maire Tahiti	Yes**

Notes: Mama Lucie did not want to give any particulars on how she uses these plants.

* Uses it to treat children's ailments and sometimes adults.

** The hair from the mid-segment of this fern is put in the baby's belly button right after the umbilical cord is cut to heal the wound.

Additional questions and answers

Do you use the leaves or roots of these ferns?

Sometimes I use the roots and sometimes the leaves, but I never use them together.

Is one stronger?

They have the same strength

How do you keep insects from eating the plants in your garden?

I use a product of "human making." (Human urine is used around the base of plants to keep ants away.) Also, smoke from fire scares insects away. Only use natural insecticides because the plants will be used for medicine. You can also use the smoke from a mosquito coil.

Have you heard of Horo Papua being used as an insecticide?

It is used to kill fish. I know a man who uses it but it is bad. Small amounts of it build up, and some people probably use it but I don't.

Do you make new medicines?

I experiment with new formulas on myself. If it works on myself then I give it to others.

What kind of ailments do you treat?

I treat almost all ailments.

IMPRESSIONS:

Mama Lucie was reluctant to talk to us at all. When we arrived, very late, she was sleeping and so we waited for half an hour for her. She talked to us as if she was very suspicious. She did not want to give away any particulars about her medicine. She did not even want to give her full name when I asked it. By the end of the interview she was much more at ease and had her grandson pick and give me different plants from her garden. She told me their names and uses. Val said she did not want to give out the secrets to her medicine because she had done the tests and her own experiments.

I asked at the end if I could take pictures of her garden and she obliged. I asked if we could take picture with her and she put on a fancy dress and hat instead of her daily pareo and emerged from inside her house.

APPENDIX C
Interview with Rita You-Sing Moorea, French Polynesia October 2005

Scientific Name	Given Name	Used?
A. plantaginuem	Maire Oviri	None
A. australasicum	Oaha	For decoration
A. evecata	None	None
A. trapeziforme	----	----
B. lonchophora	None	No
D. solida	Titi	Yes*
D. linearis	Anuhe	For decoration
L. cernua	Mou tafai	For decoration, wreaths
L. reticulatum	None	For decoration
M. commutatum	Maire	None
M. grossum	Metuapuaa	Yes**
N. hirsutula	None	For decoration

* Used for decoration and also used medicinally. If there is a bad accident and part of the body is broken, use a section of the root the length from your first finger to the end of your palm (5inches). Mash the root and mix with other roots and leaves in an Umete (a long wooden bowl) with green coconut water.

Three inches of liquid in a cup is drunk three times a day. The medicine is made in a gallon jug and when the jug is half empty then it is filled up with more coconut water to dilute it now that it has strengthened from time brewing. This medicine is drunk until the person is better.

Leaves of Titi are used to treat babies

When a baby is first born and it wakes in the night excited and shivering unable to sleep (a condition called "ira" in Tahitian) a bath is prepared using Titi. Leaves are boiled and the excited baby is calmed by bathing in this tea.

** Roots are used to treat broken bones (along with Titi)

Leaves are also used in the baby bath with Titi—this mixture is not drunk by the baby because the "poison is very strong."

Also used for sinus headaches, and if you are bit by a wasp in the mountains you take the root of Metuapua mash it up and put it on the sting to stop soreness and pain immediately after the sting.

Metuapua with "3 leaves" is used to treat babies and in the broken bone treatment, and with "7 leaves" it is used in the baby bath.

Additional questions and answers:

Have you heard of using Horo papua as an insecticide?

People used it a long time ago to kill fish.

What do you use in your garden to keep the insects away?

I don't use anything in my garden to keep bugs away. You could use Soursop seeds mashed up, mixed with water(15 seeds in 2 liters water). Spray on flowers and it kills insects sticking under the leaves.

What kinds of leaves do you pick for medicine making?

For medicine, leaves must be uneaten and clean. The younger leaves are mashed for drink, but older leaves are boiled and used in baths.

Why do you think ferns are not used as much as other plants?

Knowledge is passed down from person to person and people use what works. Healers don't necessarily explore other plants. Those who do, will experiment with new plants on themselves preparing medicines to what effects they notice on themselves.

HABITAT SELECTION AND SPATIAL DISTRIBUTION OF TWO FIDDLER CRAB SPECIES (*UCA CRASSIPES* AND *UCA TETRAGONON*)

Alicia Bihler

Department of Integrative Biology, University of California, Berkeley
Berkeley, California 94720

Abstract: *Uca crassipes* (Adams and White, 1848) and *Uca tetragonon* (Herbst, 1790) are the only two fiddler crab species found on Moorea, French Polynesia. This study addresses the spatial distribution of the crab burrows of these two species within the intertidal mudflats as a means of understanding their habitat selection. Sampling took place at four mudflat intertidal zones in Moorea using randomly determined transects with 0.25m² quadrats. Number and species were counted as well as percent vegetation cover. Soil samples were collected from each quadrat and analyzed for particle size. *U. crassipes* had a strong non-random spatial distribution. Through ANOVA analysis these crabs were shown to occur predominantly at higher elevations. These results support that which was suggested by Ens and Klaassen in 1993 in the fiddler crab *U. tangeri*. The crabs burrows are densest at higher elevations along the tidal gradient.

Keywords: mudflats, non-random distribution, clustered distribution, elevation, French Polynesia

INTRODUCTION

In taxa that excavate holes, spatial arrangement and habitat selection are controlled by many factors (Begon *et al* 1986). Biotic factors, such as proximity to other species, and abiotic factors must be closely considered (Schroder 1987, ENS 1993, Jianping 2005). In this study fiddler crabs are used as a representative of a burrowing animal to address the effect of resource availability on burrowing density and patterning.

Fiddler crabs are found throughout the world at both tropical and temperate latitudes. They are often associated with mangroves and mudflats, or other muddy substrates in the intertidal zone. Fiddler crabs remain in their burrows when submerged at high tides and emerge to forage, mate and defend their burrows during the ebb when their burrows are exposed.

Studies on the distribution on other species of fiddler crabs in mangrove inter-tidal areas have suggested that the distribution is predominantly influenced by shore level, temperature, salinity, sediment grain size, and organic matter content (Castiglioni 2005, Koch 2005, Litulo 2005). This study aimed to add information about the spatial pattern of fiddler crab holes. I evaluated some of the biotic and abiotic factors that influenced habitat selection. Understanding the effects of these factors on density-dependent distributions helped to better understand how the previously addressed variables interplayed with the crabs' location.

Uca crassipes (Adams and White, 1848) and *Uca tetragonon* (Herbst, 1790) are the only two

fiddler crab species found on Moorea (F. Branwell, pers. comm.). These two species have received relatively little study. Previous work of *U. crassipes*, and *U. tetragonon* on Moorea was restricted to the Temae mudflats (F. Barnwell, pers. comm.). Other studies of the two species in the greater Pacific area have focused on the defensive and mating behavior, and tidal influences (Murai *et al* 1995, Goshima *et al* 1996). An ecological study of *U. crassipes* and *U. tetragonon* combined with previous work will add to the understanding and interpretation of their biology.

Both *U. crassipes* and *U. tetragonon* have overlapping distributions throughout the Indo-Pacific, including the island of Moorea (www.fiddlercrab.info). Although strongly associated with mangroves (Gershman 1997, Costa 2003, Castiglioni 2005, Koch 2005, Litulo 2005, Khan 2005) these fiddler crabs also occur in mudflats with no mangroves. Their behavior is closely tied to the tidal cycle. They are affected by both the daily highs and lows, and the lunar cycle. Both species are very active and interact at low tide. They can be seen feeding, fighting, and the males can be seen moving their large claw for mate attraction. At high tide, or when startled, they remain in burrows that can be more than one meter in depth.

Koch *et al* (2005) found significant differences in the community structure of four fiddler crab species in Northern Brazil. Their study included predetermined, distinct zones based on observation. To avoid possible artificial limitations of the crabs' distributions, I wanted to examine the length of the

intertidal mudflats without assigning zones within the habitat. Due to differences between the two species on Moorea, and because they are inhabiting the same area, I predict that there will be distinct clustering of each species according to the competitive exclusion principle. This states “two species may not occupy the same niche at the same time in the same place” (Becby and Brennan 1997).

This study considered the following factors involved in the spatial distribution, and ultimately the habitat selection of the crabs: elevation, percent of vegetation coverage, sediment size, and spacing of the crabs. Spacing of the crabs was particularly interesting. In a previous study on a different fiddler crab species *U. tangeri*, Klaassen and Ens (1993) found three primary factors that contributed to the burrow location: (1) the ability to excavate deep burrows, (2) locations where there is more time available for digging burrows, and (3) and substrate better suited for courtship (drier substrate allows for better drumming by the male during courtship behavior). These findings suggest a clustered, non-random spacing of the crabs. There were equal numbers of male and female crabs within a given mudflat (Castiglioni 2005, Cesar, Ines *et al* 2005, Jaroensutasinee and Jaroensutasinee 2004, Koch 2005). I predict there will be no statistically different distribution of intraspecific males and females, and that the distribution of crabs will be correlated with sediment size, as past literature has suggested (Koch 2005, Khan 2005).

MATERIAL AND METHODS

Study Site

This study sampled four mudflat sites on the island Moorea, French Polynesia. The first and third sites are located just north of Haapiti. Site 1 (UTM 0194886, 8056563) is a mangrove site about a kilometer north of Haapiti, and Site 3 (UTM 0194355, 8056752) is the mangrove site about 2 kilometers north of Haapiti. Site 2 (UTM 0193098, 8063682) is another mangrove area located half a kilometer east of the entrance to the beachcomber. The final site is the Temae mudflats (UTM 0205035, 8065387) located on the island side of the conglomerate platform of the Temae motu (Fig. 1).

Field Sampling

I sampled each site using 4 transects with 10 quadrats per transect. This totaled 16 transects and 160 plots. Four randomly placed transects at each

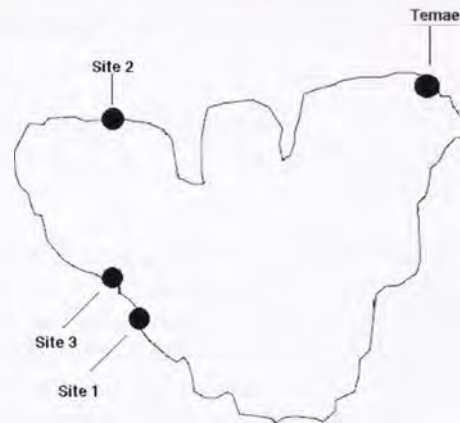


Figure 1. Map of Moorea showing the 4 study sites.

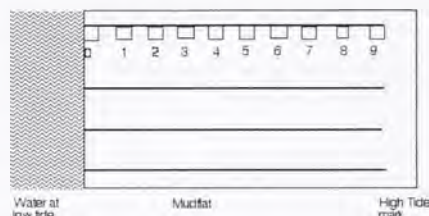


Fig. 2. Diagram of transects at a site. One transect displays the plots.

site ran from the low tide mark to roughly the high tide mark, perpendicular to the water. Each transect had 10 plots regardless the length of the transect. Transect lengths ranged from 10m to 50m depending on the site, but always ran from low tide to high tide. Plot 0 marks the low tide mark, and Plot 9 marks the high tide mark. The quadrats were 0.25m². In order to eliminate possible effects of disturbance from the quadrat on the crabs, the corners of each plot were marked with flags, then the quadrat removed. The whole transect was set up, with the tape and plot removed, before I began to sample (See Figure 2).

For each plot I recorded the number of burrows, number of burrows with ovigerous females (recognizable by the chimney-like structure of the burrow), and the percent of vegetation ground coverage. After these initial observations, I would stand or sit a few meters back from the quadrat for three to five minutes. Preliminary field sessions showed 3 to 5 minutes to be sufficient for most crabs to emerge. I recorded the number of males and females of each species, and noted which species came out of the ovigerous burrows. After sampling was complete, I would then go back and collect a small zip-lock bag full of substrate from each quadrat to be taken back to the lab for further analysis. All sampling occurred within 2 hours of low tide.

All transects were randomly selected allowing me to compare quadrats within sites, as well as

between sites. Photographic vouchers were taken of each species.

Soil Analysis

120 randomly selected soil samples were analyzed. I dried each soil sample in plastic cups at 65°C for at least two days. Each soil sample was then removed and broken up. A 40g sample was passed through 6 sieves. The mesh sizes were: 3.5mm, 2.0mm, 1.0 mm, 0.5mm, 0.25mm, and 0.1mm. Because water was used to remove the soil from the sieves, the 6 sieved fractions were dried again in the oven at 65°C for eight hours and then weighed.

Statistical Analysis

Data were analyzed using JMP software and Excel to run regressions, ANOVAs, Tukey-Kramer comparison, and χ^2 tests. The spatial pattern of the crabs was analyzed using a χ^2 test the fit against a Poisson distribution. The relationship between the number of holes observed and the number of *U. crassipes* and *U. tetragonon* present was analyzed with using a regression. The ANOVAs and Tukey-Kramer comparisons were used to analyzed the number of crabs present at different elevations. A p value of less than 0.05 was considered to be significant.

RESULTS

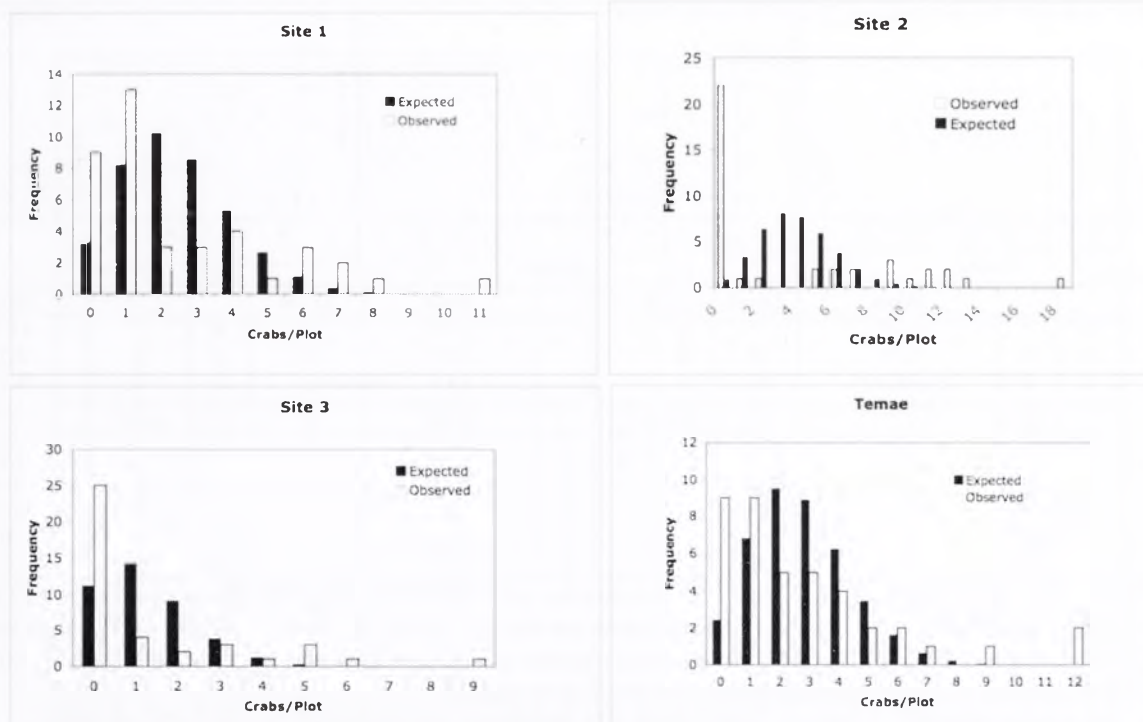


Fig. 3. The observed distribution of *U. crassipes* (in white) compared to the Poisson distribution (in black). Each of these distributions are significantly different from the Poisson with a power of 0.05

Transect and plot data

At all sites there was a positive linear

Table 1. The linear relationship of the observed number of crabs by the total number of holes seen. *Uc*=*U. crassipes*, *Ut*=*U. tetragonon*.

Site	Relationship	p value	R ²	slope
1	<i>Uc</i> total	<0.0001	0.55	0.24
1	<i>Ut</i> total	0.0006	0.27	0.12
1	Total Crabs	<0.0001	0.71	0.37
2	<i>Uc</i> total	<0.0001	0.69	0.26
2	<i>Ut</i> total	<0.0001	0.59	0.19
2	Total Crabs	<0.0001	0.79	0.44
3	<i>Uc</i> total	<0.0001	0.73	0.37
3	<i>Ut</i> total	<0.0001	0.7	0.24
3	Total Crabs	0.0282	0.12	1.02
Temae	<i>Uc</i> total	0.0003	0.29	0.39
Temae	<i>Ut</i> total	0.0353	0.11	0.12
Temae	Total Crabs	<0.0001	0.42	0.51

relationship between the total number of holes counted per plot and the number of crabs recorded per plot. A positive relationship also existed between number of crabs of each species and the total number of holes per plot (See Table 1).

For all sites, a χ^2 test of the observed number of *U. crassipes* per plot compared to a random Poisson distribution was significantly different

($p=0.05$). The data supported the hypothesis of a clustered distribution (Figure 3). I was unable to reject the null hypothesis that *U. tetragonon* exhibited a random distribution due to inconclusive data. Site 2 and Temae were the only sites to support a non-random distribution of *U. tetragonon* ($p=0.05$). For all sites, a χ^2 test of the observed total number of crabs per plot compared to a random Poisson distribution was significantly different.

A χ^2 test of the distribution of *U. crassipes* per plot compared to the distribution of *U. tetragonon* per plot was inconclusive. Only half of the sites (Site 1 and Temae) yielded significantly different

($p=0.05$) results. All sites yielded no significantly different results when comparing the distributions of males and females of the same species using a χ^2 analysis.

An ANOVA of the total number of *U. crassipes* by elevation showed a significant difference at Site 1 ($p=0.0223$), Site 2 ($p<0.0001$), and Site 3 (0.0013). There was no significant difference for Temae. Following each ANOVA with a Tukey-Kramer test for means comparison yielded no difference between means for Site 1. At Site 2, the test confirmed that plots at lower elevations (those labeled 0 through 4) have fewer crabs than those plots at higher elevations (plots 6

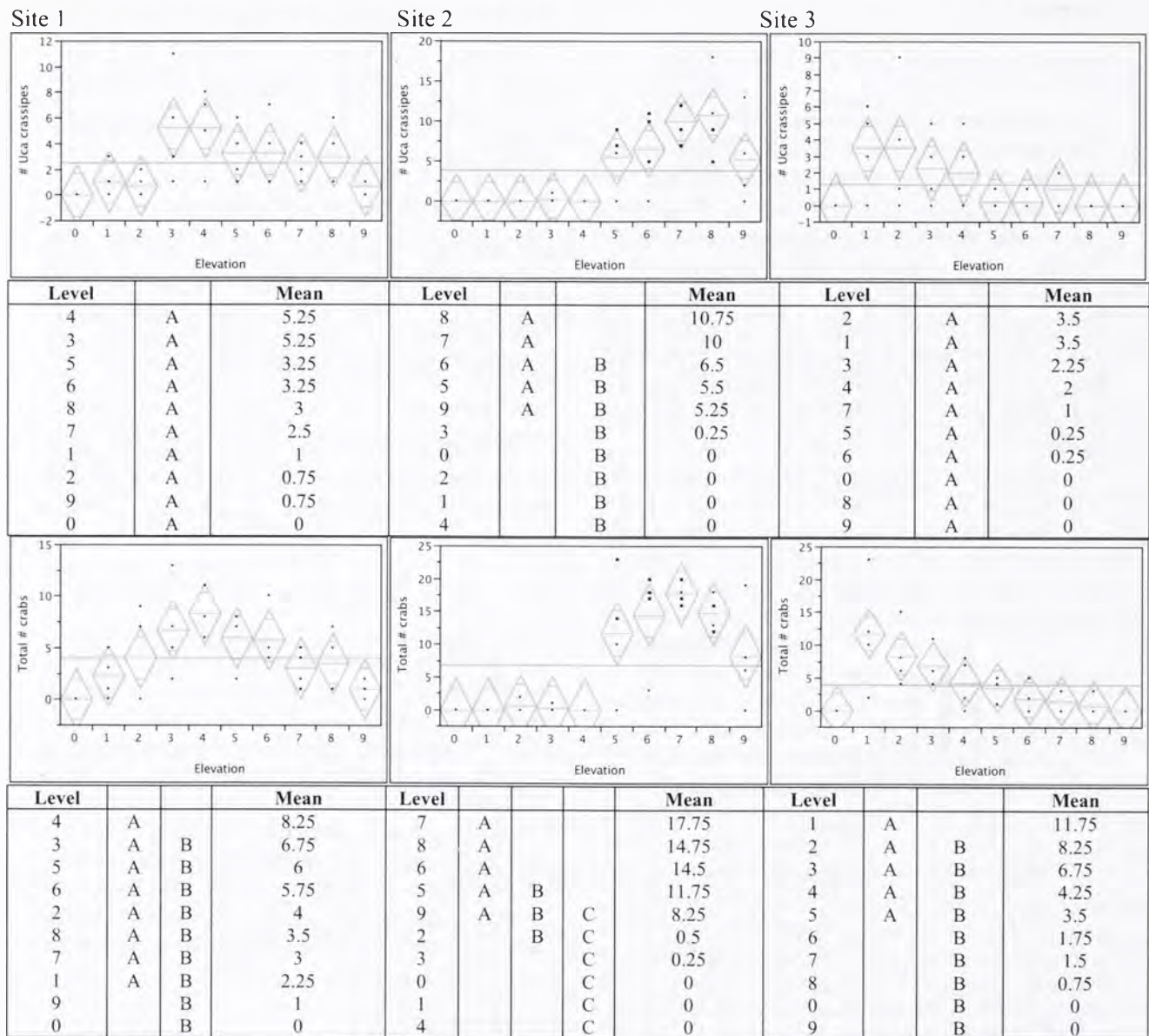


Fig. 4. ANOVA results from Sites 1, 2, and 3 for the number of *U. crassipes* by elevation. p values are 0.0223, <0.0001, 0.0013, and 0.587 respectively. Directly below are the Tukey results for each ANOVA. The next row is the ANOVA results from Sites 1, 2, and 3 for the total number of crabs by elevation. p values are 0.0056, <0.0001, and 0.0007 respectively. Again, Directly below are the Tukey results.

and 7). At Site 3, the Tukey test showed there to be no difference (See Figure 4).

An ANOVA of the total number of crabs per plot by elevation showed a significant difference at Site 1 ($p=0.0056$), Site 2 ($p<0.0001$), and Site 3 (0.0007). There was no significant difference for Temae. The Tukey-Kramer comparison yielded the following: at Site 1 a mid elevation plot (4) had more crabs than plots marking the extremes, at Site 2 higher elevation plots (6, 7, and 8) have significantly more crabs than lower elevation plots (0 through 4), and at Site 3 a low elevation plot (1) had more crabs than extreme elevation plots (0, 8, and 9) (See Figure 4).

Combined data

Because Temae showed no significant results in the ANOVA tests, the data for the first 3 sites was combined and analyzed.

After combining the data from the Sites 1 though 3, an ANOVA and Tukey-Kramer analysis yielded similar results. An ANOVA of the number of *U. crassipes* by elevation is not quite significant ($p=0.0587$), and the Tukey test yields no significant differences either. However a Student's *t* test revealed that plots at a higher elevations (7 and 8) have more crabs than plots at lower elevations (0, 1, and 2) (Figure 5).

An ANOVA of the combined data for total number of crabs by elevation had significant results ($p=0.027$). A Tukey-Kramer test supported that a plot at higher elevation (plot 7) had more crabs than the plot that represents the lowest elevation (Figure 5).

Soil Analysis

There is no measured difference in vegetation or sediment size by elevation.

DISCUSSION

The regression analysis showed several positive relationships between the observed number of crabs and the number of holes, supporting this sampling technique for this environment and system. It was impossible to observe all the crabs, so it is important that the data reflects the environment. Therefore the observed positive relationships are important.

The data strongly support my hypothesis for a non-random spatial distribution for the species *U. crassipes*. Based on the shape of the curve (See Figure 3) it appeared that *U. crassipes* has an aggregated, or clumped, distribution. Although there were inconclusive results for *U. tetragonon*, it is possible that these results are due the low frequency of this crab. This data, however, although results were inconclusive, lends supports that both of these crabs have independent distributions. They are not clustering in the same areas. This is as the competitive exclusion principle would have predicted. Because these are two closely related species they cannot occupy the same niche at the same time and place.

As was predicted males and females of the same species showed no difference in their distributions. This is in agreement with previous work done. Other studies have shown that in

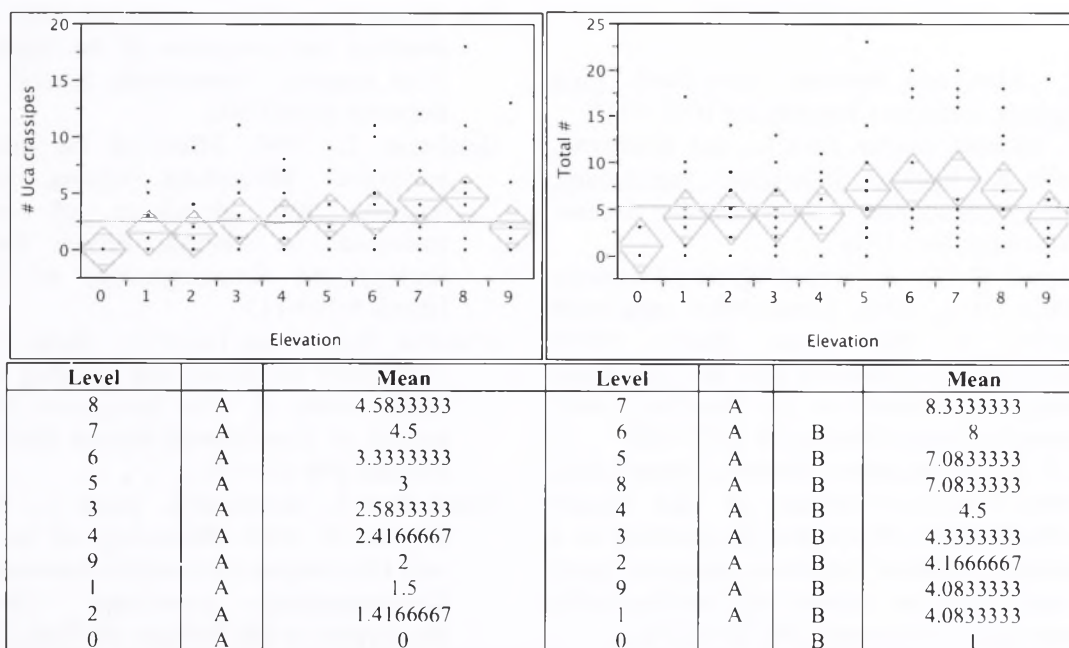


Fig. 5. The ANOVA results for the combined data from Sites 1, 2, and 3. The chart on the left is the number of *U. crassipes* per plot by elevation ($p=0.0587$). The chart on the right is the total number of observed crabs per plot by elevation ($p=0.027$). Below each chart is its respective Tukey results

populations of fiddler crabs males and females are relatively equal in number (Castiglioni 2005, Koch 2005). Due to the equal sex ratio that has been reported in past studies, the males and females have overlapping distributions. This study could not find significantly different male and female spatial distributions.

The results for the ANOVA showed interesting patterns. Across three of the four sites *U. crassipes* demonstrated a strong clustering by elevation. This was even further supported when the data for the three sites was combined. Higher elevation plots were consistently showing to have more crabs of *U. crassipes* than lower elevation plots. Further analysis was done looking at other factors, such as sediment size and vegetation, but there were no other significant relationships to demonstrate what it is the crabs are clustering around. It is possible that elevation, and all the factors that come with a higher elevation (such as depth of aerobic sediment, tidal coverage time, salinity or water in burrows of a the day, etc) determine the location of the crab burrows. If this is the case, it is these alternative factors that could be determining the burrow location.

The question still remains what is different about Temae. Why does the data from Temae agree that *U. crassipes* has a clustered distribution, but is in conflict with the ANOVAs for elevation? The data I collected does not answer this question. Perhaps it is due to some inherent difference in the makeup and geology of Temae. The site at Temae

is part of a motu, and it is the only site where there were no mangroves present.

The question still remains as to why these crabs only occur predominantly in this small region in the intertidal zone. As Ens and Klaassen (1993) suggested in their study on *U. tangeri*, fiddler crabs look for three things in their environment. And it is at the higher elevations in the intertidal zone that all three requirements (deep burrows for protection, more time available for digging burrows, and substrate suited for reproduction) can be met.

The species *U. crassipes* does show a marked non-random distribution; however, *U. tetragonon* does not display this pattern consistently. Both species are shown to occur predominantly at plots marking higher elevations within the high to low tide region. The exact factors involved in this preference are unknown. It could be that this elevation is possibly be more accommodating to the factors like those that Ens and Klaassen (1993) suggest occur at higher elevations and are preferred by the fiddler crab *U. tangeri*.

ACKNOWLEDGEMENTS

I would like to thank all the professors and GSI's of the course, but especially J. Bartolome, and G. Roderick for helping me with my experimental design and data analysis; and R. Caldwell and M. Weber for their help with the final paper and presentation.

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COMPARISON OF MACROFAUNA DECOMPOSER COMMUNITIES BETWEEN AN URBAN AND FOREST ENVIRONMENT ON MOOREA, FRENCH POLYNESIA

JENNIFER M. BREWER

Department of Integrative Biology, University of California, Berkeley, CA, 94720

Abstract. This study was conducted on Moorea, French Polynesia to investigate the contrasting arthropod decomposition communities in a forest and urban habitat. Decomposition substrate consisted of six species of tropical fruit to additionally observe whether arthropods exhibit food specificity. After one month of daily observation, the results showed significant differences in the abundance of Hymenoptera, Diptera, Diptera larvae, Diplopoda, Dermaptera, Isopoda, and Coleoptera between the two locations, Gump and Belvedere. In addition, mean numbers of individuals of several taxa differed significantly among fruit species. Diplopoda and Isopoda were found significantly more on soursop, and Dermaptera were found significantly more on soursop and star apple. The significant difference in decomposition communities between the two locations could be due to the combined influence of contrasting habitats and the effect of urban development on arthropod niches. Fruit specificity exhibited by some arthropod taxa appears to be most likely related to external fruit morphology and arthropod species competition, though chemical makeup of the fruit is a possibility. On an island where there are large numbers of nonnative species and low species richness, it is important to investigate the decomposition communities which are available in varying environments in order to estimate the health and stability of the entire living system. This study reveals that while the decomposer communities are significantly distinct, urban development does not appear to negatively impact the level of species abundance and diversity in these communities.

Key words: Arthropod; species richness; detritivore; tropical fruit

INTRODUCTION

Regulation and maintenance of proper ecosystem function depends on how efficiently nutrients are regenerated from detritus into new plant growth (Ricklefs, 1973). Factors influencing the rate of nutrient recycling include temperature, humidity, and light in the micro-environment, as well as the abundance and diversity of organisms available for detritus consumption (Ricklefs, 1973). Arthropods make up a significant part of the decomposition community which facilitates the return of organic material to the ecosystem (Tullis, Goff 1987).

The study of functional groups, or groups of taxa which fill analogous niches, has received increased attention due to the interest in species richness and its effect on ecosystem function (Hulot et al, 2000; Petchey & Gaston 2002).

Studies have shown that functional diversity influences how an ecosystem responds to disturbance (Hulot et al, 2000).

Decomposition on remote islands is of pertinent interest since islands are known for having a large number of nonnative species and low species richness (Gillespie, Roderick 2002). Thus observing what arthropods are available for the crucial process of decomposition might shed light on species richness and the level of biodiversity on the island.

This study investigates arthropod communities available for the decomposition of six species of cultivated tropical fruits between an urban and forest setting on Moorea, French Polynesia. The nature of any decomposer community depends on the micro-environment in which the organisms live. Factors such as light, humidity, water film layer, and temperature can determine the habitat suitability for a given organism (Begon et al, 1990). On Moorea, the micro-environment within the

dense *Inocarpus* forest at higher elevations contrasts sharply with urban developed areas at sea level.

Two hypotheses were tested: First, the two sites were expected to have distinct decomposer communities reflecting their contrasting habitats. Second, arthropod species richness and abundance would be greater in a nonurban location in the tropical forest, where abiotic factors are ideal for rapid nutrient turnover and arthropod community niches are more removed from modern human impact. A significant increase in the species richness and abundance from the urban to nonurban site might indicate that urban development has disrupted the necessary conditions for certain detritivores to thrive. In contrast, greater diversity and abundance of arthropod species in the urban setting could suggest that humans have introduced many nonnative arthropod species which have outcompeted earlier introduced decomposers in the lower elevations.

In addition, scientists have long studied whether the presence or absence of a particular taxa is indicative of food preference or stage specificity and have found evidence to support both phenomena (Usher, Parr 1977). By choosing six species of tropical fruit as decomposition substrate, this study also investigates whether arthropod decomposers are food specific by observing whether particular taxa prefer one fruit species over another.

METHODS

Study sites

This study was conducted on the island of Moorea, in the Society Island chain of French Polynesia. Two sites were chosen for the purpose of comparing decomposition between an urban and nonurban environment (Figure 1).

The urban site was on the landscaped grounds of the Gump station, on the lawn between the classroom and first bungalow, partially shaded by an *Artocarpus altilis* tree. The comparison site representing a less disturbed environment was chosen in the hills behind the Belvedere lookout, approximately 300 meters south of the parking lot, in a predominantly *Inocarpus* canopy forest. Most of the trees in this region were probably introduced by Polynesians

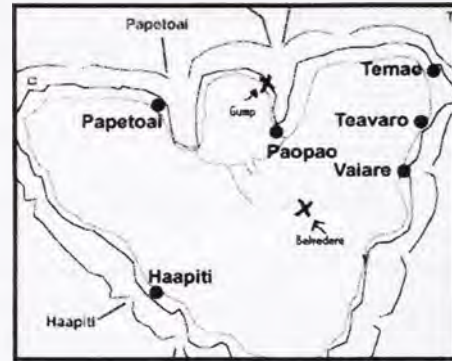


FIGURE 1. Rough location of study sites on Moorea, marked with an X and labeled as Belvedere and Gump.

several hundred years ago, and have not been disturbed for at least the last hundred years. The substrate at the Belvedere site is covered in roots and leaves, the dirt soft and a rich deep brown. Organic matter on the ground is soft and brittle, indicating the fast turnover rate typical of tropical forests (Ricklefs, 1973).

Decomposition Substrate

Six fruits of varying morphology and periods of introduction were chosen as the substrate for decomposition via arthropods. Papaya and banana, *Carica papaya* and *Musa paradisiacal* L. respectively, were introduced by ancient Polynesians, whereas mango, *Mangifera indica*, pineapple, *Ananas comosus*, star apple, *Chrysophyllum cainito*, and sour sop, *Annona muricata*, were introduced at various points since European settlement (Welsh, 1998).

Experimental design

Four fruit containers were constructed from sturdy fine poultry wire on a wood frame to be placed over the decomposing substrate to ensure that only arthropods would have access to the fruits. Each site contained three replications of each fruit species, so that in all 36 fruits were included in the experimental design, 18 at the Gump site, 18 at the Belvedere. Fruits were assigned random locations within the two cages using a random numbers table to account for the edge effect. Fruits were arranged identically at the two sites.

Distributions of Arthropods on Fruit Species

Numbers of individuals of distinct arthropod taxa, including species in the orders Isopoda, Hymenoptera, Diptera, and Dermaptera, were recorded on each fruit once a day every day for one month at both sites. Arthropods were counted on, underneath, and if visible within the fruit itself. At the end of one month, arthropod species diversity and abundance based on recordings were compared between

- 1) Belvedere and Gump sites, and
- 2) fruit species within each site, to determine differences in decomposition communities between an urban and nonurban setting, and between separate fruit species.

Statistical Analysis

In this experiment, fruit species and site location were fixed variables, and numbers of individuals of each distinct arthropod species were response variables, with data being compiled in a time series. 12 graphs were set up, one for each fruit at each location, with arthropod taxa numbers on the y axis and date on the x axis, to observe diversity and distribution of arthropods over time on each fruit. ANOVA was then used in conjunction with Tukey Kramer HSD test looking at data points from five random days to compare arthropod species abundance and diversity among fruit species. T tests were run comparing the total numbers of each distinct taxa over those five random days to determine whether population sizes of each taxa significantly differed between the Gump and Belvedere sites.

RESULTS

Gump vs. Belvedere Decomposition Communities

Arthropods found at decomposition sites between October 13, and November 9, 2005, identified to the level of order are listed in Table 1. Hymenoptera formicidae and drosophila fruit flies from the order Diptera were the first to arrive at the Belvedere, followed by Coleoptera and Diplopoda on day 5, and several other taxa much later. The order of arrival of taxa was similar at the Gump

TABLE 1. A summary of Orders of taxa found on first day of appearance at the forest and urban sites. Multiple taxa from the same order but distinct unidentified families are distinguished by 1, 2, etc. in the "Taxa Order" column. X signifies that no taxa were present at that location.

Taxa Order	Day arrival at Gump	Day arrival at Belvedere
Hymenoptera	1	1
Diptera	9	1
Coleoptera 1	12	5
Diplopoda 1	10	5
Isopoda	10	X
Diptera larvae	11	13
Dermaptera	12	14
Diplopoda 2	15	13
Coleoptera 2	12	X
Coleoptera 3	6	X
Chilopoda	13	X

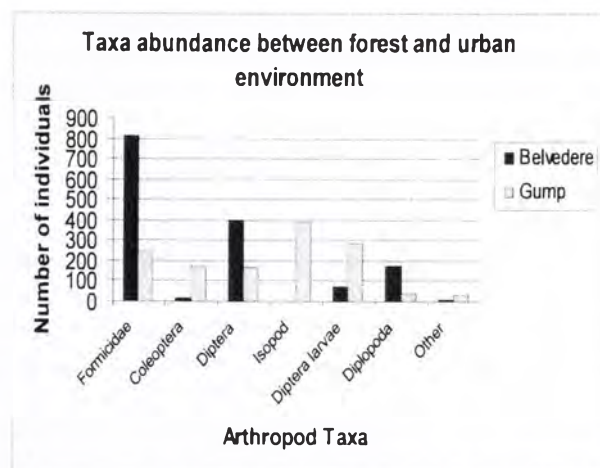


FIGURE 2. Difference in total abundance of arthropod taxa between the Gump and Belvedere sites over an entire month of observation. Black columns represent the Belvedere, gray columns represent the Gump station.

TABLE 2. A summary of t values revealing the significant differences in taxa abundance between the Gump and Belvedere with an alpha value of .05.

Arthropod Taxa	T Ratio	Degrees of freedom	Prob > t
Diptera	2.427799	214	.0160
Ants	4.235297	154.6335	<.0001
Coleoptera	-3.98938	111.7092	.0001
Isopod	-5.52424	107	<.0001
Diptera larvae	-3.32158	159.5636	.0011
Other	-3.13348	148.3078	.0021
Diplopoda	2.783066	129.1845	.0062

station in that Hymenoptera and Diptera were among the first to arrive, but overall the majority of arthropods appeared much later than at the Belvedere. Decomposers at the Gump station then persisted longer in greater numbers whereas the numbers of arthropod individuals at the Belvedere had dropped off. Graphs were constructed to illustrate how the number of individuals of each taxa on each species of fruit varied over time (See appendix).

Figure 2 illustrates the difference in taxa abundance between the two sites. While the Belvedere had significantly more individuals from the Formicidae family, Diptera and Diplopoda, the Gump station had greater numbers of all other taxa. Table 2 summarizes t values to reveal that differences between all of the identified taxa are statistically significant between the two locations.

Thus this first series of t tests comparing taxa populations between the two sites supports the hypothesis that the decomposition communities at the two locations differ significantly. Data was compiled for this t test from five random days throughout the

month so that data series were independent from one another.

Food specificity: Arthropod distribution among fruit species

When comparing arthropod distribution over fruit species, pineapple was eliminated from the comparison because pineapples at the Belvedere disappeared on day five of the study due to demonic intervention. Data was compiled from five random days, and only those taxa whose distribution varied significantly among fruit species are elaborated in the table below. Abundance of Coleoptera, Diplopoda, Hymenoptera, Dermaptera, and Isopoda all varied significantly among fruit species according to the ANOVA and Tukey Kramer means tests, with an alpha value of 0.05. Table 3 summarizes the analysis of variance of taxa populations among fruit species. Table 4 illustrates how means of arthropods differed significantly among fruit species. Only those taxa which are not connected by the same letter are significantly different. Only Diplopoda and Isopoda populations differed

TABLE 3. Analysis of Variance of taxa among fruit species. All of the above P values reveal that the mean abundance of the above taxa varied significantly among different fruit species, with an alpha level of .05.

Taxa	Source	Degrees of Freedom	Sum of squares	Mean square	F ratio	Prob >F
Diptera	Fruit species	4	521.9111	130.478	2.9108	.0230
	Error	175	7844.3333	44.825		
	C. total	179	8366.2444			
Isopoda	Fruit species	4	771.5222	192.881	8.0925	<.0001
	Error	175	4171.0278	23.834		
	C. total	179	4942.5500			
Diptera larvae	Fruit species	4	289.1444	72.2861	4.7221	0.0012
	Error	175	2678.9167	15.3081		
	C. total	179	2968.0611			
Hemaptera	Fruit species	4	4.522222	1.13056	5.4537	.0004
	Error	175	36.277778	0.20730		
	C. total	179	40.800000			
Hymenoptera	Fruit species	4	1675.644	418.911	4.6893	.0013
	Error	175	15633.306	89.333		
	C. total	179	17308.950			
Coleoptera	Fruit species	4	43.07778	10.7694	4.5105	0.0017
	Error	175	417.83333	2.3876		
	C. total	179	460.91111			
Diplopoda	Fruit species	4	505.7000	126.425	12.1924	<.0001
	Error	175	1814.6111	10.369		
	C. total	179	2320.3111			

TABLE 4. Tukey Kramer Means Comparison of Taxa populations among fruit species. Levels not connected by the same letter are significantly different.

Level	Fruit flies		Isopoda		Diptera larvae		Earwig		Ants		Coleoptera		Diplopoda	
		Mean		Mean		Mean		Mean		Mean		Mean		Mean
Mango	A	5.5833	B	1.0833	B	0.7500	B	0.0	A B	6.6667	A B	0.6667	B	0.4167
Papaya	A B	3.8055	B	0.3333	A B	1.8889	B	0.0277	A B	6.4444	B	0.2778	B	0.2778
Banana	A B	1.8055	B	0.0556	B	0.8055	B	0.0	B	2.222	B	0.1111	B	0.0
Sour sop	A B	1.4166	A B	5.7500	A B	3.6666	A	0.3888	A	9.8056	A B	0.4167	A	4.500
Star apple	B	1.1111	B	2.1944	B	0.0278	A B	0.2500	B	1.6111	A	1.3889	B	0.6944

significantly on one fruit species over others, both feeding disproportionately more on sour sop.

DISCUSSION

It was not surprising to find that the decomposer communities were distinct at the two locations, since the two habitats are so different. While both sites have taxa from many of the same orders of arthropods, these members vary on the level of family or species between the two sites, as if different species are available to fulfill the same task in fruit decomposition. This was most notable with taxa of the order Coleoptera. While one distinct species was identified at the Belvedere, two separate and distinct species were consistently found at the Gump site. The primary factor most likely responsible for this phenomenon is the difference in microhabitats created in an *Inocarpus* forest versus a landscaped urban area near sea level. These results support the practice of organizing taxa with analogous roles into one functional group.

However, the second hypothesis which predicted greater abundance and diversity of arthropods at the forest location was not supported by the results. While the Belvedere site attracted predominantly ants and fruit flies, the arthropod assemblage at the Gump station included an abundance of more diverse taxa, suggesting that perhaps human development has opened up many more niches for more recently introduced arthropods to colonize the area and thrive. Since human development facilitates the introduction of new species, perhaps whole communities of invasives have already adapted to human-

disturbed environments and constitute the diversity seen at the Gump station. Another potential explanation is that the habitat at the Gump station is more suited to support a greater diversity of taxa, since the large populations of ants and fruit flies at the Belvedere dominate resources and make settlement by other arthropods more difficult.

In addition to decomposer communities differing between large geographic locations, arthropod species abundance varies between different decomposing fruits within the same environment. Because detritus, like islands, constitutes a microhabitat which is isolated by contrasting surroundings, their study is similar conceptually to that of island biology with respect to the presence of distinct species communities from one island to the next (Begon et al, 1990). Several factors might influence fruit selection by arthropods, such as biochemistry or external morphology. In a study of rotting fruits, five different species of *Drosophila* fruit flies exhibited significant preferences for which fruit they chose to feed on. One fruit fly species preferred melons while another strongly preferred pears and grapes, a trend which was eventually attributed to the various amounts of ethanol produced by the yeast that had first colonized the rotting fruit (Begon et al, 1990). Another potential factor that would account for fruit preferences would be the presence of natural pesticides. One study showed that birds avoid fruits which have been damaged by insect pests, greatly hindering the seed dispersal and proliferation of the offended tree (Garcia et al, 1999). If this were to persist over time, those fruits containing natural pesticidal properties would be proliferated until eventually arthropod pests would avoid the specific fruit altogether. Thus two separate species of fruits in close vicinity might recruit contrasting decomposer communities as a

result of differences in biochemical or morphological makeup.

Although the biochemical makeup of the fruits is an important consideration with regard to fruit specificity, in this study it appears as though morphological makeup of the fruits is the leading determinant of which taxa fed on which fruits. Spinier, tougher-skinned fruits such as star apple and soursop generally recruited a disproportionate amount of beetles, millipedes, and earwigs in their decomposition, whereas fleshier fruits attracted mostly ants and fruit flies. It could be that because ants and fruit flies preferred the fruits which were easier to break down, arthropods with the necessary mouthparts to tackle fruits with tougher exteriors were driven by competition to soursop and star apple. It would be fascinating to study further the behavior of beetle 3 at the Gump station that was found primarily on star apple, because its dapple gray exterior camouflaged well with the surface of the fruit. Overall the results of the Tukey Kramer HSD tests do not provide strong evidence for the presence of food specificity in this case, since only Isopoda and Diplopoda population means were significantly different on one fruit compared with all others.

Successional patterns observed at the Gump and Belvedere appear to loosely coincide with results of previous succession studies, as ants and fruit flies arrived first, with coleoptera, dermaptera, diplopoda, and fruit fly larvae appearing later (De Jong & Chadwick, 1999). Overall, arthropod arrival was delayed at the Gump station compared to the Belvedere, and arthropods invaded the fruits primarily from underneath.

A study that is designed more to observe the difference in efficiency of these two distinct decomposer communities would be beneficial for determining the relationship between species richness and diversity and ecosystem function (Bjelke, Herrmann, 2005).

Since ecosystem function is vital to the integrity of island biota and decomposition is crucial for a stable and healthy ecosystem, studying the diversity of organisms available as decomposers is of utmost importance. While this study focused on the impact of urban development on decomposer communities, several more studies are necessary in order to address the larger issues pressing field science,

such as species conservation and island ecosystem preservation.

ACKNOWLEDGEMENTS

I would like to thank Jacques and Tony for supplying the fruit cages necessary for my experimental design. I would also like to thank George Roderick and Brent Mishler for help with developing my project idea, Jaime Bartolome for assistance with data organization and statistics, and Carol Hickman and Rosemary Gillespie with my final paper. The GSI's, Scott Fay, Michele Weber, and Matt Medeiros were very helpful and supportive throughout in all areas, including experimental logistics, statistics and identifying arthropods. Lastly I would like to thank the class of 2005 for keeping me safe out in the field and providing consultation and support.

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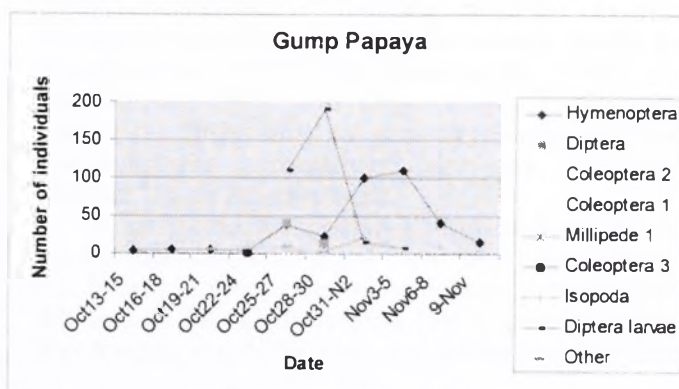
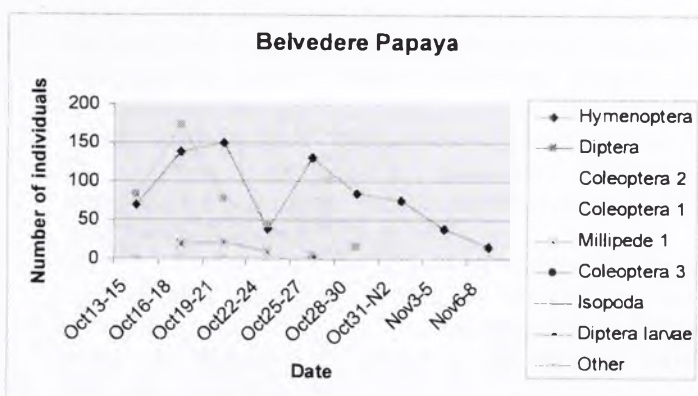
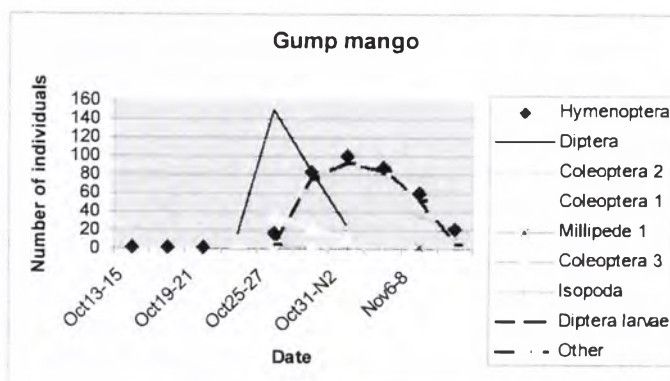
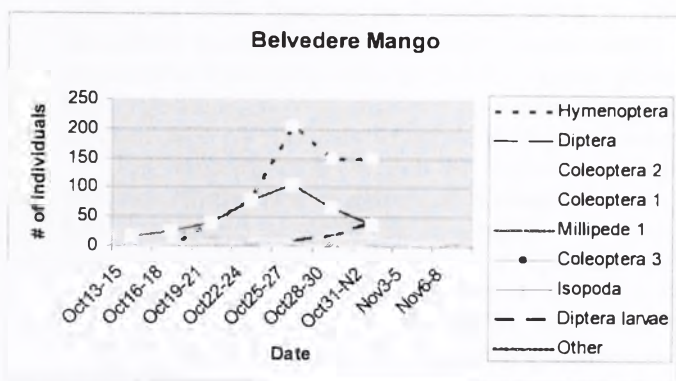
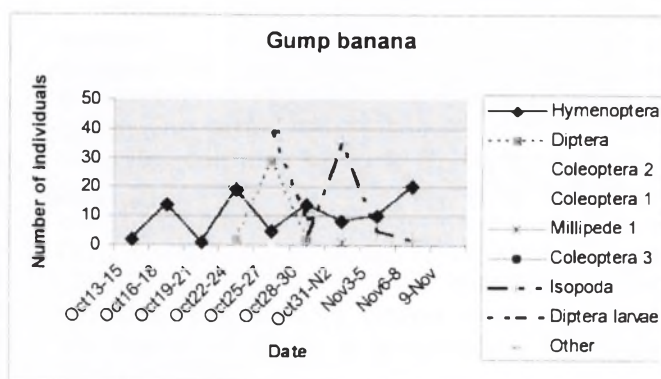
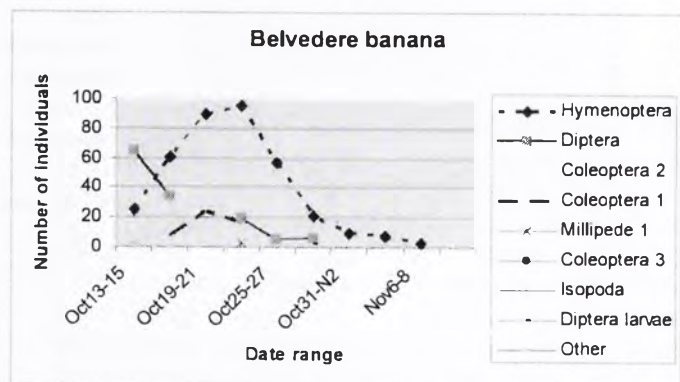
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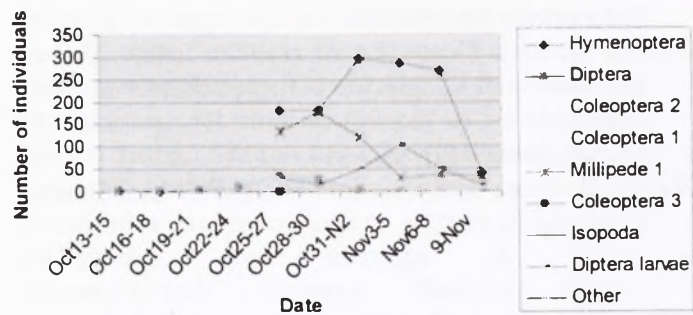
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APPENDIX

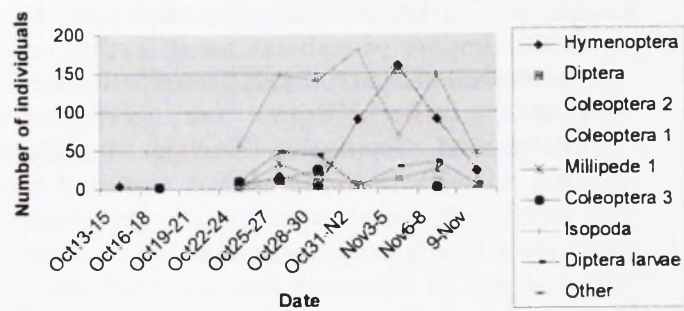
NUMBER OF INDIVIDUALS OF EACH ARTHROPOD TAXA ON EACH FRUIT OVER TIME



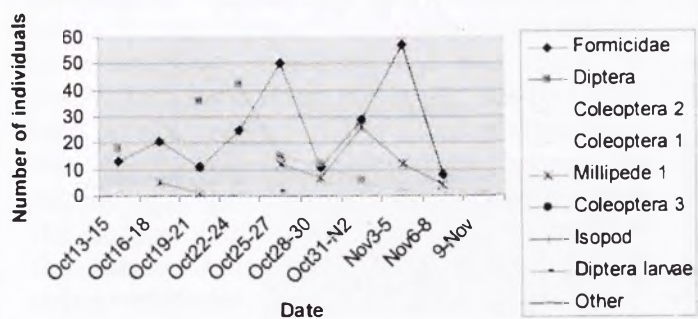
Belvedere Sour sop



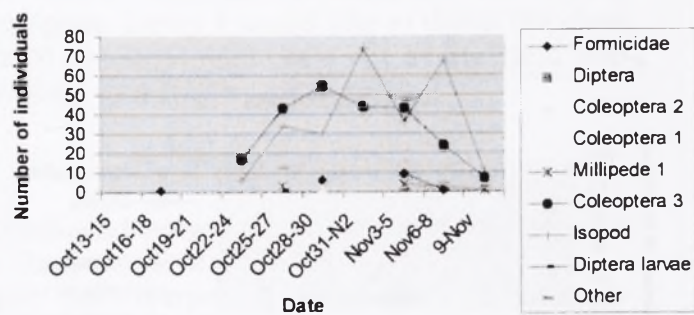
Gump Sour sop



Belvedere Star apple



Gump Star apple



SUCCESSION IN THE PHYLLOSPHERE: A CASE STUDY ON THE GIANT FERN (*ANGIOPTERIS EVECTA*)

INGRID BURKE

Environmental Sciences, University of California, Berkeley, California, 94720, USA

Abstract. Ecologists have studied community succession intensively, predominantly on a large scale. However, change occurs at every spatial and temporal level, and analysis of each level informs the larger theory. This case examines community change on a small scale by comparing epiphylls and invertebrates on *Angiopteris evecta* fronds of varying relative ages. No overall predictable successional patterns were identified, but opportunistic association patterns suggested that variable dispersal and habitat use are driving factors in this community. A fiddlehead measurement regime revealed that new *A. evecta* fronds unfurl in 24-36 days.

Key words: *dispersal, habitat use, invertebrates, community change, fern development, fiddlehead growth, epiphytes, Moorea, French Polynesia*

INTRODUCTION

Change in ecological communities over time has been studied for the past century. When the theory of community succession was pioneered, two conflicting mechanistic views dominated. Clements, in 1916 and 1936 (quoted in Ricklefs 1990, p. 174), described stages, or seres, which lead to a stable climax community. He hypothesized that climax community composition was defined by regional characteristics, and thus could be predicted (Emlen 1973). In contrast, Gleason, in 1926 and 1939 (quoted in Ricklefs 1990, p. 174), described succession as convenient associations of organisms that were able to live in similar places. He hypothesized that changes in communities are random and opportunistic rather than predictable.

The debate over which of these two basic theories dominates in different ecological settings has continued since their inception. For many years, Clements's theory of predictable progressions was widely accepted as fact (it is the only theory cited in Emlen 1973), however, as more studies are conducted regarding community change, it is becoming more apparent that the complexity of succession mechanics goes far beyond both initial theories. Disturbances within a community allow many different stages of succession to coexist and lead to the "niche disturbance model." This creates a mosaic pattern, and climax communities are now generally described as dynamic mosaics rather than stable structures (Ricklefs 1990, p. 677).

The first work on succession addressed large spatial and temporal scales by describing the progression of stages in forest communities. Since then, the majority of successional studies have

been done on similar situations, though community change, of course, occurs on every scale. The term "micro-succession" is used to describe community change which occurs on smaller, faster scales; for example, in the decay of wood (Emlen 1973). Cyclic succession has also been recognized, in which some kind of regular disturbance prevents the community from reaching any kind of climax state, and so the succession of stages is perpetuated indefinitely (Ricklefs 1990).

One community type that operates on very small spatial and temporal scales is made up of plants and animals that live on the surface of leaves. This community has been termed "the phyllosphere" and was widely ignored until about 50 years ago (Ruinen 1961). There is some disagreement among descriptions of community change in the phyllosphere (Benzing 1990 in Zotz and Vollrath 2003). This community exists in a cyclic pattern as new leaves develop and old ones deteriorate, but it has not been determined whether or not it exhibits cyclic succession. Richards (1932) observed the formation of a climax community in the phyllosphere, and Winkler (1967) described a "settling rhythm" in the changes over time in the community (both in Olarinmoye 1975). Yet Harrington (1967, in Olarinmoye 1975) and Olarinmoye (1975) both found no systematic or orderly changes in the phyllosphere community, nor any "definite rhythm" in its development.

A particularly extensive phyllosphere exists on the leaves of the giant fern *Angiopteris evecta* (G. Forst.) Hoffm., where it grows on the island of Moorea in French Polynesia (for a description of the species, see NSW National Parks and Wildlife

Service 2001). The Tahitian people call the fern “Nahe” (H. Murphy, personal communication). Because most *A. evecta* plants include fronds of various ages at the same time, and because the fern leaves are large enough to examine in the field, this species offers an excellent opportunity to study community change in the phyllosphere. An analysis of the changes in the communities as the fronds age will provide data for a discussion of micro-succession and its mechanisms on tropical ferns, and will, in turn, inform the larger theories of succession.

The phyllosphere on *A. evecta* includes both epiphylls (fungi, liverworts, and lichens), and invertebrates (insects, snails, and spiders). An exploration of the change in both of these areas as fronds age is presented in this study. The specific operating hypothesis is that communities of both epiphytes and invertebrates will quantitatively differ according to relative frond age.

METHODS

Site description

Moorea is one of the Society Islands of French Polynesia. It is classified as a high island, and, in its moderately advanced age and stage of erosion, it exhibits rugged topography and lush vegetation. Many tropical islands have fallen victim to the decimating effects of introduced species which obliterate most native organisms, but Moorea supports a relatively intact native community in a few areas, especially at higher elevations.

In order to avoid the introduction of conflicting variables by sampling from many locations around the island, all observations were made near the Belvédère lookout (222 m elevation, UTM 6K 199874/8058435, FIGURE 1). A narrow, rocky trail leads east from the parking lot to Marae Titiroa, and within 300 m, it reaches a stream. All of the plants measured were found within 100 m of this trail, in the stretch between the parking lot and the stream. The trail is often used recreationally, but the forest it leads through is considered relatively undisturbed (Coote et al. 2004). The habitat is characterized by a high *Inocarpus fragifera* and *Hibiscus tiliaceus* canopy and a fairly open, rocky forest floor which supports small herbaceous species. This particular area also contains hundreds of *Angiopteris evecta* individuals, in every stage of growth.

Plant location

A semi-random technique was used to locate ferns to observe, in order to ensure that there was no bias in the selection process. I approached an



FIGURE 1. Site location on the island of Moorea in French Polynesia. Observations were performed near the Belvédère lookout.

area in which many ferns were growing (non-random), then used blind spinning of a compass bevel to select an individual (random). The first spin of the bevel determined the direction in which I would pace, and the second spin determined the number of paces in that direction, based upon the last digit of the indicated compass bearing. When I had progressed the specified number of paces, I located the nearest fern that had at least two fronds which were not over two meters above the ground (those over two meters high were impossible for me to examine them closely enough without a ladder). Observations began on October 13, 2005 and ended on November 23; they were all performed between 8 in the morning and 3:30 in the afternoon.

Each fern plant had between two and four fronds, which are the unit of measurement. (Each frond is further divided into leaflets and then lobes, see FIGURE 2.) The percentage of physical deterioration (such as torn or yellowed leaf sections) was estimated, and fronds were placed in discrete deterioration classes as follows: no

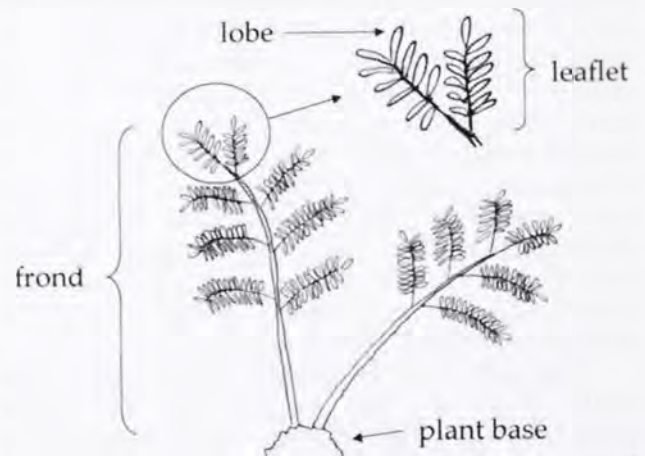


FIGURE 2. Morphology of *Angiopteris evecta*.

deterioration was defined as class 1, between 1% and 4% deterioration as class 2, 5% to 9% as class 3, and 10% and above as class 4. Because deterioration of a material increases with exposure to the elements, deterioration is used as a proxy for relative frond age. Thus, deterioration class 1 represents fronds of the youngest relative age.

Epiphyll cover

For each frond, estimates of the leaf cover of six epiphyll categories were recorded. Time and logistic restraints prevented the examination of every specimen under a microscope, so categories were formed based on morphology recognizable in the field (TABLE 1). Voucher specimens of leaves supporting each category of epiphyll were prepared for identification and storage in the Jepson Herbarium at UC Berkeley.

The statistical programs JMP and Excel were used to perform analyses of variance and Tukey-Kramer tests attempting to reject the null hypotheses that epiphyll cover and community composition do not change with relative frond age class.

Fungus #1	wavy runners radiating from a central point
Fungus #2	dark, sooty stain
Fungus #3	single small, thick, white, irregular spots
Liverwort #1	short lines of overlapping lobes
Liverwort #2	lines of tiny upright leaves
Lichen	congregations of pale spots

TABLE 1. Epiphyll categories on *Angiopteris evecta* with morphological field identification.

Invertebrate observations

For each frond, the topside and underside of every lobe was closely examined and every invertebrate found was recorded. Taxonomic groupings were used based on morphology easily recognizable in the field, as logistic and permit restrictions prevented collection of invertebrates. Identification to taxonomic group was often provided by experts at the Gump Biological Research Station.

The statistical programs JMP and Excel were used to perform analyses of variance and Tukey-Kramer tests attempting to reject the null hypotheses that invertebrate numbers and community composition do not change with frond age.

Fiddlehead growth

In the same area, 15 developing *A. evecta* fronds, or fiddleheads, were chosen to measure growth rates. The fiddleheads were at a variety of stages of development: some still exhibited a single coiled head, while others had already unfurled to reveal separate leaflet coils (FIGURE 3). Fiddlehead measurements were made from October 16 to October 28, 2005, and fiddleheads were revisited every three days. When fiddleheads were in early stages consisting of a dominant apical coil, flagging tape was tied to the main stalk under the coiled head, lined up with the lower edge of the coil. Upon revisitation, the distance traveled by the lower edge of the coil was measured. Photographs were taken every three days as well.

To analyze growth rates, all growth measurements in cm were averaged over the number of days between measurements. To estimate the total time required for frond emergence, the time in days required to reach each development stage (FIGURE 3) was estimated, then added to the times for the other stages.

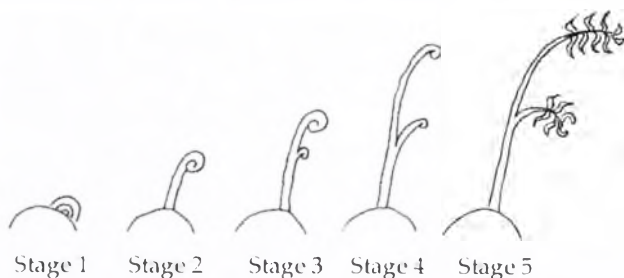


FIGURE 3. Stages in the development of *Angiopteris evecta* fronds. In Stage 1, the fiddlehead is still within the plant base, in Stage 2, the main head is unfurling, in Stage 3, individual leaflet coils are separating, in Stage 4, the leaflet coils are unfurling, and in Stage 5, individual lobes are unfurling.

RESULTS

Epiphyll cover

Total frond epiphyll cover percentages plotted against frond deterioration class shows a general upward trend as age progresses, and a marked difference in the spread of the data between the

various classes (FIGURE 4). The program JMP was used to perform an analysis of variance on the data (ANOVA, $F=12.3211$, $p=0.0001$, $DF=3$). Next, a Tukey-Kramer test was performed to reveal the location of the significant difference located by the ANOVA. The results of this test indicate that each deterioration class differs significantly from all categories not adjacent to it. For example: 1 differs from 3 and 4, but not from 2.

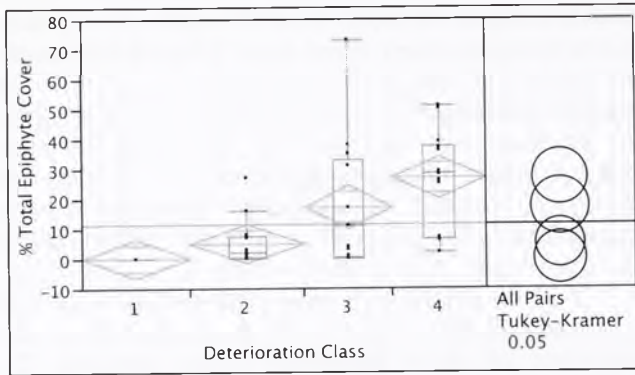


FIGURE 4. Total epiphyll cover in percentage of leaf surface of *Angiopteris evecta* fronds plotted by deterioration class, 1 representing youngest relative age. Data are displayed with quantiles, and analyzed with an ANOVA and a Tukey-Kramer test.

When each individual epiphyte type was plotted against deterioration class, three out of the six showed significant differences. FIGURE 5 is the graph for Fungus #1 (ANOVA, $F=5.9209$, $p=0.0014$, $DF=3$). The Tukey test reveals the values in class 4 to be distinct from those in the other three classes. The Tukey test delivers the same result for Fungus #3 (ANOVA, $F=6.7260$, $p=0.0006$, $DF=3$), and it shows that values in class 4 are distinct from those in classes 1 and 2 for Liverwort #1 (ANOVA, $F=5.3057$, $p=0.0027$, $DF=3$).

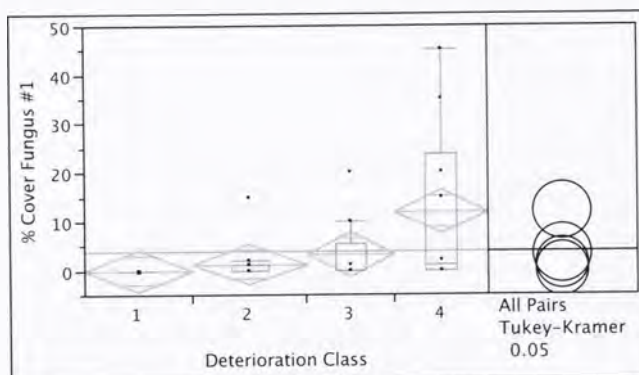


FIGURE 5. Cover of Fungus #1 in percentage of leaf surface of *Angiopteris evecta* fronds plotted by deterioration class, 1 representing youngest relative age. Data are displayed with quantiles, and analyzed with an ANOVA and a Tukey-Kramer test.

The number of different invertebrate taxa present on each frond plotted by deterioration class is illustrated in FIGURE 6 (ANOVA, $F=14.218$, $p=0.0001$, $DF=3$). The number of invertebrate individuals on each frond plotted by deterioration class is shown as FIGURE 7 (ANOVA, $F=6.1966$, $p=0.0011$, $DF=3$). In both graphs, the youngest age category exhibits the lowest numbers, with a large spread for taxa and a small one for individuals. In both graphs, the other three categories are similar in values and spread. The program JMP was used to perform analyses of variance and Tukey-Kramer tests on both data sets. The results of the Tukey-Kramer tests were the same for both taxa and individuals: they indicated that the values in class 1 were significantly different from those in classes 2, 3, and 4, which were not different from each other.

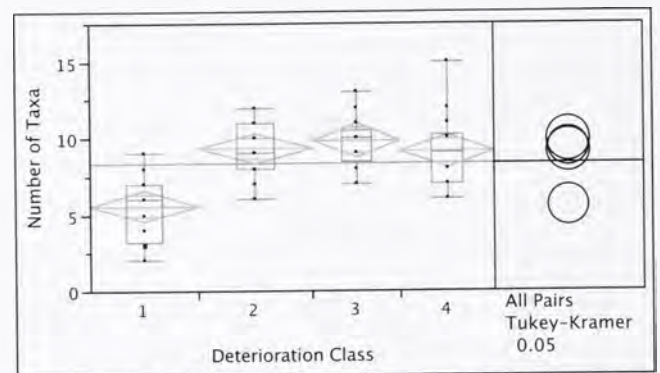


FIGURE 6. The number of invertebrate taxa on each *Angiopteris evecta* frond by age category, 1 being youngest. Data are displayed with quantiles, and analyzed with an ANOVA and a Tukey-Kramer test.

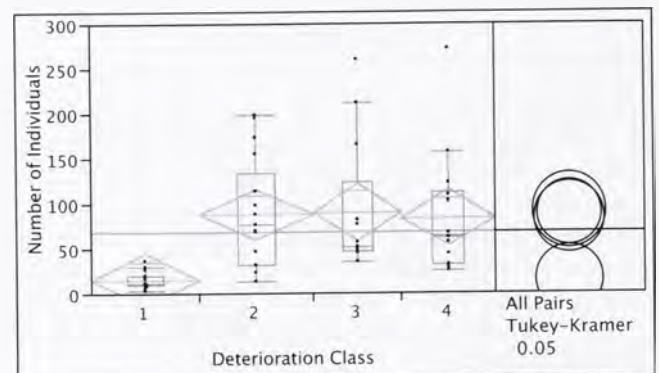


FIGURE 7. The number of invertebrate individuals on each *Angiopteris evecta* frond by age category, 1 being youngest. Data are displayed with quantiles, and analyzed with an ANOVA and a Tukey-Kramer test.

When each of the individual 19 taxonomic groups (Acaria, Aranaea: Pholcidae: Pholcus, Aranaea: Salticidae, Aranaea: Tetragnathidae: Leucauge, Aranaea: Uloboridae: Tangaroa, Coleoptera, Diptera, Hemiptera, Homoptera,

Homoptera: Aphididae, Homoptera: Coccoidea, Homoptera: Fulgoroidea, Hymenoptera, Hymenoptera: Formicidae, Lepidoptera, Microgastropods, Neuroptera, Orthoptera, Orthoptera: Gryllidae) were plotted against deterioration class, only three showed any significant differences: Aphididae (FIGURE 8; ANOVA, $F=5.1173$, $p=0.0034$, $DF=3$), Microgastropods (ANOVA, $F=3.0097$, $p=0.0377$, $DF=3$), and winged Homoptera (ANOVA, $F=3.6557$, $p=0.0177$, $DF=3$). The Tukey-Kramer test reveals a difference between values in classes 1 and 4 for Microgastropods, between 1 and 2 for winged Homoptera, and between 1 and both 2 and 4 for Aphididae.

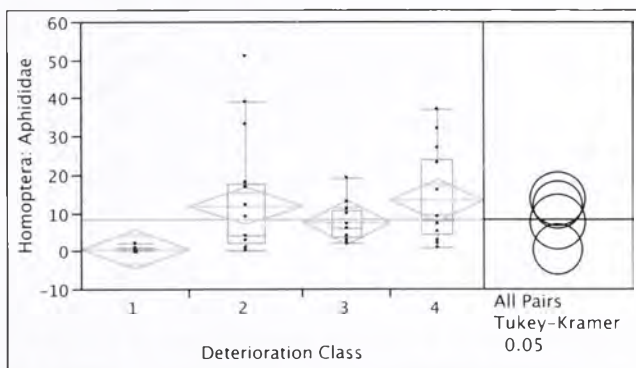


FIGURE 8. The number of Aphididae individuals on each *Angiopteris evecta* frond plotted by deterioration class, 1 referring to youngest relative age. Data are displayed with quantiles, and analyzed with an ANOVA and a Tukey-Kramer test.

Fiddlehead measurements

The average growth rate for unfurling fiddleheads in stages 2 and 3 is 3.33 cm per day, with a low measurement of 0 cm per day and a high measurement of 6 cm per day. Stages 2-5 each lasted approximately 6-9 days, so once the fiddlehead leaves the plant base, a new *A. evecta* frond can be completely developed in about 24-36 days.

DISCUSSION

Epiphyll cover

The increasing trend in total epiphyll cover as frond age increases is not surprising, since a greater length of time spent exposed to epiphyll propagules results in greater epiphyll colonization (Roskoski 1981). Lichens have been found to cover leaves more completely as the leaves age (Moore 2003), biomass of seagrass epiphylls is strongly related to leaf age (Reyes and Sansón 2001), and numbers of both epiphyll species and individuals increase as palm trees grow (Zotz and Vollrath

2003).

However, there does not appear to be any progressive pattern to the increase in cover. No epiphyll type shows a strong initial presence, which would indicate that it holds the role of primary colonizer. No type decreases with age, which would point to some kind of competitive succession in which certain epiphylls outcompete others, as described by Olarinmoye (1975). Three of the six types show no difference according to relative age at all, which supports the argument that community change among the epiphylls could be completely random.

One reason that no overall pattern is discernable may lie in the fact that individual ferns support very different epiphyll communities. For example, Fern #5 and Fern #7 both have four fronds, with one in each deterioration class. If there were some predictable epiphyll colonization pattern, then both of these plants should have about equal epiphyll percentages, since they both exhibit the entire range of succession. And yet, Fern #5 supports 0% Fungus #1, 45% Fungus #2, and 25% Lichen, while Fern #7 supports 37% Fungus #1, and none of Fungus #2 or Lichen. Perhaps individual associations of epiphylls undergo minute patterns of change (for example, Fungus #2 and Lichen could be competing on Fern #5), but no overall patterns can be identified from data sets that combine so many different individual communities.

Individual *A. evecta* plants could easily end up with completely different epiphyte associations because colonization by epiphytes depends on proximity to propagule source, ability of propagules to grow on leaves, and the number of propagules produced (Olarinmoye 1975). There is a great deal of microsite variation in epiphyll propagation (Zotz and Vollrath 2003), so different plants can be colonized differently. For example, perhaps a spore cloud of Fungus #1 was blown into Fern #7, but away from Fern #5. Epiphyte communities are also highly sensitive to their immediate environs (Olarinmoye 1974). Roskoski (1981) points out that overstory plants change temperature and wind levels, and capture propagules which can be washed down later by rain. Thus, tiny variations in habitat, such as fern proximity to shade plants, and in atmospheric variables, such as fern proximity to a water source, can result in strong variations in epiphyte growth.

Invertebrate observations

It is not surprising that fronds of the youngest relative age class supported so few invertebrate taxa and individuals, because they represent new habitat that has not been exposed long enough to

be fully colonized. However, it is surprising that the other three relative age classes are so similar and that frond diversity, calculated by the Shannon-Weiner Diversity Index, shows no significant difference between relative age classes (ANOVA, $F=0.5758$, $p=0.6333$, $DF=3$). If the data followed the classic succession pattern, described by Elston (1995), they would exhibit both numbers of taxa and numbers of individuals going through an increasing stage, a leveling-off, or climax, stage, and finally, a decreasing stage.

It can be argued that a pattern of succession does exist here, and that the relative age classes are not the right size to observe it properly. For example, some predictable increasing pattern may be occurring between fronds of class 1 and fronds of class 2, and the similarities between classes 2, 3, and 4 may simply represent the climax stage where the community is stable. However, as with the epiphylls, individual ferns support very different invertebrate communities. For example, Fern #14 and Fern #18 both have four fronds, which cover the entire range of relative age. If there is a predictable pattern of establishment, then the invertebrate totals for both ferns should be similar. However, Fern #14 supports 0 Acaria and 29 Aphididae, while Fern #18 supports 299 Acaria and 7 Aphididae. If such different communities exist on individual plants, then these data sets cannot reveal any overall progressive patterns.

Differential habitat use may also explain why so much of the invertebrate data appears to support a random association model of succession. Out of all 19 taxa, the only three that exhibited significant differences according to relative age, Aphididae, Microgastropods, and winged Homoptera, are taxa that rely on the fern lobe material, or on the biofilm that coats it, for their sustenance throughout life. While previous studies have not necessarily implied that succession of invertebrate communities on leaves exists, the structure of insect assemblages has been found to depend upon leaf structure (Peeters 2002) and herbivorous insects have been shown to distinguish patterns within a single plant, and exhibit a marked preference for leaves in certain stages of development and of certain ages (Lawrence et al. 2003). Thus, perhaps taxa like these, that are strongly tied to the *A. evecta* habitat, would show predictable patterns of change when studied in more detail.

Most of the other taxa found on the ferns, though, are mobile hunters or are using the frond habitat more briefly, perhaps to rest or as protection. A study on spiders demonstrated that habitat structure exerted more influence on spider diversity than did successional age of the habitat

(Hurd and Fagan 1992). The fact that *A. evecta* fronds are relatively uniform may be a reason that these more mobile taxa form only random associations that do not depend on relative frond age. Because the majority of the data describe these random associations, any patterns that do exist among certain taxa are obscured.

Conclusions

Communities of epiphytes and invertebrates on *A. evecta* fronds do not exhibit predictable successional changes between fronds in 4 relative age classes. However, analyzing the changes in these small, ephemeral communities focuses attention on dispersal and specific habitat use. These are two factors that are not often emphasized in succession work on larger scales, yet they play integral roles in the mechanics of community change. Greater consideration of these factors in the future will lead to further insight into the general concept of community change.

Further research

Because each *A. evecta* plant apparently supports such a unique assemblage of epiphylls and invertebrates, more information could be gathered by a revisitation study, in which changes over time on an individual plant were monitored. More specific studies on epiphytes, herbivorous invertebrates, or carnivorous invertebrates may yield more detailed data within which succession patterns may possibly be recognizable.

ACKNOWLEDGEMENTS

Thank you Nicky and Tanya for being the best roommates ever! My eternal gratitude goes to Nicky for her project suggestion when I was desperate. Thanks to Matt Medeiros for advice and entomological assistance, and to Matthew Ebright for technical and emotional support. Thanks to all the Moorea students, staff, and faculty for making the 2005 class great!

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HOST SPECIFICITY OF WOOD INHABITING MACROFUNGI ON THE ISLAND MOOREA

TANYA CHAPPLE

Plant and Microbial Biology, University of California, Berkeley, California 94720 USA

Abstract. Host specificity of wood inhabiting fungi is a rare occurrence in tropical ecosystems. High diversity of host species usually limits distribution of the fungi. However, in low diversity tropical situations, the occurrence of host specificity is much higher. Moorea, a remote oceanic island, in French Polynesia supports a lower diversity than a tropical mainland ecosystem. This study examined host specificity of wood inhabiting fungi in a low diversity situation. Host species and diameter of the woody substrate were recorded for each occurrence of fungi in order to examine host specificity and size specificity. A wood inhabiting fungus was shown to have possibilities of host specificity by greater than 50% of its occurrences on one host wood. Specificity for both host species and in size of host was found to occur. 58 distinct macrofungi were found, 32 of which were found only once. Of the 17 fungi that were found more than twice, 10 were possibly host specific.

Key words: Basidiomycota; Ascomycota; wood decay; Society Islands, French Polynesia

INTRODUCTION

Wood inhabiting macro-fungi occur throughout the world. Their spores are wind dispersed and capable of traveling for thousands of miles (Wong 2005). Wind dispersal could bring the spores to remote islands, such as Moorea. Wood inhabiting fungi could also have been inadvertently brought to islands by humans.

It is important to understand the mechanisms of host specificity in fungi to create a more accurate evaluation of the actual global diversity of fungi (Hawksworth 2001). Islands, as centers of speciation, are very important to estimates of global biodiversity (Whittaker 1998), which makes Moorea an interesting study location to examine host specificity in wood inhabiting fungi. Host specificity in wood inhabiting fungi occurs rarely. It is usually more advantageous to have generalist characteristics, to grow and reproduce on a number of substrates/hosts rather than be restricted to one (Gilbert, Ferrer, and Carranza 2002). The limiting factor for a host specific relationship is availability of the host (Gilbert and Sousa 2002). In a low diversity forest with a high density of only a few tree species it is much simpler for fungi to maintain a host specific relationship because of the ready availability of a host. In a high diversity forest it is less advantageous and therefore less likely to maintain the host specific relationship because the host is less common (Gilbert, Ferrer, and Carranza 2002).

The island of Moorea formed as part of a hot spot archipelago, in a location remote from continental sources for plant immigrants, and thus

supports a low diversity ecosystem. Due to the low diversity and higher density of available hosts, it was expected that there would be more incidences of host specificity in wood inhabiting fungi than occurs in mainland situations. Specificity is not limited to host species, but can also be based on the size of the substrate. A fungus may be specific to logs, or smaller branches, or twigs (Lodge 1997). The purpose of this study was to examine the occurrence of specificity in both size of substrate, and host species.

Initial observations suggested differences in fungal diversity in different habitats. If diversity was dependent on the availability of substrate; then the habitat with the most wood availability should have the most diversity. The broader objective of this research was to understand the



FIG. 1. Map of Moorea showing the 4 study areas for the host specificity survey. Line transects were done at Gump Hill, Marae Tetiroa, and the Belvedere to examine the differences in wood availability and fungal richness between these three habitats.

diversity and distribution of the wood decay macrofungi found on Moorea.

METHODS

Host Specificity Survey

Macrofungi fruiting bodies were surveyed on the north central portion of the island of Moorea (Fig. 1), starting September 29, 2005 and ending November 15, 2005, during the transition period from the dry to the wet season. Voucher specimens were collected for each distinguishable taxon found, and will be deposited into the University Herbarium, UC Berkeley. Macrofungi taxa were identified to the most specific level of classification possible (Alexopoulos 1996, Hemmes and Desjardin 2002), considering the obscurity of any taxonomic references for the fungi of French Polynesia. For each occurrence, the diameter of each wood piece was measured, and the species of the host wood was determined if possible (based on branching morphology and composition of the surrounding habitat). The habitat (dominant tree species (Murdock and Hinkle 2005; USDA, NRCS 2005) and GPS coordinates were also recorded.

Specificity for host species was evaluated for all taxa found on more than two instances. Possible host specificity of a fungus was shown by

greater than 50% of its occurrences on one host species.

Specificity for size was examined by measurements of wood diameter that were placed into size classes:

- less than 1 cm
- 1 to 2.99 cm
- 3 to 4.99 cm
- 5 to 6.99 cm
- 7 to 9.99 cm
- 10 to 14.99 cm
- 15 to 19.99 cm
- 20 to 24.99 cm
- 25 to 29.99 cm
- greater than 30 cm.

Habitat Variations in Wood Availability

The variation in wood availability for three habitats was investigated.

1. Low elevation *Hibiscus tiliaceus* L. and *Cocos nucifera* L. (Coconut), sampled near the Gump station (UTM: 06K 0199684 8063942, elevation: 9 meters)

2. *Inocarpus fagifer* (Parkinson) F.R. Fosberg forest, including *Neonauclea forsteri* Merrill, *Hibiscus tiliaceus*, and *Aleurites moluccana* (L.) Willd. (Candlenut), sampled near the Marae Tetiaroa (UTM: 06K 0199552 8058855, elevation: 208 meters)

3. High elevation mixed forest, sampled near the Belvedere (UTM: 06K 0199703 8058280, elevation: 256 meters). The mixed forest type is dominated by *Metrosideros polymorpha* Gaud., *Angiopteris evecta* (J.R. Forst.) Hoffman, *Hibiscus tiliaceus*, and *Freycinetia impavida* (Gaudich ex Hombr) B.C. Stone.

Two 25 meters long line transects were sampled per site. Every piece of wood crossing the transect was measured.

The diameter of each wood piece was measured at the point of intersection with the line transect, and placed into size classes:

- less than 1 cm
- 1 to 2.99 cm
- 3 to 4.99 cm
- 5 to 6.99 cm
- 7 to 9.99 cm
- greater than 10 cm

Wood pieces of diameter 3 cm and larger were measured for the entire length of the transect, 25 m. But smaller branches (1 – 2.99 cm) and twigs (<1 cm) were measured until there were more than 20 samples for each of these size classes. The frequency (number of wood pieces per meter) of occurrence for each size class was determined.

In addition to wood size, species of wood was noted if determinable, as well as fungus presence.

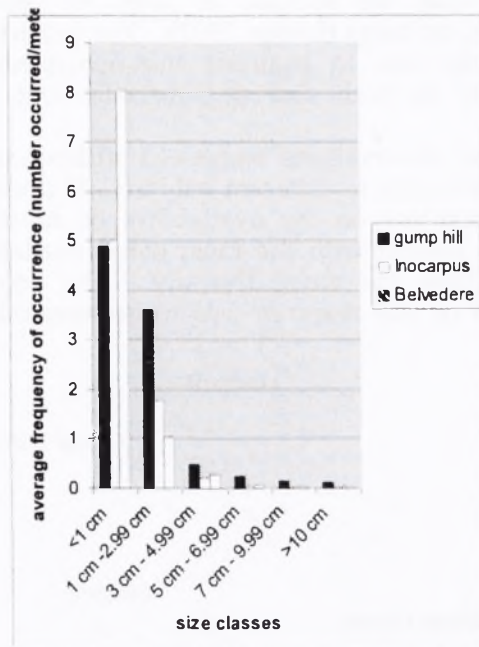


FIG. 2. The difference in size class distribution of down woody debris in three different habitats: 1. Gump hill (*Hibiscus tiliaceus* and *Cocos nucifera*), 2. Marae Tetiroa (*Inocarpus fagifer*), and 3. Belvedere (*Metrosideros polymorpha* and *Angiopteris evecta*). ($P = 1.557E-48$, from a chi square test)

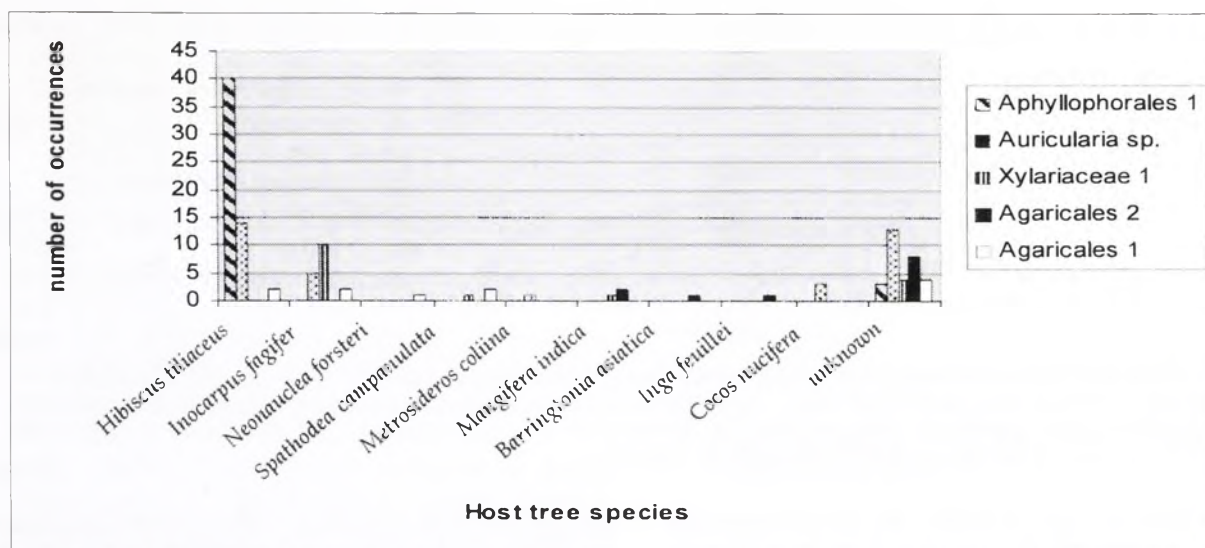


FIG. 3. Figure shows the difference in host specificity between the five most abundant fungi taxa. *Aphyllophorales 1* was only found on *Hibiscus tiliaceus*. The null hypothesis that fungi occurrences would be equal for all different hosts was rejected with a chi square test ($P = 3.42E-15$) (Appendix B: Table 1).

Fungus fruiting body presence and taxa were recorded along the entire transect for all size classes of wood.

Analysis

Fungi with possible host specificity were determined from those found 3 or more times, and that occurred more than 50% on one host species.

To determine the significance of the role of host specificity and size specificity in Moorea, a chi square test was used. The chi square test used the fungi that occurred more than ten times (*Aphyllophorales 1*, *Auricularia sp.*, *Xylariaceae 1*, *Agaricales 2*, and *Agaricales 1*).

Differences in richness between the three habitats were determined with an ANOVA test. Differences in wood availability were determined with a chi square test.

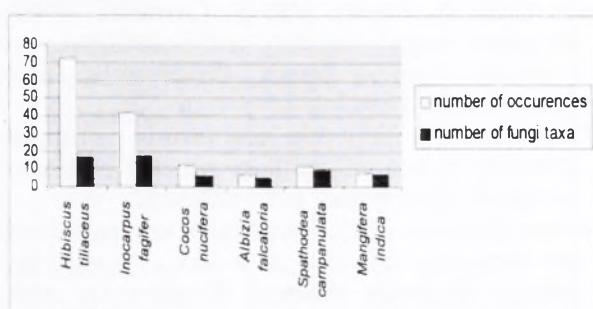


FIG. 4. Figure shows the difference in the number of fungi taxa found on six different host wood types. ($\chi^2 = 13.6$, with 5 degrees of freedom and a critical value of 11.1)

RESULTS

Host Specificity Survey

A total of 58 distinct macro-fungi were found, 32 of which were found only once (Appendix A). 17 fungi were found more than twice. From these 17, 59% showed signs of host specificity. There were three Ascomycetes (*Xylariaceae 1*, *Xylaria 1*, and *Xylaria 2*) found more than twice, two of them showed signs of host specificity (Table 1). There were 14 Basidiomycetes found more than twice, eight of which showed signs of host specificity (Table 1).

Specificity of both host species and of size of substrate was significant. Chi square analyses were done using the five most common fungi: *Aphyllophorales 1*, *Auricularia sp.*, *Xylariaceae 1*, *Agaricales 2*, and *Agaricales 1*. In a chi square test, significance for host specificity was $P = 3.42E-15$ (Fig. 3, Appendix B: Table 1). The null hypothesis of no host specificity (equal occurrences of a fungus on every different host species) was rejected.

In a chi square test, significance for size specificity was $P = 2.77E-12$ (Fig. 5, Appendix B: Table 2). The null hypothesis of equal occurrences of a fungus on all sizes of wood was rejected.

The null hypothesis that richness of fungi is the same on all host species was rejected using A chi square one sample test for goodness of fit which showed significant differences in the number of taxa found on different host species ($\chi^2 = 13.6$, which is greater than the critical value, 11.1, with 5 degrees of freedom) (Fig. 4).

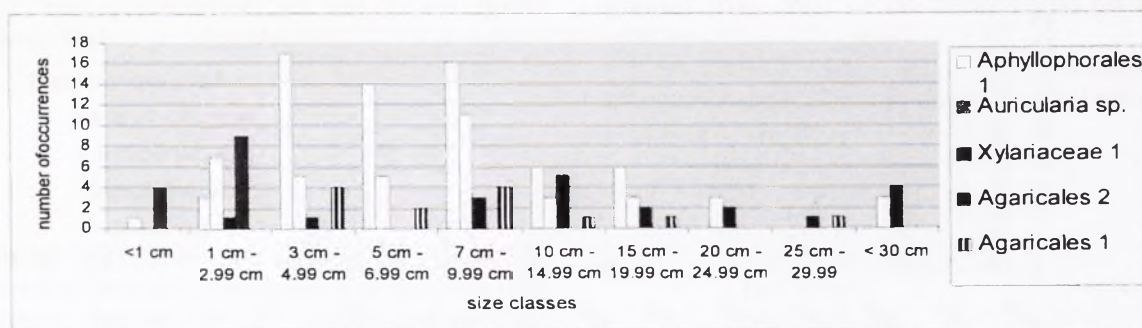


FIG. 5. Differences in size specificity of five fungi found most frequently. *Agaricales 2* was only found on twigs, and small branches. *Aphyllophorales 1* was mostly found on intermediate sized branches. Size specificity was broader for *Auricularia sp.*, *Xylariaceae 1*, and *Agaricales 1*. ($P = 2.77E-12$, from a chi square test) (Appendix B: Table 2).

Host preference can actually be environmental requirements of both the fungi and its host (Ferrer and Gilbert 2003). The presence of *Hibiscus tiliaceus* in a variety of environmental conditions suggests that the host specificity of *Aphyllophorales 1* is required because of the qualities of the host, such as wood density and chemical properties, and not the environmental conditions.

Inocarpus fagifer hosted the most taxa of fungi (Fig. 4). Of 16 taxa four had possible host specificity (Table 1). My observations suggest that *Inocarpus fagifer* grows in moist conditions, often near streams. Host specificity on this wood may be due more to environmental restrictions, such as a requirement for constant moisture, rather than properties of the host.

The majority of wood inhabiting fungi was found on branches, 3 cm to 10 cm. However the strongest size specificity was shown by *Agaricales 2*, which was only found on twigs and small branches, less than 3 cm diameter.

This study occurred through October and November, during the transition from the dry season to the wet season. To appropriately examine the fungal diversity of Moorea, the whole effect of seasonal changes should be considered for future studies.

There was very significant indication of host specificity and size specificity occurring on Moorea. There also was a higher percentage of host specificity occurring on Moorea than in a high diversity tropical forest, however this question should be further examined in future studies with more comparable methods between the different ecosystems.

ACKNOWLEDGMENTS

I would like to thank the Professors, George Roderick, Brent Mishler, Carole Hickman, James Bartolome, Roy Caldwell, and Rosemary Gillespie, and the GSIs, Michele Weber, Matt Medeiros, and Scott Fay for direction and help in experimental

design and with statistics. I also thank Tom Bruns, for help with fungi identification and for encouragement to pursue this project. I would also like to thank the class of 2005 for help in the field and general companionship. I am also particularly indebted to Marimari Kellum for the use of her property for the survey, as well as for information about the edible rat ear mushroom, *Auricularia sp.*

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APPENDIX A

MACROFUNGI AND HOST SPECIES OCCURRENCES

fungus name	number of occurrences														
	total	unknown	<i>Hibiscus tiliaceus</i>	<i>Inocarpus fagifer</i>	<i>Neonauclea forsteri</i>	<i>Spathodea campanulata</i>	<i>Albizia falcataria</i>	<i>Cocos nucifera</i>	<i>Metrosideros collina</i>	<i>Mangifera indica</i>	<i>Barringtonia asiatica</i>	<i>Inga feuillei</i>	<i>Syzigium cumini</i>	<i>Miconia calvescens</i>	<i>Syzygium sp</i>
<i>Aphyllophorales</i> 1	43	3	40												
<i>Auricularia</i> sp.	36	13	14	5				3	1						
<i>Xylariaceae</i> 1	16	4		10		1				1					
<i>Agaricales</i> 2	12	8								2	1	1			
<i>Agaricales</i> 1	11	4	2	2	1	2									
<i>Polyporaceae</i> 37	7	3		4											
<i>Xylaria</i> 1	6	3	1	2											
<i>Polyporaceae</i> 1	4	1		3											
<i>Polyporaceae</i> 30	4	1					2						1		
<i>Agaricales</i> 46	4	3	1												
<i>Agaricales</i> 3	3		3												
<i>Agaricales</i> 29	3					1	2								
<i>Aphyllophorales</i> 40	3	0	2	1											
<i>Agaricales</i> 4	3							1		1				1	
<i>Polyporaceae</i> 49	3					3									
<i>Xylaria</i> 2	3			2		1									

<i>Polyporaceae</i> 68	3							3							
<i>Polyporaceae</i> 8	2							2							
<i>Polyporaceae</i> 50	2							2							
<i>Polyporaceae</i> 57	2					1				1					
<i>Coprinus</i> sp.	2	1		1											
<i>Xylariaceae</i> 2	2	1		1											
<i>Polyporaceae</i> 48	2	1	1												
<i>Basidiomycota</i> 76	2	1	1												
<i>Polyporaceae</i> 6	2	2													
<i>Polyporaceae</i> 3	1		1												
<i>Polyporaceae</i> 12	1						1								
<i>Polyporaceae</i> 13	1						1								
<i>Tremellales</i> 1	1	0		1											
<i>Agaricales</i> 15	1	0	1												
<i>Aphyllophorales</i> 2	1		1												
<i>Aphyllophorales</i> 39	1			1											
<i>Polyporaceae</i> 42	1			1											
<i>Tremellales</i> 2	1												1		
<i>Polyporaceae</i> 51	1					1									
<i>Aphyllophorales</i> 52	1		1												
<i>Aphyllophorales</i> 54	1		1												
<i>Polyporaceae</i> 55	1			1											
<i>Polyporaceae</i> 56	1					1									
<i>Basidiomycota</i> 58	1					1									
<i>Aphyllophorales</i> 61	1			1											
<i>Polyporaceae</i> 64	1									1					
<i>Polyporaceae</i> 66	1									1					
<i>Polyporaceae</i> 67	1									1					
<i>Hydnaceae</i> 71	1						1								
<i>Polyporaceae</i> 72	1							1							
<i>Xylaria</i> 75	1													1	
<i>Polyporaceae</i> 77	1		1												
<i>Agaricales</i> 78	1		1												
<i>Agaricales</i> 11	1	1													
<i>Polyporaceae</i> 27	1	1													
<i>Polyporaceae</i> 34	1	1													
<i>Agaricales</i> 35	1	1													
<i>Polyporaceae</i> 59	1	1													
<i>Aphyllophorales</i> 62	1	1													
total found	210	55	72	36	1	12	7	12	1	8	1	1	1	2	1

Notes: This table shows all the macrofungi found and what host species they were found on. Macrofungi were identified to the most specific taxonomy possible (Alexopoulos 1996, Hemmes and Desjardin 2002)

APPENDIX B

CHI SQUARE ANALYSES TABLES

TABLE 1: chi square table for host specificity. Shows observed and expected values for the five most common macrofungi found.

	unknown	<i>Hibiscus tiliaceus</i>	<i>Inocarpus fagifer</i>	<i>Neonauclea forsteri</i>	<i>Spathodea campanulata</i>	<i>Metrosideros colina</i>	<i>Mangifera indica</i>	<i>Barringtonia asiatica</i>	<i>Inga feuillei</i>	<i>Cocos nucifera</i>
observed										
<i>Aphylllophorales</i> 1	3	40	0	0	0	0	0	0	0	0
<i>Auricularia</i> sp.	13	14	5	0	0	1	0	0	0	3
<i>Xylariaceae</i> 1	4	0	10	0	1	0	1	0	0	0
<i>Agaricales</i> 2	8	0	0	0	0	0	2	1	1	0
<i>Agaricales</i> 1	4	2	2	1	2	0	0	0	0	0
expected										
<i>Aphylllophorales</i> 1	11.66	20.41	6.19	0.36	1.09	0.36	1.09	0.36	0.36	1.09
<i>Auricularia</i> sp.	9.76	17.08	5.19	0.31	0.92	0.31	0.92	0.31	0.31	0.92
<i>Xylariaceae</i> 1	4.34	7.59	2.31	0.14	0.41	0.14	0.41	0.14	0.14	0.41
<i>Agaricales</i> 2	3.25	5.69	1.73	0.10	0.31	0.10	0.31	0.10	0.10	0.31
<i>Agaricales</i> 1	2.98	5.22	1.58	0.09	0.28	0.09	0.28	0.09	0.09	0.28

TABLE 2: Chi square table for size specificity. Shows observed and expected values for the five most common macrofungi found.

	<1 cm	1 cm - 2.99 cm	3 cm - 4.99 cm	5 cm - 6.99 cm	7 cm - 9.99 cm	10 cm - 14.99 cm	15 cm - 19.99 cm	20 cm - 24.99 cm	25 cm - 29.99 cm	>30 cm
observed										
<i>Agaricales</i> 1	0	0	4	2	4	1	1	0	1	0
<i>Auricularia</i> sp.	1	7	5	5	11	3	3	3	0	3
<i>Aphylllophorales</i> 1	0	3	17	14	16	6	6	0	0	0
<i>Agaricales</i> 2	4	9	0	0	0	0	0	0	0	0
<i>Xylariaceae</i> 1	0	1	1	0	3	5	2	2	1	4
expected										
<i>Agaricales</i> 1	0.44	1.76	2.37	1.84	2.99	1.32	1.05	0.44	0.18	0.61
<i>Auricularia</i> sp.	1.39	5.54	7.48	5.82	9.42	4.16	3.32	1.39	0.55	1.94
<i>Aphylllophorales</i> 1	2.09	8.38	11.31	8.80	14.24	6.28	5.03	2.09	0.84	2.93
<i>Agaricales</i> 2	0.44	1.76	2.37	1.84	2.99	1.32	1.05	0.44	0.18	0.61
<i>Xylariaceae</i> 1	0.64	2.57	3.47	2.70	4.36	1.93	1.54	0.64	0.26	0.90

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ARE CURRENT REEF FISHING PRACTICES SUSTAINABLE? AN ASSESSMENT OF ONGOING MARINE CONSERVATION EFFORTS AND MANAGEMENT PRACTICES ON THE ISLAND OF MOOREA, FRENCH POLYNESIA

SARIKA CULLIS-SUZUKI

Integrative Biology, University of California, Berkeley, California 94720 USA

Abstract. The decrease of fish catches is occurring on a global scale. The need for effective regulations and realistic management goals is becoming more urgent if sustainable fishing is to be attained. This study focuses on the island of Moorea in French Polynesia and assesses the state of its current reef fishing practices and management efforts. Research focused on three aspects. Firstly, population abundance of three commonly eaten and sold reef fish families was studied (lutjanids, scarids and serranids) at three differently regulated zones around the island: a marine protected area, an area regulated by size restriction and a non-regulated zone. Results illustrate significant differences in abundance for each fish family depending on site. Secondly, by-catch survival rates of lutjanids were monitored by using two methods of capture: hand line caught fish and gill-net caught fish. Results show a significant difference in mortality rates depending on method of capture: gill-net caught snapper were more likely to die than hand line caught snapper. Lastly, a survey was conducted within the fishing community to assess their current awareness of fishing regulations and willingness and ability to abide by them. Results show fisher people have witnessed a decline in fish within their lifetimes. Results also suggest an increased communication between management officials and fisher people would increase effectiveness of current regulations and marine protected areas on Moorea.

Key words: *by-catch; conservation; gill-nets; lutjanid; marine protected area; scarid; serranid*

INTRODUCTION

Fish populations are decreasing on a global scale at an unprecedented rate (Watson and Pauly 2001, Pauly et al. 2002.). Developing sustainable fisheries has become an issue of universal concern. Over the years, many different management plans have been formulated in attempts to regulate fishing (Kennelly and Broadhurst 2002). One currently debated fisheries management tool is the marine protected area, or MPA. Marine protected areas have both advantages and disadvantages which must be weighted when considering implementation (Yasutoshi and Yasushi 2003).

French Polynesia is no exception to the reality of perceived over-fishing (Walker 2001). On the island of Moorea, sister island to Tahiti, I observed local fisher people experiencing this crisis. Most natives of Moorea, including resource elder P. Mape, agree that in their lifetime, they have witnessed a decline in fish stocks. For a community that, to my perception, relies fundamentally on fishing, there is a pressing need for conservation policies and management plans that promote sustainable fishing practices.

In October of 2004, in an attempt to increase reef fish abundance, the PGEM (Plan de Gestion de l'Espace Maritime) established eight marine protected areas (MPAs) around Moorea (Temaru 2004). Each MPA has specific regulations depending on what the fisher people of that area requested, but all either limit fishing or disallow it completely (Temaru 2004). Every community within Moorea, Paopao, Haapiti, Teavaro and Afareaitu has accepted the installation of MPAs in their region and possess at least one MPA to date, *except* for the community of Papetoai (Fig. 1). Instead, Papetoai has asked to have restricted fishing *by size*, rather than complete areas of coasts where fishing is not allowed, such as MPAs (Temaru 2004). This is significant because such a method exists nowhere else in French Polynesia (C. Monier, *pers. comm.*). If this technique for increasing reef fish abundance does, in fact, prove successful, it could have profound implications on other areas, illustrating the potential to adopt laws that aren't as invasive or restrictive as MPAs.

The fundamental question therefore remains: is either of these methods, MPAs or marine conservation by fish size restrictions, effective?

And if so, which method works best? In order to assess if such methods are having an effect, reef fish population abundance must be measured at each different location. In addition, it is critical that there be a process by which the impact of such methods on each community is measured. This study focuses on the current conservational solutions to the decline of fish on Moorea, and their effectiveness to date.

MATERIALS AND METHODS

All data for this project were collected from the beginning of October to the beginning of November in 2005. The research center used was the University of California, Berkeley Gump Station in Paopao. There were three parts to this study, and each required different materials and methods.

Fish Population Abundance

The first part was a survey recording population abundance of three different reef fish families at three distinct sites. Snappers (including: *Lutjanus fulvus* Forster 1801, *Lutjanus gibbus* Forsskal 1775, *Lutjanus kasmira* Forsskal 1775, *Lutjanus monostigma* Cuvier in C & V 1828), Parrotfish (including: *Scarus psittacus* Forsskal 1775, *Scarus frenatus* Lacepede 1802, *Scarus ghobban* Forsskal 1775, *Chlorurus sordidus* Forsskal 1775), and Groupers (including: *Epinephelus hexagonatus* Forster 1801, *Epinephelus merra* Bloch 1793, *Cephalopholis argus* Bloch et Schneider 1801- all fish were identified by photographs from Randall 2005) were chosen because of their popularity amongst reef fishermen (V. Brotherson and J. You-Sing, *pers. comm.*) and the given time frame for collection of data, which limited the possible number of families to study. The three sites surveyed were chosen because each is regulated differently: a marine protected area (MPA) in Paopao, a non-regulated zone in Paopao, and an area of Papetoai, the region of Moorea that has fishing restrictions through size limits. Another determining factor for site selection was proximity of locations to each other and to the Gump Station, and similarity of sites to each other. Underwater observations (in this case by snorkeling), were used to determine which area contained a greater abundance of fish. These three sites were measured twice each day over the course of 12 days for a total of 24 times each: 12 times at a shallow depth of 1 m – 2 m, and 12 times at a greater depth of 3 m – 4 m. In total, there were 72 transects completed. Sites were visited at rotating times of the day to reduce potential bias. Transects were 20 m long, the same length used for



Fig. 1. Map of Moorea illustrating five regions (including Paopao and Papetoai) and all eight Marine Protected Areas (depicted in red). Image used with permission from C. Monier.

underwater transects in the study by Victor, 1986, and stretched parallel to the shore. After waiting approximately five minutes, the snorkeler, equipped with mask, snorkel, fins and a dive slate, would slowly float over the transect, recording the number of fish from each of the three specified families observed along the 20 m transect, and within 2 m to the right and left of the transect.

By-catch Survival Rates

The second part of this study focused on the collection of undersized lutjanids from two methods of fishing: hook and line, and gill-nets. The purpose was to monitor and to compare survival rates of the fish to determine which method of fishing was more harmful to snapper by-catch. In the context of this paper, 'by-catch' refers to undersized fish caught in the region of Papetoai that, according to recent regulations, must be released. Lutjanids were selected because of their high catch rate both by net and by hand line, and because of their fishing popularity (V. Brotherson and J. You-Sing, *pers. comm.*). Twenty two snapper were collected by net and 22 by hand line. The lutjanids were obtained from five different fisher people on Moorea and all came from either Paopao or Papetoai regions because of the sites' proximity to the Gump Station in Paopao (where all subsequent fish observations were made in aquaria). Obtaining lutjanids was achieved by accompanying the fisher people either on their fishing boats (motor boats or pirogues) or from shore. It was important to measure the fish to determine their size before confirming their status as by-catch. The undersized snapper were

collected in large buckets in which the water was changed frequently, and then brought back to the wet lab at the Gump Station. Fish were never kept longer than 30 minutes in a bucket. Fish were released into aquaria of length 80 cm, width 50 cm and depth 43cm, with a fresh, constant sea water flow and a stable temperature of 28 degrees Celsius. The fish were held for a period of four days. Feeding of fish was attempted but rarely successful, most likely due to high levels of stress on the fish upon being held in captivity. During those four days of captivity, the snapper were monitored for change in behavior, change of health, or, death. All fish that died while in captivity were labeled and frozen for possible future autopsies. All fish that survived captivity for four days were then released back into the ocean where they were caught. The health of the fish was described upon their release, including any noticeable changes in their behavior or physical state.

Fishing Community Survey

The last part of this study focused on the Moorean social awareness regarding fishing practices in the community. In order to determine the effectiveness of new conservation methods, research on the societal aspect is needed in addition to direct fish population analyses (Pinkerton and Weinstein 1995). To accomplish this, a survey of 25 questions were posed to 30 fisher people, most from the regions of Paopao and Papetoai (again, these locations were chosen for reasons of proximity to the Gump Station in Paopao). This survey was similar in design (though not in length) to the ranching survey conducted by Liffman et al., 2000, in California. Fisher people were contacted for surveying at random, sometimes by recommendations and other times by chance introductions. The surveys were conducted principally to understand the local fisher people's level of awareness of current fishing regulations and the level of community compliance with these new laws, as these factors are pivotal to the success of fishing regulations.

RESULTS

Fish Population Abundance

According to the probability value (Table 1), it is suggested that there is a significant difference in parrotfish abundance between certain sites. Table 2 and Fig. 2 show results of the Tukey-Kramer Multiple Comparison Test: Parrotfish populations vary significantly between the MPA site and the

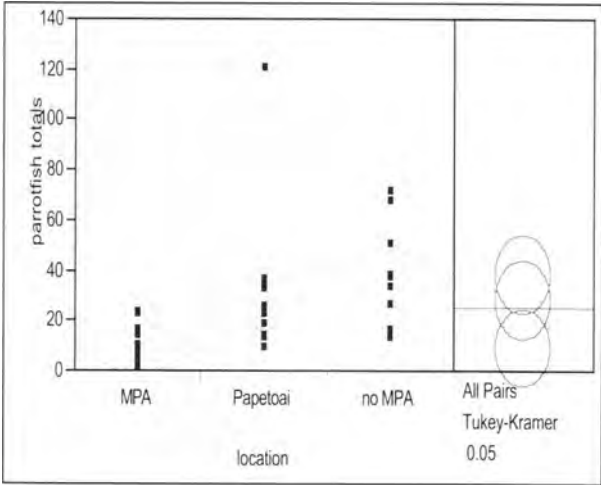


Fig. 2. Analysis of parrotfish abundance at three different sites: MPA, Papetoai and an area of Paopao with no fishing regulations (no MPA). A Tukey-Kramer test was conducted to determine significance.

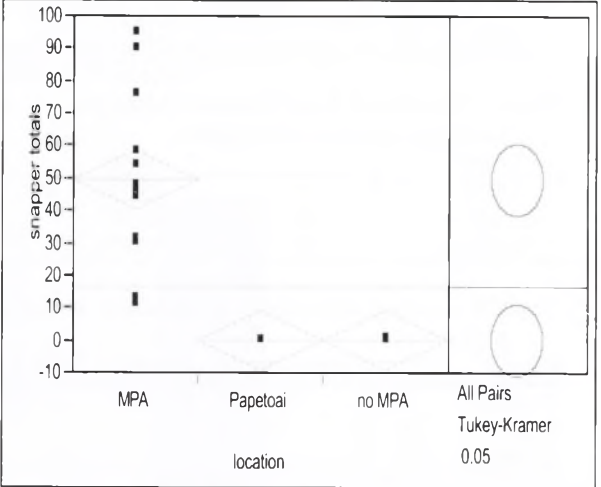


Fig. 3. Analysis of snapper abundance at three different sites: MPA, Papetoai and the site with no MPA). A Tukey-Kramer test was conducted to determine significance.

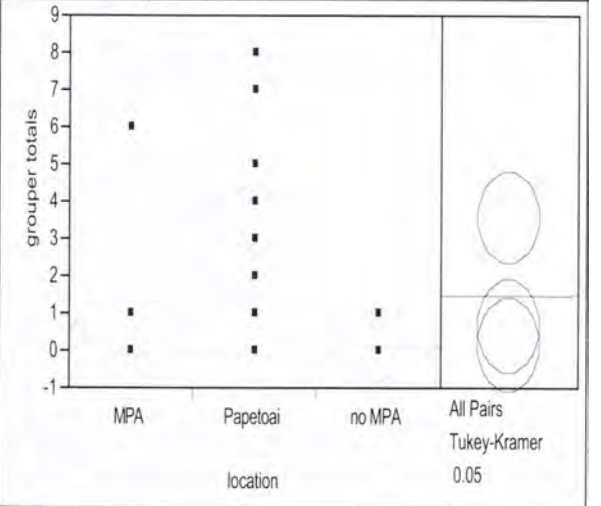


Fig. 4. Analysis of grouper abundance at three different sites: MPA, Papetoai and the site with no MPA. A Tukey-Kramer test was conducted to determine significance.

By-catch Survival Rates

TABLE 1. Analysis of variance for parrotfish abundance at three sites.

Source	DF	Mean Square	F Ratio	Prob > F
location	2	2594.08	5.4325	0.0091
Error	33	477.51		
C. Total	35			

TABLE 2. Results of Tukey-Kramer Multiple Comparison Test for parrotfish.

Level	Mean
no MPA A	38.2
Papetoai A B	28.4
MPA B	9.3

TABLE 3. Analysis of variance for snapper abundance at three sites.

Source	DF	Mean Square	F Ratio	Prob > F
location	2	9817.53	40.0179	<.0001
Error	33	245.33		
C. Total	35			

TABLE 4. Results of Tukey-Kramer Multiple Comparison Test for snapper.

Level	Mean
MPA A	49.583
no MPA B	0.083
Papetoai B	0.000

TABLE 5. Analysis of variance for grouper abundance at three sites.

Source	DF	Mean Square	F Ratio	Prob > F
location	2	40.8611	13.5861	<.0001
Error	33	3.0076		
C. Total	35			

TABLE 6. Results of Tukey-Kramer Comparison Test for grouper.

Level	Mean
Papetoai A	3.583
MPA B	0.666
no MPA B	0.166

non-MPA site. The probability value (Table 3) suggests there is a very significant difference in snapper abundance between certain sites. After conducting a Tukey-Kramer Multiple Comparison Test (Table 4, Fig. 3) we see that the significance lies between snapper abundance in the MPA site and Papetoai, as well as between the MPA site and the site without an MPA. The probability value (Table 5) suggests there is a very significant difference in grouper abundance between certain sites. The Tukey-Kramer Comparison Test (Table 6, Fig. 4) establishes that the significant difference between sites appears to be between Papetoai and the MPA, and Papetoai and the site without an MPA.

From Table 8 we see that the Chi-square value is 3.97; this is greater than the critical value of 3.84,

TABLE 7. Confidence Intervals.

Level	Count	Prob	Lower CI	Upper CI	1-Alpha
hand	3	0.23077	0.081795	0.502564	0.950
line					
net	10	0.76923	0.497436	0.918205	
Total	13				

TABLE 8. Chi-Square Test results.

Test	Chi-Square	DF	Prob>Chisq
Likelihood Ratio	3.9765	1	0.0461

deeming these results significant. The Likelihood Ratio Test clarifies that gill-net caught snapper have a higher mortality rate than hand-line caught snapper.

Fishing Community Survey

It becomes clear from figures 5 and 6 that there could be a dilemma with the current management system of Moorea. Figure 5 shows us that most of the fisher people are indeed aware of MPAs on Moorea, and very few do not know or aren't sure of what they are. This does not seem to match up to figure 6, which illustrates that over half of the surveyed fishing population at times fish within MPAs. I deduced that fifteen out of 30 people (half of all surveyed) said they knew about MPAs but still fished within MPAs.

Regarding current regulations of the region of Papetoai, where size restrictions rather than MPAs are used, results showed that few people claimed they were aware of the fishing restrictions of that area (Fig. 7). Interestingly though, a very high number of those surveyed fished within Papetoai (Fig. 8). These two graphs together present a powerful account of the fishing reality of this surveyed population: awareness is low but fishing is high in the region of Papetoai.

The last figure (Fig. 9) presents the reality of decreasing fish stocks on the island of Moorea. Here we see that out of the 30 people surveyed, 29 responded that they have witnessed declines in fish over the years. Some fisher people noticed only certain species declining, but of those 29, all agreed that within their lifetime, fish abundance has indeed been diminishing.

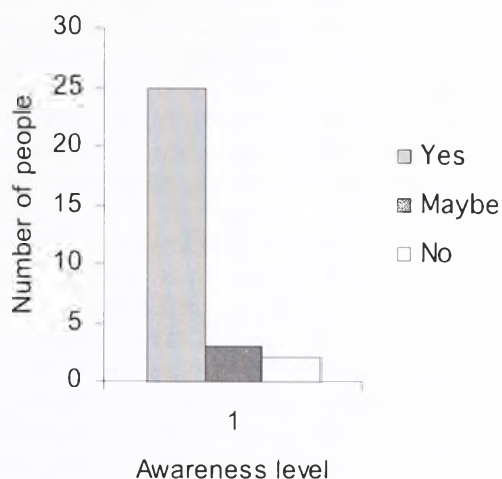


Fig. 5. Graph represents number of fisher people (out of 30) who indicated that they were currently aware about marine protected areas on Moorea.

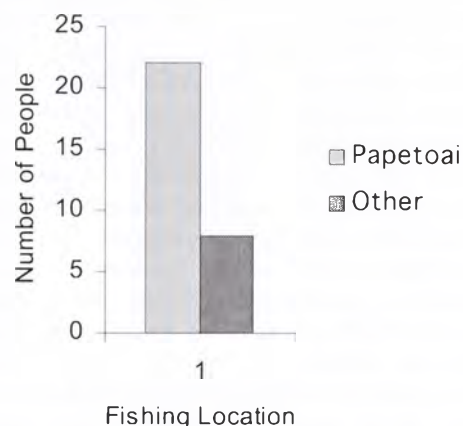


Fig. 8. Graph represents number of fisher people (out of 30) who currently fish within the region of Papetoai.

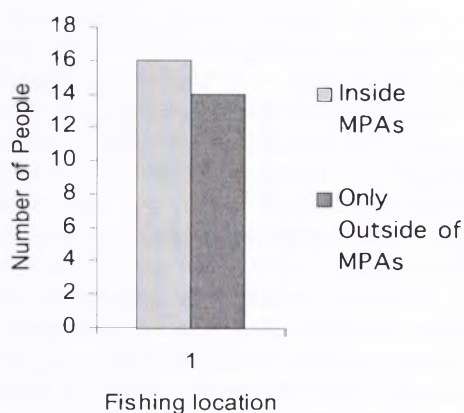


Fig. 6. Graph represents number of fisher people (out of 30) that currently fish in MPA zones.

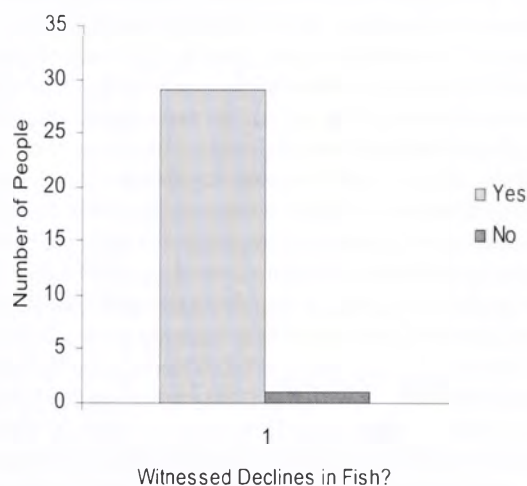


Fig. 9. Number of fisher people (out of 30) who have witnessed a decline in fish (of any species) over the years.

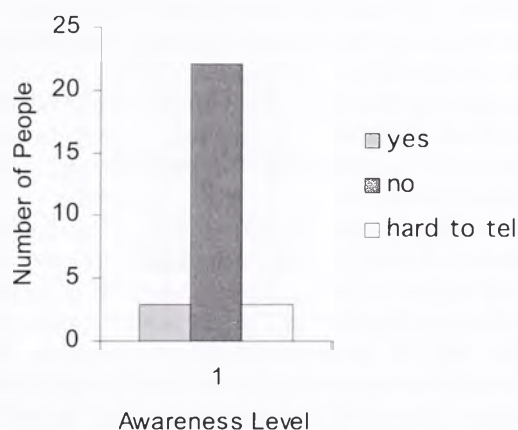


Fig. 7. Graph represents current awareness of 30 fisher people on Moorea regarding fishing restrictions of Papetoai.

DISCUSSION

Fish Population Abundance

The first part of this study, the population abundance analysis, produced unexpected results. All three fish families had highest abundance in different locations. This implies that either habitat preference differs amongst fish families, or fishing pressure differs for each fish family. Parrotfish had a significant difference in abundance between MPA sites and non-MPA sites, yet the higher

abundance occurred surprisingly in the non-MPA zone (Fig. 2). In contrast, snapper had a significant difference in abundance between MPA sites and non-MPA sites, as well as MPA sites and Papetoai (Fig. 3). There are several possible reasons for such results: perhaps snapper preferred the MPA habitat over the other locations for reasons unaffected by fishing pressure; or, perhaps fishing pressure of snapper was indeed lower in the MPA site than the other sites thereby accounting for the much larger number of snapper within the MPA. Lastly, the studied site within Papetoai appears to have the greatest population abundance for grouper over both the MPA site and the non-MPA site (Fig. 4). This could be attributed to either the higher desirability of habitat for grouper within Papetoai to that of other locations, or it could indicate that fishing regulations within Papetoai are indeed effective for grouper, and thus account for their higher population abundance within the area.

These results could be interpreted in different ways: perhaps MPA sites are working best for snapper whereas the fishing regulations in Papetoai mostly benefit grouper. Finally, parrotfish could prefer the habitat of the non-MPA site over other sites to such an extent that their abundance is higher at this site.

It is difficult to determine from these results the significance of MPAs or size restrictions in Papetoai as effective management tools for fishing on Moorea. More detailed studies must be done on population abundance of fish in each of these areas, as well as habitat preference of specific fish families, to be able to come to distinct conclusions. It must also be noted that the installation of these MPAs and fishing restrictions was very recent, in 2004, therefore it is very feasible that changes in fish abundance have not yet been detected between sites.

Human error accounts for the largest potential source of error for this part of the project. When counting fish it is virtually impossible to make an exact tally, as fish are fast and easily scared off. Certain families of fish could be more easily frightened, creating a bias in favor of bold or territorial fish. Also, bias can occur because certain fish tend to hide under or within coral and are thus harder to distinguish (Lipej et al. 2003). Failure to identify fish families accurately is yet another possible source of human error. Finally, poor site selection could have potentially contributed to sources of error: fish abundance correlates to habitat preference, therefore if the selected sites differed significantly in habitat, fish abundances could differ accordingly.

This study was important because it monitored current methods of fish capture within the reef for sustainability. The concept behind fishing size restrictions in the region of Papetoai depends on the assumption that fish will survive upon release. If, in actuality, released fish do not survive, there would be no point in restricting fishing by size. However, if it is proven that certain methods of fishing are less damaging than others, perhaps Papetoai could enhance its regulations by also including certain gear restrictions. The results from Table 7 show a significant difference in survival rate between snapper caught by hand line and those caught by gill-net: the Chi-square value from the Likelihood Ratio is greater than the critical value, deeming the difference between mortality rates significant.

The biggest cause for death amongst net-caught snapper was most likely due to deep cuts they suffered around the head and belly, and often gills. Another probable cause of death was the pressure from the fisher person's hands on the fish while attempting to disentangle the fish from the net: internal organs were compressed, potentially causing irreversible damage to the fish. For hand line-caught fish the cause of death was less evident, but hypotheses include hooks being deeply imbedded in the fish's throat or gills, 'snagging,' whereby the fish is hooked on vulnerable areas such as the stomach, and aggressive handling of fish by the fisher person upon catch.

Another monitored aspect of the by-catch study was the condition of fish upon release. In general, snapper that had been caught by net and released were in worse physical condition than those caught by hand line: open sores or lacerations were common and visible on the released netted fish, but not on those caught by hook.

These results are valuable as they indicate differing levels of harm inflicted on fish depending on what fishing method is used. Management within Papetoai could consider requiring the most sustainable fishing methods *as well as* size restrictions; this would enhance their current regulations, potentially increasing fish abundance.

This by-catch study was limited to holding fish in captivity for a maximum of only four days. Although a significant difference was found between those fish caught by hand-line and those by net, it is more than conceivable that there would be more deaths for fish caught by net after four days. This conclusion is based on the condition of the fish once they were released. Had

fish been in captivity longer, the mortality rates would have likely increased for snapper caught by net, as upon release they were often in very bad condition. Other possible sources of error could be length of time between capture and release into aquarium; unavoidable changes in aquarium water; degree of disturbance while in captivity, and inevitable range of harm when handling fish depending on fisher person. It should also be noted that eight hand line caught snapper contracted what appeared to be a fungal infection, and half were dead after one night of captivity, the others being mortality ill. Five snapper caught by net also suffered similar symptoms, but were released before signs worsened. Symptoms appeared as a thick, white film covering the entire body, blinding the fish. Although it cannot be proven that this infection was not the result of the method of capture, evidence suggests infection caused by close confinement in captivity to be the most probable cause of contamination, and hence these infected fish were not included in the study's data.

Fishing Community Survey

The main focus of the fishing survey was to determine a) if fisher people were aware of fishing regulations, b) if fisher people followed fishing regulations, and c) if in fact there is cause for concern: have fisher people witnessed a decline in reef fish catch over the years? The results to these questions, posed to 30 random fisher people, gave profound results. From Figs. 5 and 6 we see that although the majority of people surveyed said they were aware of MPAs on Moorea, more than half fished within MPA zones nevertheless. In fact, exactly 50% of those surveyed fished within MPA zones while supposedly knowing about the protected areas. There are many possible reasons for such results. In some cases, although aware of MPA sites, the fisher person could not justify stopping fishing in such areas, as the basic need to feed one's family was the priority. In other cases, it was evident that although the surveyed said they were aware of MPAs on Moorea, it was more probable that they actually did *not* know, judging by the way in which they answered more detailed questions pertaining to protected areas. However, the most common situation was that the surveyed knew about only *certain* MPA sites on Moorea, and therefore fished in other MPA zones unknowingly. Finally, there were those who were aware of only *certain* rules regarding the MPAs, such as limited boat restriction within the protected area. These results were undoubtedly the product of both misunderstandings and lack of awareness; such consequences could be avoided if

communication between those in charge of making the regulations and those that must follow them are strongly improved.

In Figs. 7 and 8, results regarding the region of Papetoi are examined. In terms of awareness, we see that the vast majority of fisher people were not aware of the fishing regulations of Papetoi. Furthermore, we see that most of those surveyed fished in Papetoi. The conclusion drawn from these figures is that although the majority of people surveyed do fish in Papetoi, most are not aware of the fishing restrictions. Once again, this lack of awareness can be attributed to the poor communication between those managing and those being managed. Finally, Fig. 9 shows that 29 out of the 30 surveyed disclosed that they have witnessed a decline in fish over the years. This result simply intensifies the call to increase effective management on the island.

The most obvious source of error lay in the language barrier between the surveyor and the surveyed, occasionally resulting in failure to communicate and understand the other clearly. Another impediment was unforeseen cultural differences that could have affected the survey responses. However, the most notable source of error in the survey lay in its failure to discern if in fact the surveyed was answering truthfully. The survey relies on honest information, without which such information would prove irrelevant. One example of a source of error was blatantly apparent when surveying one fisher person in particular. She was fishing as she responded to the survey, and, upon being asked whether she released her undersized fish, she answered 'Always;' yet she was keeping all her undersized fish as she answered the questions. Such unexpected incidences are perhaps inevitable, yet remain to the study a grave source of error.

This study is but an introduction to the current fishing reality of Moorea; such an investigation is by no means closed. At present, little research exists regarding the current state of affairs of reef fishing on Moorea, simply increasing the urgency for more such studies. This topic is extremely broad and much more time is required to study it comprehensively. From this study it can be concluded that annual surveys on population abundance of fish within differently regulated sites would benefit research considerably, hopefully establishing which management plans are most effective. Fishing regulations and management plans must be created according to ecological health as well as the needs of the people. It is also evident that many aspects must be taken into consideration when creating management regulations, even

basic factors such as gear selectivity. In addition, this study reinforces the necessity for communication between those that create managing plans and those that are to follow them. All these factors, ecological, economical and social concerns, must be addressed when considering performance of management tools such as MPAs (Bohnsack 1998).

ACKNOWLEDGMENTS

The author gratefully recognizes the fundamental support from her UC Berkeley professors: the patience, guidance and time provided by professor G. Roderick; the enthusiasm and comments offered by professors B. Mishler and R. Gillespie; the vital suggestions from professor R. Caldwell; the meticulous assistance of C. Hickman in revisions as well as her keenness and good nature, and the statistical knowledge of J. Bartolome as well as his help in the field. Information provided by F. Murphy as well as advice and knowledge gained from H. Murphy are also very much appreciated. The author would also like to express deep appreciation to the fisher people who were part of the by-catch study, J. You-Sing and L. Brotherson, without whom such a study would prove impossible. Those who took time to complete the fishing surveys must be thanked for their patience and good-nature, and are greatly appreciated, as is V. Brotherson for her input on the survey. The author also thanks the resource elder P. Mape for his time and consent to conduct such a study on Moorea. This study was deeply enriched by I. You-Sing who contributed by translating, setting up appointments and offering her own personal knowledge. This project must also be attributed in part to C. Monier who helped give birth to the principle focus of this research and whose key input and knowledge were invaluable, and to *Service de la Pêche* of the *Ministère de la Pêche de la Polynésie Française*, for useful information. The author would also like to thank S. Fay for his support and help with statistical software and survey planning, M. Weber for her interest and advice on survey graphing as well as personal approachability and field knowledge, and M. Medeiros for his continuous support and encouragement. Finally, the author would like to thank her peers and partners from the Moorea Class of 2005 without whom this project would not be possible.

Although the reality of over-fishing is undeniably daunting, there is reason to remain optimistic: the fishing community of Moorea remains open and eager; more research and more

communication can potentially change this island's over-fishing into a sustainable way of life.

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FACTORS INFLUENCING DISTRIBUTION PATTERNS OF EPIPHYLL COLONIZATION AND HERBIVORY ON DECAYING POPULATIONS OF THE INVASIVE SPECIES *PSIDIUM GUAJAVA*

SARAH I. DANIELS

*Environmental Science Policy and Management, University of California
Berkeley, California 94720 USA*

Abstract Presence of one invasive species can facilitate the presence of other introduced or invasive species within a community, the presence of this secondary introduction, can become a resource for further propagation by other non-indigenous species. On the tropical island of Moorea, in French Polynesia, the distribution of a non-native epiphyte, guava leaf spot fungus, on a dying population of the invasive plant species, *Psidium guajava*, was observed. A non-native scarab beetle, responsible for eating guava leaves within the garden, was also studied to see if the degree of foliage consumption was dependent upon the amount of non-native epiphyllic coverage. Along with this, the patterns of colonization of two other epiphylls, a sooty mold fungus and a lichen species, were observed to find any additional relationships between microenvironment and epiphyte growth. Epiphyte growth and herbivory of each leaf was measured as a ratio of leaf area covered. Significant differences in abundance of the epiphylls were found among the four locations in the garden and among different tree sizes. Curiously, no positive correlation was found between herbivory and ratio of the non-native epiphyllic growth on each leaf in this field study. A negative correlation was found between herbivory and amount of lichen on each leaf. Further experimental studies were executed to control for lichen coverage and deduce if the herbivores preferred the leaves with greater proportions of the leaf spot fungus. These tests provided significant results that leaves with 100% coverage of the leaf spot fungus were favored the most by the scarab beetles. Thus, even though this population of *P. guajava* did provide a habitat for more non-native epiphyllic species to thrive, the potential of the non-native leaf spot fungus to increase the presence of the non-native beetle within the garden cannot be determined by field studies due to confounding effects by another epiphyll, the lichen. This study further explains the implications of critically analyzing changes within an invasive species community by recognizing both biotic and abiotic factors through both field and experimental studies.

Key words: Epiphylls, invasional meltdown, French Polynesia, herbivory, leaf spot fungus, sooty mo(u)ld, lichen

INTRODUCTION

Introduced species have been widely known to out-compete native species for resources and survival, causing the extinction of the native species. However, not only can invasive species cause immediate harm to ecosystem diversity, but under certain circumstances, they can also have beneficial effects for other introduced species causing further harm to ecosystem dynamics and the environment. This process of one introduced species facilitating the presence of another can lead to an "invasional meltdown" (Simberloff and Von Holle 1999). Generally,

ecological networks on oceanic islands are vulnerable to destruction by non-native species. (Loope and Mueller-Dombois 1989) Invasive flora species have become successful on the tropical island of Moorea, a Society Island of French Polynesia (Meyer 2004).

The invasive species, *Psidium guajava* (Kellum, per. com.), on Moorea is widely recognizable by its typically smooth red bark, uninhibited by any massive epiphytic growths on the trunk and leaves. After a brief evaluation of guava plants on the western side of the island at the lowest and highest elevations, it appears that this trend is consistent—with the striking

exception of trees found on the property of Marimari Kellum, owner of 60 hectares of land in Opunohu Valley (8061376 UTM).

Natural history of Marimari Kellum's garden dates back to 1925. According to Marimari, her father was once able to cultivate enough guava fruit to sell to the local fruit juice factory. Since 1992, her fruit-bearing trees have decreased in productivity and she nurses a few of her guava plants in order for them to bear a fair number of fruit each season. Without clear external symptoms of plant-death, Kellum presumes that a root rot fungus is a likely candidate for a decrease of guava fruit yield, as well as her lime and soursap fruit. Interestingly, *P. guajava* plants have become the most susceptible to secondary symptoms of decline in health fitness, such as epiphytic growths and herbivory, as compared to the other unhealthy fruit-bearing plants on her property.

These epiphytic growths were specifically found on the leaves of the plant, and thus termed as epiphylls. Study of these epiphylls adds to increased knowledge of the phyllosphere, communities of micro-organisms that grow on or inside of leaves (Ruinen 1961). Even though overriding trends among epiphytic communities have been observed, the patterns within a narrower spectrum, such as among leaves in different environmental conditions of a localized plant community, has not yet been thoroughly researched. It is important to recognize these patterns for a more complete understanding of how these epiphyllic organisms proliferate in their environment, especially in the case of this study, where two of the species are threats to the success of an agriculturally useful plant.

Many damaging fungal species were identified on the surface of the leaf and given plausible genus names including black spot fungus (*Phyllosticta* sp.), anthracnose *Pestalotia* sp.), black sooty mold (*Phaeoscardinula* sp.) and guava leaf spot disease (*Cercospora* sp.) (Chen, pers. com.). The three epiphylls that were the most common and easiest to identify were placed under strict examination; the leaf spot fungus, a lichen, and sooty mold.

The guava leaf spot fungus was also chosen because it is presumed to be an obligate parasite on the plant and, hence, was introduced along with *P. guajava* (Chen, pers. com.). The black sooty mold is more than an ornamental, it can have harmful effects on the plant when in high

frequencies. Therefore, patterns of growth for this epiphyll are also revealed by this study.

The black sooty mold is found most commonly in the tropics (Mibey 1997). Its growth is caused by soft scale insects within the family Homoptera (Mibey 1997) that excrete a trail of sugary residue that coats the leaves. This fungus develops on top of the leaf surface by exploiting the "honeydew" medium as nutrients (Nameth et al. 1996). Even though the leaf is not directly harmed by this fungus, the leaf's productivity is reduced; as sun exposure decreases, so does the plant's ability to photosynthesize (Wood et al. 1988). In one study of this "sooty mold" on pecan leaves, it was shown that the fungus shaded 98% of the light penetrating the leaf and reduced photosynthesis by 70% (Wood et al. 1988). Furthermore, this saprophyte can cause premature senescence of leaves, (Laemmlen 2003), stunt fruit growth, (Rajak and Diwakar 1987) as cited by Mibey 1997) and yield loss (Hughes 1976), as cited by Mibey 1997). A separate study on the

This paper attempts to uncover patterns in the presence and degrees of infestation of three different epiphylls (one which is introduced) on the invasive *P. guajava* plants within Kellum's property according to two discrete variables: location within the garden and size (age) of the plant. The first variable was chosen because all of the guava plants within the same general area have either arisen from the same root foundation, or the trees are related through generations of seedling offspring (Kellum, per. com.). Due to these extreme similarities in heredity, the distribution of epiphytic organisms on trees within the same area is even more dependent on the environmental conditions. The second variable was chosen because it has been shown that differences in abundance of vascular epiphytes varies between host tree size (Schmidt et al. 2001), however fewer studies have determined whether the same applies for nonvascular epiphylls.

This investigation also examines whether the non-native leaf spot fungus increase or decrease leaf susceptibility to herbivory by a non-native insect. The only herbivore culprit found between the months of October and November was the non-native scarab beetle. It is within the subfamily Rutelinae and the genus *Adoretus* (Ratcliffe, per. com.). Most likely originating from Japan and Taiwan, *Adoretus* sp. is now widely distributed throughout Hawaii and

the Pacific Islands (Mau and Kessing-Martin 1991). Even though this taxonomic group of insects is well known as leaf-feeders, they can also favor fungal-damaged leaf material as well (Borrer et al. 1989). These beetles, commonly known as shining leaf chafers, are particularly harmful agricultural pests on a variety of cultivated crops (Borrer et al. 1989, Mau and Kessing-Martin 1991). I am looking at whether the beetles favor leaf foilage with epiphytes coverage over uninhibited leaves. The hypothesis is that the non-native insects prefer leaves covered with the non-native leaf spot fungus over the sooty-mold and lichen. The null hypothesis is that the herbivores have no preference over leaf type depending upon type of epiphytic growth. This may give insight as to how a third introduced species is enhanced by the presence of two others (the invasive guava plants and its non-native, obligate epiphyll).

MATERIALS AND METHODS

Locations

Kellum’s garden was divided into four distinct regions each containing patchily dispersed guava groves. The first location is in the northern-most part of her property, closest to Opunahu Bay. The second location is on the lower eastside of the garden, which is in a moist canopied region. The third location is in a more sun-exposed area, west of the second location. The forth location is in the coconut grove on the far west of Kellum’s property. Most plants sampled in this location were small and found beneath a cover of coconut palms. A map of these locations on Kellum’s property is shown in Figure 1.

Leaf Sampling and Leaf Scoring for Epiphyte Coverage and Herbivory

After surveying Marimari Kellum’s property for *P. guajava*, I restricted this study to plants that could be feasibly sampled using a 1-meter ladder. Plants that were not firmly rooted in the soil were also eliminated from my study. (For example, some trees have fallen over due to their dwindling health or other environmental factors.) A minimum of a 50 leaf sample size was needed per plant, thus small plants or plants with very few leaves were also exempt. I divided the property into 4 separate regions, each with different environmental conditions.

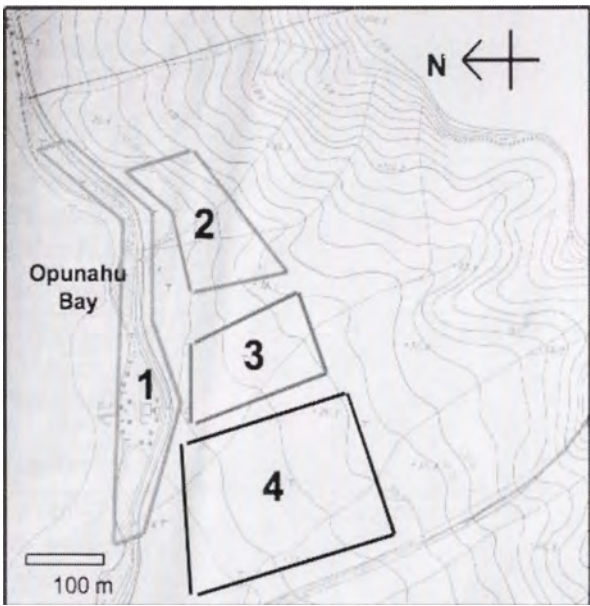


FIG. 1. Topographic map of Marimari Kellum’s garden. Four *P. guajava* sampling sites are marked. Site 1 corresponds to the front yard, site 2 is in the west side of the garden, site 3 is in the central location, and site 4 is to the east side.

Utilizing a random number table, I systematically chose four branches on each tree and picked 6-7 leaves from each of those four branches to accumulate a total of 25 leaves from each tree. A total of 37 trees were sampled. To guarantee that all chosen leaves contained a minimal amount of epiphylls, new budding leaves were purposefully ignored. Abundance (leaf coverage) of each major epiphyll and amount of herbivory per leaf was quantified as a proportion of leaf area. A ¼ x ¼ sq-inch wire grid was placed over each leaf and the number of squares with the given epiphyte/bite marks on the leaf were counted. This number was divided by the number of squares occupied by leaf surface area.

Taxonomic Groupings of Epiphytes

The three most prevalent epiphylls are described in Table 1. Each species was examined under high resolution microscopes with assistance by fungal or epiphyll specialist at UC Berkeley in order to acquire taxon descriptions of each.

Tree size measurements

Plants over 2 meters tall were measured by finding the circumference of the trunk at breast height, which was later converted into diameter

TABLE 1. Three epiphylls of study. Even though a proposed genus name is given, the name was debated among specialists who identified the organisms. The general term is referred to throughout this paper because further DNA analysis is necessary in order to determine the true identity of these species.

Common name of epiphyll	Description
Black "Sooty Mold" fungus	Ascomycete. Saprophytic. Generalist. Mycelium start growth between central leaf vein. Can be composed of multiple species. Proposed genus name: <i>Phaeoscardinula</i> sp.
Guava leaf spot disease	Ascomycete. Raised, long conidiophores. Arranged in clumps, found sporadically throughout leaf. Hyphae penetrate through surface. Proposed Genus name: <i>Cercospora</i> sp.
Lichen (nonspecific)	Greenish white. Grows in circular cluster-patterns over leaf surface. Bright green juvenile thalli found along central leaf vein, bright green.

at breast height. Plants shorter than 2-meters were measured directly by height.

Experimental Test of Adoretus Leaf Preference

Beetles were collected from *P. guajava* trees in the garden during the early evening and transferred to small containers, four beetles per container. Two experimental designs were executed on these herbivores. For each test, total number of herbivore bites was counted on each leaf. Any prior herbivory on the leaves was accounted for before the experimental trials. The containers were placed in a cool and dark environment (a cardboard box) for 24 hours and afterwards the amount of leaf material consumed was recorded. For all tests, each set of leaves were picked from the same branch to limit any confounding variables, including the relative health of the tree branch and age of the leaves.

The first design was used to test whether the beetles preferred a leaf with complete coverage of the black fungus, the leaf spot fungus, or no epiphyllic growth. (The "clean" leaves were used as an internal control.) Two trials were performed for this test.

The second test was used to determine which gradient of leaf spot fungus the beetle preferred. The leaf choices were; complete leaf spot coverage, partial leaf spot coverage, and no leaf spot coverage. Ten trials were performed for this test.

Statistical analysis

Data collected was analyzed using JMP software. The distribution of the epiphytes based on the two discrete variables was analyzed using a parametric ANOVA test followed by a nonparametric Krustal-Wallis Test. The same process was done to analyze the amount of leaf foliage consumed by the chafer beetles when given selections of leaves with varying coverage of epiphytic growth. A Olmstead-Tukey corner test of association was used to find correlation on a non-linear relationship between herbivory and one of the epiphyllic organisms.

RESULTS

A total of 925 leaves, 25 from each of 37 trees, were collected and scored for overall coverage of the leaf spot fungus,, sooty mold, and lichen, as well as a few other species including a liverwort and two more lichen species. These were later disregarded in statistical analysis due to low frequencies of presence. The orange leaf spot fungus was found in the highest frequency, followed by the sooty mold, and then the lichen (Figure 2). For the analytical tests done in this section, an ANOVA test was first used to analyze the difference between mean ratios according to the two discrete variables. If the ANOVA test showed significant differences among the mean frequencies, this test was followed by a Krustal-Wallis test because all distributions of data sets for epiphytes and herbivory on each leaf were not normal and could not be altered using numerous transformations. (Excessive zeros were used to represent absence of a species on the leaf's

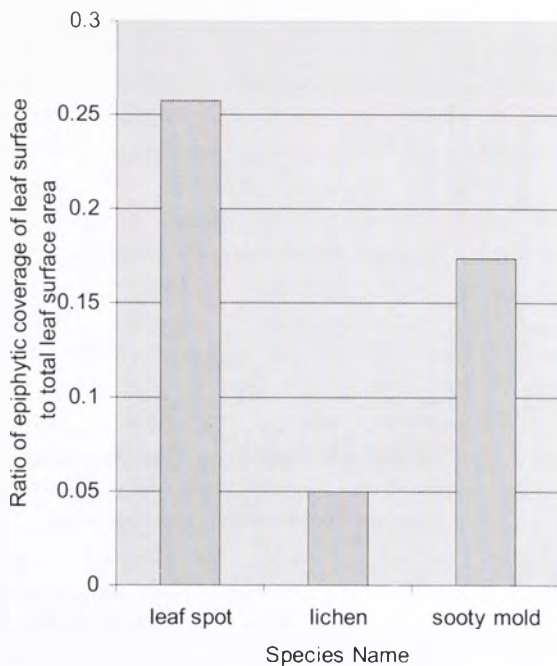


FIG. 2. Overall average amount of each epiphyll coverage per leaf.

surface, which skewed the distribution). A non-normal distribution violates one of the assumptions of an ANOVA test. Therefore, the P values were obtained from a nonparametric Krustal-Wallis test instead.

Epiphytic distribution and amount of herbivory associated to location

The results of the Krustal-Wallis test showed that there is a significant difference between the average amounts of the leaf spot fungus, lichen, and black sooty mold according to locations within Marimari Kellum's garden. According to Figure 3, the leaf spot fungus was found in a significantly higher concentration (an average of 50% coverage per leaf) in the moist canopy side of the garden. The same fungus was found in significantly lower amounts in the coconut grove. The lichen was found in the highest proportion in the moist canopy region and in the front yard. The sooty mold was found in a significantly higher proportion in the coconut grove and significantly lower proportions in the moist canopy region.

Epiphytic distribution associated to plant size

The trees were organized into 3 discrete groupings (or bins) according to natural breaks in

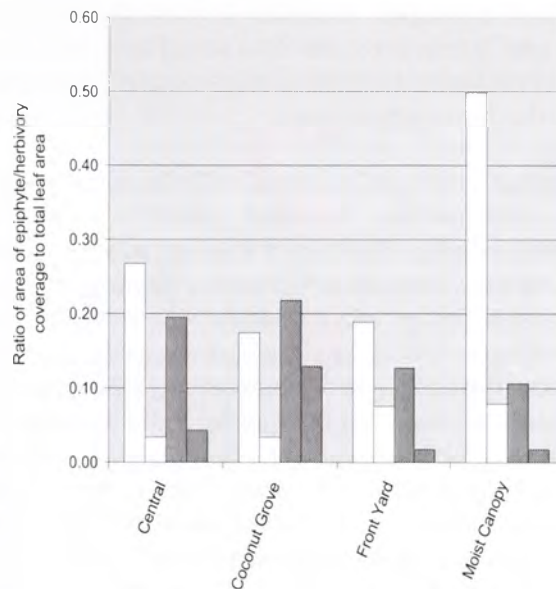


FIG. 3. Average amount of each epiphyll growth.

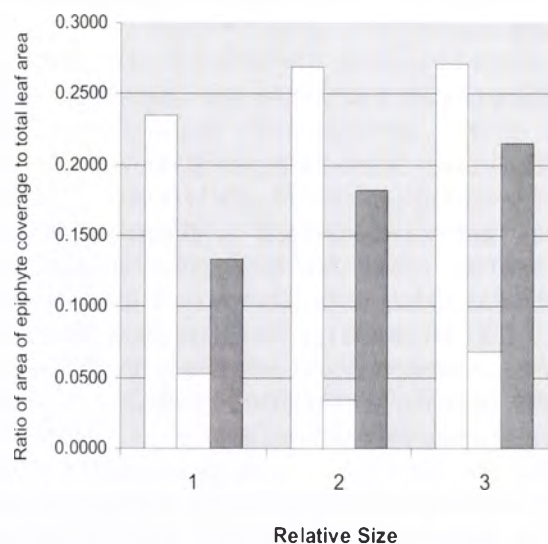


FIG. 4. Comparison of epiphytic distribution according to plant size. Unfilled bar represents leaf spot fungus. Striped bar represents lichen. Shaded bar represents sooty mold (df=2, $P<0.0048$ for leaf spot fungus and $P<0.0001$ for lichen and the sooty mold.)

the size measurements: short (including all plants below 2 meters high, and with trunk diameter between 0.96 cm and 1.75) intermediate (plants with trunk diameter between 2.23 cm and 4.24 cm) and tall (trunk diameters above 5.41 cm). The results of the Krustal-Wallis test showed that there is a significant difference between the average amounts of the three epiphytic growths per leaf within three relative sizes of plants (Figure 4). The average proportion of leaf spot fungus per leaf increased slightly over the three

plant sizes. The average proportion of the lichen per leaf decreased from the first to second plant size and increased from the second to third plant size. The amount of sooty mold steadily increased over the three plant sizes.

Epiphyll coverage vs. amount of herbivory per leaf

The results of a simple regression analysis to compare the amount of herbivory per leaf against the amount of epiphyte coverage per leaf showed that there was no strong correlation between the two variables for the leaf spot fungus. Also, no correlation was found between the amount of herbivore bite marks and the amount of sooty mold on each leaf. However, there was a non-linear relationship found between the amount of herbivore bite marks and the amount of lichen on each leaf (Figure 5). Thus, the Olmstead-Tukey corner test of association was used to prove that this negative, non-linear correlation is significant.

Experimental Test of Adoretus Leaf Preference

The tests were performed using non-ratio data, meaning each bite mark was counted on the leaves and not calculated as a ratio over total leaf surface area. This is because all leaves in each trial were approximately the same size, hence the denominator was unnecessary in order to compare results from each trial. It is important to note that the amount of lichen was kept at a bare minimum for each of the tests.

For the first test, two trials were used. Due to complications in finding beetles and appropriate leaves, only two trials of this experiment were successful. The presence of complete leaf spot fungal coverage on the leaf surface did show more herbivore bites than a leaf with complete sooty mold coverage or no epiphyte coverage at all. However, because only two trials were successful, an ANOVA test could not prove that these differences were statistically significant (Figure 6.).

Still, utilizing the information gained from this first experiment, a second test (which looked at gradients of leaf spot fungus) showed that leaves with complete coverage of the epiphyll leaf spot fungus were eaten (on average) significantly more than leaves with partial coverage or little coverage of the same species (Figure 7). Ten trials were successfully completed for this test.

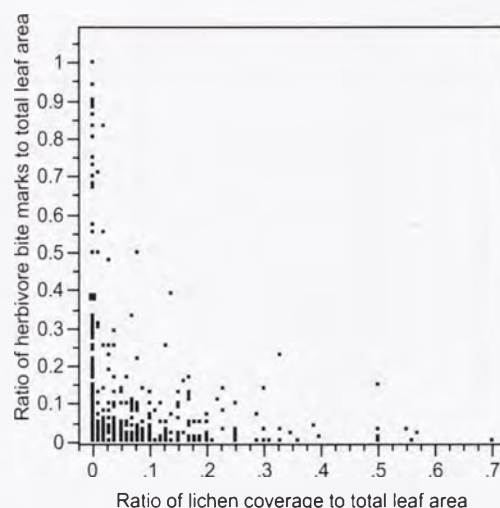


FIG. 5. Correlation of epiphyll coverage per leaf against amount of herbivory per leaf. (Test of association $df=3$, $P<0.0001$)

Trial	100% Cover of Leaf Spot Fungus	100% Cover of Sooty Mold Fungus	0% Epiphyll Cover
1	37	13	5
2	20	3	6

FIG. 6. First Experimental Test Results. Number of herbivore bite holes on each leaf type. ($df=2$, $P<0.1135$)

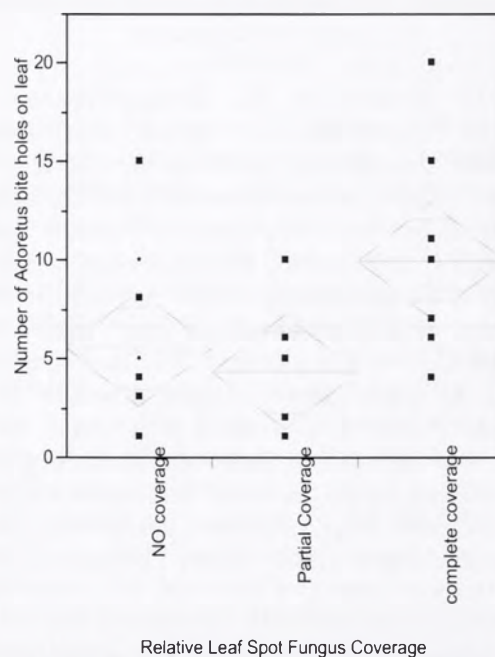


FIG. 7. Second experimental test results. Leaf coverage against number of herbivore bite holes per leaf. ($df=2$, $P<0.0216$)

DISCUSSION

In the observational study of epiphyte growth in different locations within Marimari's garden, results show that the lichen was most abundant on the eastern side of the garden, which is also the moistest location of the garden. Returning to Figure 1, the topography of her garden shows a steeper slope down to the eastern side of the garden than the other sites, which leads to a greater build-up of run-off during rain storms. With a large tree canopy covering many of these guava plants, the leaves are generally shaded throughout the day. Interestingly, the greatest proportion of lichen populations found in this extremely moist area does coincide with the greatest proportion of the parasitic leaf spot fungus. Lichen as well as other epiphylls increases the moisture content on the leaf surface (Gregory 1971, as cited by Coley and Kursar 1996). Furthermore, it has been shown that for temperate crops, increasing the moisture content on the leaf surface also increases the probability for infection by pathogenic fungi; the damp leaf surface is more conducive to colonization, infection, and sporulation (Huber and Gillespie 1992). This study may suggest that the same occurs on tropical crops, such as guava, as well. The parasitic leaf spot fungus may be in such a distinctively larger proportion on the east side of the garden because of the two moisture contributing factors: the abiotic factor of location and the biotic factor of large lichen populations on the leaves. This correlation between lichen and the parasitic leaf spot may not be found in other locations, such as the northern (front-yard) sector of the garden, due to differences in microclimate. It is important to highlight that in the central location of the backyard and the coconut grove, the average frequencies of lichen per leaf are significantly lower and the average frequencies of the leaf spot fungus are significantly lower as well.

When looking at the three plant sizes, the average amount of the leaf spot fungus does not correlate well with the average frequencies of parasitic fungi per leaf. This may be due to a variety of morphological and physiological properties associated with plant size including differences in CO₂ uptake, transpiration, (and stomatal closure), and the anatomy and morphology of the leaf (leaf thickness and photosynthetic capacity) all which have shown to influence the growth of epiphylls (Gerhard

Zotz 2001, Schmidt and Zotz 2001). Because of these disparities among tree size, it is essential to take these factors into consideration when investigating patterns among epiphyllic growth (Zotz et al. 2001).

The correlation between herbivory by *Adoretus* sp. and the frequencies of lichen per leaf shows a strong negative correlation. This compliments prior knowledge on the chemical components of lichen. This epiphyll can be toxic to certain herbivores, indirectly protecting the leaf against insect attack (Coley and Kursar 1996). Further evidence to support this theory can be provided by Figure 3, which shows a maximum amount of herbivory per leaf with the lowest amount of epiphyllic lichen in the coconut grove site. The results of the negative correlation imply that the beetles evaded leaves that have already been colonized by the lichen.

From these results, the native lichen has two possible affects on the invasive *P. guajava* leaf foliage; (1) inadvertently reducing the leaf-susceptibility to herbivory, and (2) increasing the leaves' vulnerability to infection by the parasitic leaf spot fungus. The lichen, acting as a confounding variable, may help explain why a positive correlation between the amount of herbivory and the amount of the leaf spot fungus per leaf was not found through observational studies (due to the fact that lichen and fungus colonized the same leaf).

When the amount of lichen per leaf was controlled in the experimental setting, a positive correlation was found. The experimental study showed that the beetles favored the leaves with full coverage of the leaf spot fungus significantly more than that with some or no fungal growth. This provides evidence that the non-native parasitic fungus may enhance the presence of the non-native beetle species within Marimari's garden. If the guava leaf spot fungus is a true contributor to the *Adoretus* sp diet, then the non-native leaf spot disease may play a major role in the population dynamics of the introduced beetle.

In contrast to the lichen and leaf spot fungus, the sooty mold was found in the lowest quantities per leaf in the moist canopy area. One hypothesis for why this occurred is that the damp abiotic factors of this area along with the moist characteristics of the epiphyllous lichen keeps the leaf surface wet, preventing the honeydew residue from coating the leaf and inhibiting the growth of the saprophytic sooty mold.

Sooty mold was found in highest frequencies in the coconut grove. All *P. guajava* found in this drier area were sheltered by the small coconut trees. In the drier microclimate, the shade may have encouraged the growth of sooty mold. Prior studies have shown that rain does decrease the mold's ability to proliferate on the leaf surface (Wood et al. 1988). Therefore, a low protective canopy from small rain showers and drier environmental conditions could cause these *P. guajava* to be more contaminated by the mold.

In the observational study of epiphyll growth and plant size, it appears that sooty mold may be best described by this discrete variable. This could provide evidence that younger *P. guajava* have a defense against the saprophytic fungi. Or it is possible that the scale insects (that create the honeydew surface) tend to colonize on older plants. For purposes of early detection of sooty mold infestation, this correlation with plant size should be further researched.

An important question that arises from this study is whether to remove these trees, despite their mediocre productivity. *P. guajava* is a host for more detrimental species, such as the guava leaf spot disease and the sooty mold, along (along with the black spot fungus and anthracnose). There is evidence that the leaf spot fungus may cause the leaves to be more susceptible to attack by non-native, herbivorous insect populations. By allowing harmful species such as these scarab beetles to thrive on the invasive guava plants may encourage this insect populations to increase, which could have side effects on other native species. Under these circumstances, uprooting of the diseased *P. guajava* plants should be considered instead of allowing natural decay.

Changes within a population of invasive species can expand the ecological niches for other harmful non-native species. This population of *P. guajava*, in an unhealthy state, became relatively defenseless against these epiphyllic secondary symptoms of disease, one being a non-native fungus. Under experimental conditions, the non-native beetle favored the damaged leaf material (caused by the fungus) over the undamaged leaf material. Even though there is a positive relationship found among these invasive and non-indigenous species in a controlled setting, the same pattern is not found in the field. The positive relationship between the non-native species is hidden by the presence of another species—the lichen. The lichen may be inadvertently blockading the “invasional

meltdown potential” of the *Adoretus* sp. to a certain degree, by discouraging the beetle from specific leaves on the trees. If there are enough other favorable leaves for the beetle to choose from, then the presence of the lichen may not be such a large deterrent. At any rate, recognizing the lichen as a possible intermediary shows the importance of taking all abiotic and biotic factors surrounding the issue of the invasive species into consideration as well.

The delicate ecological balance of species on oceanic islands are easily manipulated by non-native species and have a higher probability of attack by invasive species than mainland communities (Loope and Mueller-Dombois 1989). It is crucial that ecologists recognize changes in health status among invasive species within an ecological community in order to determine the influences of this change on other non-native species. Moreover, looking at patterns within the island community network as a whole may unveil a more complete picture of how other non-native species are proliferating from the changes caused by one invasive species.

Future studies could reveal whether the beetle species is already invasive to Moorea and causing problems for native species. Another inquiry to be investigated is if damaged leaf foliage has important nutritional value for this beetle species. This would help predict whether a large increase of parasitic epiphylls would influence beetle populations as well. The lichen's dual roles on the leaf surface (encouraging a parasitic fungal epiphyll while discouraging herbivory of the damaged leaves) is an interesting model that should be looked at in other island communities.

ACKNOWLEDGEMENTS

Firstly, my deepest gratitude goes to the gracious Marimari Kellum who allowed me to spend endless hours (day and night) in her exotic garden. Utilization of her ladder and other tools were very much appreciated. A special thanks to my nighttime bug catchers and vehicular chauffeurs, as well as my official ladder holder. To all my professors and GSIs, thank you for your helpful guidance and patience. A special thanks to the following professors for their assistance with statistical analysis: George Roderick, Jamie Bartolome, and Brent Mishler. Thank you to Brent Mishler, John Taylor, Dr. Mo-mei Chen, Matt Medeiros, and Brett Ratcliffe (at

University of Nebraska, Lincoln) for their respective epiphyte and arthropod expertise. I appreciated all of your time and effort. And thank you to the Moorea Class of 2005 for all the fun, best of luck to all of you with your future endeavors.

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THE DISTRIBUTION, ARTHROPOD COMMUNITIES, AND LARVICIDAL PROPERTIES OF THREE SPECIES OF MYRTACEAE ON THE ISLAND OF MOOREA, FRENCH POLYNESIA

DANIELLE F. FUCHS

*Department of Environmental Science, Policy, and Management
University of California, Berkeley*

Abstract. Plants produce secondary compounds that humans use in medicine, cosmetics, sanitation, fine chemicals, pharmaceuticals, perfumes, and nutraceuticals. For example, several species of the genus *Syzygium*, family *Myrtaceae*, have antibacterial, antifungal, and antioxidant properties that could be used to fight cancer and some neurological disorders. This study looks at three species of the family *Myrtaceae* (*Syzygium malaccense*, *S. cuminii*, and *Myrtaceae* 1) found on the island of Moorea, French Polynesia for distribution and larvicidal effects on *Aedes polynesiensis*. Six sites were established at different elevations throughout the island. *S. malaccense* was observed to grow at high elevations in less disturbed areas, and *S. cuminii* was observed to grow at low elevations in highly disturbed areas, whereas *Myrtaceae* 1 was observed to grow at all elevations and in mildly disturbed areas. Eight orders of arthropods were found between the six sites. Arthropod diversity increased as elevation increased, possibly due to a larger human impact at lower elevations. To determine each species' effect on mosquito larvae, extracts were made by blending leaves with distilled water and then straining. After 24 hours, *S. malaccense* had killed 95% of the mosquito larvae, whereas *S. cuminii* had killed 14% and *Myrtaceae* 1 had killed 18%. Future studies would be necessary to isolate and extract the active compound or compounds in *S. malaccense*.

Key words: *Syzygium malaccense*, *Syzygium cuminii*, *Aedes polynesiensis*, secondary plant compounds

INTRODUCTION

Plants have had billions of years to evolve into complex and highly specialized organisms that do much more than simply grow and reproduce. Beyond the primary metabolic processes that control respiration, cell growth, storage, and reproduction, most plants produce secondary compounds that shape how they interact with their environment. Secondary compounds are produced in low abundance, sometimes less than 1% of the total carbon mass of an individual, and are often stored in specialized cells or organs (Bourgaud et al. 2001). However small in abundance, these compounds are important for human as well as plant use.

Secondary plant compounds can interact with all aspects of a plant's ecosystem to help increase the fitness of the plant. Some plants produce anti-germinative compounds (allelopathy) to reduce competition for water and soil nutrients. Plants prevent leaf damage from sunlight by producing UV absorbing compounds. Other

secondary compounds act in numerous ways to deter herbivores or attract predators which prey on herbivores. Plants protect themselves from pathogens, or phytoalexins, such as bacteria, fungi, and viruses, by producing antibiotic, antifungal, and antiviral compounds. These properties are also important to humans, who have used plants and their secondary compounds in traditional medicine for thousands of years. In addition to pharmaceuticals, secondary plant compounds are used in cosmetics, fine chemicals, perfumes, and nutraceuticals (Bourgaud et al. 2001).

There are some problems associated with harvesting large quantities of secondary plant compounds, however. Many plant species are difficult to cultivate outside of their natural biome. The uptake of nutrients that are used in the production of secondary compounds is easily affected by light, heat, and other environmental factors (Moyes and Raybould 2001). Although some secondary compounds are more efficient in conjunction with other compounds in the plant, it is often more practical to artificially synthesize

a chemical for wide-spread use (Bourgaud et al. 2001).

One plant genus that has recently been getting attention for its beneficial properties is the genus *Syzygium*, of the family *Myrtaceae*. Besides being important food sources, several species of *Syzygium* are known to have medicinal properties. *S. aromaticum*, or clove, is found to have antibacterial and antioxidant properties and may help to fight diabetes (Abu-Shanab et al. 2004, Abdel-Wahhab and Aly 2005, Bafna and Balaraman 2005, Moreira et al. 2005, Prasad et al. 2005). *S. cordatum*, *S. samarangense*, and *S. cuminii* are also used to treat diabetes (Hanshella et al. 2005, McCormack 2005, Musabayane et al. 2005). *S. jambolana* is used in South Africa to treat infectious diseases and is found to have antimicrobial properties (Djipa et al. 2000). *S. cuminii* is shown to suppress aggressive behaviors in animals (Chakraborty et al. 1986). These two species are also known to have larvicidal properties against mosquitoes (Cavalcanti et al. 2004, Sharma et al. 2005).

This study looks at the genus *Syzygium* on the island of Moorea, French Polynesia. Although the genus *Syzygium* is one of the most speciosa in Polynesia (Whistler 1988), only three species are currently found on Moorea. These are *Syzygium malaccense* (L.) Merr. & Perry, *Syzygium cuminii* Skeels, and *Syzygium jambos* Alston (Murdock). A native of South-East Asia, *S. malaccense*, also known as the Malay apple or rose apple, was brought to the Society Islands by ancient Polynesians as a food source. *S. cuminii*, known on Moorea as pistash, is native to India, and *S. jambos* is a native of Indonesia (Murdock).

I wanted to study *Syzygium* in its natural habitat and see how each species' secondary compounds affects the community around it. Differences in secondary compounds evolve from selective pressures from herbivores and pathogens that differ between spatial and temporal ranges (Harvey et al. 2003). Herbivorous insects such as weevils and aphids that frequent a limited range of plants probably locate their hosts by recognizing specific plant-derived volatiles (Moyes and Raybould 2001, Tosh et al. 2003). I hypothesize that the different *Syzygium* species will be found in different habitats on the island and host different insect communities. I also want to test whether the species of *Syzygium* on Moorea have active secondary compounds that will kill mosquito larvae. I hypothesize that they will be

able to kill mosquito larvae, as studies have shown several *Syzygium* species do so. I chose to focus on mosquito larvae because mosquitoes are not only a nuisance but a threat known to carry Dengue fever. They breed easily in the fallen coconuts that liberally litter the island of Moorea (Neil Davies, pers. com.). Cleaning up fallen coconuts helps to control mosquito populations but cannot entirely eradicate them. Pesticides are harmful and can be expensive and uneconomical to apply in large quantities. I hope to find that the extracts of common, local plants can be used as safe, inexpensive larvicides against mosquitoes. *Syzygium* species have the potential to affect human communities as well as the plant and insect communities around them.

MATERIALS AND METHODS

Study sites

This study was conducted between October 7 and November 15, 2005. I chose six sites around the island of Moorea, French Polynesia, in which to survey *S. malaccense*, *S. cuminii*, and *S. jambos*. These species were initially identified in the field with the help of Professor Brent Mishler, who is the Director of the Jepson Herbarium and the University Herbarium at UC Berkeley. After the field survey was conducted, I referenced *Flora Societensis* by Stan Welch to confirm the identity of each species and found that *S. jambos* was misidentified (Welch 1998). However, the species I surveyed is still in the family *Myrtaceae* and is morphologically similar to *Syzygium*. Henceforth the third species will be referred to as *Myrtaceae* 1. Voucher specimens are available in the Jepson Herbarium at the University of California, Berkeley.

I chose the six sites based on preliminary observations of the island. A student research paper from 2000 also listed *Syzygium* forests (Bechtel 2000). If an area of the island had at least five individuals of *Syzygium* within a thirty meter radius than I chose that area as a survey site. Figure 1 is a map showing the locations of each site on Moorea. Site #1 was at the Belvedere (lat 17°33'40" long 149°49'35"), about 240 meters above sea level and four kilometers from the ocean. Site #2 was at Marae Titiroa (lat 17°33'20" long 149°49'45"), about 140 meters above sea level and approximately three and a half kilometers from the ocean. Site #3 was along the west side of the Vaiare-Paopao

route (lat 17°33'05" long 149°48'40"), which increased in elevation from about 250 to 350 meters and is less than two kilometers from the ocean. Site #4 was in an abandoned coconut plantation near Haapiti (lat 17°34'15" long 149°52'05") half a kilometer from the coast and approximately 25 meters above sea level. Site #5 was across the street from the Gump Station (lat 17°29'05" long 149°49'25"), about ten meters above sea level and less than half a kilometer from the ocean. Site #6 was a residential area on the North-East side of Opunohu Bay (lat 17°29'45" long 149°48'50"), five meters above sea level and less than half a kilometer from the ocean.

Field survey

At each site I used psuedorandomization to establish three separate plots. Once at a site, I walked along established trails for a little ways and stopped in an area where I could see at least one species of *Syzygium* present. Then I spun a compass to establish a random direction and looked at the second hand of a watch for a random number of paces. If the second hand was between one and nine I multiplied the number by ten and walked that many paces in the direction of the random compass bearing, but if the second hand was between ten and sixty I used that number without multiplying. I established a plot center with that random distance and direction. To establish the next two plot centers at the site, I used the same compass-and-watch technique, starting from the previously established plot center.

Once I had established a plot center, I took the aspect and slope of the plot. I estimated the percent canopy cover at plot center using a circular, mirrored densitometer. Then I found the North bearing on a compass and drew a field tape out five meters in that direction. Going clockwise, I tallied the number of individuals of *S. malaccense*, *S. cuminii*, and *Myrtacae* 1 in the circular plot of five meters radius. I also recorded the height of each individual using a two-meter height pole. I used a clinometer to get an angle measurement that would allow me to calculate the height for trees that were taller than 3.5 m.

Next I swept the entire plot for insects and arthropods for five minutes using an insect net. After five minutes I tallied the number of individuals of each Order that I collected with my net. Lastly, I secured two 7x12 cm double-sided sticky papers in the plot, preferably near



FIG. 1: Map of Moorea showing six study sites. Site 1 was at the Belvedere, site 2 was at Marae Titiroa, site 3 was on the Paopao side of the Vaiare-Paopao route, site 4 was at pk 21.3 near Haapiti, site 5 was the Gump Hill, and site 6 was a residential area at pk 8.3 on Cook's Bay.

Syzygium species. After at least two days, I revisited each plot. Again I swept for insects for five minutes, and I also recorded the number of arthropods of each Order that had landed on the sticky traps. I left the traps up and revisited the plots one last time after at least two more days. I tallied the total number of arthropods caught by the traps before removing them and also swept with an insect net for five minutes.

Bioassay

I performed a bioassay to test whether or not the extracts of *S. malaccense*, *S. cuminii*, or *Myrtacae* 1 induced mortality in mosquito larvae. I needed to breed mosquito larvae so I collected fallen coconuts that had holes in them, filled them with water, and placed them in a shady area where mosquitoes would be likely to breed. After several days I checked the cocoanuts for mosquito larvae. I identified the larvae as *Aedes polynesiensis* and put 20 larvae each into 24 clean containers. This gave me six replicates for each plant extract plus six replicates of a control.

In order to make the plant extracts, I collected young leaves from each of the three species and weighed out 10 grams of each. Then I blended each species separately with 180 mL of distilled water. Finally I strained each mixture to obtain the desired extracts. I filled six containers of mosquito larvae with 30 mL each of *S. malaccense* extract, six with *S. cuminii* extract, and six with *Myrtacae* 1 extract. The remaining six containers were each filled with 30 mL of distilled water for a control. I left the containers

uncovered at room temperature (22°C) under ambient lighting.

I recorded the number of mosquito larvae that had died in each container after every hour for the next twenty-four hours.

Data analysis

I performed several Spearman's Rank correlations to see if the average density of individuals of *Syzygium* in each site correlates with either the average height of individuals at the site, the number of arthropod Orders found at each site, the average percent canopy cover of each site, or the elevation of each site. I also used the Spearman's Rank test to see if the average canopy cover of each site correlates with the average height of individuals of *Syzygium* at the site or if the number of arthropod Orders found at each site correlates with the elevation of the sites. I performed a chi-square test for independence to see if the distribution of arthropods differs per site. Lastly I used the Friedman test to determine whether or not the *Syzygium* extracts actually killed more mosquito larvae than the control.

RESULTS

Field survey

Syzygium is found all over the island of Moorea, although the species are more prevalent in some areas than others. *S. malaccense* grows in the *Inocarpus* forest in less disturbed areas on the island, and also at higher elevations, from about 140 to 350 meters above sea level. *Myrtaceae* 1 has a wider range than *S. malaccense*, as it can grow at much lower elevations (5-10m), but it is still only found in less disturbed areas. *S. malaccense* and *Myrtaceae* 1 can grow on very steep slopes (up to 79%) and need very little direct sunlight to grow. The average canopy cover at the sites where *S. malaccense* grows is 88%. *S. cuminii* is found only at low elevations and can thrive in highly disturbed areas. All three species are reproducing successfully in their habitats, with a higher concentration of seedlings than mature adults at most sites. See Figure 2 for histograms of the heights of each species surveyed.

I found eight Orders of arthropods between the six sites (Araneae, Blattodea, Coleoptera, Diptera, Homoptera, Lepidoptera, Neuroptera, and Orthoptera). These include spiders, flies,

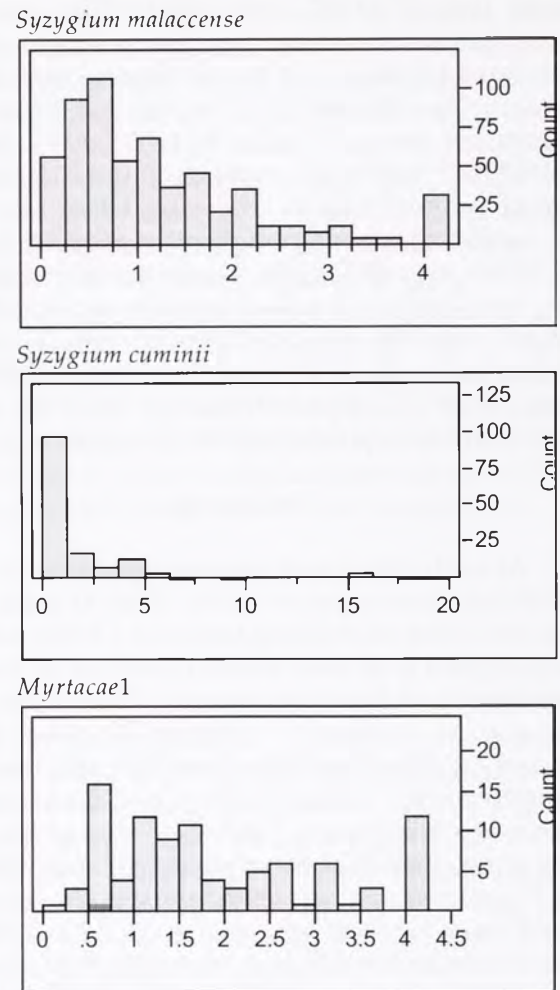


FIG. 2: Histograms of the heights (m) of *S. malaccense*, *S. cuminii*, and *Myrtaceae* 1.

lacewings, aphids, moths, crickets, mosquitoes, weevils, leaf hoppers, scale insects, and cockroaches. Figure 3 shows the distribution of arthropods at each site. This distribution was not the same between each site ($X^2 = 376.3$, $df = 25$).

The average density of individuals of *Syzygium* in each site correlates negatively with the height of individuals at each site and positively with the average canopy cover of each site ($r_s = -0.96$ and 0.9 , respectively; critical value = 0.886 at the 0.05 alpha level). Also, the number of arthropod orders found at a site correlates positively with the elevation of the site ($r_s = 0.91$). The average density of individuals in each site does not correlate significantly with either the number of arthropod orders found at each site or the elevation of each site ($r_s = 0.64$ and 0.76 , respectively). There is no significant relationship between average canopy cover and

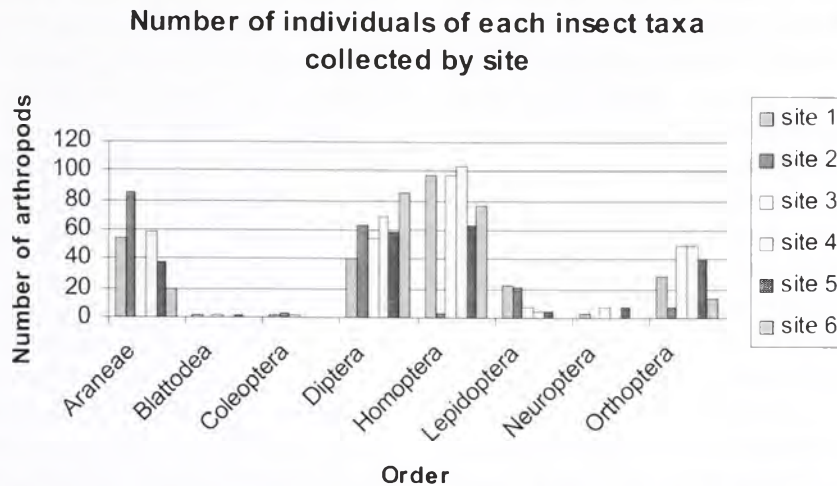


FIG. 3: Number of arthropods of each Order collected at each site.

average height of individuals in a site ($r_s = -0.083$).

Bioassay

In the bioassay, the *S. malaccense* extract killed significantly more mosquito larvae than the control after 24 hours ($X^2 = 10.85$; critical value = 7.81 at the 0.05 alpha level). The extracts of *S. cuminii* and *S. jambos* did not kill significantly more than the control. Figure 4 shows a graph of larvae mortality over time with each treatment. After six hours the number

of larvae that had died was still statistically similar for each extract, but after twelve hours *S. malaccense* had killed significantly more larvae. At 24 hours, *S. malaccense* extract had killed 95% of the mosquito larvae. The *Myrtaceae* 1 had killed 18% and *S. cuminii* had killed less than 14%.

DISCUSSION

Field survey

This survey found that *S. malaccense* and *S. cuminii* do indeed have different habitats, but

Average number of live mosquito larvae per treatment over time

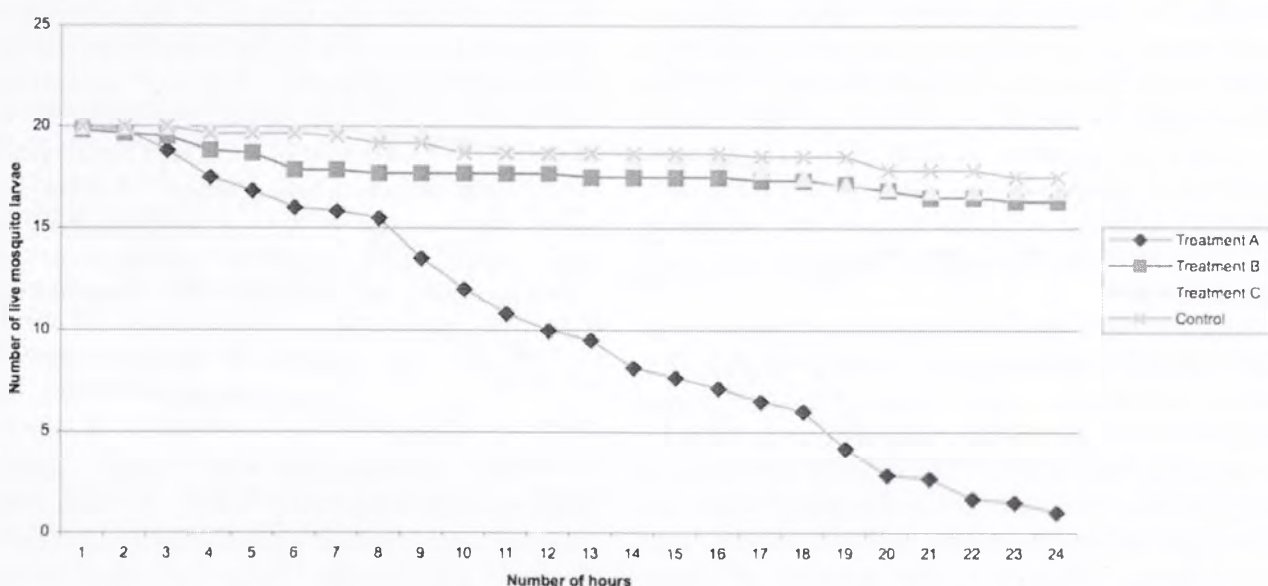


FIG. 4: Number of live mosquito larvae per treatment over time. Treatment A used *S. malaccense* extract, Treatment B used *Myrtaceae* 1 extract, and Treatment C used *S. cuminii* extract.

Myrtaceae 1 grows over a very broad range that overlaps both of these habitats. Perhaps *S. malaccense* and *S. cuminii* were introduced into different areas of the island when they first established a population, and certain factors have kept them from spreading to other areas of the island. *S. malaccense* was thought to have been planted at sacred sites by the ancient Polynesians (Brent Mishler, pers. com.). Since it has had the most time to adapt, perhaps *S. malaccense* has naturalized to the *Inocarpus* forest and cannot get the nutrients or moisture it needs at lower elevations. *S. cuminii* is the most recently introduced of the three species and is considered invasive on Moorea (Brent Mishler, pers. com.). Invasives must be hardy, and *S. cuminii* thrives at the highly disturbed lower elevations.

I was not surprised to find a low diversity of insects and arthropods on Moorea because of the idea of taxonomic disharmony. While few species of insects can even make it to an island, even less can survive to produce viable populations. Once an insect or arthropod does colonize an island, it often undergoes adaptive radiation to fill broader niches. Most sites had a high abundance of spiders, flies, crickets, and true bugs (Homoptera). Sites that did not have a high diversity of arthropod orders still had a high number of these four taxa. These sites mostly contained *S. cuminii*. I had thought the low diversity of arthropod orders associated with sites dominated by *S. cuminii* could be due to the fact that these sites are less dense than the other sites, but there is no correlation there. However, insect diversity does increase significantly with increasing elevation, and this could be explained by human activity. Human activity and its impacts are concentrated around the coast of Moorea at low elevations, and disturbance decreases as elevation increases. Perhaps farming monocultures and spraying pesticides are the cause of the depauperate insect populations.

Since *S. malaccense* and *Myrtaceae* 1 were both found liberally at the same sites, perhaps the insects associated with them have a more generalist strategy and do not associate with only one specific host plant. These sites attracted a more diverse amount of arthropods, such as lacewings, beetles, weevils, aphids, moths, and scale insects. However, the scarcity of these arthropods at lower elevation sites could again be explained by human disturbance. Since I did not find a specific insect associated with any of

the three study species, I am assuming that none of the plants produce specific secondary compounds to attract them. The *Syzygium* and *Myrtaceae* on Moorea did not evolve with the arthropod communities that they now associate with. In fact, most of the arthropods that I observed were introductions to Moorea, too. This is probably why most of the plant-insect interactions were general.

One disadvantage of this study is that I was not able to observe the *Syzygium* and *Myrtaceae* when they were flowering or fruiting. Perhaps the insect communities around them differ when they need to attract pollinators. These species may also produce different compounds while fruiting. Studies have shown that the fruit of *S. malaccense* is very fragrant and emits an odor similar to that of the rose (Pino et al. 2004). However, this still may not make a difference in Moorea if the local insect populations are not what *Syzygium* evolved with.

Bioassay

This study showed that one species of *Syzygium* on Moorea can intentionally help eradicate insects. Some compound or combination of compounds in the leaves of *S. malaccense* proved to be toxic to mosquito larvae. I am not sure why *S. malaccense* killed the larvae when *S. cuminii* and *Myrtaceae* 1 did not. *S. malaccense* more likely developed this toxicity during its evolution in South-East Asia than during its period of naturalization in Moorea. It would be interesting to test whether the fruit of *S. malaccense* can kill mosquito larvae at a higher rate than the leaves. Although eventually highly effective, *S. malaccense* may not be a practical larvicide because of the amount of time it takes to work and the high concentration needed. It is a highly abundant species, however, and probably would have much less negative consequences on the environment than pesticides if it were to be used in larger quantities.

CONCLUSION

The distribution of, and arthropod communities associated with, *S. malaccense*, *S. cuminii*, and *Myrtaceae* 1 seem to be more affected by human interference than by any secondary compounds that they may emit. It is difficult to observe the effects of secondary compounds when a species is not in its native habitat. Invasive

plants and insects often have to develop more generalist strategies in order to survive. However, plants that are beneficial to humans can increase their range if they become widely planted. We may see this in the future with *S. malaccense* if the residents of Moorea are informed of its toxic effects against mosquito larvae. Of course, more laboratory research could be done to isolate and extract the compounds in *S. malaccense* that kill mosquito larvae to make this pesticide even more practical for widespread use.

ACKNOWLEDGMENTS

I would like to thank my professors George Roderick, Brent Mishler, Jamie Bartolome, Rosemary Gillespie, Carole Hickman, and Roy Caldwell; and my GSIs Matt Medeiros, Michele Weber, and Scott Fay for their help and guidance. I would also like to thank Neil Davies for his help with identifying the mosquito larvae and Frank Murphy for managing the Gump Research Facility for our class use.

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SUBSTRATE PREFERENCE OF FUNGIA ON PROTECTED REEFS ON MOOREA, FRENCH POLYNESIA

COURTNEY E. HART

Department of Earth and Planetary Science, University of California, Berkeley, California 94720 US

Abstract: *Fungia* are individual free-living corals that tend to form local aggregations. Although they are typically noted for living on coral reef slopes near heavy wave action, they are also commonly found on fringing reefs in the lagoons of Moorea, French Polynesia and many other tropical islands in the Pacific. This study investigates the factors involved in determining the spatial distribution of *Fungia*. Initial observations suggest that *Fungia* tend to live on substrate that shelters them from the sun in the shallower and wave-protected areas parts of the lagoon. In this study substrate type, substrate condition and shelter type of the available substrate were compared to the substrate preferred by *Fungia*. The data revealed that *Fungia* and the three subgenera present in Moorea preferred to live on dead coral covered in algae that provided shelter from direct sunlight. This distribution was non-random – statistical analysis showed that the preferred location of *Fungia* was significantly different than the available distribution of substrate. The findings of this study provide a cornerstone for future research on the life history of *Fungia* and possible reproductive and maturation strategies.

Key words: *Fungia*, substrate, habitat preference, coral life cycles, *Scleractinia*, *Fungiidae*, reef growth

INTRODUCTION

Around the world coral reef ecosystems are threatened by overfishing, development, pollution, possible climate change, human encroachment and many other factors. At a time of worldwide decline of coral reef health, it is important to understand the basic biology and interactions of the individual coral species as well as the complexes that comprise an entire reef. A key aspect of life history in corals is habitat selection and preference. Habitat selection may involve imprinting a form of associative learning characterized by a rapid establishment of a perceptual preference for an object (Smith, 1990). It is not rigid; it is ever changing throughout time, space and species type. There is an urgent need for natural history in confronting the plight of marine ecosystems (Dayton, 2003). In order to better understand the processes and enhance the basic knowledge of *Fungia*, it is important to amass natural history information about coral reef habitats. This particular study examines the habitat preference of species within the four groups of *Fungia* present in protected lagoons of Moorea, French Polynesia from October thru November.

Species of the genus *Fungia*, (*Scleractinia*, *Fungiidae*) are the most abundant and widespread of all the mushroom corals (See FIG. 1). Of all the major genera, *Fungia* are the most restricted to tropical waters (Veron, 2000). *Fungia* are individual free-living corals and tend to form local aggregations. Morphologically, *Fungia* species have similar short tapering tentacles with batteries of stinging nematocysts that extend in the night and retract when disturbed (Veron, 1986).

Mobility is particularly highly developed in the family *Fungiidae*, and free-living adults are documented in 36 out of 41 species (Hocksema 1989, Hocksema and Dai 1991). Their life history strategies include both asexual and sexual reproduction. Asexual reproduction occurs from the acanthonauti stalk or from a damaged parent coral that can not repair its tissues by regeneration (Kramarsky-Winter, Loya, 1996). Sexually, different sexes release gametes into the water column which eventually fuse, develop into planula larvae, and settle on a hard surface after taking up zooxanthellae. This process can take last from two weeks to two years (Grant & Makcenzie, 2000). After settlement, the single stalk that attaches the larva to a substrate dissolves and the coral becomes mobile and begins to grow and mature.

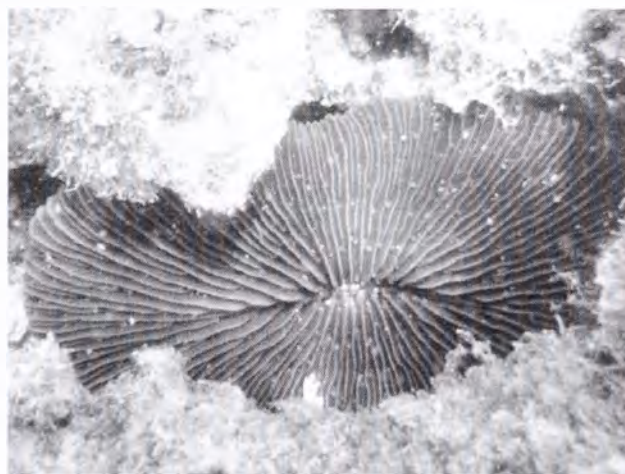


FIG. 1 Example of a *Fungia* sp. living in the shelter of dead sub-massive coral at Papetoai Beach.

All *Fungia* occur in a wide variety of habitats on Indo-Pacific reefs (Pichon 1974, Hoeksema and Moka 1989). They are found often on reef walls where wave action is present (Veron, 1986) and lagoons. Benefits of living near heavy wave action may be the rapid dispersal of larvae and immature polyps after they become detached from substrate. Because *Fungiids* are able to locomote, they have the ability to choose their substrate and to migrate from one substrate to another. They move over short distances by nocturnal expansion of tissues that push against adjacent surfaces (Chadwick, 1988). Members of the genus *Fungia* appear to interact with the environment and with other organisms (Chadwick, 1988); furthermore, their ability to move enables them to escape detrimental interactions on the reef. Since they cannot build their own colonial structures for protection, they must move in order to avoid over-competition for space and light. Advantages that arise from this characteristic are: (1) mobile corals extend reefs onto sand and serve as nuclei for the establishment of new reefs (Sheppard 1981); (2) corals may retreat from competitive contact with large colonial corals (Maragos 1974, Chadwick 1988), and (3) avoid bleaching by migrating downslope (Hoeksema 1991).

Fungia are seldom found on exposed reef fronts or in temperate, non-reef localities, both of which are common environmental extremes for other corals (Veron, 2000). In fact little is known concerning the dispersion patterns of *Fungia* on sheltered reefs (Chadwick-Furman & Loya, 1992). This study is based on the premise that details such as habitat preference are central to modern evolutionary and environmental biology (Greene, 2004). This particular project follows these ideals of natural history by exploring natural habitat preferences of *Fungia* on protected reefs on Moorea. It also identifies the sorts of habitats *Fungia* select, and this in turn helps to determine how the reef community in these areas is structured.

Mobility in corals may have many important implications for a coral reef composition and for corals in general. The lack of a strong fossil record (Veron, 2000) in all *Fungia* species may be suggesting a shift towards mobile corals in the future. The ability for an organism to adapt in a way that allows it to escape detrimental effects may prolong the existence of corals far into the future despite natural and human disturbances. It is possible that *Fungia* have no preference for living on certain substrates but initial observations suggest that they tend to live on substrate that shelters them from the sun in the shallower and wave-protected areas parts of the lagoon. This study tests whether a preference for sheltered habitat occurs randomly or is part of the life history choices of *Fungia*. Knowledge like this may be important in the future for amounting knowledge of *Fungia* with the hope of increasing possible restoration efforts.

MATERIALS & METHODS

All studies were conducted from the UC Berkeley Gump's Marine Station on the island of Moorea in French Polynesia in October and November of 2005. Not all species were present in study area (see Table 1) however for the purpose of this study species were not distinguished from each other because the point was to study the habitat preference of all *Fungia* present in the protected reef areas.



FIG. 2. Study sites on Moorea. (i) Papetoai Beach, (ii) Gump Station, (iii) Temae Beach.

Study Sites

Three locations with protected reef were chosen as study sites, Papetoai Public Beach in Opunohu Bay, Temae Public Beach and the reef in front of Gump Research Station in Cook's Bay (See FIG. 2). Each site was divided into three areas and transects were used to arbitrarily ascertain the available substrate. An observation snorkel was also conducted at each area for the purpose of counting *Fungia* and marking down their substrate. Site descriptions are as follows:

i. Papetoai Beach is a shallow, flat, sandy reef area situated just before the mouth of Opunohu Bay on the east side of the pass, 900 meters from the crest. Between the beach and the beginning of the fringing reef, a fine-grained sandy beach extends for about 5 meters into the water. Close to shore the coral appears to be living and healthy but in deeper waters, much of the coral is dead with conspicuous algal growth. The coral patches become increasingly sparse towards the channel and sand and coral rubble make up most of the substrate. A current is usually present running parallel to the beach in an east to west direction but wave action is minimal due to protection from the barrier reef. Depth of the area studied ranged from 0 meters to 5.9 meters.

ii. The Gump Station is located on the northwest side of Cook's bay near the pass in the reef. All of the shoreline north of the station until the green channel marker consists of a reef flat and wall of a fringing reef. The wave action is minimal; on windy days swells enter the bay but rarely break on the shore with any major force. The currents are also minimal. Distances from the shore to the wall of the reef average at about

100 meters. Depth of the reef ranged from 0 meters at shore to 5.4 meters.

iii. Temae Beach is located on the eastern corner of the island where the barrier reef comes closest to shore. Bordering the corner of the island the reef moves further and further offshore from the island in the direction of the Sofitel La Ora Resort. There is little fringing reef as the lagoon widens, only a massive sandy beach, a lagoon with coral and large sandy patches, and dense coral cover closer to the algal ridge. Wave disturbance is prominent near the crest of the ridge and effects can be seen in the massive coral rubble. But the lagoon is large enough that wave action dissipates near shore. Studies were conducted in an area where the distance from shore to the algal ridge ranged from 220 m – 450m even though the distance between algal ridge and shore continues to grow. Depths in the lagoon ranged from 1.2 meters to 2.4 meters.

Field Observations

Substrate was determined by sampling transects from the reefs and recording substrate type and availability every five meters. A 50 meter long tape was stretched out perpendicular from the shore. At Papetoai beach and the Gump Station, transects started at or near the wall of the fringing reef and extended all the way back to the beach. At Temae beach the transect tape started about 50 meters from the ridge where wave action was minimal and extended to shore or to a point in the sandy lagoon where corals were no longer present. The transects were a way to arbitrarily determine the types and percent cover of substrate available for *Fungia*.

Type of substrate was categorized as massive coral (CM), sub-massive coral (CS), branching coral (CB), coral rubble (CR), or sand (SD). The condition of the substrate was marked as: alive (A), dead (D) or dead with algae (DWA). I also noted whether or not the substrate was sheltered (SH) or not sheltered (NSH). A sheltered substrate was defined as anything that provided cover and protected from direct sunlight. Most dead corals were deformed enough to provide areas with filtered sunlight. Sand was considered sheltered if it was under or partially under a coral overhang. Six transects were sampled at each locations, two in each station (See Appendix 2 for GPS coordinates of locations).

Observational snorkels were also conducted in each station. The snorkels consisted of two researchers spending an hour at each station looking for *fungiids*. For each specimen found we recorded the number per area, what substrate it was on, what condition the substrate was in, and whether or not it was sheltered. Two research hours were devoted to each area in the three different study locations (9 sessions total). All information was recorded in the water on a dive slate and then transferred to paper and Microsoft Excel immediately following the field session.

Statistics

Differences in the distribution of naturally available substrate and the type of substrate that *Fungia* tended to prefer were tested for significance using the Chi-squared statistic. Comparisons were performed for each category of data (substrate type, substrate condition and shelter type) between each area in each location as well as between the totals numbers from each location.

RESULTS

Field Observations

Substrate Type

In all three locations, *Fungia* tended not to reside in or on the substrates that were most common. At the Gump station, 50% of the substrate available consisted of sand (SD) and only 4% consisted of sub-massive

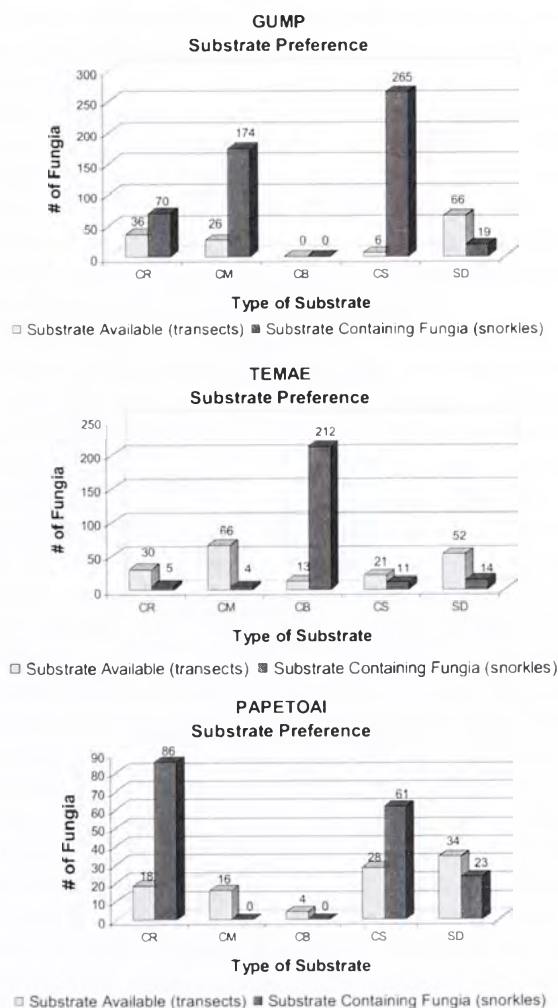


FIG. 3. These graphs show the difference between types of substrate available (based on arbitrary transects) and the types preferred by *fungia* species. Statistics showed that the probability of this pattern occurring naturally was as low as $2.7E-24$. The corals actively selected one type of substrate per location to the others. In all cases that type of substrate offered the most shelter.

coral (CS). However 50% of the *Fungia* counted at this location were found to be living on sub-massive coral while only 4% lived on the sand. The rest of 46% of substrate was occupied but the combination of coral types. At Papetoai Beach 50% of the *Fungia* lived on coral rubble (CR) which only represented 18% of the available substrate. And at Temae Beach branching coral (CB) only made up 7% of the available substrate yet 86% of all *Fungia* were found living on it. When comparing all three sites, there was not a preference for type of substrate, however it was obvious that the fungia were not living equally among all substrates. On average, 62% of all *Fungia* lived on substrate that only represented 10% of what was available (FIG. 3). A Chi-squared statistic performed on this data showed that distribution was non-random ($p < 0.0001$)

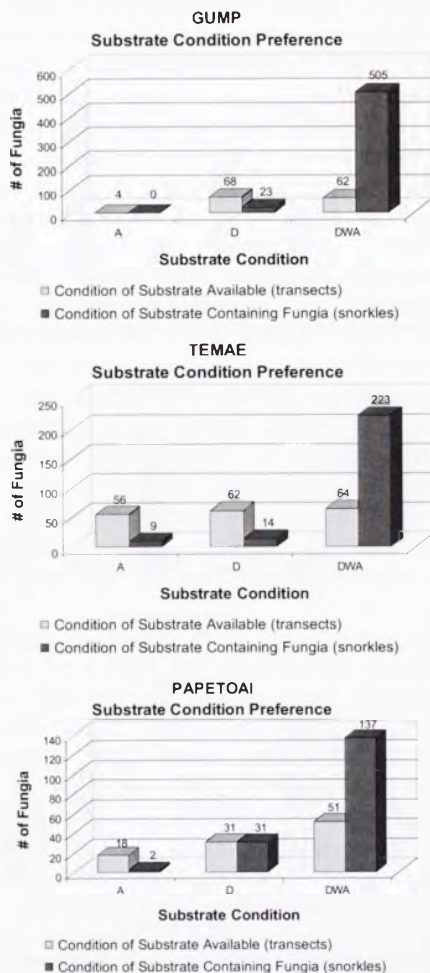


FIG. 4. Bar three graphs show the availability of substrate condition based on the arbitrary transects versus the preferred condition. In most cases the most abundant substrate condition was dead with algae (DWA) but often not by much. However, the *Fungia* preferred to live on coral that was dead with algae is it often provided the most sheltered habitat.

Substrate Condition

In all three areas, the majority of substrate was dead coral covered in living algae (DWA). It ranged from 35% to 51% of what was available. For the most part, the *Fungiids* preferred to live on dead coral covered in algae. At Temae and Papetoai beach, 31% and 18% respectively, of all substrate was categorized as alive (A). But *Fungia* was rarely found on living coral. Only 4% of the *Fungia* counted in Temae and 1% in Papetoai preferred to be on living substrate. At the Gump Station there was very little living coral available compared to the other types of substrate (FIG. 3). A Chi-squared statistic showed that the distribution of *Fungia* was non-random ($p < 0.00001$).

Shelter Type

Although *Fungia* showed a preference for certain substrate types (CM, CB, CS, CR, SD) in certain areas, there was not one type of substrate that they seemed to prefer across the board at all three sites (See

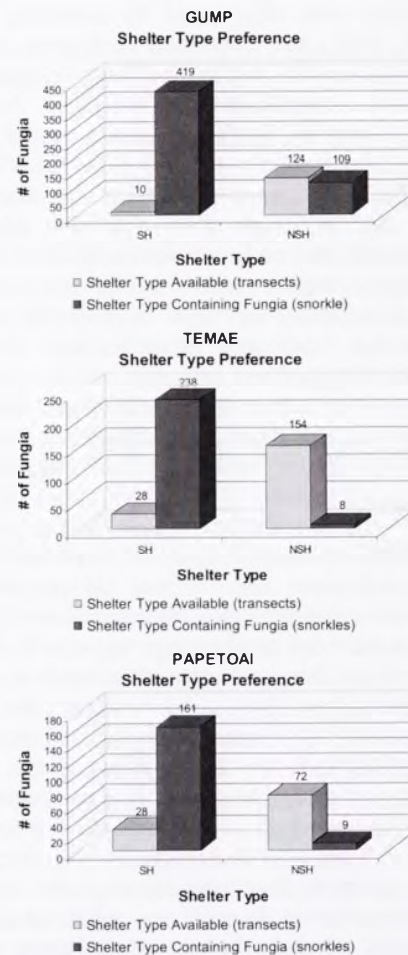


FIG. 5. These three graphs show ratio of available shelter types based on the arbitrary transects and the number of *Fungia* that actually preferred sheltered substrate. The majority of substrate available was not sheltered yet the majority of fungia counted were found living on or in sheltered areas.

FIG. 5). However there was a connection between substrate preferences noticeable at Temae Beach, Papetoai Beach and Gump Station. All *fungiids* preferred sheltered substrates (living on or in) at all protected lagoon sites. At Temae, Papetoai and Gump the percentage of available sheltered habitat was 15%, 28% and 7%. However at all three locations at least 79% of all *fungiids* studied lived in or on a sheltered substrate. From site to site that type of substrate – CR, CB, CM, etc. – differed. At Temae Beach, branching coral offered the most sheltered substrate but there was very little branching coral at the other two locations. Sometimes a *Fungia* would be living on sand but was still sheltered by a coral overhang. The healthiest coral was usually living in, on, or near a sheltered substrate. A Chi-squared statistic for shelter type also showed that distribution of *Fungia* compared to distribution of substrate conditions was not random ($p < 0.00001$)

Statistics

Chi-squared tests showed that the substrate preferred by all *Fungia* was significantly different than what was available in the protected lagoon areas of Moorea. For all substrate types in the three locations p-values ranged from 1.40902E-05 to 2.66902E-24. Chi-tests for substrate condition revealed p-values ranging from 9.24824E-08 to 1.02926E-41 among the three locations. And for shelter type in the locations, p-values ranged from 7.34768E-31 to 5.51796E-66 ($p < .05$ is significant). The data showed that the probability for mushroom coral to be dispersed equally among the substrates available is extremely low. *Fungia* prefer substrate to be dead, covered in algae, and offering shelter.

DISCUSSION

Although there is documented data describing the habitat of most *Fungia*, specific substrate preferences of these corals has not been well documented within protected lagoon habitats until now. Migration from reef to sand and from shallower to deeper habitats, in both sheltered and exposed environments, it has important implications for the initiation and extension of coral reefs (Chadwick-Furman & Loya, 1992). Therefore, understanding the natural history of their life patterns – where they start their lives, how they migrate, and what areas they prefer – will be important to fully comprehending *Fungiids* role in coral reef health and growth.

This study found that the *Fungia* present in Moorea prefer to live in or on sheltered substrate within protected reef areas (protected with reference to heavy wave action). In many cases huge congregations of *Fungia* species were found living together. The healthiest specimens, ones containing the most color and least bleaching, were observed in these huge

congregations and they always lived on sheltered substrate.

It has been thought in the past that some mushroom coral lived on reef walls near heavy wave action. A few observational snorkels to the outer reef of Cooks Bay confirmed this fact. Many *Fungiids* were living about 10 meters deep amongst swaths of healthy coral reef. Scuba divers who went as deep as 40 meters on Moorea reported seeing hundreds of mushroom corals during their dives. However the deepest depth of the lagoons and fringing reefs studied was about 5 meters. The study was limited to this depth because it relied solely on snorkeling as a research technique. At shallower depths such as these, the sun light is much more intense often affecting the temperature of the water.

In an environment such as the ones studied, mushroom coral must be able to avoid the intense sunlight at such shallow depths. Thermal stress during exposure to high irradiances of solar radiation, or irradiances higher than the current photoacclimatization state, causes damage to both photochemistry and carbon fixation at the same time in zooxanthellae, while DNA damage, apoptosis, or necrosis are occurring in the host tissues of symbiotic cnidarians (Lesser & Ferrell 2004). In order to avoid stresses they must find shelter when in the protected lagoon areas. This study shows that mobility of the coral species allowed them to seek out sheltered habitat to protect them from the elements in the shallow waters.

Other sessile coral are unable to accommodate any changes in water temperature or light intensity because they lack the ability to move away from it. Although there was a proportion of *Fungiids* that were found on the sand or not on protected substrate, they were often bleached and small. No numbers were taken for this observation; however, an elder in the community who lived his life fishing on Moorea, conveyed that the coral presently studied is never found on the sand, it is always found on dead coral rubble (Papa Mape translated by Hinano Murphy, *personal communication*). Although the study showed that some coral are found on sand, there numbers are significantly lower than those who sought out sheltered substrate.

There are many factors that might explain the non-random distribution of *Fungia*. Not only does a sheltered substrate help to protect mushroom coral from direct sunlight that could increase the rate of bleaching and overall coral death, but it also created a haven for juvenile *Fungia*. During the study, many of the most sheltered areas such as dead coral heads at the wall of the fringing reef or masses of branching coral in the lagoon were home to many *Fungia* still attached to the substrate. This observation implicates that younger *Fungia* in the protected lagoon need a more sheltered area than on the walls of reefs in order to start their lives. These may be important findings in terms of understanding the life cycles of mushroom coral and their contribution to reef growth.

CONCLUSION

Like all corals, *Fungia* have a very interesting life history. In order to understand how their life cycles function and how they factor into the health and reproduction of coral reefs, researchers must provide ample data on every aspect of the organism. Identifying the trends in habitat preference of *Fungia* in protected lagoons will play a role in helping to understand the early life stages of mushroom corals. The next step may be to compare a variety of different locations on the island to compare life history strategies based on environmental constraints. Because their ability to locomote may provide hard substrate for new coral recruitment, the complete life history of *Fungia* will prove to be helpful in solving future coral reef issues.

ACKNOWLEDGEMENTS

I would like to thank all of the Professors and GSI's that coordinated the 2005 Moorea program. Thanks to Scott Fay, Michele Weber, George Roderick and Jaime Bartolome for helping me to understand statistical inferences. A big thank you to all my fellow students who assisted me in my ocean excursions. Special thanks to all the employees at the Gump Station: Irma and Jocques Yu-Sing, Frank and Hinano Murphy and family, Valentine and Maneo, and Tony for acquainting us American students to life on Moorea; an experience we will not soon forget.

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APPENDICES

APPENDIX 1: Classification of *Fungia* worldwide. The genus *Fungia* is divided into four groups based on morphological features and location. This table shows the species that are known to be present in Moorea according to Veron (2000). All those present in Moorea were studied in this study.

Group	Species	On Moorea?
Group 1: Species with large septal teeth	<i>danai</i>	Yes – common
	<i>corona</i>	No
	<i>scruposa</i>	Yes – uncommon
	<i>horrida</i>	Yes – common
	<i>valida</i>	No
	<i>klungzingeri</i>	No
Group 2: Species with saw-like teeth and usually with a central rib	<i>fungites</i>	Yes – common
Group 3: Species with middle sized rounded teeth	<i>concinna</i>	Yes – abundant
	<i>fralinae</i>	No
	<i>rapanda</i>	Yes – common
	<i>scabra</i>	No
	<i>spinifer</i>	No
	<i>granulosa</i>	Yes – uncommon
	<i>puishani</i>	No
Group 4: Species which have distinctly non-circular polyps	<i>scutaria</i>	Yes – common
	<i>taiwanensis</i>	No
	<i>seychellensis</i>	No

APPENDIX 2. GPS locations for transect sites.

LOCATIONS	STATION	GPS	ACCURACY
Papetoai Beach	1A	0197308, 8063868	13 m
	1B	0197391, 8063961	13 m
	2A	0197348, 8063910	11 m
	2B	0197369, 8063925	9 m
	3A	0197411, 8063967	18 m
	3B	0197311, 8063873	19 m
Gump Station	1A	0199851, 8064024	10 m
	1B	0199850, 8064007	8 m
	2A	0199823, 8064141	14 m
	2B	0199833, 8064080	10 m
	3A	0199851, 8064212	10 m
	3B	0199858, 8064245	10 m
Tema Beach	1A	0207272, 8063295	10 m
	1B	0207320, 8063281	8 m
	2A	0207123, 8063308	9 m
	2B	0207179, 8063307	10 m
	3A	0207071, 8063279	10 m
	3B	0207033, 8063265	7 m

The first thing I noticed when I stepped out of the plane was the cold air. It was a sharp contrast to the warm, humid air of the tropics. I had heard that the weather in the north was harsh, but I didn't realize how cold it would be. The wind was biting, and the sun was a pale, distant orb in the sky. I wrapped my coat around myself and tried to ignore the shivers running down my spine.

As I walked through the airport, I noticed the different people. There were no more people in traditional dress, but instead, I saw people in coats and hats. They looked at me with curiosity, and I felt a bit out of place. I had never been to a place like this before. The architecture was different, too. The buildings were tall and made of stone, with many windows. I had never seen anything like that before.

I found a small, quiet room where I could sit and rest. The room was simple, with a wooden table and a few chairs. I sat down and looked out the window. The view was beautiful. There were mountains in the distance, and a river flowed through the valley. I had never seen anything like that before. I felt a sense of peace and tranquility.

I had heard that the people in the north were different, but I didn't realize how different they would be. They were more reserved and formal than the people in the tropics. I had to learn to be quiet and polite. I had to learn to respect their customs and traditions. It was a challenge, but I was determined to succeed.

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PHYLOGENETIC DISTRIBUTION OF *HALIMEDA* (BROPSIDALES) ON MOOREA

Stephen M. Hatosy

*Environmental Science, Policy, and Management, University of California,
Berkeley, California 94720 USA*

Abstract. Coral reefs are species rich ecosystems and although much work has been dedicated to their conservation, they remain threatened by overexploitation. To examine evolutionary trends in reef distribution 20 individuals representing six *Halimeda* species (*H. distorta* (Yamada) Colinvaux, *H. minima* (Taylor) Colinvaux, *H. opuntia* (Linnaeus) Lamouroux, *H. borneensis* Taylor, *H. discoidea* Decaisne, and *H. taenicola* Taylor) were collected from algal ridge, back reef, lagoon, fringing reef, bay and motu environments on the island of Moorea, French Polynesia. The variations in morphology were studied, morphological characters were developed and a phylogenetic analysis was inferred from the morphological characters using PAUP and McClade. A tracing of reef distribution on the most parsimonious tree inferred from fourteen morphological characters showed little phylogenetic conservatism with reef distribution.

Key words: *phylogenetic conservatism; phylogenetic repulsion; reef conservation; reef zonation*

INTRODUCTION

Covering an area of approximately 600,000 km² (Smith 1978), coral reefs have been the focus of many studies dealing with species assemblages and ecosystem function (Peres and Picard 1969, Pratchett et al. 2001, Aronson et al. 2005, Bozec et al. 2005, Connolly et al. 2005). This research has made it clear that coral reef communities are important because they have rich biodiversity (Ray 1988), protect coastlines from severe weather (Sheppard et al. 2005), are responsible for 9%-12% of the world's fisheries' productivity (Smith 1978, Birkeland 1997), and provide approximately \$375 billion per year in ecosystem services (Costanza et al. 1997, West and Salm 2003). This knowledge has thus generated much needed interest in coral reef conservation and management (Ray 1988, Begger et al. 2003, West and Salm 2003, Aronson et al. 2005), as well as public education of coral reef biology (Armstrong 2005). The best way to focus conservation efforts is to examine the ecological and evolutionary patterns of species distribution within coral reef ecosystems. In this way, a better understanding of species distribution can aid in better understanding the complex community associations that occur on coral reefs and highlight

areas that require specific and immediate attention.

The green calcareous alga, *Halimeda*, can serve as an excellent model organism to investigate such patterns because (1) *Halimeda* has a worldwide tropical distribution (Hillis-Colinvaux 1980, Hillis 1999, Bandeira-Pedrosa et al. 2004, Kooistra and Verbruggen 2005) so it can serve as a useful model in any tropical ocean, (2) it occurs on all reef zones providing representation of all reef habitats, and (3) it has a rich fossil history. It first appeared in the fossil record 260 million years ago during the Permian, achieved circumtropical distribution during the Cretaceous, survived the mass extinction at the Cretaceous-Tertiary boundary, and underwent an evolutionary radiation resulting in the 33 extant species distributed world wide (Hillis et al. 1998, Hillis 2001). Three extant lineages of *Halimeda* are also represented in the fossil record dating back to the late Triassic and Cretaceous Periods (Dragastan and Soliman 2002). This fossil history can lend itself to comparative analyses between ancient and current reef distributions of *Halimeda*.

To investigate the use of *Halimeda* as a model of species distribution, this study will examine the six species that occur on Moorea, French

Polynesia. By constructing and using a phylogeny of the *Halimeda* present on Moorea, the distribution of *Halimeda* on six reef habitats (algal ridge, back reef, lagoon, fringing reef, bay and motu) will be analyzed. There are three possible outcomes (B. Mishler pers. comm.):

(1) The distribution of *Halimeda* among the six habitat types is random (the null hypothesis).

(2) Related species are distributed ecologically closer together than would be expected for a random distribution (Hypothesis 2). This hypothesis follows the philosophy of phylogenetic conservatism.

(3) The distribution among the six habitat types is overly dispersed (Hypothesis 3). This follows the philosophy of phylogenetic repulsion and in the case of ecological distribution it is synonymous with competitive exclusion (the terms phylogenetic repulsion and overly dispersed are used interchangeably in this paper).

METHODS

Sampling

Sampling was done between September and November 2005 on the island of Moorea, French Polynesia. By free diving and reef walking, *Halimeda* species were collected at six sites along the north coast between Motu Tiahura and the Temae public beach (Fig. 1) at the algal ridge, back reef, lagoon, fringing reef, bay, and at a motu.

Motu Tiahura was predominantly a shallow (<1m) sand flat (composed of biogenous sediment) that gradually sloped down into a lagoon. Collection was done south of the conglomerate coral platform. Collections were made to a depth of one meter.

Papetoai public beach and the derelict pier were fringing reef environments. The areas within three meters of shore were mostly composed of loose biogenous substrate and large coral rock formations. Past three meters, the substrate was mostly coral rock and coral rubble. The limit of the collection zone was a large channel frequented by boat traffic. Collections were made down to a depth of about seven meters.

The area off the Gump Station was a fringing reef within a bay. The substrate was a mix of sediment (mostly terrigenous) and coral rock formations. The depth of the area collected was usually two to three meters; however, collections

Fig. 1. Location of collection sites are marked with black dots.



were made to a depth of about seven meters were the bottom sloped down the bay floor.

The Maharepa fringing reef was composed of large coral rock formations near shore out to a point where depth was about four meters and the bottom was composed mostly of biogenous sediment and coral rubble with sparse large coral rock. The latter environment was considered the lagoon which was about five meters deep. The back reef had much the same composition as the fringing reef except that the back reef also had considerable water movement. The algal ridge was identified as the point where water depth was near 0.5 meters and substrate was mostly conglomerate coral rock and dominated by algae. Collections were made to a depth of five meters.

Temae public beach started with a large lagoon composed of biogenous sediment and sparse coral rock. Depth of the lagoon was about four meters. The back reef was composed of large coral rock and coral rubble and was two to three meters deep. The algal ridge was composed of conglomerate coral rock and dominated by algae. The depth was less than 0.5 meters. There was very noticeable water movement throughout the lagoon, back reef, and algal ridge. Collections were made to a depth of four meters.

The samples collected in clear zip-lock baggies, transported back to the Gump Station and maintained in the seawater system at the station for character development.

Character development

Characters were developed by reviewing past taxonomic work (Hillis et al. 1998, Hillis 1999,

Bandeira-Pedrosa et al. 2004, Kooistra and Verbruggen 2005) and a field guide (Payri et al. 2000), and by studying and comparing the samples collected. Species were identified by using Payri et al. (2000) and through correspondence with Antoine d.R. N'Yeurt at the University of French Polynesia. This information was also used to create a photographic field guide (Appendix A) and species key (Appendix B) of *Halimeda* present on Moorea.

Characters 1 through 12 were adapted from Hillis (1998). Character 13 was developed by examining a change in morphology between segments proximal to the central thallus and segments distal to the central thallus. Character 14 was developed by examining the extent of calcification of individuals. Calcification was characterized qualitatively by looking at the rigidity of the thallus. Character 15 was developed by taking digital photographs (using a Canon PowerShot S70) of surface utricles under 100X magnification on a Nikon compound microscope. Utricle diameter was determined by converting digital photographs to PDF files then using the measurement tool on Adobe Acrobat. Ten utricles from each individual were measured in pixels (pt), and an average of those ten measurements was used.

Measurements of the lengths and widths of segments and lengths of holdfasts and thalli were made to the nearest millimeter. For characters resulting from segment measurements, ten segments were measured from each individual and averages were taken.

Variations of the ratio segment-length-to-segment-width and utricle diameters between individuals were analyzed using the Kruskal-Wallis Test. Individual samples were treated as "sample groups" and the ten measurements taken from each sample were considered "treatments."

Characters were then evaluated using three criteria. (1) Characters needed to be independent from each other. (2) Characters needed to be inheritable. (3) Characters needed to have discrete states. Character 16 (ecological distribution) was the trait being investigated and was excluded from the data matrix before the phylogeny was inferred.

Character states were assigned to each sample of algae (see RESULTS for listed characters and character states) and this data was entered into a

character matrix (Appendix C) using McClade 4.0 (Maddison and Maddison 2000). The tree was rooted to *Halimeda discoidea* based on previous molecular work done where *H. discoidea* was more closely related to the outgroup compared to the other *Halimeda* species (Hillis et al. 1998).

The character matrix used for a parsimony analysis which was performed using PAUP* 4.0 for Macintosh (Swafford 2001) using the heuristic search method searching for the most parsimonious tree. Maximum parsimony trees generated from the heuristic search were used to construct a strict consensus tree by using the "calculate consensus" option in PAUP*. The data analysis from PAUP* was then transferred to McClade 4.0 (Maddison and Maddison 2000), and Character 16 (ecological distribution among the reef zones) was traced onto the maximum parsimony tree to investigate patterns of reef distribution.

Voucher specimens

Voucher specimens were prepared by washing the algae in fresh water, then drying between card stock and wax paper. Vouchers will be deposited at the Jepson Herbarium at the University of California-Berkeley.

RESULTS

Characters

1. Thallus mostly:

Erect (0); sprawling or pendant (1)

Erect thalli stood tree-like from their substrate. Sprawling or pendant thalli formed mats or draped over the substratum.

2. Axis of growth:

Mostly in one axis (0); both horizontal and vertical axes present (1)

For most *Halimeda* species, the growth axis of the thallus tends to occur either horizontally as with *H. distorta* or vertically as with *H. borneensis*. However, some species, particularly in Section *Opuntia*, the axis of growth occurs in both the vertical and horizontal directions as with *H. minima*.

3. Basal segments:

No special structure (0); Fan-like structure (1); Pillar-like structure (2)

Basal segments were the source of peripheral branching segments. Fan-like structure was a basal stalk segment joined to two or three segments that formed a half-circle. Pillar-like structure consisted of cylindrical basal segments from which branching segments arose. Basal segments with no special structure looked like the rest of the segments on the thallus.

4. Rhizoids:

Usually amassed in a single region (0); not restricted to a single region (1)

Amassed in a single region required no other rhizoids had emerged from peripheral segments. Very distinct is the large bulbous holdfast of *Halimeda borneensis*, but also seen in *H. discoidea*, and *H. taenicola*. Not restricted to a single region required rhizoidal tufts emerging from multiple segments.

5. Holdfast length/mature thallus height:

20% or greater (0); less than 20% (1)

For a score of 0, the length of the holdfast had to be more than 20% of the mature thallus height.

6. Holdfast with:

Adhering sediment particles (0); no attached sediment (1)

Sediment particles that had to be manually removed from rhizoids were considered adhering.

7. Branching pattern:

Essentially in one plane (0); frequently in different planes from proximal segments (1)

Thalli that branched in different planes had a "bushy" appearance. Thalli branching in one plane had a palmate appearance.

8. Segment shape:

Rarely contorted (0); commonly contorted (1)

When looking at a segment's upper margin, commonly contorted segments appeared wavy in their shape. Rarely contorted segments were flat.

9. Segment shape:

Lacking pronounced stalk (0); with basal margin often prolonged into short stalk (1)

The short stalk protrudes out two or more millimeters from the center of the segment.

10. Segment shape:

Upper margin with groove from which new segments emerge (0); one central and commonly two or more lateral pits from where new segments arise (1)

Small depressions can be seen along the upper margin of the segments. A groove has to run along the entire upper margin to be scored 0. Any separations in the depression was scored 1 (as lateral pits)

11. Segment length/segment width in upper half of thallus:

0.6-0.9 (0); 1.0 (1); < 0.6 (2)

Ten segment lengths and ten segment widths were measured and divided. The quotients were then averaged for character scoring. Variations between species is greater than variations within species (Kruskal-Wallis; $X^2=30.8$; d.f.=6; $p<0.0001$).

12. Breadth of largest segments:

< 5 mm (0); 6 mm-9 mm (1); >10 mm (2)

The Kruskal-Wallis Test shows a significant variation between species ($X^2=44.7$; d.f.=6; $p<0.0001$).

13. Change in segment morphology:

No change (0); segment morphology changes between proximal and distal segments (1)

Excluded: Studies (Hillis et al. 1998, Kooistra and Verbruggen 2005, Verbruggen et al. 2005) have discussed the morphological plasticity of *Halimeda* species. Due to this plasticity, the change in morphology does not occur due to genetic variation.

14. Calcification:

Slight (0); moderate (1); heavily (2)

Slight calcification requires that all parts of the thallus are very flexible (fleshy-like) and do not tend to break when bent. Moderate calcification results with a thallus that is very sturdy yet allows some motion of the thallus. Heavy calcification has the thallus very compact and immobile to motion.

15. Average peripheral utricle diameter (Fig. 2):

<9 pt (0); 9 pt- 14 pt (1); >14 pt (2)

The Kruskal-Wallis Test shows a significant variation of utricle diameter between individuals ($X^2=143.6$; d.f.=15; $p<0.0001$). Fig. 2 shows the grouping of the character states.

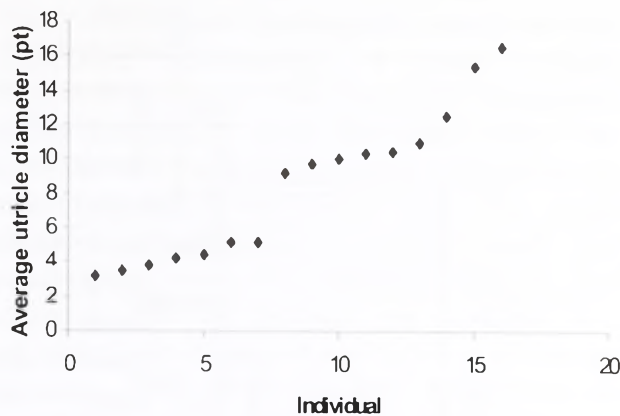


Fig. 2. Discreteness of utricle diameter character states based on mean utricle diameter of ten utricle diameters taken from 16 individual *Halimeda* samples. Discrete breaks can be seen at utricle diameters of 9 pt and 14 pt.

16. Ecological distribution:

Algal ridge (0); back reef (1); lagoon (3); fringe reef (4); motu (5)

Algal ridge was considered the area on the barrier reef that lies between 0m and 0.5m. The back reef was the area of barrier reef that extended out to a depth of four meters. The lagoon was any area of reef that was deeper than four meters. The fringing reef was the area of reef adjacent to shore that was shallower than four meters. The bay was the area of fringing reef within Cook's Bay. The motu was the fringing reef on the east side of Motu Tiahura.

Excluded: This character was excluded in the phylogeny because the phylogeny was used to investigate any historic distributional patterns.

Phylogeny

Phylogenetic analysis of fourteen morphological characters resulted in 52 equally parsimonious trees with a tree length of 36 steps and a Consistency Index (C.I.) of 0.50. The strict consensus tree (Fig. 3) and one of the maximum parsimony trees (MPT) (Fig. 4) both resolve two clades: the Borneensis clade containing *Halimeda borneensis* and the Minima-Opuntia-Distorta (MOD) clade, which includes *H. minima*, *H. opuntia*, and *H. distorta*. The strict consensus tree leaves both clades unresolved while the MPT shows good resolution in the MOD clade but still leaves the Borneensis clade unresolved. The

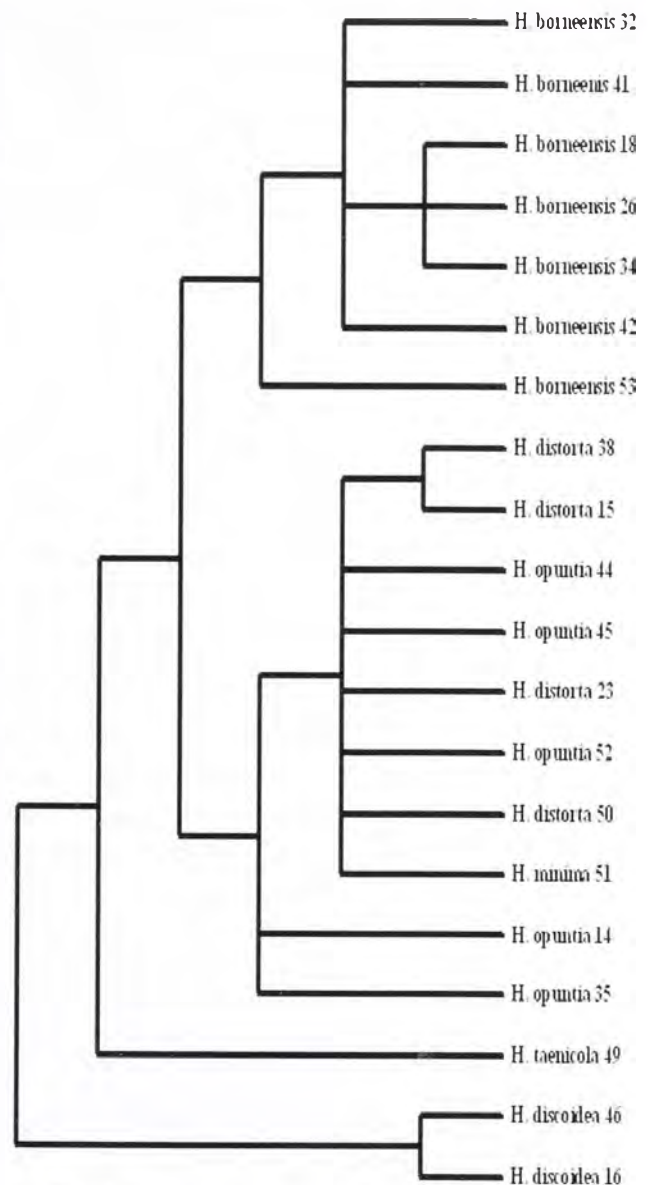


Fig. 3. Strict consensus tree inferred from 52 equally parsimonious trees with 36 steps and a Consistency Index (C.I.) of 0.05. The tree was rooted to *Halimeda discoidea* based on Hillis (1998). Two clades, one containing *H. borneensis*, the other containing *H. minima*, *H. opuntia*, and *H. distorta* have been resolved. Numbers after species name represent sample identification numbers.

Borneensis clade retains the same resolution in both the strict consensus tree and MPT.

Ecological distribution traced on the MPT (Fig. 5) shows clades are not restricted to any specific reef zone. Three species (*H. borneensis*, *H. opuntia*, and *H. distorta*) were distributed over four of the six zones.

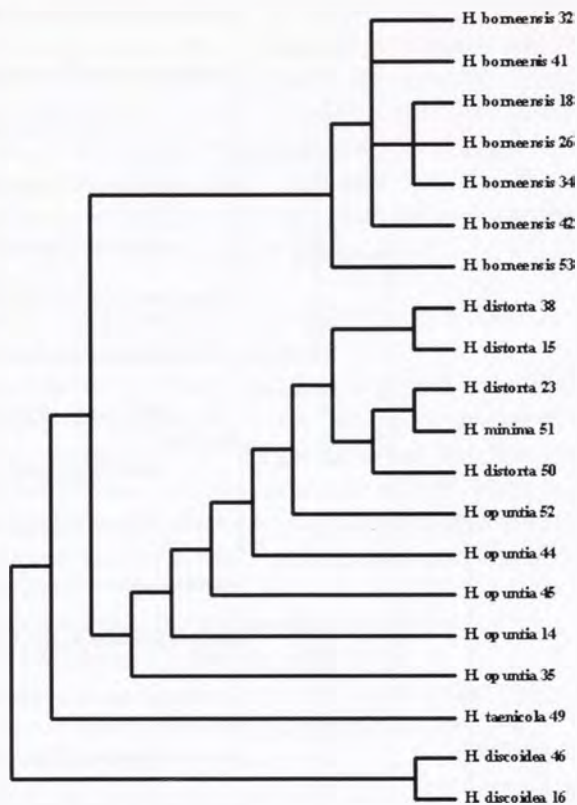


Fig. 4. Maximum parsimony tree inferred from fourteen morphological characters. One of 52 equally parsimonious trees with 36 steps and a Consistency index (C.I.) of 0.5. The clade containing *H. minima*, *H. opuntia*, and *H. distorta* is fully resolved. The clade containing *H. borneensis* retains the same resolution from the strict consensus tree. Numbers after species name represent sample identification numbers.

DISCUSSION

The two clades resolved in this phylogeny (the Borneensis clade and MOD clade) are supported by molecular work (Kooistra et al. 2002). Within the MOD clade *Halimeda minima* is located within a monophyletic group of *H. distorta*. This is not supported by molecular work (Kooistra et al. 2002, Kooistra and Verbruggen 2005). This could be due to misidentification; however, the morphology of *H. minima* was consistent with that described in Payri et al. (2000). Kooistra et al. (2002) have also described another clade that includes *H. taenicola* and *H. discoidea*; however, that relationship was not resolved here.

The tracing of character 16 (ecological distribution) (Fig. 5) does not appear to support Hypothesis 2. Based on Hypothesis 2, one would

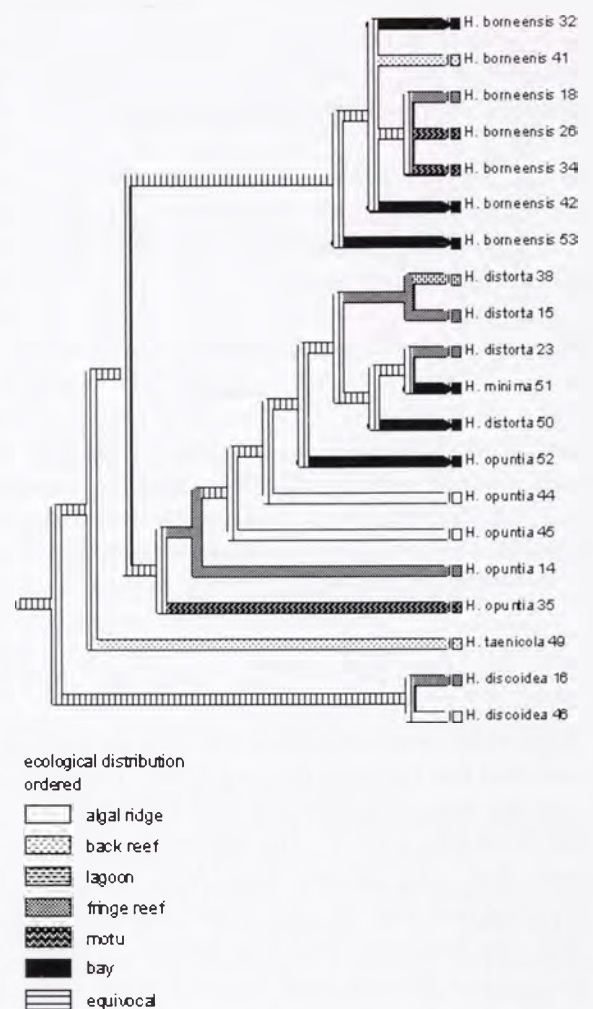


Fig. 5. Character 16 has been traced on the maximum parsimony tree from Fig. 4. There are no reef zone distributions that are monophyletic. Numbers after the species name represent sample identification number.

expect nearest neighbors to exist on the same reef zones, but as can be seen in Figure 4 no such situation exists. In this phylogeny, nearest neighbors often occupy different reef zones. This could lend support to the idea of competitive exclusion in Hypothesis 3. However, Hypothesis 2 or Hypothesis 3 cannot be considered unsupported or supported due to lack of a complete null hypothesis. In order to generate a null hypothesis, simulations need to be run by randomly assigning ecological character states to the MPT so an expected random distribution is available as a null hypothesis (B. Mishler pers. comm.). The null hypothesis that is generated can then be tested against the actual distributions found.

Besides distribution among reef zones, another avenue of investigation could be the phylogenetic distribution of more refined ecological characters. The ecological characters used in this study were described under the assumption that each reef zone is a unique habitat with its own abiotic and biotic factors that could limit species distributions. However, each reef zone can have a complex of habitats within it. For example, as a whole, the back reef is subject to a lot of water movement and herbivory is a common occurrence. But within the back reef, there are exposed rocky terraces, sheltered rocky crevices, and open sand flats. Each of these also has unique biotic and abiotic factors that can limit the distribution of certain species. It is possible adaptation has occurred in relation to these more local habitat types.

This has been observed in Atlantic and Indo-Pacific *Halimeda* in which species either occupying sandy substrate or high hydrodynamic areas are monophyletic (Kooistra et al. 2002). If habitat specificity is phylogenetically conserved, as it was in Kooistra et al. (2002), then those traits could be used to examine which habitat parameters (temperature, hydrodynamics, herbivore pressure, substrate, etc.) are most important in defining distributional boundaries. Habitat parameters can be considered important if they are specific within a species, yet the species differs in reef zone distribution. This combined information of habitat specificity and reef distribution could begin to construct a better understanding of reef algal assemblages.

Another useful approach would include a wide spread study investigation distribution patterns over entire oceans. Some work has already been done molecularly using *Halimeda* distributed throughout the tropical oceans (Kooistra et al. 2002, Kooistra and Verbruggen 2005). By using a large scale study, coral reef conservation efforts could further be focused by using evolutionary distribution patterns to help locate marine biodiversity hotspots and evolutionary sources (Briggs 2005). Once found these areas could be the focus of more intense conservation efforts.

The model proposed in this paper requires some refinement. Computer analyses are needed to further investigate which processes are occurring, and addition of more defined ecological character states are needed. However, coral reefs

are vital ecosystems (Smith 1978, Ray 1988, Birkeland 1997, Costanza et al. 1997, Briggs 2005, Sheppard et al. 2005) not only to biodiversity but to those who depend on them for sustenance. With the current state of coral reefs, anything that can limit wasted effort is worth investigating further.

ACKNOWLEDGMENTS

I would like to thank Frank Murphy and Neil Davies for the use of the Gump Station. I also thank Brent Mishler, Carole Hickman, Rosemary Gillespie, Scott Fay, Michele Weber, and Matt Medeiros for their help with project focus and phylogenetic work. I also thank Sara Lopus, Sara Steidl, Alicia Bihler, Courtney Hart, and Kristin Tremain for transportation to and from the study sites. I thank Carmen Yeung, Hampton Smith, Ingrid Burke, and Ali Kattan for their assistance in the field and I thank Antoine de R. N' Yeurt at the University of French Polynesia for his help with species identification and current information on taxonomic changes.

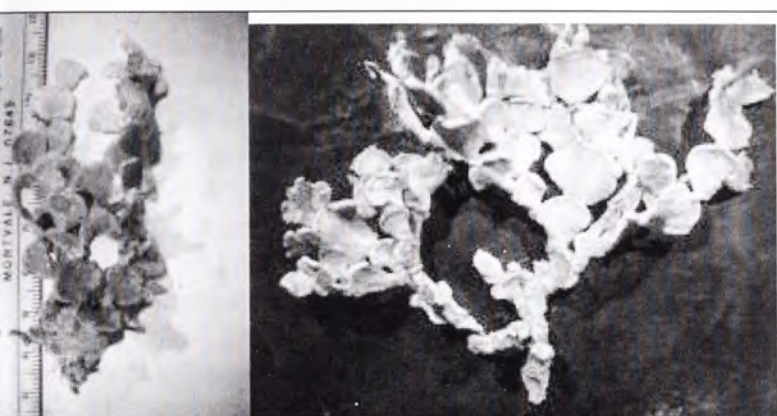

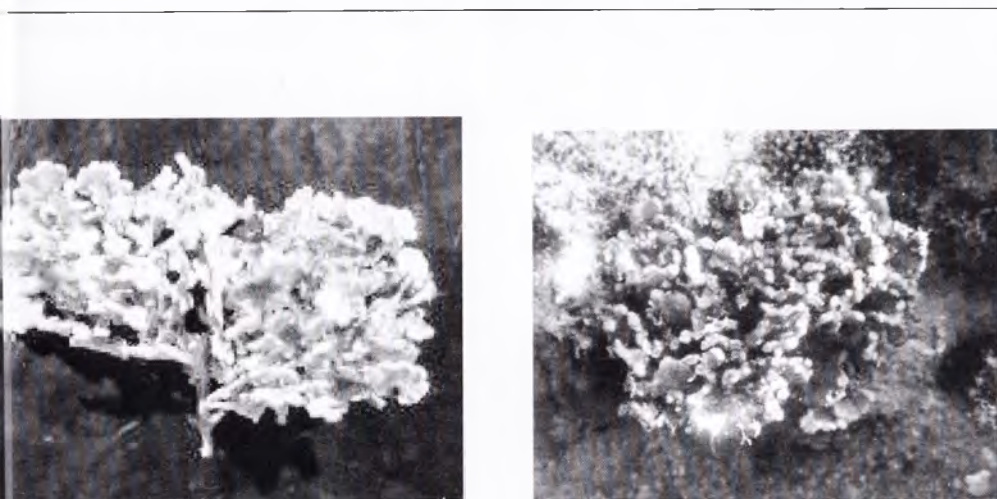
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APPENDIX A

Halimeda of Moorea

	<p><i>Halimeda taenicola</i></p> <p>Attached to rock. Basal segments forming a pillar-like structure. Segments large (8-10mm) with little calcification. Thallus is very flexible. Specimens were found on the back reef (specimens were also found on Tetiaroa forming dense mats on the seaward facing reef).</p>
	<p><i>Halimeda discoidea</i></p> <p>Very fleshy species found attached to rocky substrate. Segments highly convoluted, and broad (8-10mm). Basal segments form fan-like structure. Specimens found on algal ridge had holdfasts attached in small holes in coral rock. Specimens found in fringing reef were attached in crevices of coral bommies.</p>
	<p><i>Halimeda minima*</i></p> <p>Can be differentiated from <i>H. opuntia</i> and <i>H. distorta</i> by its reduced segments (2-3mm). Thallus forms compact bushy structure attached to rocky substrate with a single holdfast. Unlike <i>H. opuntia</i> and <i>H. distorta</i>, multiple rhizoidal attachments are often absent.</p>



a



b

Halimeda borneensis (formerly *Halimeda incrassata* (A. d. R. N'Yeurt personal communication))

Occurs in loose sediment. Large bulbous holdfast firmly attached in sediment. Thallus is erect with calcification moderate to heavy. Morphology is very plastic as can be seen from the photos to the left. (a) The specimen pictured was found on the fringing reef in Cook's Bay. It was originally classified as *H. macroloba*, but correct identification was assessed by A. d. R. N'Yeurt (personal communication). Segments are broad (width: 8-10mm). Thallus branches in almost one dimension and is flexible. (b) The specimen pictured was found on the shallow (<1m) sandy shelf on the windward side of Motu Tiahura. Segments are smaller than (a) (width: 3-5mm) and the thallus has a compact bushy form and is very rigid.



*Halimeda distorta**

Small segments (width: 3-5mm). Thallus is sprawling forming dense mats on rock and in rock crevices. Typically starts from one main holdfast, although, other small rhizoids have been found on peripheral segments.



*Halimeda opuntia**

Small segments (width: 3-5mm). Thallus is sprawling on rocky substrate and forms loose bushy clumps (Hillis (1980) also describes *H. opuntia* occurring on sandy substrate). Attached to substratum via small multiple rhizoidal holdfasts.

**Halimeda minima*, *H. distorta*, and *H. opuntia* can often be difficult to separate. Consult Payri et al. (2000) for further information.

APPENDIX B

Key for the *Halimeda* of Moorea*

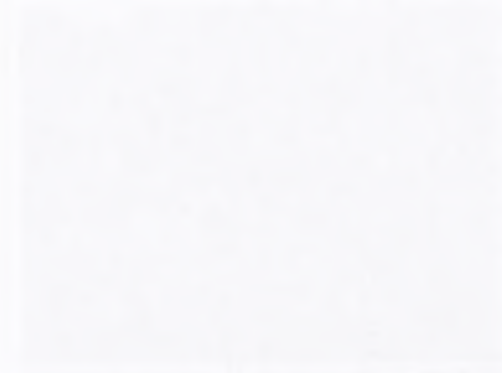
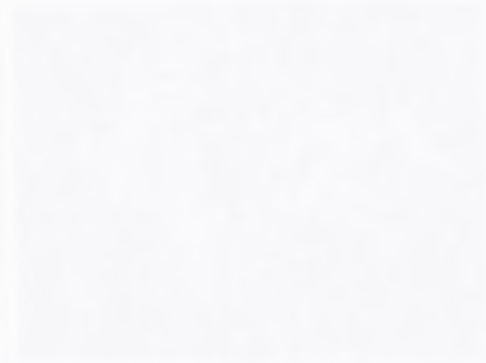
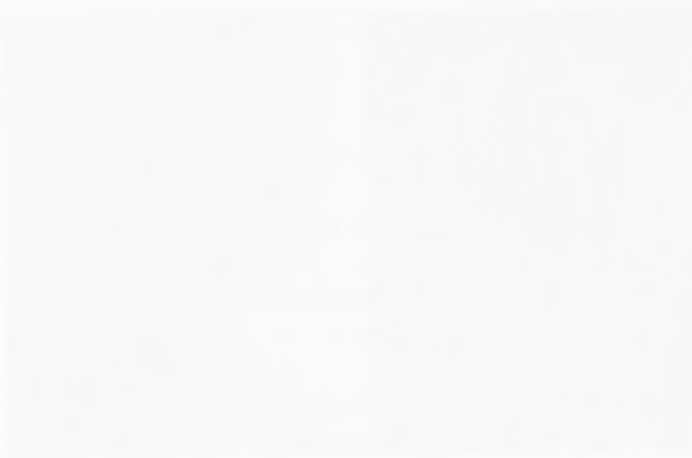
- 1.a. Thallus predominately erect.....go to 2
- 1.b. Thallus sprawling forming mats or
draping over the substrate.....go to 4
- 2.a. Thallus growing on loose substrate, large bulbous holdfast.....*Halimeda borneensis*
- 2.b. Thallus growing on rocky substrate.....go to 3
- 3.a. Thallus segments highly contorted, very lightly calcified (almost fleshy).....*H. discoidea*
- 3.b. Thallus segments not contorted, lightly calcified.....*H. taenicola*
- 4.a. Thallus segments very reduced (2mm-3mm wide),
growing in very compact clumps.....*H. minima*.
- 4.b. Thallus segments more
than 3mm wide.....go to 5
- 5.a. Thallus growing in loose clumps, many rhizoidal attachment points.....*H. opuntia*
- 5.b. Thallus growing as loose mats attached to rock, one main holdfast.....*H. distorta*

*This is only meant as a quick reference to the *Halimeda* found on the reefs of Moorea. The taxonomy of *Halimeda* in French Polynesia is currently in a state of change (A. N'Yeurt pers. comm.). Please consult Payri et al. (2000) for further information.

APPENDIX C

Character matrix used to infer phylogeny

character	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
<i>H. borneensis_32</i>	0	0	1	0	0	0	1	0	0	1	0	0	0	?	?	4
<i>H. borneensis_41</i>	0	0	1	0	0	0	0	0	0	1	0	1	0	1	2	1
<i>H. borneensis_18</i>	0	0	1	0	0	0	0	0	0	1	0	0	1	2	1	3
<i>H. distorta_38</i>	1	0	0	0	1	1	1	0	1	1	0	0	1	?	?	2
<i>H. opuntia_14</i>	1	0	0	0	1	1	0	1	0	1	0	1	1	1	0	3
<i>H. distorta_15</i>	1	0	0	1	1	1	1	0	1	1	0	0	1	?	?	3
<i>H. borneensis_42</i>	0	0	1	0	0	0	0	0	0	1	0	1	0	1	1	5
<i>H. discoidea_46</i>	0	0	1	0	0	1	0	1	0	0	2	1	0	0	1	0
<i>H. opuntia_44</i>	1	0	0	1	1	1	1	1	0	1	0	0	1	1	0	0
<i>H. taenicola_49</i>	0	0	2	0	1	1	0	0	0	0	0	1	1	0	2	1
<i>H. opuntia_45</i>	1	0	1	1	1	1	0	1	0	1	0	0	1	2	0	0
<i>H. discoidea_16</i>	0	0	1	0	0	1	0	1	0	0	2	1	0	?	?	3
<i>H. distorta_23</i>	1	1	0	?	?	?	1	0	0	1	2	0	1	1	1	3
<i>H. opuntia_35</i>	1	1	0	1	1	1	0	1	0	1	0	1	0	1	1	4
<i>H. borneensis_26</i>	0	0	1	0	0	0	0	0	0	1	2	0	0	1	1	4
<i>H. borneensis_34</i>	0	0	1	0	0	0	1	0	0	1	0	0	1	1	1	4
<i>H. opuntia_52</i>	1	1	0	1	1	1	1	1	0	1	0	0	1	1	1	5
<i>H. distorta_50</i>	1	1	0	1	1	1	1	0	0	1	0	0	1	2	2	5
<i>H. minima_51</i>	1	1	0	1	1	1	1	1	0	1	1	0	1	1	0	5
<i>H. borneensis_53</i>	0	0	1	0	1	0	0	0	0	1	0	1	0	1	1	5



1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90
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THE EFFECTS OF HERBIVORY ON ALGAL SUCCESSION ON DEAD CORAL SUBSTRATE

Ali Kattan

Integrative Biology, University of California, Berkeley, California 94720 USA

Abstract. The effects of herbivory and presence of fish and urchins, *Diadema savignyi* and *Echinometra mathaei*, on dead coral substrates was studied on the island Moorea in French Polynesia. Herbivore exclusion cages were designed to test the effects on algal succession on two dead coral substrates, *Acropora hyacinthus* and *Pocillopora verrucosa*. Five different treatments were observed for percent coverage of algal growth and data was recorded 6 times over a 31-day period. Growth of filamentous algae, *Polysiphonia scopulorum*, was seen growing the sixth day of treatment in all designs. Significant difference in percent coverage of separate types of algae was not observed until day 27 of the study between the caged *Diadema s.* and closed cage treatments in percent coverage of fleshy algae on corals. Herbivory and presence of urchins showed greater effects on algal succession than that of fish. Algae were sampled and identified to create a conceptual model of algal succession on both coral substrates over study period. Transects were established at two sites to determine types of algal growth in relation to the density of urchins per unit area. Transect data showed correlations between high urchin density and high percent coverage of filamentous and low percent coverage of fleshy algae. Transect results supported findings in cage experiments showing that urchin species are vital to maintaining balance in reef ecosystems by halting succession of macro-algal dominance.

Key words: *Diadema savignyi*, *Echinometra mathaei*, *dominance*, *phase shift*, *predator*, *reef ecosystem*, *herbivory*

INTRODUCTION

Herbivory in ecosystems is integral to maintaining a balance in species distribution. The removal of predators in a system can cause a skew in the distribution of taxa and can lead to the extinction of a species. Coral reef systems are subject to ecological changes due to factors, biotic and abiotic, which can lead to changes in reef species dominance. This loss of dominance is termed a phase shift and is detrimental to the survival of coral species. Previous studies examined the factors that affect algal growth on coral substrate and has shown that the main factor retarding live coral growth is macro algal cover (McClanahan, 1997; McCook, 2002; Wilder,

2003). Macro algae are able to grow on a number of different substrates, and thrive on the rugosity of dead coral substrate calcium carbonate. Light, nutrients, sedimentation and water flow are significant factors in the growth and dispersal of algae (McClanahan, 1997). Herbivory, by fish and urchins is a major biotic factor that controls the growth of macro-algal species (McClanahan, 1997; Wilder, 2003). The main herbivorous species found in coral reef systems, are the Surgeonfish, *Ctenochaetus striatus*, Parrotfish, *Acanthurus nigrofusus* and urchins *Diadema savignyi* and *Echinometra mathaei*. (Carpenter, 1986; Hay 1985; Wilder, 2003). The availability and density of these species

determine coral vs. algal dominance in reef systems (Hay 1985; Hay, 1997).

Decline in fish populations is occurring globally due to overfishing, industrialization, and elimination of habitat. The reduction of herbivore predation allows for algal growth and eventually climax communities of macro-algae to exist (Carpenter, 1986; Hay, 1997; Wilder, 2003). The phase shift to a macro-algal dominant system occurs through the successional growth of algae on dead coral substrate (McClanahan, 1997). Herbivores play a vital role in allowing or arresting the successional pathways to algal growth (Carpenter, 1986; Hay 1985; Hay 1997; McClanahan, 1997). The primary objective of this study was to determine the effects of herbivores on successional growth of algae on two abundant species of coral, *Acropora hyacinthus* and *Pocillopora verrucosa* in Moorea, French Polynesia.

McClanahan's study (1997) of herbivory on successional algal growth on the reefs of Eastern Africa, determined that endolithic "turf" algae are the initial colonizers of calcium carbonate plates in back reef sites. However, in certain environments with stronger water movement along with certain grazers allowed for the removal of sediment particles and growth of coralline red algae (McClanahan, 1997). This successional growth then led to the establishment of calcareous algae, *Halimeda* sp. (McClanahan, 1997). Wilder (2003) performed a similar study in the Vaipahu lagoon, Moorea, French Polynesia, to determine effects of herbivores on algal growth. In Wilder's (2003) study relative effects of urchins on algae were related to availability of algal patches within mobile distance <1m. His study also determined that fish had greater effects on algal growth when urchins were absent in the treatment. Wilder (2003) focused his study on the herbivorous effects on the ash-free dry

weight and biomass of the algae through different caged designs.

In the current study I used similar cage designs, but rather than strictly observing algal coverage, my experiments focused on two different coral species while monitoring primary and secondary succession of algal growth on each coral head. The main goal of this study was to monitor successional growth of algae on the substrates stated. Given that herbivory plays a vital role in maintaining coral reef dominance in reef ecosystems (Carpenter, 1986; Hay, 1997; Knowlton, 2001; McClanahan, 1997; Wilder, 2003) my study looked closely at the response algal growth to the removal of major predators. Specifically I address these four questions, Does herbivory by urchins and fish deter the successional growth of algae that is detrimental to the survival of coral species? If so, is one predator's presence more vital to arresting the growth of macro-algae, or fleshy algae? Is there a relationship between the number of urchins present in a system to the percentage of benthic or macro-algal growth on dead coral substrate? Finally, does the species of coral substrate affect the succession of algae? This study aimed to answer these questions by testing these hypotheses: (1) Treatments will display differences in percent coverage of groups of algae. (2) The availability of fish will deter growth of "turf" algae on substrates studied and allow for the growth of red corallines. This will then lead to higher succession in algae eventually leading to macro-algal growth of brown algae. (McClanahan, 1997) (3) The herbivory of urchins will "arrest" growth of algae to a benthic state (McClanahan, 1997). (4) The two coral substrates of *Acropora hyacinthus* and *Pocillipora verrucosa* will show differences in percent coverage and succession of algae.

Expected outcomes were that algal growth would reach climax state under caged

conditions and patterns would emerge in the different designs showing the effects of herbivory of fish and urchins on algal growth. Differences in algal coverage would show the significance of herbivory in maintaining a natural coral reef system.

METHODS

Study site: Herbivore exclusion experiment

The cage experiments were conducted in 25 meters off shore at the Gump Research Station in Moorea, French Polynesia ($17^{\circ}29.417'$ S, $149^{\circ}49.574'$ W). The environment where cages were placed representative of the island bay ecology with its variable water flow and bottom substrate of sand, silt and rocks (Payri, R. N'Yeurt, Orempuller, 2000). The algae that thrive in this environment create a soft moving substratum allowing for the growth of a number of different green algae and fleshy red and brown algae. Herbivore presence and predation is moderate with species of the surgeonfish, *Ctenochaetus striatus*, parrotfish, *Acanthurus nigrifuscus*, territorial damselfish and urchins *Diadema savignyi* and *Echinometra mathaei* (Kattan, *pers. obs.*). Coral cover is quite similar to the fringing reef environment on the island with abundant coral species of *Porites*, *Pocillipora*, *Acropora* and *Montipora*. Depth of the study site averaged at 1.3 meters and sunlight exposure to cages was approximately 11-12 hours a day.

Study site: Urchin presence and algal coverage on calcium carbonate substrate

Two transects were established and sampled at both Temae Airport and Maharepa for the correlation study of algae coverage and urchin presence (Temae Airport: $17^{\circ}28.396'$ S, $149^{\circ}46.537'$ W; Maharepa: $17^{\circ}28.882'$ S, $149^{\circ}47.642'$ W). Transects were sampled

from shore to algal ridge encompassing typical lagoon environments of the island. Transect data were grouped for both sites along with replicates from each site. Map of Moorea with sites of both studies are in Figure 1.



Fig. 1. Study sites

Herbivore exclusion experiment

Two species of dead coral substrate were used for the cage experiments to determine differences on both substrates and for replication. Coral heads of coral species, *Acropora* and *Pocillipora*, were used for a prior experiment testing effects of sedimentation on re-growth of live coral. These coral heads were collected on August 11, 2005 and “were placed for 31 days outside the lagoon on the reef slope (20 feet deep), inside the reef crest (9 feet), mid lagoon (not in sandy area, 10 feet), and along the shore on the fringing ref (20 feet)” (Lenihan, *pers. comm.*). Initial conditions were recorded for each of the corals by percent coverage of coloration, brown sediment coverage, bare calcium carbonate and any endolithic algal “turf” growth. Coral heads showed no sign of live substrate with percentage of white calcium carbonate coverage over 90% for all corals. A nested one-way ANOVA test was performed to show no statistical difference in initial conditions of coral heads. In this study

coral heads were placed in treatment for ~31 days.

Cages were designed to create four different treatments of herbivore exclusion. Cage material consisted of .65 cm² wire mesh held together with zip ties. Bottoms of cages were constructed with green mesh and chicken wire. Cage sizes were either 30x30x30 cm or 35x35x35 cm according to treatment used. Cage designs were adapted from designs used in previous studies specifically, Wilder (2003) and McCook, Diaz-Pullido (2002). Five treatments of herbivore exclusion were designed including one control.

A full cage was constructed to exclude both urchins and fish and was used as a control to allow for full potential of algal growth. Treatment code: “-Fish, -Urchin”. Another full cage was designed for fish exclusion and urchin inclusion by caging separate species of urchins. Thus, two separate cages were constructed for this treatment for each of the *Diadema* and *Echinometra* species. This was done to exclude the factor of competitive animal behavior. Treatment codes: “-Fish, +*Diadema*” and “-Fish, +*Echinometra*”. A fourth treatment was no cage, which left coral heads open to allow for predation by fish and urchins. Treatment code: “-Fish, -Urchin”. A fourth treatment consisted of an open top cage that excluded urchin predation, but allowed fish to graze on algal growth “+Fish, -Urchin”. All four designs were used for each coral substrate at a site with each cage separated ~1.5 m. A coral substrate from each of the two species was placed in each treatment for a total of five different designs. Three replicates of each treatment were constructed allowing for replication for statistical tests. A total of fifteen cages were placed at the study site.

Cages were visited daily for observation and cage cleansing. Cages were cleaned with a brush to allow for full sunlight exposure and to reduce sediment accumulation. Data were collected every six

days from the start of the treatment for percent coverage of algal growth and coloration. Percent coverage was only recorded on the heads of corals and excluded any algal growth on the bottom of the coral. Algae that could not be identified was recorded and later identified in the lab. Specific green, yellow, and red coloration percent coverage was recorded for each head of corals. Percentages were determined using square plots, .65 cm², from the cage material to observe presence of algal growth.

Succession of Algae on Coral Substrate

Primary and secondary growths of algae were sampled from corals for identification. New algal growths were coded and recorded for date and presence in treatment. Slides were prepared with samples to determine characteristics microscopically. Algae were identified to genus and species when possible using an algae field guide (Payri, R. N'Yeurt, Orempuller, 2000). Samples that could not be identified in Moorea were preserved as voucher slides and taken back to the herbarium at UC Berkeley for identification by Professor Richard Moe.

Urchin presence and algal coverage on calcium carbonate substrate

Algal growth on calcium carbonate substrate and the presence or absence of urchins, *Diadema* and *Echinometra* species were observed along each of 2 transects at Temae and Maharepa. Transects were swam from shore to algal ridge using a 1 m² quadrat to estimate percent coverage. Transect length varied depending on the distance between algal ridge and shore. The purpose of transects was to find a relationship between urchin presence/herbivory and structure of algal community on dead coral substrate.

Statistical Analysis

In both studies coverage on corals was described using 5 functional groups: (1) bare calcium carbonate, (2) filamentous turf, (3) calcareous algae, (4) encrusting coralline algae and (5) fleshy brown algae (McLanahan, 1997). Percent coverage of categories was recorded at the end of the experiment on each of the corals. To obtain the absolute effects of herbivory from fish and urchins on each treatment I performed one-way nested ANOVAs on each response variable at the end of the cage experiment. Final conditions were tested against initial conditions of corals. Statistical tests were performed with each data set at 6-day intervals to show progressive effects of herbivory. A Tukey-Kramer “Honestly Significantly Different” test was performed on data that displayed significant differences in percent algal coverage among treatments.

Diadema and *Echinometra* species were grouped into “Total Urchin Presence” for transect data to view correlation between urchin presence and certain algal growths. Percent coverage of algae on dead coral and rubble substrate was recorded to compare with experimental study of dead coral substrates. Regression tests were performed on transects’ data after data was pooled and urchin presence was root transformed for normal distribution.

RESULTS

Herbivore Exclusion Experiment

Initially bare corals showed no differences in coverage prior to treatments (one-way ANOVA, $p = .1728$). One-way ANOVA test was performed to show differences in algal coverage among the five different treatments. Differences in algal coverage were statistically insignificant among treatments during the first ~26 days of treatment. Significant differences were only

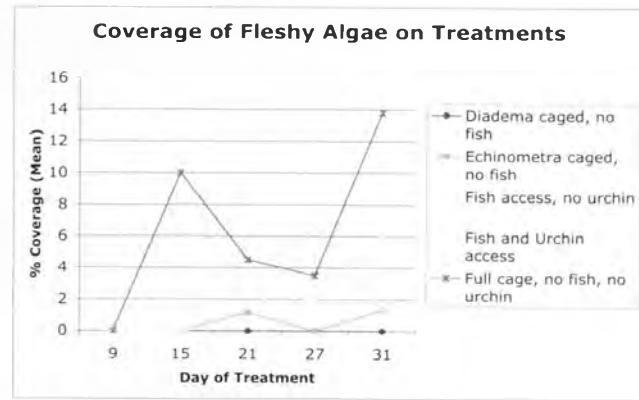


Fig. 2. Mean percent coverage of fleshy algae in treatments during 31-day study.

apparent in the last two sub samples of data, day 27 and 31 as shown with fleshy algae in Figure 2.

The ANOVA results showed differences in percentage of fleshy algal coverage among treatments in sub sample data of day 31 ($F\text{-val.}=3.313$, $p\text{-value}=.0262$). Fleshy algae included red algae of the genus *Laurencia*, brown algae of species *Dictyota*, and green algae that could not be identified. Tukey-Kramer HSD results showed significant difference in percent coverage of fleshy algae in the caged *Diadema* treatment. Positive values in the Tukey-Kramer HSD table showed pairs of means were significantly different between the caged *Diadema* and closed-cage treatments. Appendix A shows ANOVA results for day 31 of fleshy algae coverage between treatments.

One-way ANOVA test showed a significant difference in percent coverage of filamentous algae among treatments for Day 27 of treatment, (ANOVA, $F\text{ val.}=11.47$, $p<.0001$). Positive values in the Tukey-Kramer HSD table showed pairs of means were significantly different between “-Fish, +*Diadema*” and “-Fish, -Urchin” treatments. However, $p\text{ value}=.153$, for ANOVA for day 31 of treatment showed no significant difference in percent coverage of filamentous algae. Filamentous algae were mainly species of *Polysiphonia scopulorum* and a small percent was a green filamentous alga that

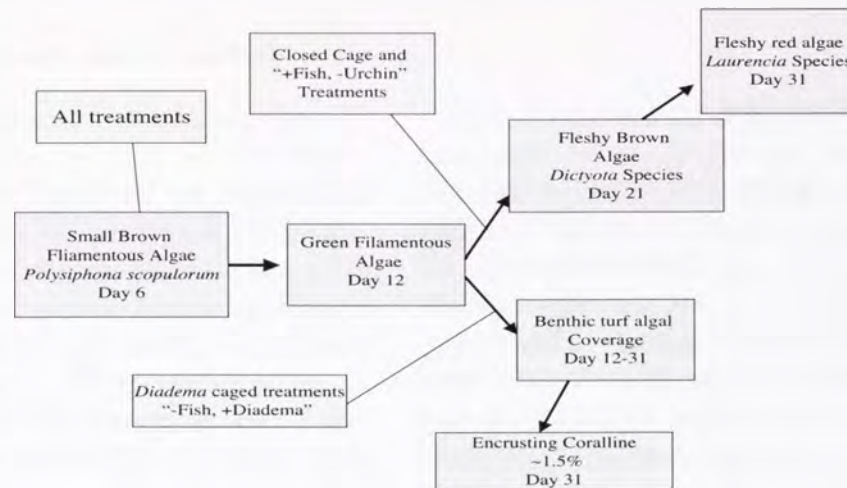


Fig. 3 Conceptual model of succession of algae on studied coral substrate

could not be identified; it closely resembled with cyanobacteria.

Visual observation of coverage of encrusting coralline algae was higher in the urchin-caged treatments, though ANOVA tests did not show statistical difference in day 31 of treatment (F val.=1.38, p val. = .36).

Succession of Algae on Coral Substrate

According to the ANOVA results, differences in algal growths in separate treatments were not significant until the 27th day of treatment. Prior to significant differences in growth, the brown filamentous algae *Polysiphonia scopulorum*, covered most of the corals in all treatments. Algae were sampled at day 9 of treatment and was observed at 43x and identified with field guide (Payri, R. N'Yeurt, Orempuller, 2000). New growth of a filamentous alga, a new species relative to *P. scopulorum*, was not recorded until day 15 of treatment. In the "+Fish, +Urchin" treatment allowing access to both fish and urchins, the new filamentous green algae was sampled and observed at 43x for identification; however I was not able to identify it. Fleshy algal growth was not observed until the 21st day of treatment and composed mainly of brown algae, *Dictyota* sp.

(R. Moe, *pers. comm.*) Fleshy red algae were also observed and sampled on day 31 of treatments and identified as belonging to the genus *Laurencia* (R. Moe, *pers. comm.*). Fleshy alga was juvenile and when present coverage ranged from ~5-7%. Presence of encrusting coralline algae was observed from day 9 of the treatment thru the end of the study. Percent coverage was greatest in all treatments at day 31 of the study. The greatest mean coverage of coralline growth occurred in the *Diadema* caged treatment, "-Fish, +Diadema", at ~1.5%. A conceptual model of the successional pathways of algal functional groups under different treatments was constructed and is shown in Figure 3.

Urchin presence and Algal coverage on calcium carbonate substrate

Transect data showed a significant relation between high urchin density and high percent coverage of benthic filamentous algal cover on dead coral substrate as shown in Figure 4 (Regression, r^2 value = .06043). Graph results showed a linear relationship between high urchin density and low percent coverage of fleshy algae on substrate, though regression test did not produce as significant

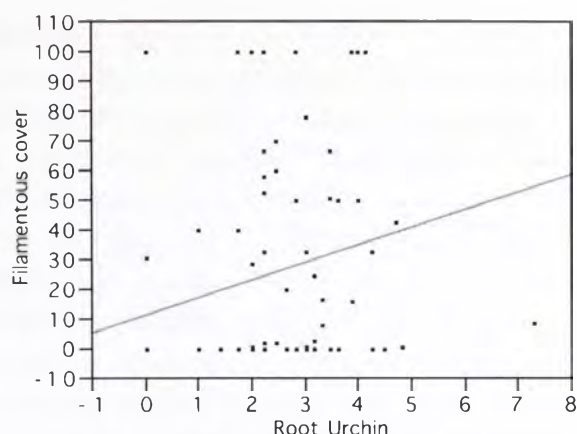


Fig. 4 Linear Regression of Root Urchin Density vs. % Coverage of Filamentous algae

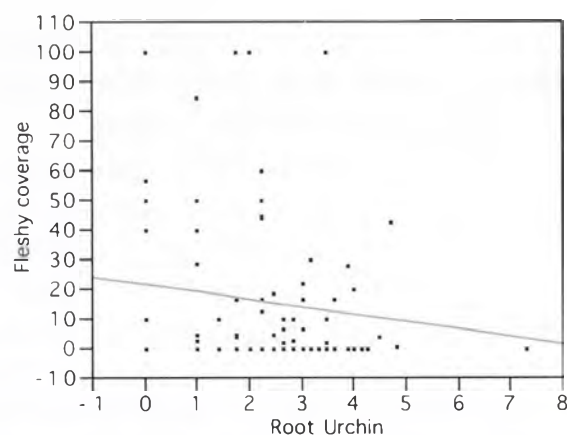


Fig. 5 Linear Regression of Root Urchin Density vs. % Coverage of Fleshy algae

results as shown in Figure 5 (Regression, r^2 value = .020122)

DISCUSSION

The two species of dead coral substrate, *Acropora hyacinthus* and *Pocillipora verrucosa* used for this study showed no differences in algal coverage throughout cage experiment. The rugosity and surface of these corals differed in their polyp structures; however, this did not create differences of growths on each species. This leads to the view that all coral species are susceptible to algal growths and dominance. Benthic algae in filamentous form is quick to grow on dead coral substrate yet can be sustained in that stage through the herbivory of urchins, especially *Diadema* species (Hay 1997). The type of algae that is responsible for the phase shift in the reef system cannot be described by this study or by previous studies. Though the arrival of benthic filamentous algae does lead to growths of different types of algae as shown in this study.

Filamentous turf cover on coral substrates occurred almost immediately as coverage was up to ~5% on some corals by the 6th day of treatment. This supports the rapidity of benthic algae to grow on such

substrate and as being primary in growth in the chain of succession (Bruggeman, 1994; McClanahan, 1997). Succession of turf forming algae on the corals showed growth of brown filamentous *Polysiphonia scopulorum* as primary in the turf assemblage. Secondary growth of the small green filamentous algae was seen mid-way through the study and occurred first in the open treatment. Coralline growth was observed early in the study but not sufficient to consider in the succession of algae on substrates studied. Coverage that was observed, <3%, was seen mainly on the *Pocillipora verrucosa* substrate. Reasons for such low growth are due to time constraints with previous studies showing significant growth of encrusting coralline occurring after 60-90 days (McClanahan 1997; Diaz-Pullido, McCook, 2002). Growth of juvenile fleshy algae was observed on day 21 in treatments that excluded urchin and allowed for fish grazers, mainly of *Dictyota* species. Growth of fleshy red algae under the genus *Laurencia* were first observed in the same treatments at day 31 of the study. Fleshy brown and red algae were observed to be the climax stage of succession in this period of study on substrates used. This climax state supports a number of studies portraying fleshy brown macro-algae to be the climax stage of succession over longer time periods, as great as 30 months in

Diaz-Pullido and McCook's study (Bruggeman, 1994; McClanahan, 1997; Diaz-Pullido, McCook, 2002; Wilder, 2003).

The effects of herbivory were shown to be stronger for urchins, especially the *Diadema* species. The caged *Diadema* treatment, "-Fish, -Diadema", showed a significant lower percent coverage of fleshy algae than the closed cage treatment. This result supports conclusions made in Kenya that urchin grazing is different from fish grazing due to the ability of urchins to deter growth of fleshy algae and maintain succession in a benthic state (McClanahan, 1997). The treatment that allowed *Diadema*, along with other herbivores, "+Fish, +Urchin", did not show differences in percent coverage of juvenile fleshy algae. Though the open treatment allowed urchins to graze, the caged treatment kept *Diadema* and *Echinometra* species within proximity of corals.

The reasoning for minimal effects of fish grazers on algal growth can be attributed to their feeding preferences, most importantly that territorial damselfish prefer later successional species of algae to graze on, allowing for juvenile growths of macro-algae (Sammarco, 1980). McClanahan suggested in her study that large roaming herbivorous fish allow turf-forming algae to pass through more successional stages allowing for growths of corallines and fleshy algae (1997). This study supports this view by showing succession of juvenile fleshy algae in treatment "+Fish, -Urchin", allowing herbivory by fish and excluding urchins.

Greatest mean value of encrusting coralline percent coverage was seen in day 31 of data in the caged *Diadema* treatment at ~1.5%. Bruggemann showed that coralline growth is greatest with lower coverage of benthic turf and filamentous algae, with data suggesting that urchin grazing allowed for the greatest growth of coralline (1994). Data from this study is not significant to support this

view; however, further study with less time constraint can establish supporting results.

Transect results provided supporting evidence to the above findings in relation to density of both *D. savignyi* and *E. mathaei* in the field. Observation of algal growth was similar to observations made in cage experiment by examining growth on dead calcium carbonate substrate. Results of linear regression show that greater urchin presence and herbivory maintains succession of algae in a benthic filamentous form. In contrast, low urchin density and herbivory show higher values of percent coverage of fleshy algae. These results strengthen the importance of urchins in halting succession of macro-algae.

Reef ecosystems are a diverse balance of live healthy corals, herbivores, algal communities, and a number of different organisms (Whitaker, 2002). The disturbance of any of these species upsets this balance creating a phase shift leading to the non-existence of certain species in the system (Hay 1997). The disturbance of large herbivores through over-fishing and water pollution causes this disturbance by offsetting a chain of events that leads to the death of corals and macro-algal dominance in these systems (Carpenter 1983, Knowlton 2001, Wilder, 2003). As shown in this study, urchins, especially *Diadema savignyi*, are vital in maintaining the balance and health of live coral species.

ACKNOWLEDGMENTS

I would like to thank the following people for their gracious help in making this study possible:

Brent Mishler for assistance with experimental design and procedure. George Roderick and Jamie Bartolome for assistance in ecological concepts. Richard Moe for identification of algae and assistance in preserving slide vouchers. Hunter Lenihan for

the use of corals for the study.

Graduate Student Instructor, Scott Fay, for assistance with statistics.

Fellow Colleague, Carmen Yeung, for assistance with transects.

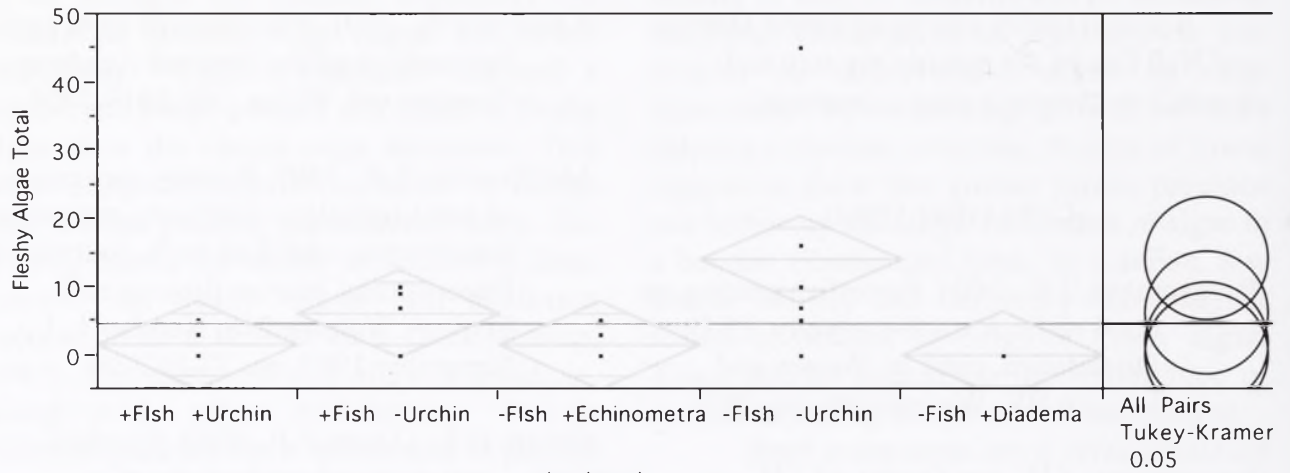
I would like to also thank Frank Murphy and Neil Davies for making my stay and research in Moorea a great experience.

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APPENDIX A

ANOVA AND TUKEY-KRAMER HSD RESULTS FOR DAY 31 FLESHY ALGAL COVERAGE



Abs(Dif)-LSD	-Fish -Urchin	+Fish -Urchin	+Fish +Urchin	-Fish +Echinom.	-Fish +Diadema
-Fish -Urchin	-12.991	-5.158	-0.491	-0.491	0.842
+Fish -Urchin	-5.158	-12.991	-8.324	-8.324	-6.991
+Fish +Urchin	-0.491	-8.324	-12.991	-12.991	-11.658
-Fish +Echinom.	-0.491	-8.324	-12.991	-12.991	-11.658
-Fish +Diadema	0.842	-6.991	-11.658	-11.658	-12.991

Positive values show pairs of means that are significantly different.

THE RELATIONSHIP BETWEEN A PARASITE AND ITS HOST: AN INVESTIGATION OF *CASSYTHA FILIFORMIS* (LAURACEAE) AND *PEMPHIS ACIDULA* ON MOOREA, FRENCH POLYNESIA

SARA LOPUS

Environmental Science Policy and Management, University of California, Berkeley, California 94720 USA

Abstract. The relationship between *Pemphis acidula*, a parasitic angiosperm, and *Cassytha filiformis* (Lauraceae) was investigated on the Tiahura Motu, Moorea, French Polynesia. Comparisons were made between the architectural structure, resource allocation, and germination rates of parasitized and unparasitized plants. Parallel comparisons were made between parasitized and unparasitized branches on parasitized plants. Parasitized *Pemphis* specimens had larger branches, less mature fruits, and lower germination rates of ripe seeds than did unparasitized plants; similar trends were exhibited between branches of parasitized plants, although germination rates of seeds from parasitized branches were higher than those from unparasitized branches. Differences in fruit maturities could indicate that a high proportion of fruits fall from parasitized plants before reaching maturity. Among parasitized plants, the lower germination success of seeds on unparasitized branches may indicate selection by the parasite for resource-rich host branches.

Key words: *Pemphis Acidula*; *Cassytha filiformis* (Lauraceae); *parasite*; *host fitness*; *Moorea*, *French Polynesia*

INTRODUCTION

Relationships between parasites and hosts can be complex, and the effects of a parasite on its host are often difficult to predict. While it is widely acknowledged that the presence of a parasite can decrease the hosts' rates of survival and reproduction (Gomez 1994, Koskela et al. 2002), there is some debate as to the most advantageous adaptive strategy for a parasite to develop over time.

The negative effect of a parasite on the fitness of its plant host can vary greatly in degree, from a case of mild parasitism in which the host plant maintains its ability to perform photosynthesis and produce fertile fruits to a case of total parasitism in which the parasite feeds on its host's supplies of water and nutrients until the host is unable to reproduce and dies (Gomez 1994, Koskela et al. 2002). While some parasites require their host plant to stay alive until they are able to release their own seeds for reproductive purposes, other parasites are able to survive on a number of host plants at once, so the death of a host does not represent the death of the associated parasite (Satterthwaite 1998).

In order for a parasite to prevent total deterioration of its host species, it may be evolutionarily advantageous for the parasite to

decrease in virulence over time (Gomez 1994), taking what it needs without eliminating its host. Theoretically, a state of zero virulence will occur if there is no lower constraint on virulence, if resistance has no cost to the host, or if the parasite benefits from increased host reproduction (Toft and Karter 1990). In the classic biological control case of myxomatosis, a viral disease released to control rabbits, populations of rabbits exhibited an almost immediate decline after introduction of the virus. Although the rabbit populations began to climb after a few years, the virus proceeded to increase in virulence and now maintains a balance of intermediate virulence (Aparaicio et al. 2004). The case demonstrates the dynamic relationships and delicate balances between parasites and hosts that may develop over time.

Because of their geographic isolation from other land masses, tropical islands provide ideal habitats for the investigation of complex biotic relationships between organisms that have evolved in isolation. Communities of native organisms present on islands are comprised not only of those organisms that were able to reach the island but also of those that were able to survive alongside the other species present (Roughgarden 1989 in Whittaker 1998). Interspecific relationships such as parasitism

between native plants on a tropical island therefore represent a long-standing balance.

While many scientific studies have investigated the relationship of the parasite *Cuscuta europaea*, or dodder, (Gomez 1994, Kelly 1992, Koskela et al. 2002) with its plant hosts, markedly less research has been devoted to the similar parasite *Cassytha filiformis* (Das 2000, Satterthwaite 1998). *Cassytha* is a total twining parasite without foliage leaves or chlorophyll (Das 2000) that has been suggested to reduce the fitness of its host plants (Burch 1992 in Satterthwaite 1998), removing nutrients through points of attachment called haustoria. Haustoria provide a parasite with roughly equal areas of contact to the host's xylem and phloem and allow it to take up photosynthetic products as well as water (Room 1971).

Pressure by a parasite leading to reductions in host fitness could be represented by changes in the host's architectural structure (Witham and Mopper 1985) as well as in the way a plant allocates its resources. There are tradeoffs associated with the development of tolerance to parasites, such as decreases in a host's reproductive biomass while total vegetative biomass remains the same (Koskela et al. 2002). A stressed plant may also devote an unusually high proportion of its resources to reproduction in an act of overcompensation (Lennartsson et al. 1998), possibly as a last-ditch effort to reproduce before dying, while limiting its production of branches and leaves.

The problem of identifying a parasite's effect on host fitness is complicated by the fact that not all unparasitized plants are equally fit. Dodder, for instance, is more likely to coil on hosts of high nutritional status than those of poor quality (Kelly 1992). Since a lag time may occur between parasitic infection and exhibition of a parasite's deleterious effects on its host (Preston 1977), what may appear to be a healthier population of parasitized than unparasitized plants could actually be the result of recent infestation upon healthy hosts by the parasite.

Less research has been dedicated to investigation of relationships involving parasitic angiosperms than other types of parasites, and of that research, very little has been conducted in the field (Gomez 1994). For the purposes of this study, I focused upon the relationship between *Cassytha filiformis* (Lauraceae) and *Pemphis acidula* on the Tiahura

Motu in Moorea, French Polynesia. *Cassytha* is a parasite with a very wide host range (Das 2000), and it was recently found to grow upon 19 of 21 possible host species on the Tiahura Motu (Satterthwaite 1998). *Pemphis* was an ideal study organism for this project because it is the only shrub present across a large region of the Tiahura Motu, providing a relatively homogenous study habitat.

In this study, I investigated the differences in resource allocation between parasitized and unparasitized *Pemphis* individuals within a parasitized zone. Since past research has shown that herbivory leads to more frequent destruction of term shoots than lateral shoots, causing host plants to take on a short, dense appearance (Witham and Mopper 1985), I expected to find similar results among parasitized *Pemphis* individuals. I also expected to detect differences in the resource allocation of parasitized and unparasitized *Pemphis* individuals, as indicated by differences in the ratio of mass and number of fruits to leaves to primary branches, indicating either increased or decreased devotion to creation of reproductive material by parasitized individuals. I expected ripe seeds from parasitized individuals to have lower germination rates than those from unparasitized individuals, indicating reduced fitness among fruits that survive to ripeness.

In addition to investigating differences between parasitized and unparasitized plants, I also compared the above factors between parasitized and unparasitized branches on parasitized individuals. I expected unparasitized branches on parasitized plants to exhibit different types of resource allocation and germination rates than parasitized branches. Existence of such differences could indicate that a host plant's reaction to infestation by *Cassytha* is a relatively local one. I expected a parasitized host to exhibit decreased fitness only locally, allocating a disproportionately high number of its resources to certain portions of the plant, depending upon the parasite's presence or absence. Differences in branch fitness could also indicate that the parasite has preferentially selected resource-rich host branches on which to attach.

METHODS

I conducted an investigation of resource allocation by *Pemphis acidula* individuals, some of which were hosts to the parasite *Cassytha filiformis*, on the Tiahura Motu, a small island off the northwestern coast of Moorea, French Polynesia (S 17° 29', W 149° 54'). The motu's northern shore provides an ideal site upon which to study the two species because of the homogeneity of the region's plant populations. The shoreline is composed of a conglomerate region approximately 10 m in width, with sandier substrate inland. *Pemphis* is the only

and one unparasitized branch from each specimen. I measured the length and basal diameter of each branch before removing the 75cm of the branch closest to the tip.

I separated each branch tip sample into segments of quaternary branches. A quaternary branch is classified as the branch segment from the highest point where two tertiary branches meet to the point where two quaternary branches meet (Figure 2) and become a 5° branch. Any number of tertiary, secondary, and primary branches could stem from a single quaternary

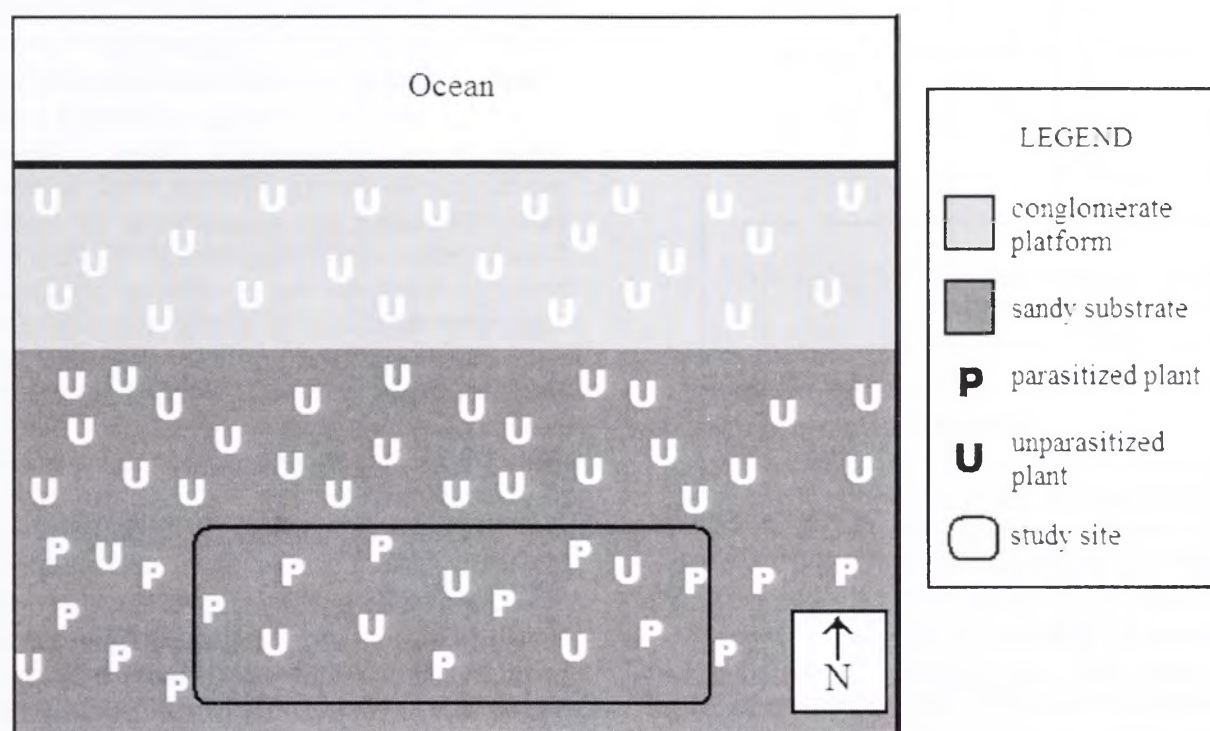


FIG 1. Approximate composition of study site on Tiahura Motu.

shrub growing in the region and is relatively evenly distributed across the shoreline. *Cassytha* is present only on shrubs located in a zone approximately 20 m from shore (Figure 1).

I sampled ten randomly-selected *Pemphis* individuals, four of which were unparasitized and six of which were parasitized to various degrees. All *Pemphis* specimens were located in close proximity to one another within the zone of parasitized individuals (Figure 1) and appeared to have similar access to physical resources. From each specimen, I randomly selected four branches to sample; in the case of parasitized individuals, I selected at least one parasitized

branch. I randomly selected a tertiary branch segment (along with a secondary and primary branch, if present) to sample from each quaternary segment (Figure 2). From each tertiary segment, I randomly selected a secondary branch (and a primary, if present) to sample. From each secondary segment, I randomly selected a primary branch to sample. From each primary branch, I also counted the numbers of fruits and leaves present. I found the dry masses of all fruits. In addition to finding the dry masses of leaves, I used the computer program ImageJ to approximate the leaves' surface areas.

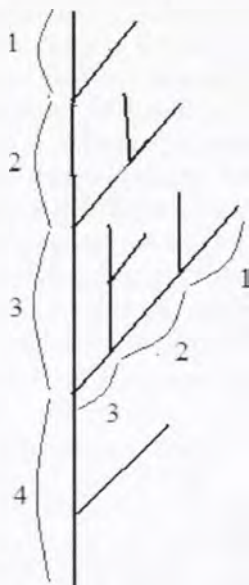


FIG 2. Classification of branch types from primary (1) to quaternary (4).

Plant architecture

I performed a t-test to compare the ratios of branch lengths to basal diameters in order to identify whether branches on parasitized plants are shorter than those with comparable basal diameters on unparasitized plants; I performed the same test to compare parasitized and unparasitized branches on parasitized plants. Additionally, I performed t-tests to compare mean basal diameters and lengths of branches on parasitized and unparasitized plants as well as parasitized and unparasitized branches on parasitized plants.

Resource allocation

To identify differences in plants' resource allocation strategies, I performed t-tests to compare the number of leaves per primary branch, the mean weight of leaves per primary branch, the mean total leaf surface area per primary branch, and the mean individual fruit weight.

Germination rates

In order to determine whether fruits from parasitized and unparasitized *Pemphis* specimens have equal rates of germination, I collected ripe seeds from each specimen, keeping separate the seeds from parasitized and unparasitized branches within the same organism. I ran germination trials with 20 seeds from each unparasitized specimen and 40 seeds (20 from unparasitized branches and 20 from parasitized branches) from each parasitized specimen. The seeds were placed in ziploc bags between two sheets of filter paper moistened with 10 cc water. A t-test was used to compare seed germination rates of various branch types. Since seed production is nearly always used in studies of plant tolerance as an estimate of total plant fitness (Strauss and Agrawal 1999), I performed a t-test to compare the number of seeds in each randomly-selected ripe fruit. All statistical tests were performed using the computer software program JMP.

RESULTS

Plant Architecture

There was no significant difference in the ratio of branch length to basal diameter between parasitized and unparasitized plants (p-value: 0.8792, $\alpha=0.10$) or between parasitized and unparasitized branches on parasitized plants (p-value: 0.3931, $\alpha=0.10$).

Mean basal diameters of branches on parasitized plants were significantly larger than diameters on unparasitized plants (p-value: 0.0310, $\alpha=0.10$, Table 1), and within parasitized plants, mean basal diameters on parasitized branches were significantly larger than those on unparasitized branches (p-value: 0.0095, $\alpha=0.10$, Table 1). Mean branch lengths on parasitized plants were significantly greater than those on unparasitized plants (p-value: 0.0076, $\alpha=0.10$, Table 1), and within parasitized plants, mean lengths of parasitized branches were significantly greater than those of unparasitized branches (p-value: 0.0377, $\alpha=0.10$, Table 1).

TABLE 1. Mean branch basal diameters (mm) and lengths (m) for unparasitized plants and parasitized plants and between unparasitized and parasitized branches on parasitized plants.

	U. plant	P. plant	U. branch on p. plant	P. branch
Branch basal diam.	22.0**	28.7**	23.2**	33.4**
StDev	8.2	9.9	9.1	8.3
Branch length	1.69**	2.29**	1.96**	2.56**
StDev	0.55	0.71	0.72	0.61
n	16	24	11	13

Notes: T-tests used to compare means between columns; * $p < 0.10$; ** $p < 0.05$.

Resource allocation

Parasitized plants had significantly more leaves per primary branch than did unparasitized plants (p-value: 0.0521, $\square=0.10$, Table 2). There were no significant differences between parasitized and unparasitized plants' mean leaf dry weights per primary branch (p-value: 0.6689, Table 2) or mean leaf surface areas per primary branch (p-value: 0.8755, Table 2). Among parasitized plants, there was no significant difference between parasitized and unparasitized branches in mean number of leaves (p-value: 0.9440, $\square=0.10$), mean leaf dry weight (p-value: 0.3873, $\square=0.10$), or mean leaf surface area (p-value: 0.6725, $\square=0.10$) per primary branch.

There was no significant difference between parasitized and unparasitized plants in the mean number of fruits per primary branch (p-value: 0.6955, Table 2). Individual fruits from unparasitized plants weighed significantly more than did fruits from parasitized plants (p-value: 0.0734, $\square=0.10$, Table 2). Among parasitized plants, there was no significant difference in the mean number of fruits per primary branch between parasitized and unparasitized branches (p-value: 0.8374, $\square=0.10$) or in individual fruit weights between parasitized and unparasitized branches (p-value: 0.8159, $\square=0.10$).

Between parasitized and unparasitized plants, there were no significant differences in the ratios of leaf mass to primary branch mass (p-value: 0.9300), fruit mass to leaf mass (p-value:

0.9486), or fruit mass to branch mass (p-value: 0.3046).

TABLE 2. Resource allocation data for unparasitized plants and parasitized plants: mean number leaves, leaf dry weight (g), and leaf surface area (cm²) per primary branch; mean number fruits per primary branch; and mean mass per fruit (g).

		Unparasitized plants	Parasitized plants
Leaves	# Leaves/ 1st branch	7.47*	8.19*
	StDev	2.81	2.94
	n	123	119
	Dry weight/ 1st branch	0.069	0.063
	StDev	0.045	0.042
	n	20	39
	Surface Area/ 1st branch	3.63	3.73
	StDev	2.47	2.14
	n	20	39
Fruits	# Fruits/ 1st branch	1.81	1.92
	StDev	0.85	1.1
	n	26	24
	Mass/ Fruit	0.019*	0.013*
	StDev	0.002	0.002
	n	26	24

Notes: T-tests used to compare means across columns; * $p < 0.10$; ** $p < 0.05$.

Germination rates

Germination rates were significantly higher for ripe seeds from unparasitized plants than for those from parasitized plants (p-value: 0.0275, $\square=0.10$, Table 3). Among parasitized plants, germination rates were significantly higher among ripe seeds from parasitized branches than from unparasitized branches (p-value: 0.0969, $\square=0.10$, Table 3). There was no significant difference in the mean number of seeds per ripe fruit between parasitized and unparasitized branches (p-value: 0.8617, Table 3) or parasitized and unparasitized branches among parasitized plants (p-value: 0.2869, Table 3). The very small sample size of randomly-selected ripe fruits from

TABLE 3. Germination rates of ripe seeds; mean number seeds/fruit for unparasitized and parasitized plants and unparasitized and parasitized branches on parasitized plants.

	U. plant	P. plant	U. branch on p. plant	P. branch
Germ rate	0.125**	0.038**	0.008*	0.067*
StDev	0.031	0.018	0.023	0.023
n	4	12	6	6
Seeds/ Fruit	21.2	21.6	20.0	22.3
StDev	1.4	1.7	5.3	1.4
n	14	10	3	7

Notes: T-tests used to compare rates and means between columns; * $p < 0.10$; ** $p < 0.05$.

parasitized plants may be responsible for the lack of significant results.

DISCUSSION

Although I expected branches on parasitized *Pemphis* individuals to be shorter than branches on unparasitized individuals with comparable basal diameters, there were no significant differences in ratios of branch basal diameter to branch length. The results do not support my hypothesis that parasitized *Pemphis* individuals would be shrubbier than unparasitized plants and therefore do not suggest that the effects of *Cassytha* on its host are similar to those of herbivory described by Witham and Hopper (1985).

Branches of parasitized *Pemphis* individuals were significantly longer and had significantly larger basal diameters than branches of unparasitized plants, indicating that parasitized plants' branches are larger than those of unparasitized plants. The same is true for parasitized plants on parasitized branches in comparison with unparasitized branches. It is possible that the parasitized plants are older than unparasitized plants; the parasite may not have attached itself to the younger plants in the region yet, explaining why the unparasitized plants are smaller. It is also possible that the parasite has selected for larger host plants, particularly if branch size is an indicator of plant fitness. This would support the results observed by Kelly (2002), in which dodder individuals selected for resource-rich plants when choosing hosts.

Although parasitized plants had significantly fewer leaves per primary branch than did unparasitized plants, the total weights and surface areas of leaves per primary branch were not significantly different between the two plant

types. Parasitized plants must therefore produce smaller leaves than do unparasitized plants.

The comparable masses of leaves per primary branch indicate that approximately equal amounts of resources are allocated to the production of leaves among both plant types, parasitized and unparasitized, and both branch types within parasitized plants. The photosynthetic potential of the plants and branches is also roughly the same, since there is no significant difference between plant types in total leaf area per primary branch. In this sense, there is no apparent difference in plant fitness between parasitized and unparasitized plants and branches.

Fruits on parasitized plants weighed significantly more than did fruits on unparasitized plants. Since the plants were only sampled once during a season, it is possible that fruits from parasitized plants reach maturity earlier in the season than do fruits from unparasitized plants. If so, the difference in fruit size does not indicate differences in plant fitness. There is also the possibility that a high proportion of the fruits on parasitized plants fall before reaching maturity, as was observed by Gómez on *Hormathophylla spinosa* infected by dodder (1994). If so, the smaller fruits on parasitized plants are indicative of lower fitness among parasitized individuals. Further experimentation over a longer period of time could indicate which scenario is occurring on the Tiahura Motu.

The lack of significant differences in ratios relating leaf masses, fruit masses, and primary branch masses indicates that there is not a difference between plant types in the amount of resources allocated to photosynthetic, reproductive, and structural materials at the primary level. Further analyses could indicate whether differences exist at higher structural levels.

Since the number of seeds per fruit and the number of fruits per primary branch are approximately equal between plant and branch types, differences in germination rates are representative of plants' overall reproductive fitness. The rates indicate that unparasitized plants produce more viable reproductive material than do parasitized plants. The opposite trend is true within parasitized plants, with the higher germination rates of parasitized branches indicating better reproductive fitness than unparasitized branches on parasitized plants.

Using reproductive success as an indication of plant fitness, the results of this study suggest parasitized *Pemphis* individuals are less fit than are unparasitized plants, as indicated by the lower germination rates of ripe seeds. The reduced fitness of parasitized plants indicates that *Cassytha*

is a virulent parasite that reduces the fitness of its host plant.

The very small sample size associated with the counts of seeds per fruit may be responsible for the lack of observed differences, so repeating the study with more fruits could confirm whether parasitized branches actually produce a greater number of viable seeds than do unparasitized branches on parasitized plants. Additionally, germination trials were conducted using only ripe fruits, although counts of fruits per primary branch include fruits at all stages of maturity; classifying fruits by stage of maturity when counting would eliminate the possibility of artificially high germination rates among plants with high proportions of immature fallen fruits.

In a number of investigated parameters, parasitized *Pemphis* branches exhibited characteristics that may indicate higher fitness levels than unparasitized branches on parasitized plants. Parasitized branches were larger and had higher germination rates among ripe seeds than did unparasitized plants. It is possible that this trend is an indication of low virulence by *Cassytha*, in which the parasitic relationship has become one of commensalism (Gómez 1994), through evolutionary advantages to *Cassytha*. Based on visual observations in the field, however, this theory seems unlikely, since there were many dead *Pemphis* branches with dead *Cassytha* attached. Such observations imply that the presence of *Cassytha* on *Pemphis* leads to at least the localized death of the host plant. A future study comparing the percentage of dead branch material between parasitized and unparasitized plants could serve to confirm whether this is the case.

More likely, I believe that the high fitness of parasitized branches on parasitized plants indicates that *Cassytha* selects for fitness when attaching itself to a host plant. There were very few examples of production of fruit by *Cassytha* in the field, so I assumed the parasite reproduces primarily by attaching itself to nearby plants, and I did not consider the possibility of fruit dispersal patterns affecting the parasite's distribution on host plants. A controlled study of *Cassytha*'s host preferences when attaching to plants could confirm whether distribution is non-randomly in favor of resource-rich branches. Since it is possible that there is a lag time between attachment of *Cassytha* to *Pemphis* and the exhibition of deleterious effects, the parasitized *Pemphis* individuals may display reduced fitness in coming years.

It is also possible that parasitized *Pemphis* plants disproportionately allocate resources to parasitized branches, which in turn have higher

reproductive fitness as a result of overcompensation. Research tracking the reproductive health of parasitized branches over time could provide more information about *Cassytha*'s virulence and the parasite's long-term effects on *Pemphis*.

ACKNOWLEDGMENTS

Thank you to the faculty members and graduate student instructors who offered their support at the University of California Gump Station. Thank you in particular to Brent Mishler for his suggestions regarding research methods and to Jaime Bartolome for his guidance with research methods and statistical analyses. Thanks also to David Ackerly and Ellen Simms for their advice about project structure and past research on the subject.

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THE EFFECTS OF COMPETITION ON THE DISTRIBUTIONS OF THREE GECKO SPECIES ON MOOREA, FRENCH POLYNESIA

Natalie M. M. Reeder

Integrative Biology, University of California, Berkeley, 94720 USA

Abstract. The introduction of *Hemidactylus frenatus* to oceanic islands has been documented to cause the decline of native species in urbanized areas in several instances. *H. frenatus* was recently introduced to the island of Moorea, French Polynesia. A distributional study using transects showed that *H. frenatus* is only found on and around buildings while both *Gehyra oceanica* and *Lepidodactylus lugubris* can also be found in the forests. Behavioral experiments designed to test competition for insect prey were conducted on each species pair to determine their effect on one another. *G. oceanica* and *L. lugubris* seem to avoid one another while neither reacts to the presence of *H. frenatus*. The lack of direct effect of *H. frenatus*' presence may support an exploitation competition mechanism for displacements this species has caused elsewhere. A guide to the Gekkonids of Moorea was also constructed.

Key words: *Gekkonidae*; *Gehyra oceanica*; *Lepidodactylus lugubris*; *Hemidactylus frenatus*; French Polynesia; Moorea; introduced species; exploitation competition

INTRODUCTION

The classic image of natural science is one of bug collections and field notes cataloguing the diversity of animals to be found in the field. More recently, the focus has shifted to understanding the underlying mechanisms of that diversity so global biodiversity can survive the current extinction crises resulting from increasing human influence (Gosling, 2003).

Competition is a mechanism that has a profound effect on community diversity but is often overlooked because of its complexity. Competition can result in a clumped distribution and limit population size leaving the population more vulnerable in the event of habitat destruction or disturbance (Bustard 1970; Dugatkin 2004). Introduced species have caused the loss of many indigenous species by out-competing them. Increased human mobility and habitat disturbance has resulted in a dramatic rise in non-indigenous species introductions. In Florida in the United States, the green anole, *Anolis carolinensis*, was introduced and out-competed a native anole species, eventually replacing it (U.S. Congress 1993).

Tropical islands are particularly sensitive to these introductions. Because of their isolated nature, they support a small number of highly specialized indigenous species that tend to be less able to adapt to changes in the environment (Whittaker 2002). In the Mascarene Islands the gecko *Hemidactylus frenatus* has caused the near extinction of two endemic species of the genus *Nactus* by excluding them from refugia (Cole et al. 2005). Behavioral experiments on the geckos of Hawaii have shown that *H. frenatus* displaces *L. lugubris* populations there by means of exploitation and interference competition (Case et al. 1996; Case 1993).

In locations where multiple gecko species co-exist, one species usually dominates and monopolizes the prime foraging spots and refugia. Wherever *H. frenatus* is present, it is thought to displace *Lepidodactylus lugubris* and *Gehyra oceanica* while *L. lugubris* will displace *G. oceanica* from urban environments when *H. frenatus* is not present (Case 1993; McKeown 1978; Zug 1991). It is unlikely that a factor other than the introduction of *H. frenatus* could explain these displacements since introductions have occurred on hundreds of oceanic islands under different

conditions but with the same result (Case et al. 1996).

There are at least 5 species of gecko on Moorea in the Society Islands: *Phelsuma* sp., *Gehyra oceanica*, *Gehyra mutilata*, *Lepidodactylus lugubris* and *Hemidactylus frenatus*. All of these geckos are common human commensals so it is widely assumed that each species was carried by a human vector, but the timing of their introductions is unclear (McKeown 1978). It seems likely that *H. frenatus* is the most recent introduction since it was not found in a 1988 survey of the lizards of Moorea (Ineich 1988).

The relatively recent introduction of *H. frenatus* to Moorea provides an intriguing opportunity to investigate the effects *H. frenatus* is having on the distribution of the resident gecko populations. If *H. frenatus* is having a similar displacement effect on Moorea as elsewhere, the behavior of the resident geckos in the presence of *H. frenatus* may support hypotheses of interference or exploitation competition as a cause. A behavioral experiment will add to our understanding of the mechanisms of species displacement prevalent in this system.

Determining whether the distribution of Moorean geckos follow a similar pattern to those documented elsewhere can illuminate the processes that establish species dominance as well as how introduced species affect native species. If the distributions follow the same patterns, then the future effects of *H. frenatus*' introduction may be inferable from the events that have transpired on other tropical islands and conservation efforts can be made.

The purpose of this study is to conduct a behavioral experiment to determine how three of the Moorean Gekkonid species affect each other's behavior, to make a preliminary comparison of their distributions, and to compare these results to the associations found elsewhere in the hope of identifying a mechanism for native species displacement by invasive *H. frenatus*.

MATERIALS AND METHODS

Distribution

An equal number of transects were established in each of three elevation zones, below 100 m, between 100 and 200 m, and above 200 m. A transect 20 paces long was sampled every 100 paces along the trail in a random direction determined by a coin flip. Every tree

along the twenty pace transect was inspected for geckos. Geckos were identified to species by observation of dorsal patterns, size and toe pad and claw morphology either on the tree or upon capture. The number and species of geckos found was recorded as well as the dominant vegetation and conditions of the transect area. The elevation of each transect was recorded by GPS where possible or determined by locating it on a topographic map of the island. The transects were established on the Cross-Island trail and unnamed trails at the Gump Station and in Mari Mari's garden (fig 1). All transects were done between 16:00h and 19:00h, September 18 to November 19, 2005.

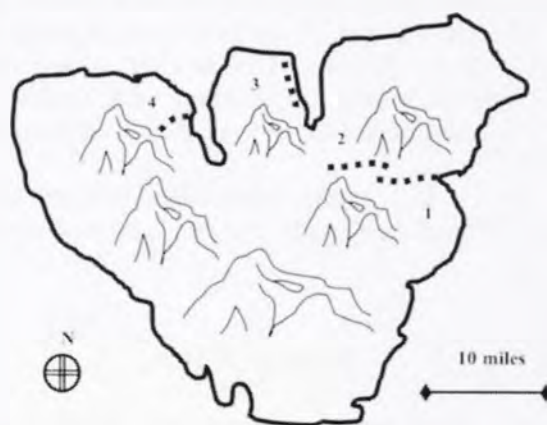


FIG. 1. The four transect sites on the island of Moorea, French Polynesia where all research was done. The transects encompassed three elevation zones reflecting levels of human disturbance and urbanization.

Competitive interactions

Three geckos, one each of *G. oceanica*, *L. lugubris*, and *H. frenatus*, were captured each night of the study on the U.C. Berkeley Richard Gump Research Station on Moorea, French Polynesia between 18:30 and 19:30h, September 18 to November 19, 2005. Upon capture, each gecko was identified to species. Its environment, including substrate on which it was found and height from the ground was recorded. I also noted its behavior if I observed it eating, drinking or interacting with other geckos before capture or if it behaved interestingly upon capture. Each captured gecko was placed in a holding terrarium with shelter and water. Once one of each species was obtained, a tubular black light was turned on at one end of the experimental tank to attract insects. The

experimental tank was a plastic aquarium 62.1 cm long by 31.8 cm wide by 26 cm tall fitted with a top made of wire mesh of ¼ inch squares stapled to a wooden frame that fit snugly on the aquarium (fig 2). The light attracted a constant presence of 5 to 6 glassy-winged sharp shooters, 4 to 5 moths, and 1 to 2 other insects on average. The mesh allowed the insects to fly through into the tank.

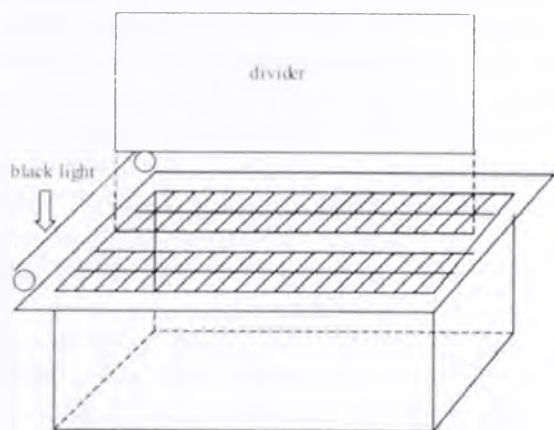


FIG. 2. The test terrarium with a metal mesh top and a removable, opaque divider was used to test each gecko's access to insect prey attracted to the black light at the left end both alone and in the presence of another species.

At the start of each trial a divider made of aquarium plastic wrapped in masking tape to form a visual barrier was placed in the test tank to divide it down the center the long way so each side had a lighted end and a dark end. Two geckos of different species were placed on either side of the divider. They could not see one another. They were then observed in this condition for a half an hour. Their distance from the lighted end as well as side of the cage was recorded every three minutes using a centimeter ruler attached to both sides of the top. After a half an hour, the divider was removed through a slit in the top screen that was then resealed with Velcro. Once again, their distance from the lighted end and the side of the cage they were on was recorded every three minutes for a half hour. Their behavior and any conflict or insect predation was recorded during the hour trial. These trials were then repeated in the same manner until all three combinations of gecko species had been tested.

After each trial, the geckos were returned to the holding terrarium. Each gecko was then sexed and measured for snout to vent length, tail condition, total tail length, length of tail

regeneration where applicable, and weight. Sex was determined by looking for enlarged femoral and pre-anal pores for males and the presence of eggs and lack of pores for females. Snout to vent length was measured using a clear, plastic millimeter ruler from the tip of the nose to the cloacal opening. Tail condition was determined by checking visually for a point of regeneration. The tail length and point of regeneration were measured with the ruler starting at the vent. The weight was obtained by suspending the gecko in a pre-weighed bag from a spring scale. Any scars that could be used for individual recognition were drawn and a representative digital image was recorded. Finally, the gecko was returned to its exact location of capture.

Statistical analyses

In order to determine if the distributions of the three gecko species are significantly different, the transect data were analyzed with a Chi-Square test of independence. A significant result suggests different habitat preferences or uses and when used in conjunction with data from other locations it could indicate possible interspecific effects on the geckos' distributions.

The raw data from the behavioral tests were used in three ways to determine what effect the presence each gecko species had on the behavior of other two. First, the raw distance data were analyzed with a Kruskal-Wallis non-parametric test to determine if there was a significant difference in the distance of the gecko from the lighted end of the cage when it was alone and with another species. If the test found a significant difference, then we could conclude that the presence of a different species has an effect on the behavior of the other. From my hypotheses I would expect that whichever species remained close to the light in the presence of another would be the dominant species.

Secondly, the raw data were used to obtain the relative distances of each lizard from the other during the trial. These data were tested with a Kruskal-Wallis non-parametric test to determine if any of the species moved significantly farther away from another when the divider was removed. Such a result would support an avoidance mechanism for competitive displacement.

Lastly, the size differences between the geckos in each test were plotted against the average relative distances from the period of the

trial without the divider. A regression line was fitted to this graph to determine if there was a correlation. A significant correlation would suggest that size might play an important role in the outcome of interspecific competition of these geckos.

RESULTS

Distribution

Transects revealed a significant difference in the distributions of the three species among three elevation zones reflecting the level of human disturbance (Chi-Square, $\chi^2=16.683$, $CV=9.49$, $DF=4$). *H. frenatus* was found exclusively below 100 m and always either on or very near a building. *G. oceanica* and *L. lugubris* were both found in all three zones, in the high elevation, native forest, in the mid-elevation disturbed forest, and in the urbanized zone below 100 meters on and around man-made structures (table 1).

TABLE 1. Transects were sampled in three elevation zones to establish the distributions of the three gecko species in the study. The total number of each species found is shown for each zone.

	<i>G. oceanica</i>	<i>L. lugubris</i>	<i>H. frenatus</i>
below 100m	4	5	9
100-200m	5	3	0
above 200m	9	1	0
totals	18	9	9

The presence of another species does not significantly affect the distance of a gecko from its prey

None of the species pairings had a significant effect on the distance of any of the geckos from the insect-attracting light. The data were tested for a difference in proximity to the light before and after the divider was removed. A Kruskal-Wallace test yielded no significant difference among *G. oceanica* alone, with *L. lugubris*, and with *H. frenatus* (Kruskal-Wallace, $\chi^2=2.8869$, $DF=2$, $p=0.2361$). The test showed no significant difference among *H. frenatus* alone, with *G. oceanica* and with *L. lugubris* (Kruskal-Wallace, $\chi^2=0.7608$, $DF=2$, $p=0.6836$). Again among *L. lugubris* alone, with *G. oceanica* and with *H. frenatus* the Kruskal-Wallace test found no significant difference (Kruskal-Wallace, $\chi^2=4.11$,

$DF=2$, $p=0.1280$). None of the species combinations significantly altered a gecko's distance from its prey.

L. lugubris and G. oceanica avoid each other

Data from the behavioral experiment were used to obtain the relative distance of each individual from the other before and after they were aware of the other. The relative distances before and after were tested for a significant difference. A significant difference was found between the relative distances of *L. lugubris* from *G. oceanica* before and after the divider was removed (fig. 3). Since the data was not normally distributed, a Kruskal-Wallace analysis was used to determine significance (Kruskal-Wallace, $\chi^2=20.46$, $DF=1$, $p<0.0001$).

No significant difference was found between *G. oceanica* and *H. frenatus* (Kruskal-Wallace, $\chi^2=2.7556$, $DF=1$, $p=0.0969$) or between *L. lugubris* and *H. frenatus* (Kruskal-Wallace, $\chi^2=1.2092$, $DF=1$, $p=0.2715$). These pairs of geckos did not move closer to or farther away from each other after the divider was removed.

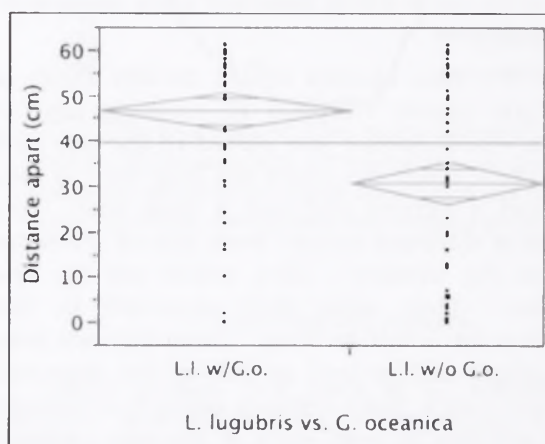


FIG 3. A one-way analysis of the distances apart of *L. lugubris* and *G. oceanica* before and after they are visually aware of one another. *L. lugubris* and *G. oceanica* are significantly farther apart once they are aware of each other.

Size differential may affect the distance of H. frenatus from L. lugubris

The average relative distance of one gecko from another was plotted against their size differential to determine if there is a significant correlation. Each species pair was plotted and fitted with a regression line. No significant

correlation was found between *G. oceanica* and *H. frenatus* (Regression analysis, $R^2=0.2501$) or *G. oceanica* and *H. frenatus* (Regression analysis, $R^2=0.118$).

A slightly significant size effect was found between *H. frenatus* and *L. lugubris* (Regression analysis, $R^2=0.6076$) with their average distance apart increasing as their size difference increased (fig. 4).

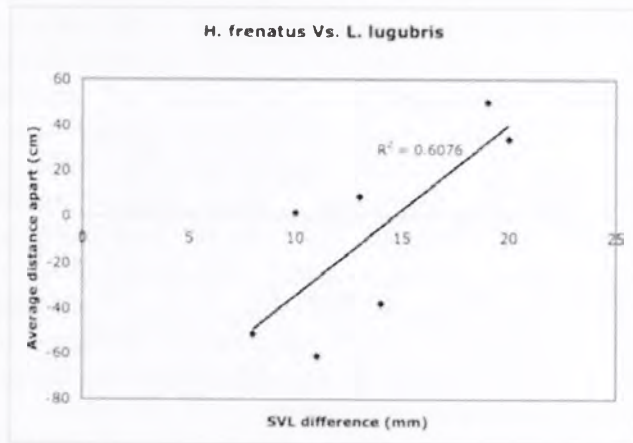


FIG. 4. The difference in size between *H. frenatus* and *L. lugubris* was correlated with their average distance apart to determine if there was a size effect.

DISCUSSION

The distributions found for *L. lugubris*, *H. frenatus*, and *G. oceanica* on Moorea are consistent with those found elsewhere. *L. lugubris* is often found in the forest as well as on or near buildings (Case 1993). *G. oceanica* is another species well known to inhabit forests as well as rural or suburban habitats with gardens near buildings (Zug 1991). *H. frenatus*' exclusion from the forest was not unexpected since they have been found to be poorly adapted to living on crumbly substrates such as the loose bark of trees in the forest (Cole 2005).

On other islands where *H. frenatus* has been introduced, it has caused a displacement of *L. lugubris* and others, including *G. oceanica* from urbanized areas (Case 1993). This study sought to determine which of several forms of competitive behavior could explain the advantage of *H. frenatus* over *L. lugubris* and *G. oceanica* in urban environments. Interference competition in which the presence of *H. frenatus* near a food resource would discourage other species from a prime foraging site results in a weakening of body

condition in the subordinate species from lack of access to high-density prey areas (Cole 2005). Direct competition in which one species fights, predares, or physically blocks another could also result in deleterious effects on the subordinate species (Case 1993; Bustard 1970). Exploitation competition in which one species has a biological advantage for capturing and consuming prey, thereby depleting the resource for other species, has emerged as the most probable explanation for competitive exclusion elsewhere (Petren et al. 1996).

The results of this study do not support a direct competition explanation. No predation and only one agonistic interaction were observed over the course of the study. None of the species appeared to defend the area closest to the insect-attracting light.

Interference competition seems to be a factor between *G. oceanica* and *L. lugubris* only. A significant increase was found in their distance from each other after the divider was removed. Such an effect was not found when either species was tested with *H. frenatus*. These results could reflect the evolutionary history of the three species on Moorea. *G. oceanica* and *L. lugubris* have been sympatric on Moorea for thousands of years, probably after being brought as stowaways on ancient Polynesian canoes (Petren 1998; McKeown 1978). *H. frenatus* has only recently arrived, not appearing until after 1988 (Ineich, 1988). As a result, *G. oceanica* and *L. lugubris* may have evolved a behavioral response to avoid one another resulting in niche separation. They may not have had time to develop such a response to the newcomer *H. frenatus*.

The lack of support for either direct competition or interference competition as a mechanism for *H. frenatus* dominance over *L. lugubris* and *G. oceanica* follows with the dominant hypothesis of exploitation competition. *H. frenatus* has been demonstrated to have a hunting advantage over *L. lugubris* due to its larger size and over both *L. lugubris* and *G. oceanica* due to its faster running speed. As a result, *H. frenatus* is more successful during foraging sessions when resources are clumped and depletes the insects causing lower prey availability for other species of geckos foraging in the same area (Petren et al. 1996).

Size is often an important factor in gecko competition with larger individuals out-competing smaller ones (Petren et al. 1996). The correlation test results do not support a size effect

on the behavior of the species combinations except for between *H. frenatus* and *L. lugubris*. The lack of a size effect on the other two species combinations could be a result of the small sample size and further investigation needs to be done to determine the role of size in this system.

Without data showing how the population densities of *L. lugubris* and *G. oceanica* have been affected by the introduction of *H. frenatus*, it is hard to discern what the behavioral effects found in this study may mean for the future of these populations. It must be considered, however, that the introduction of *H. frenatus* to other islands has almost always resulted in a decrease in the species previously inhabiting the man-made structures of that island (Cole 2005; Petren 1998; Case 1993; McKeown 1978). The possibility that the case on Moorea could follow the same pattern of species displacement is apparent. If competition is indeed observed between these species as elsewhere, a competitive displacement effect could be predicted, possibly causing the decline of an excluded species on Moorea as it did for the genus *Nactus* on Mauritius and requiring implementation of conservation efforts (Cole 2005).

Studies from other islands have resulted in a better knowledge of gecko conservation techniques. A simpler habitat structure with more clumped insect resources gives the greatest advantage to *H. frenatus*. Adding more complexity to the habitat can decrease interspecific competition and has been shown to reduce deleterious effects on *L. lugubris* in the presence of *H. frenatus* (Petren 1998). Providing artificial habitats of a more crumbly substrate can provide an *H. frenatus*-free refuge for claw-bearing species that are able to utilize such substrates (Cole 2005).

It is possible that *H. frenatus* has not yet begun to impact the resident populations of geckos, but with an increase in urbanization, *H. frenatus* may have a greater advantage. Increased urbanization means a simultaneous decrease in natural habitats. In combination with *H. frenatus*' dominance in urban areas, there could be serious implications for *L. lugubris* and *G. oceanica* if conservation efforts are not made on their behalf.

This system can be an important study model to gain much needed information on the dynamics of invasive species and competitive displacement. The interactions of these geckos reflect issues of inter and intraspecific competition,

invasive species, feeding behavior and human disturbance. Knowledge gained from study of these geckos can be applied to a variety of other systems.

Future studies

Future studies should make population estimates for all species present on the island and begin to track these populations over time. With this information we will be able to see the effect of *H. frenatus*' introduction as well as other factors affecting the herpetofauna of Moorea. A study to corroborate that *H. frenatus* does deplete insect resources can further support the hypothesis of exploitation competition. More comprehensive studies of the biology and ecology of each species are needed. Further study into the behavior of each species of gecko will give insight into their interactions with other species and information on their specific diet and refugia preference would reveal sources of competition.

ACKNOWLEDGEMENTS

Thank you to Prof. Craig Moritz for helping me in my preliminary investigations and providing me with vital field guides and relevant studies while I was in Moorea. Merci à Jacques You Sing for helping build the experimental terrarium. Thank you to Julia Vo for capturing the occasional stray gecko. Thank you to Sarah Daniels and Alicia Bihler for the use of their cameras and to Sara Lopus for lending me her GPS. I could not have had so much fun or gotten so much work done on my muddy night hikes without Danielle Fuchs. Thanks to Scott Fay and George Roderick for help with JMP and to Roy Caldwell for his help with behavioral experiment design and interpretation. Finally, thanks to Richard Gump for being such a party boy and making this wonderful experience possible.

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APPENDIX A

SPECIES OF THE FAMILY GEKKONIDAE FOUND ON MOOREA, FRENCH POLYNESIA

Gehyra mutilata

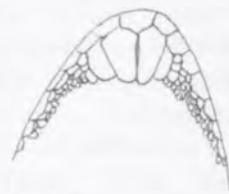
Habitat: Human commensal, forests

Description: Adult SVL: 42-50 mm. Uniformly light olive to dark olive-brown. Light and dark spots on head and body. Belly grayish olive to lemon yellow. Base of tail may be orange ventrally.

Right Foot



Mental Scales



Tail



Gehyra oceanica

Habitat: Found in the forests and rural and suburban areas

Description: Adult SVL: 59-84 mm. Uniformly light olive to dark-olive brown. Light spots all over body. Belly grayish olive to lemon yellow. Base of tail may be orange ventrally.

Gehyra mutilata



ventral

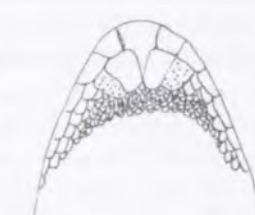


Hemidactylus frenatus

Habitat: On and around man-made structures

Description: Adult SVL: 48-58 mm. Uniform beige to grayish beige with small dark spots. Long slender toes. Makes gurgling threat call and chirping territorial call.

Gehyra oceanica

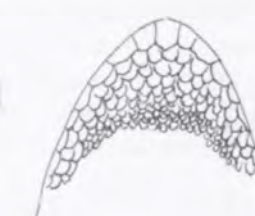


Lepidodactylus lugubris

Habitat: In the forest and on and around man-made structures

Description: Adult SVL: 34-44 mm. Grayish beige with widely spaced pairs of dark brown spots in a chevron pattern from neck to tail. Dark brown eye stripe. Belly yellowish white to light beige. Makes chirping call.

Hemidactylus frenatus



dorsal

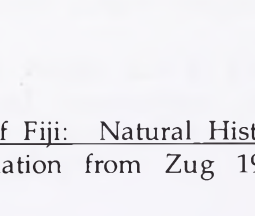
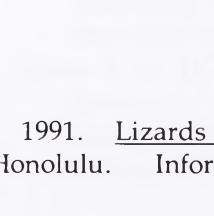


Phelsuma sp. (laticauda laticauda?)

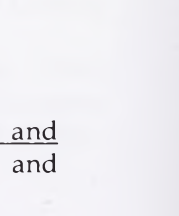
Habitat: Unknown. Sighted on the GSI bungalow and on a store shelf

Description: Adults 130 mm total. Uniform vivid green to yellow-green. Two short, bright orange stripes just anterior to the hind limbs.*

Lepidodactylus lugubris



ventral



*Figures redrawn from Zug, George R. 1991. Lizards of Fiji: Natural History and Systematics. Bishop Museum Press, Honolulu. Information from Zug 1991 and www.phelsumania.com.

KEEPING AN EYE ON YOUR NEIGHBOR: TERRITORIALITY AND HOME RANGES IN JUMPING SPIDERS

GINEVRA L. RYMAN

Environmental Science Policy and Management, University of California, Berkeley, California 94720 USA

Abstract. Territoriality plays an important role in structuring populations, but relatively little is known about the territoriality of jumping spiders (Araneae: Salticidae). Jumping spiders are charismatic and unique spiders, with their large median eyes, excellent vision, and adaptable predatory strategies. Since they do not spin webs to catch their prey it is more challenging to directly observe their territorial behaviors than with web-spinners such as orb-weavers. There has been little previous study done on the salticids of Moorea, French Polynesia. An initial survey was completed to see what species were present on the island, and to see if biodiversity varied with elevation. This study then went on to investigate the territoriality of *Thorelliola ensifera* on the large tree fern *Angiopteris evecta*. Field observations show that females have a much smaller home range than males who move large distances in search of receptive females. Females may also exhibit stronger site fidelity once they have laid eggs, and remain close to them until they hatch. In a system where a male's lifestyle emphasizes courtship and searching for females, silk draglines may prove a useful method of locating a mate. In a series of laboratory experiments, I tested whether the presence of silk from the opposite sex significantly altered behavior compared to silk from an animal of the same sex. The results showed no significant difference in behavior in either males or females. While chemicals in silk may still play a role in mate location, visual cues remain important to this highly visual animal.

Key words: *arthropods; Salticidae; Society Island; French Polynesia; pheromones*

INTRODUCTION

A territory is a site which an animal or group defends for a long period of time, compared to their lifespan (Stamps 2004). It differs from a home range in the fact that the site is virtually exclusive to an individual or group (Rolando 2004). There are numerous debates about how the adaptation of territoriality arose. It has been proposed that territoriality helps to control population densities (Kalela 1954), allocate existing resources (Brown 1964), protect individuals from predation and disease (Horn 1968), and increases an individual's relative fitness (Verner 1977). Territoriality has been shown in many taxa from vertebrates such as the red squirrel *Tamiasciurus hudsonicus* (Rusch and Reeder 1978) and the pika *Ochotona hyperborean* (Gliwicz et al. 2005), to invertebrates like the limpet *Patella longicosta* (Branch 1975), and the harvester ant *Pogonomyrmex rugosus* (Holldobler 1976). Riechart (1981) conducted a series of experiments with the desert spider *Agelenopsis*

aperta, and found that it was adaptive for animals to sometimes occupy larger territories than necessary.

Further research on territoriality has been conducted on web building spiders such as the orb weaver *Metabus gravidus* (Buskirk 1975), which lives communally but defends its own feeding area. Christianson (1984), and Riechart (1978) have also shown that other web-builders also defend their web as a territory. Wandering spiders like the wolf spider have also been shown to exhibit territoriality (Moya-Larano et. al., 2002). Gillespie & Tabashnik (1994) studied residence time and web site tenacity in the happy face spider, *Theridion grallator*. This spider uses minimal webs for prey capture, and they found that it is able to forage away from its web. Most spiders are potentially cannibalistic and aggressive toward other spiders near them (Hodge and Uetz 1994) and the role that territoriality plays in their interactions provides room for further study.

The Jumping spiders (Salticidae) are a remarkably diverse family, containing more than four thousand species (Coddington and Levi 1991). They occur on all continents except Antarctica, and have diversified widely to fill niches in many distinct habitats. Jumping spiders have the most highly developed sense of sight of all the spiders, and are characterized by their two large anterior median eyes, and an overall range of vision covering 360 degrees (Foelix 2002). Salticids are diurnal, and active hunters (Jackson 1998) and sight plays an important role in locating mates as well.

Few people have studied the Salticidae on the island of Moorea, French Polynesia. J.W. Berry and J. A. Beatty visited the Society Islands in 1987, and published a survey of the jumping spiders of the Pacific islands in a series of three papers (Berry et al. 1996, 1997, 1998) describing new species and renaming others. The primary objective of this study is to contribute to knowledge of the species present on the island, their distribution, and basic territorial behavior. In this study I surveyed variation in biodiversity with elevation. This was accomplished by sampling spiders at six sites across the island at sea level and 300 meters (See Fig. 1). I hypothesize that the higher elevation sites will be more diverse, because the habitat is less disturbed.

I then focused on the jumping spider *Thorelliola ensifera* (Thorell 1887) (Salticidae), to study its biology in detail. Unlike the jumping spider *Psecas chapoda*, (Romero & Vasconcellos-Neto 2005), *T.ensifera* has no association with a specific plant. It occurs on many different plants across the island of Mo'orea and is cosmopolitan throughout the Pacific (Berry and Beatty 1996). Initial field observations showed *T. ensifera* to be commonly found on the fern *Angiopteris evecta*, which is also known as the giant fern. It can reach heights of several meters, and provides a unique microhabitat on which to observe jumping spider behavior.

Salticids do not build webs to capture prey, but they use silk as draglines, and to construct sleeping burrows and egg cases. The jumping spider *Phidippus johnsoni* inhabits the same site for prolonged periods of time, sometimes from 28 to 33 days (Jackson 1978). The lifestyle of males focuses on locating mates, and courtship (Jackson 1979) while females lay several egg sacs on the underside of concave leaves, and construct retreats on adjacent leaves. This may be suggestive of

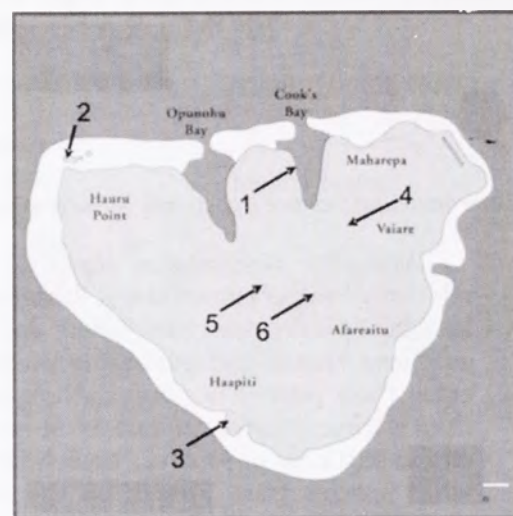


FIG. 1. Location of sites sampled in this study. (1) Hill behind Gump Station (2) Motu Tiahura (3) Mangroves (4) Peak of cross island hike Vaire-Pao Pao (5) Agricultural School (6) Three coconuts trail

maternal care of the young (Rossa-Feres et al. 2000). I hypothesize that *T. ensifera* females would have a small territory near their burrow and that males would roam across these territories in search of females. Observations of *T. ensifera* in the field suggested that the presence of silk may play a role in site selection and locating a mate. Accordingly, experiments were set up to test the role of silk in the location of habitat and mates for these spiders.

METHODS

Diversity at different elevations

To examine whether species composition, distribution and diversity of jumping spiders varied with elevation, three study sites were chosen at sea level, and three sites at 300 meters. The sea level sites consisted of the hill leading to the Gump Station, the second mangrove site south of Ha'apiti, and Motu Tiahura. The three sites at 300 meters were the Three Coconuts trail at the Belvedere, the trail from the agricultural school to the Belvedere, and the top of the pass on the Vaire- Pao Pao trail. (See Figure 1).

Spiders were found and collected using visual scan, sweep net, and beating sheet methods. The beating sheet method consists of a meter square sheet, which was held under the bush or tree to be sampled. The plant was then hit 5 times with a large axe handle, and spiders that fell were collected in plastic vials, identified, and

released. Each site was surveyed twice for approximately two hours at a time. Animals which could not be identified in the field were brought to the laboratory and identified under the microscope. Voucher specimens of each species are deposited in the Essig Museum at UC, Berkeley.

Movement and territory size

Focusing on the fern *Angiopteris evecta*, four fronds were randomly selected along the start of the three coconuts trail in the Belvedere. A thorough visual scan of each *A. evecta* was done. Animals were captured in a vial and then transferred to a small ziplock bag. A hole one to two millimeters in diameter was cut in the middle of the bag. Four different colors of Santee Plus nail polish were assigned numbers one through three (e.g. silver=0, yellow=1) and used to assign individuals a two digit identification number. The animal's abdomen was then positioned under the hole and two colored dots of nail polish were applied. While in the bag the width and length of the animal's cephalothorax were measured using calipers. The sex and level of maturity of the animal was also recorded. Animals' initial positions were marked on the *A. evecta* with a dot of black Sanford Sharpie. The four ferns were then visited a total of ten times in the following two weeks. Each visit the number of marked animals found was recorded and their positions were marked and measured. All new animals were marked and measured in the same fashion.

Site selection

Ten mature males and females were collected from *A. evecta* in the Belvedere. They were each

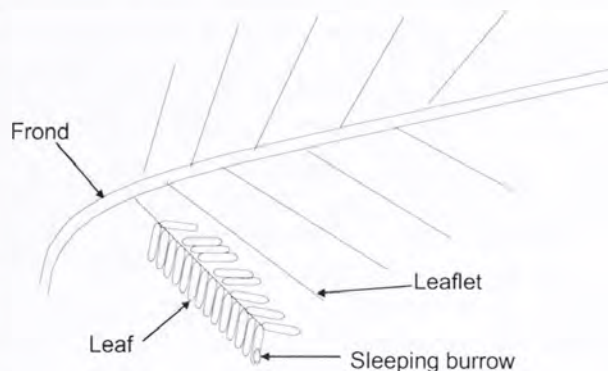


FIG. 2. Schematic diagram of *Angiopteris evecta*, and a common location of a sleeping burrow.

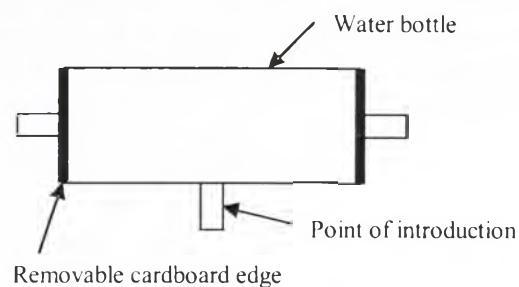


FIG. 3. Silk exposure behavior arena.

housed in a clear, cylindrical 1.5 liter water bottle, with the top cut off and replaced with fine mesh. Several centimeters of potting soil were placed on the floor, and the cage was misted to provide humidity. An arena was constructed out of a clear, cylindrical, 1.5 liter water bottle. The top and base were cut off using a razor blade, so that the length of the arena measured 20 cm long. Removable cardboard sides were cut for the ends of the arena, with holes to set vials into.

Two sets of experiments were run, to observe how silk from an unknown individual affects behavior. Animals were moved into vials the afternoon before the experiments were run. Overnight, they constructed sleeping burrows in the vials. If an individual did not make a burrow it was thrown out. The first experiment one of the vials was empty and sterile while the other contained silk from an unknown individual of the same sex. The sides of the area where they were placed were randomly selected. The individual whose silk burrow was used, was also randomly selected. The trial was run for 20 minutes and notes were taken on the behavior of the animal including the amount of time spent on either side of the area, what time it entered the vial (if at all), and what time and the duration of any mating dance that it began. The second experiment was set up in the same fashion, except that the unknown individual was of the opposite sex.

Data analysis

A Chi Square was done on the biodiversity data. The high elevation sites were compared to the low elevation sites to see whether there was a significant difference in species diversity and composition at different elevations.

A one way ANOVA was also completed to determine whether animals spent significantly longer on one side of the arena in the laboratory experiment. and to compare data from the field observations.

RESULTS

Diversity at different elevations

A total of 107 animals were collected. This sample represented a total of five species (*Bavia aericeps*, *Thorelliola ensifera*, *Plexippus paykulli*, and two unknown species). Two unidentified species were found only at Motu Tiahura. At the mangrove site only *P. paykulli* was found. A Chi Square was done to see if there was a significant difference in biodiversity among elevations and sites. The *B. aericeps* data as well as the miscellaneous data were left out of the Chi-Square because the values were less than five. The Chi Square value was 4.008 and the critical value is 5.99, so we fail to reject the null hypothesis.

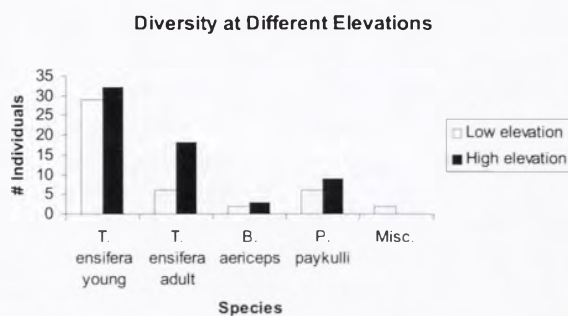


FIG. 4. Species sampled at low and high elevation.

Movement and territory size

Sixteen animals were marked (5 males, 11 females), from four *A. evecta* fronds. The males had a significantly wider cephalothorax than females $F=9.065$ $p<0.001$, and they were almost significantly longer $F=3.805$, $p>0.071$. Only one male was recaptured. Out of the eleven females, seven were recaptured a total of 20 times. The average distance moved from the original location was 0.51 meters. While observing males in the field I saw several move from one frond to the next, a distance of several meters, in a matter of minutes. The four fronds from the study site were between two and two and a half meters long. Leaflets were 20 centimeters apart on each side. Where each leaflet connects to the frond there is a

wider base or "bulb". I often observed spiders on or near these. Animals were not found in close proximity to one another, generally not closer than 50 centimeters.

Once when placing a male back onto the fern, it encountered another male. It began performing a mating dance. The other male then chased it away.

Site Selection

Only one male and one female entered the vial that contained silk from an animal of the same sex. No animals entered vials which contained silk from an animal of the opposite sex. I excluded one of the male versus male trials from the ANOVA, because it never left the vial, and spent no time on either side of the arena. The time spent on the side of the empty vial was not significantly different between the groups ($F=0.9492$, $p>0.44$). The spent on the side of the silk containing vial was not significant either ($F=0.5704$, $p>0.64$).

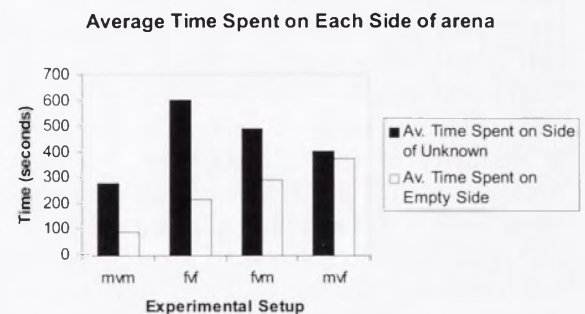


FIG. 5. Species sampled at low and high elevation.

DISCUSSION

Jumping spiders on *A. evecta* may be spaced out in home ranges to allocate food resources (Brown 1964), provide themselves more protection (Horn 1968), or to increase each individuals relative fitness (Kalela, 1954). The bulb where each leaflet attaches to the frond, may offer the spiders protection by helping them hide from predators, or allow them to catch more food by hiding from their prey. Field observations show that by building sleeping burrows near egg sacks, female *T. ensifera* exhibit parental care of the offspring (Personal Observation, Jackson 1978). It is necessary for females to establish a territory in order to adequately protect their offspring, thereby enhancing their relative fitness. Males appear to move across such vast spaces compared to their body size, that it would be difficult to defend the area as an exclusive territory.

This study has not demonstrated that jumping spiders have territories, but only home ranges which may or may not be exclusive. Since other animals were not observed inside these home ranges, it is likely that they may be defended as territories. Experimentation needs to be done to test whether *T. ensifera* actually defends these home ranges, such as the studies done by Moya-Larano (2002). A displacement study could be conducted to see how females etch out new territories or if they return to their old ones.

The results from the diversity study show how a few species have become very successful and are widespread across all elevations of the island. I initially hypothesized that diversity would be greater at higher elevations due to human disturbance around the perimeter of the island. The study showed there is no significant difference in biodiversity between elevations. This may be due to the fact that human disturbances have allowed these non-native spiders to colonize the whole island. The areas on the main island adjacent to Motu Tiahura should be further searched to see if the two unknown species occur there as well.

The results from the site selection trials showed that in my experiment, silk did not elicit noticeable behavioral changes. This may not be the case in the field as (Roberts and Uetz, 2002) have shown pheromones in silk alter wolf spider behavior. Jumping spiders are very visual animals so perhaps, chemical cues are not as important as visual ones. The spiders I used may have been at different reproductive stages, or may have already been mated with. This could be controlled by raising spiders in the laboratory to use in the experiment.

ACKNOWLEDGEMENTS

I would like to thank my field buddies Tanya Chapple, Alana Trotter, Ingrid Burke, Carmen Yeung, and Danielle Fuchs for keeping me safe and entertained. I would also like to thank Rosemary Gillespie, Carole Hickman and George Roderick for their advice, editing and encouragement.

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APPENDIX A

COMMON JUMPING SPIDERS OF MOOREA, FRENCH POLYNESIA



FIG. 6 Female *Thorelliola ensifera*. Three white spots on lower abdomen. Bands of coloration make it look like a "bee".

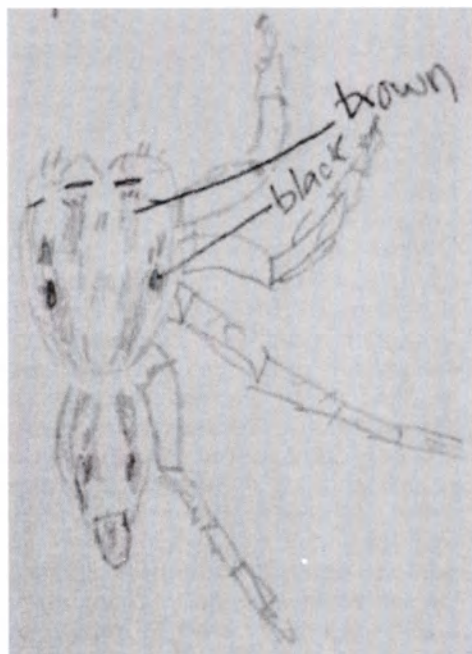


FIG. 8 *Plexippus paykulli*. Males have white "bars" on face. Both sexes have white stripe down back.

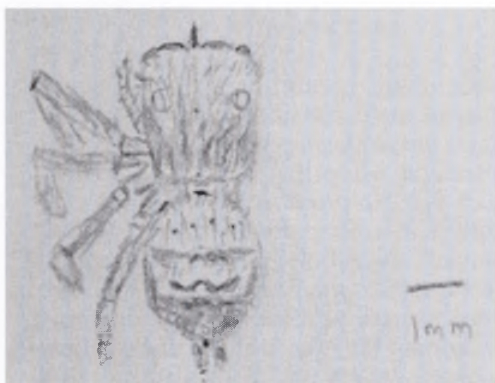


FIG. 7. Male *Thorelliola ensifera*
Much darker than female, legs same dark black as body. Three white spots on lower abdomen. Has fused setae which look like a horn between pedipalps.



FIG 9. *Bavia aericeps*. Front legs are longer than other commonly found species. Elongated abdomen with lightly colored legs and body.

There is a significant difference between the two groups in the number of children who are overweight. The difference is statistically significant at the 5% level. The results of the chi-square test are shown in the table below.

Group	Overweight	Not Overweight	Total
Group 1	15	85	100
Group 2	10	90	100
Total	25	175	200

The chi-square test results are as follows:

$\chi^2 = 1.38$

df = 1

p = 0.24

Since the p-value is greater than 0.05, we fail to reject the null hypothesis. There is no significant difference between the two groups in the number of children who are overweight.

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Since the p-value is greater than 0.05, we fail to reject the null hypothesis. There is no significant difference between the two groups in the number of children who are overweight.

INVESTIGATION OF FACTORS CONTRIBUTING TO THE DECLINE OF A NATIVE MEDICINAL PLANT, *CORDIA SUBCORDATA* ON MOOREA, FRENCH POLYNESIA

IAN SANCHEZ

Department of Integrative Biology, University of California, Berkeley, California 94720 USA

Abstract. Populations often experience bottlenecks, or extended declinations, from which each may succumb to extinction, or possibly return to a sizable, flourishing state. The current condition of the *Cordia subcordata* population on Moorea, French Polynesia is a diminishing one. Existing on many Pacific islands, *C. subcordata* mostly grows around coastal environments, where it can easily drop its fruit into the ocean so it can float along and disperse elsewhere. Because this environment describes Moorea perfectly, it seems implausible that *C. subcordata* would not be able to maintain a thriving population on the island. This study sets out to test growing and dispersal abilities of this native tree in order to gain more of an idea of light amount and soil preference, how much floating in sea water affects potentially germinating fruits, how long fruits can last in flowing sea water, and how well they can distribute themselves around specific island habitats. What is found is that not only does *C. subcordata* not have a statistically significant soil preference and its fruits' germination abilities are not explicitly affected by extended periods in flowing sea water, but its sea water dispersal abilities seem good enough to have continued the once large Moorean population. Fruits are found to have the ability to float in flowing sea water for long periods of time without sinking, with most continuing to float for over a month. A statistically significant result through one-way ANOVA analysis was that *C. subcordata* fruit prefer a higher amount of light during the germination process, but curiously only for a short period of time. Less light had a continually lessening affect on germinates as time progressed through the experiment. Each result has its own possible causal affect for the diminishment, and each is analyzed for possible contribution to the actual reason.

Key words: *coastal environment; sea water dispersal; germination; population declination; shoreline species*

INTRODUCTION

During initial human colonization, and along the way of societal advancement, native floral species play a crucial part in society both agriculturally, and culturally, no matter what area of the world is being discussed. Among the plants with a variety of uses in the societal progression on Moorea, French Polynesia was the *Cordia subcordata* Lam. (Clark et al. 2005). Not only was it used as a food source (the seeds inside the fruit are still commonly eaten today), but also in the construction of boats and crafts with its sturdy wood (Kepler 1990), and in healing, as potencies became knowledge of healers around the island (Petard 1972). Illnesses such as minor coughs, as well as other breathing sicknesses, could be cured by ingesting a mixture of leaf abstracts mixed with hot water (Petard 1972). As society advancement increased, population density, along with agricultural practice

potentially started to affect growth of *C. subcordata*.

A shoreline growing tree, *C. subcordata* is native to many Pacific islands due to its dispersal abilities. Fruiting practically year-round, *C. subcordata* drops the fruits to the sea it is usually found bending over. These fruits can then eventually reach back on shore after using their natural buoyancy to disperse out over the flowing ocean.

In the Hawaiian Islands, although once abundant along the coastlines, *C. subcordata* became a rarity for many years in the early and mid-20th century (Kepler 1990). With its fate being analogous to the Dutch elm after the Dutch elm disease caused a bottleneck in its population, *C. subcordata* now grows abundantly there (Kepler 1990). Similarly, a decline in the local Moorean population appears to be in the process (H. Murphy pers. comm.).

Certain potential causes of the evident decrease in population (H. Murphy pers. comm.) led to a variety of experiments to test

To fully test the third hypothesis, not only was an average dispersal distance and dispersal ability test created, but also a test to show how long *C. subcordata* fruit can float in flowing sea water. Each experiment was established in order to completely test each hypothesis, and to potentially answer the question of why *C. subcordata* populations around Moorea are vanishing.

Experiments for this study were conducted at the Gump Station along the shoreline of Cook's Bay, and at a nearby plantation of *C. subcordata* approximately 1 kilometer away from the Gump Station (17°30'S, 149°50'W) on the island of Moorea in the Society Islands of the French Polynesia (Figure 1).

main sand type around Cook's Bay and 3) "plain" gray sand without much extra substrate taken from a local beach. Samples were to be planted in each soil type and placed in three different light regimes: full sun, full shade, and partial sun, which was about half the light amount as the full sun examples. Mesh shade coverings were used to put over cages containing the full shade samples, while nothing was put over the cages housing the full sun sample. Partial shade samples were placed in the station's botanical garden under a shade covering that let in about half the amount of sun as the full sun samples got.

Amount of time floating in a flowing sea water tank was the final variable taken into account in this study. On a weekly basis new fruits were planted under specific light and soil treatments, with each weekly sample having one additional week of flowing sea water stress than the previous, starting with a control group having zero, the first week having one week, and so on. Fruits for these weeks' samples were taken from the flowing sea water tank used in the Flotation Ability Test. Seed pots were watered on a daily basis, unless the soil had retained enough water from the previous day's watering or from rain. For the combined four different weekly planted samples, a total of 99 pots contained fruits: 27 for each of the first three weekly sets, and another 18 for the sample that had been floating in the tank for 3 weeks.

Numbers of *C. subcordata* sprouts each planting pot contained was monitored as germination began to occur. These final numbers of total germinates were later analyzed on four separate days of the experiment using single variable ANOVA statistical analysis to test for preference.

Seeds per Fruit Data

To estimate the average number of seeds available to potentially germinate inside the walls of the fruits of *C. subcordata*, twenty fruits were taken from one specific tree in the local plantation on two separate occasions. The fruits were then opened carefully and each fruit's seed count was listed from the two twenty fruit samples to give an estimate of the average number of seeds per fruit.

Mark and Recapture of "Dispersed" Fruits

To test dispersal abilities of the *C. subcordata* fruit, a type of mark and recapture study was created in a different form than the typical test, primarily used for population size estimates. For this test fifty seeds were marked with a conspicuous color of paint. The fruits were then tossed into the ocean simultaneously in an area where a *C. subcordata* tree already existed at the time, to imitate what actually happens when that particular *C. subcordata* fruits and drops the fruit to the water. After about an hour had passed, dispersed "marked" fruits were then gathered with their respective distances traveled in the hour marked accordingly. Also noted was whether or not the fruit reached shore or was found still floating, as well as how many out of the total had been recaptured. This test was repeated in order to better the accuracy of the corresponding data.

"Gathering Area" Sample

To verify whether or not its fruits were dispersing in the area, *C. subcordata* fruit was counted among the rest of the fruits and seeds gathered in a pile on the seashore right outside of the UC Berkeley Gump Station. A total amount of all types was taken, which was used to get an average number of *C. subcordata* found in the mix.

RESULTS

Flotation Ability Test

While only twenty-eight of the original floating fruits remained in the flotation tank by the end of the experiment due to their use in the germination tests, a total of four *C. subcordata* fruits had sunk out of the possible one-hundred that spent time in the tank. The remaining twenty-four had been floating the entire experiment - approximately forty-two days.

Germination Tests

Light Variable: Of four chosen days to apply one-way ANOVA statistical analysis, days 23 and 30 yielded significant p values for an alpha of 0.05 ($p < 0.0002$ and $p < 0.05$ respectively), showing that the pots growing in full sunlight grew better than those in partial shade, which themselves grew better than those in full shade, rejecting the null

hypothesis that each light source will have the same growing rates. Days 17 and 37 yielded insignificant p values ($p < 0.32$ and $p < 0.2175$).

Soil Variable: One-way ANOVA tests for this variable were unable to reject the null hypothesis for any of the four days statistically tested ($p < 0.8385$, $p < 0.2236$, $p < 0.2360$, and $p < 0.2851$ respectively for days 17, 23, 30, and 37). This shows that *C. subcordata* grows similarly among each soil type tested.

Sea Water Variable: Also statistically analyzed with one-way ANOVA, results of the test yielded values of $p < 0.5958$, $p < 0.5756$, $p < 0.3021$, and $p < 0.7869$ for days 17, 23, 30, and 37 respectively, showing that sea water stress does not significantly affect growing rates of *C. subcordata*. See Appendix A for the mean number of seeds sprouted under each variable for each day analyzed, along with the error bars at each data point.

Seeds per Fruit Data

The average number of seeds per fruit from the first sample taken from the local plantation was 2.7, while the second sample average was a slightly higher 3.0.

Mark and Recapture of "Dispersed" Fruits

Figure 2 shows the graph of the distribution of the 100 dispersed fruits by 25 meter segments. The average distance traveled from the original point, although slightly skewed by a few outliers, was about 36 meters. Another result from this test is that out of the 100 dispersed fruits, 94 of them were recovered. Of those 94 recovered, 91 reached the shore to a potential germination site.

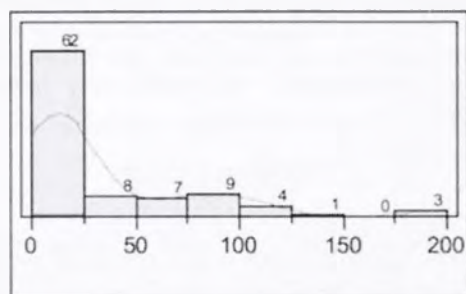


Fig. 2. Mark-Recapture study distribution of 100 fruits by increments of 25 meters.

The lack of significance in the germination test results, minus the light variable data,

shows that *C. subcordata* has no particular preference to soil type of the three tested, and that its fruits can also float through flowing sea water for a great amount of time to find germination areas, and still continue to germinate at its usual rate. Normal soil inclination of *C. subcordata* has been documented to be sandier and clay soils (Friday et al. 2005). Results of this study however show it grows just as well in darker, moist dirt found on the interior of Moorea, as well as in more rocky and coral rubble-filled sand, as it does in the fine gray sand it can sometimes be found growing in on local Moorean beaches and motus.

Although significant statistical results in the germination experiment could have shown a reason for the decrease in *C. subcordata* around the island to be caused by a having preferred soil to grow in, inability for this experiment to falsify this null hypothesis that there is no preference among the 3 soil types is interesting enough. The same idea goes for the rejection of the hypothesis that *C. subcordata* should show some sort of preference to more or less time soaking in flowing sea water. In his Origin of the Species, Charles Darwin suggests that certain plants whose fruits or seeds have the ability to float can disperse by ocean up to vast distances, but this number he mentions is only about ten percent (1859). With the combined results of the similar growth rates of the sample *C. subcordata* after all amounts of sea water flotation time, and those of the flotation buoyancy test (which shows over 90% of the fruits not sinking for up to 42 days), *C. subcordata* is among that minority of plants that can disperse large distances in order to expand their populations elsewhere.

A study to show that *C. subcordata* can not only float in sea water, but disperse and return to land after floating away from its source, was the pseudo "Mark and Recapture" study. While mark-recapture studies usually estimate population size (Budnitz 1998), this study was used to measure dispersal distance of fruits from an actual *C. subcordata* source tree. Results show that most fruits did return to shore, surpassing areas where any sort of blockade from the shore existed (none were recaptured while stuck in these make-shift blockades that include tall, thick roots and rock walls). A similar study has been done that suggests that barriers, such as piled rocks or high, long roots can be effective in fruits not returning to shore and germinating near them (Betz 2001). Many similar barriers exist in

Cook's Bay, the site of the experiment, but the study shows that these did not explicitly affect the dispersed fruits ability to return to the shoreline, as it seems most were able to surpass them and return elsewhere. As seen in the exact dispersal distances of each fruit though (Appendix B), certain lengths of areas existed where no returned fruits were recaptured, more than likely because of a barrier at those particular distances.

Along the entire distance which was searched for the 100 marked *C. subcordata* fruits were only two of these trees fully grown along with one younger tree estimated to be about 2 years old due to its size and documented *C. subcordata* growth rate (Friday et al. 2005). This, along with that fact that multiple unmarked *C. subcordata* fruit were also found along these shores, leads to the conclusion that it cannot be for lack of dispersal ability that it is not reproducing enough, but it is in fact not doing so.

A similar native tree on Moorea is the *Thespesia populnea* (L.) Soland. Ex Correa; it is similar not only because of its apparent medicinal values (Petard 1972), but because it disperses its seeds by ocean as well (Nakanishi 1988). Although not counted individually, it was apparent that less *T. populnea* were accounted for in the pile of gathered fruit and seeds in the Gathering Area Sample than *C. subcordata*. This should allow one to at least hypothesize that more *C. subcordata* exist on Moorea than *T. populnea*. Unlike the *C. subcordata* though, *T. populnea* maintains a stable population throughout the island (Betz 2001). Near the few *C. subcordata* trees seen around the island growing in its natural habitat along the beaches, *T. populnea* seemed to flourish, growing not only older trees, but young ones as well. Only one young *C. subcordata* was noted as a new generation tree growing near its probable mother. A

conclusion from this evidence is that while other trees may be growing new generations of their offspring near themselves without any secondary dispersal method such as ocean, *C. subcordata* seems to be doing both forms of dispersal, but neither form is working to continue its Moorean population, or at least not in large quantities.

Of the 3 variables tested in the Germination Experiment, *C. subcordata* only showed statistically significant preference among light amounts (See figure 3 for the day by day plot of number of sprouts for each light amount).

Its natural habitat is mostly in full sun, but can grow well enough in partial shade (Allen 2002), so the significance was expected here. Unexpected was the outcome of the fruits planted under the fully shaded cages. That they grew was surprising enough, but results of the ANOVA test telling that there is no significant preference in light amount as time goes on is very interesting. These results show that *C. subcordata* can not only grow on the shores in a lighted area, but can eventually grow just as well on the interior of the island, in the densely planted, very shaded areas were it to disperse there. Because of its seemingly inability to reproduce well enough currently, this could be a new trait it possesses in order to re-establish itself interiorly around the island and elsewhere, though this idea cannot be fully legitimized by this result alone, but perhaps from future experiments on *C. subcordata* and its growing capabilities.

When combining the two data sets in the Seeds per Fruit test, the average number of seeds in each of the 40 fruits sampled was 2.85, obviously well below the maximum amount of four. While some of the germinated samples did contain 4 sprouts, most did not. This is more than likely partially due to the inability of the fruit to

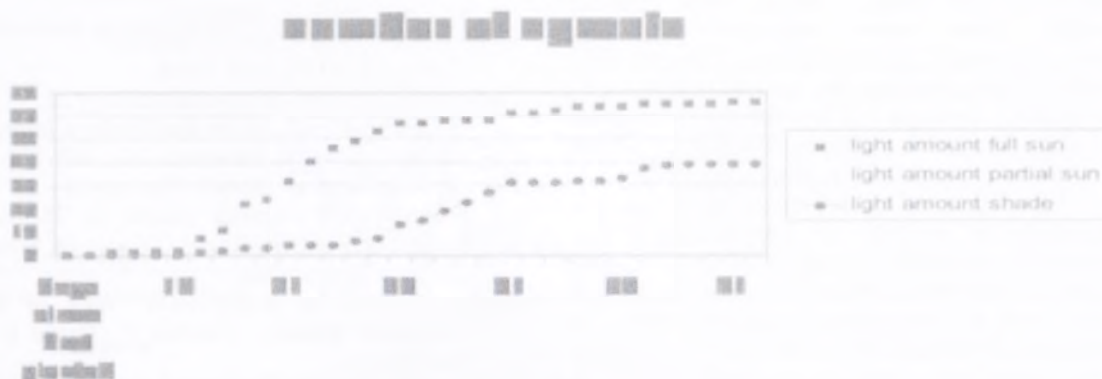


Fig. 3. Day by day sprout numbers are plotted starting with day 12 (no germination before this day).

fully open up and allow each seed to sprout, but the 2.85 average numbers of seeds in each fruit should also be considered because the fruits in both studies were taken from the same *C. subcordata* plantation. Because each fruit should potentially house 4 seeds, but rarely does, this can also be attributed in the lack of reproduction around Moorea. These data show that not only do most *C. subcordata* fruit not get fully fertilized to 4 seeds, but many even have less than 3, thus driving the average to 2.85.

What was once a successfully reproducing population on Moorea (H. Murphy pers. comm.), *C. subcordata* is now seemingly on an implausible decline among the naturally growing population. It is implausible because practically everything tested in this study shows that it should continue to thrive as it once did. Problems with pests exist on other island *C. subcordata* populations, but the pests tend to leave sort of trademarks, such as half-eaten leaves (Friday et al. 2005), none of which that have been studied and documented are seen on the Moorean trees. It could be argued that the tree's value as a wood source to natives causes the decline because all of the trees are being cut down. A problem with this theory is that *C. subcordata* has been used as a source of wood for such things as boats and crafts throughout the history of the local Polynesians (Rock 1974). This should mean that if the trees being cut down were a great problem to its reproduction, the population would have surely diminished a long time before now.

The question of why the population on Moorea is declining begs to be answered. An idea this study does not address is the affect of increasing urbanization around the island. This could definitely be a factor since it is an easy assumption that most of the population density will grow closely to the shore, in turn causing more settlements and housing set up closer to the ocean. Along with housing would come more industrial building, including hotels, grocery stores, churches, etc., each in turn affecting the natural habitat of *C. subcordata*. Perhaps it is less adaptable to these changes than such plants as *T. populnea*, whose population seems to be maintaining itself.

The future of *C. subcordata* on Moorea is currently in a state of uncertainty. The Hawaiian population was once in a similar state of diminishment but has since worked its way back to prominence (Kepler 1990). A similar fate could be possible for the Moorean trees, since they seem to maintain dispersal

and growing abilities according to the multiple experiments in this study that tested the various traits of *C. subcordata*.

ACKNOWLEDGMENTS

I would like to thank all of my professors and graduate student instructors of the Moorea class of 2005, George Roderick, Rosie Gillespie, Roy Caldwell, Carole Hickman, Matt Medeiros, Michele Weber, and especially Brent Mishler, who helped me with experimental semantics while he was on and off the island, as well as Jamie Bartolome and Scott Fay, both of whom guided me along the way of statistical analysis. Additionally I want to thank Frank and Hinano Murphy, who verified that a declination in the population of *C. subcordata* is indeed in effect.

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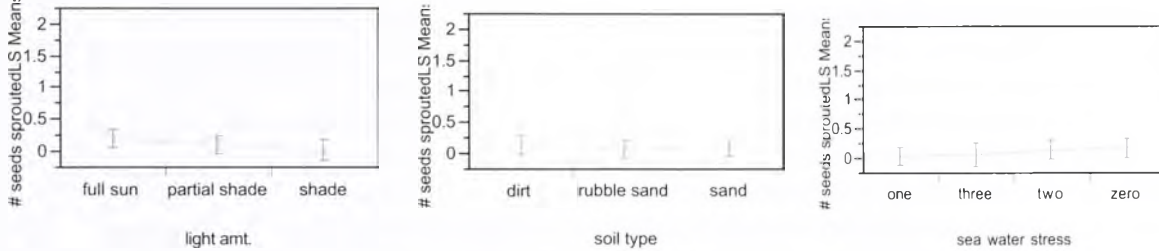
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APPENDIX A

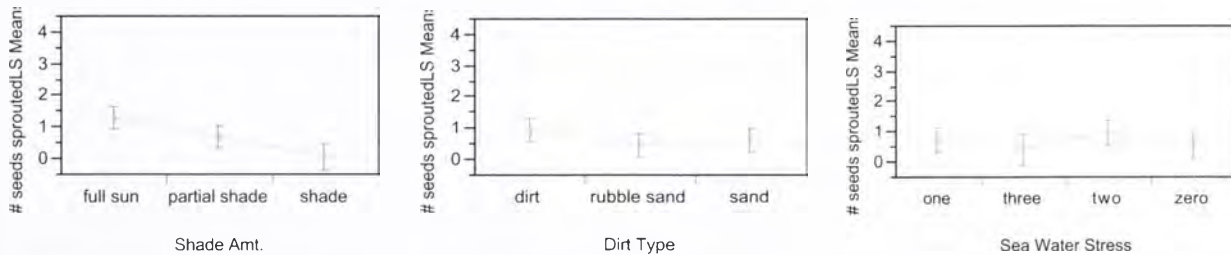
MEAN NUMBER OF SPROUTS PER VARIABLE ON STATISTICALLY TESTED DAYS

Day 17



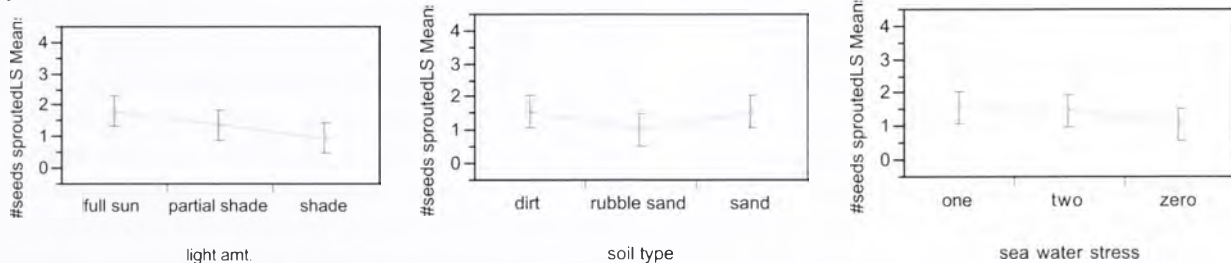
Day 17 mean plots. At this point the light variable does not show significance. Amount of sea water stress is in weeks.

Day 23



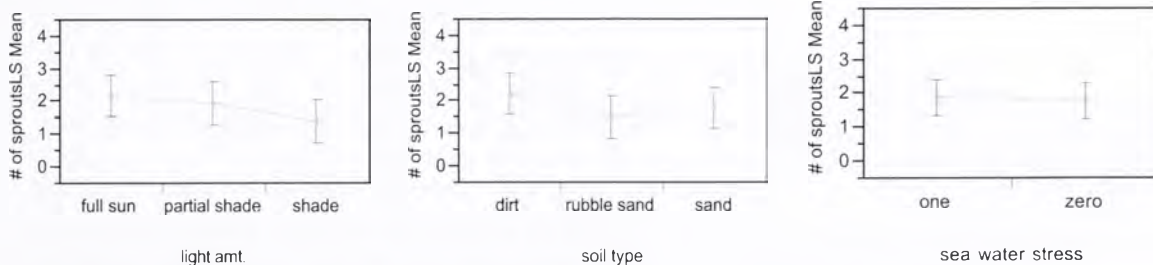
Significance among light amounts is obvious from day 23's plot.

Day 30



Significance is not as obvious on for light amount on day 30 as it is for day 23.

Day 37



C. subcordata no longer shows significant preference for light amount by day 37.

APPENDIX B

EXACT DISPERSAL DISTANCES OF EACH RECAPTURED C. SUBCORDATA FRUIT IN METERS

15	29.1	5.5	4.8	23.1
14	30.2	5.5	5.4	17
14	30.2	4.6	5.4	30.1
16.3	30.6	17.2	5.1	35.5
15.6	64.5	16.4	6.1	69.5
13.1	64.6	191	6.2	99.7
13.1	64.8	192	6.7	102.1
13.4	65.9	195	6.7	102.5
13.6	70.8	2	6.7	111
13.6	74.8	2.9	15.8	125.3
14	75.1	3.5	17.1	92
17	81.2	3.8	17.1	5.5
19	88	3.9	17.3	4.2
19.6	91.6	4	17.9	3.4
21.9	92.6	4	18.2	
22.2	98.2	4.2	18.4	
22.9	98.8	4.3	19.4	
23.1	111.8	4.4	20	
27	19.7	4.6	20.4	
27	19.7	4.6	20.5	

EFFECTS OF CURRENT AND PREY ODOR ON TWO NUDIBRANCH SPECIES, *GLOSSODORIS CINCTA* AND *CHROMODORIS LOCHI*.

HAMPTON CHAWNER SMITH

*Department of Environmental Science, Policy, and Management
University of California, Berkeley, California 94720 USA*

Abstract. This study examines the effects of current and prey odors (*Hyrtios erecta*) on the navigation and orientation behavior of the nudibranchs *Chromodoris lochi* and *Glossodoris cincta*. These nudibranchs are common subtidal predators of sessile animals in the tropical southern Pacific Ocean. In the presence of prey odor and current, *G. cincta* orients upstream and over 80% of animals successfully locate prey with indications of chemotaxis. Behavior of *C. lochi* showed upstream orientation in current but not in the presence of prey. When conspecifics were included in the odor plume with prey, *C. lochi* did orient upstream. In all cases, the presence of flow is an essential condition for locating distant prey.

Key words: nudibranchs; *Glossodoris cincta*; *Chromodoris lochi*; orientation behavior; chemodetection; Moorea, French Polynesia

INTRODUCTION

Gastropod molluscs have been used to the advantage of neurobiologists because of the relatively limited behavioral repertoires and accessible nervous systems of these animals. Both in terms of behavior and central nervous system, they are sufficiently complicated to be interesting but not so complex to be horribly complicated to describe. Important generalizations about the organization of small neural networks have emerged from such studies, including the idea that animal behaviors are organized in hierarchies of successively more dominant responses (Chase 2002). Analysis of behavior provides the background necessary for studies underlying of the underlying neural circuitry and function.

All animals rely on environmental cues using olfactory, tactile and visual stimuli to determine their surroundings. In the case of gastropod molluscs, their world has no sounds and, in most cases, no sights. Thus, the distance perception of gastropods usually depends on olfaction to detect chemical cues from waterborne molecules for information on their milieu. Chemoreception

controls or influences numerous specific behaviors including feeding, homing, aggregation, mating, escape, and avoidance (Chase 2002). Also, marine animals can use cues provided by current, the structure of the odor plume itself, or a combination of both factors to locate odorous prey in the aquatic environment (Teyke et al. 1992).

Nudibranchia (Opisthobranchia) is an order of Gastropoda that have been the examined in numerous neuro-physiological studies but their chemosensory behavior has not been well reported. Nudibranchs are sensitive to molecules exuded by prey and have relatively specialized diets (Megina et al. 2002). Experiments have

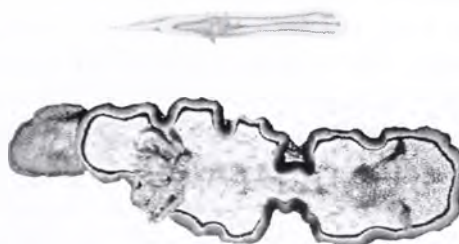


Fig. 1. Nudibranchs *Chromodoris lochi* (top) and *Glossodoris cincta* (bottom).

demonstrated their ability to locate distant food sources and differentiate between preferred prey (Elvin 1976, Rogers and Paul 1991). Unfortunately, most researchers report only orientation success rates but not path or orientation strategies. In addition Y-maze experiments are unsuitable for studying path parameters because of wall encounters (Caldwell 2005). There is little experimental evidence to elucidate the mechanisms used by nudibranchs to orient themselves toward a prey which may ultimately provide for a better model for the nudibranch nervous system.

Two particular species of nudibranch (suborder Doridina) are found in abundance along the rich tropical reefs of the Society Archipelago in the Southeast Pacific, *Chromodoris lochi*, Rudman 1982 and *Glossodoris cincta*, (Bergh 1888) (Fig. 1). These species of the family Chromodorididae are sponge feeders and share similar overlapping spatial niches. Chromodorids are characterized by their bright colors and elaborate patterns. Their colorful mantle is a form of aposomatic coloring; a warning sign to predators of their toxicity resulting from chemicals they have sequestered from the sponges of their diet (Cimino et al. 1983). They move by crawling from one substrate to another secreting a mucous layer from their large foot, allowing the animal to glide (Lambert 1991). These nudibranchs have a prominent pair of lamellated rhinophores at the front end of their bodies which have special receptors to "smell" chemical dissolved in the water (Seavy and Muller-Parker 2002).

A major problem in understanding prey detection for species in this family is that no study to date has made systematic comparisons of the behavior of nudibranchs in current and in still water with the appropriate control treatments. Therefore my research intends to establish a baseline for the navigation behavior of *C. lochi* and *G. cincta*. This investigation will examine the differences in the effects of current and prey odor on these two species. Observing the effects of presence or absence of current and presence or absence of prey odor will provide a comprehensive comparison of movement behavior. The primary objective is to quantify

their orientation and displacement in order to determine the mechanism by which they find prey. To discover prey-searching mechanisms, changes in the precision of orientation, movement speed, and linearity of movement must be measured. The observation that orientation towards prey becomes more precise with proximity would support a mechanism of chemotaxis. In addition, I am interested in the ability of these nudibranchs to locate prey in still water or whether current is an essential condition. Further, examination using two closely related species that share the same habitat may provide interesting clues as to differences in their behavior.

METHODS

Experiments were performed in an outdoor wet laboratory at the UC Berkeley Richard B. Gump South Pacific Research Station in Moorea, French Polynesia (S17° 29' 24.2", W149° 49' 34.6") in the Society Archipelago. Nudibranchs (*Glossodoris cincta* and *Chromodoris lochi*) and the preferred prey, the black sponge *Hyrtios erecta*, were collected by snorkel at depths of 1-5 meters from a lagoon adjacent to the station in Cook's Bay. The study was carried out from October 3 to November 18, 2005. The mean water temperature was 28° Celsius.

Subjects

Nudibranchs were exposed to the natural photo-period of the region and artificial low light intensity during some evenings. Specimens were acclimated to laboratory conditions over 7 to 21 days by simulating natural conditions in a large aquarium (140 liters) with continually flowing seawater pumped from a depth of 2 meters. During the study, nudibranchs were maintained on an excess of sponge but were starved at least 1 week before trials to motivate a feeding response. Before the experiments, behavior of each slug was monitored over one complete 24 hour period for baseline observations of temporal activity patterns and food preference under laboratory conditions.

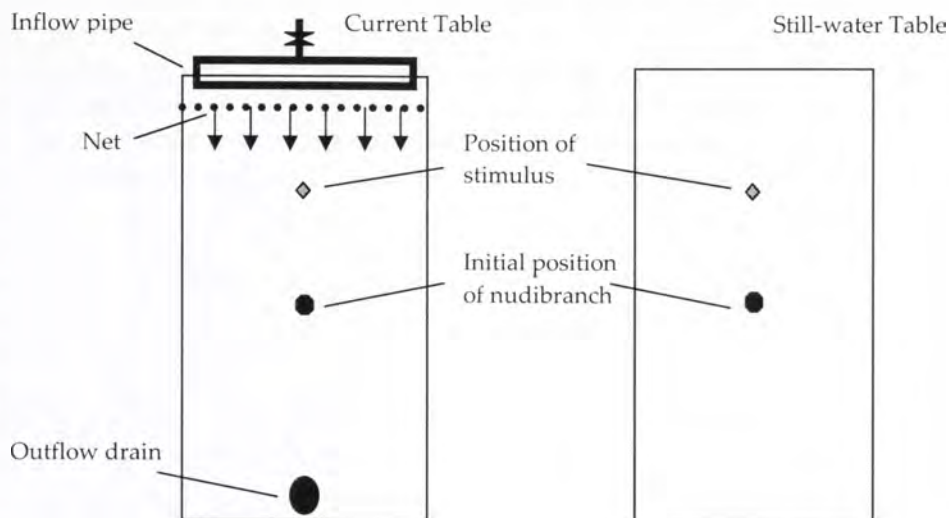


FIG. 2. Drawing of the experimental setup illustrating flow table and relative positions of nudibranchs and stimulus.

Sea table experiments

The responses of 20 individuals each of *G. cincta* and *C. lochi* nudibranchs to stimuli were examined in a 98 cm x 2.4 m water table (12 cm depth) constructed of wood and covered with fiberglass resin. A 3.3 cm diameter PVC pipe, pierced with holes at 3 cm intervals, supplied water at the head of the table. Zooplankton netting was stretched 10 cm from the source in order to create more uniform flow. A centrally located second PVC pipe (7 cm diameter) was placed on the downstream end of the table and evacuated water from the table maintaining the water level at about 6 cm. The rate of inflow and consequently the flow velocity was controlled by a valve in the tube supplying water at the head of the table (Fig. 2). During trials, the current velocity was nearly uniform across the table at a velocity of 3 cm/s (observed with florescent green dye). The current velocity selected was within the estimated range of flow at the habitat site. Trials in still water were conducted in a table of the same dimensions. Before each trial, the table was filled to a depth of 6 cm and left undisturbed for 30 minutes. Both tables were cleaned and emptied after each trial.

Preliminary experiments

Light preferences were examined using a 60 watt lamp suspended 40 cm above the center of the sea table containing 20 *G. cincta* for 3 hours during an evening. Animal movements were recorded and the trial was repeated for *C. lochi*. Next, aggregation behavior was quantified by placing 20 animals of each species in still water with observations made at the end of 12 hours. Lastly, trailing behavior of each nudibranch species was tested by first placing 4 animals at the center of a still water sea table to lay down trails for 30 minutes. After removal, a single individual from that group was placed in the table at a different starting point and left undisturbed for 30 minutes. Mucous trails were observed for path following. This was repeated for each animal of the group and for a total of 12 animals of each species.

Responses to odor and current

I evaluated the effects of current and prey odor on the behavior of *C. lochi* and *G. cincta* using combinations of presence and absence of current and presence and absence of sponge odor. Twenty replicates of each of the four treatments were performed for each of the two species using a new nudibranch for each trial. The trials were

run in pairs simultaneously, one in the flow table and the other in the still water table. The prey odor consisted of approximately 20 g of chopped sponge wrapped in black plastic screening. In trials without odor, an equivalent volume of dead coral was wrapped in the same way. In the treatments with current, the stimulus was placed in the flow for 3 minutes before introducing the nudibranch to allow a stable current and odor plume to develop. For the treatment involving prey odor in the still water table, the stimulus was put in place 20 minutes before introducing the nudibranch for a suitable odor gradient to form. In all treatments the stimulus was placed 25 cm from the head of the tables and the nudibranch 30 cm further down perpendicular to the stimuli, so as not to bias their orientation. One additional treatment was performed on *C. lochi* identical to the treatment with flow and prey odor with the addition of two conspecifics feeding on the prey. These results were analyzed separately.

In all trials for each treatment, photographs were made of the mucous trails with a digital camera after i) the odor source was reached, ii) the table wall was touched, or iii) after 10 minutes had elapsed.

For each nudibranch, orientation was evaluated by calculating the angle from which it left a 25 cm radius circle centered on the starting point, relative to a straight line toward the stimulus (Fig. 3). Individuals that did not exit the circle within 10 min were excluded from this analysis. The Rayleigh test of uniformity was performed to determine whether orientation was significant. Rayleigh testing determines if observed samples of angular data have a tendency to cluster around a given angle indicating a lack of randomness of the distribution. This circular statistic is based on the concept of the mean vector length, r , which is proportional to the strength of orientation on a scale of 0 to 1 (Batschelet 1981). To further analyze the orientation of nudibranchs that successfully located prey in the treatment involving both current and odour, and to determine whether orientation toward the prey improved as the nudibranch got closer to the odour source, I calculated mean vectors (r) as above for a series of concentric circles with radii of

5, 10, 15, 20, and 25 cm. I then applied a regression to the plot of mean vector length against distance from the starting point.

For each trial, the mean path length and travel time were measured beginning from a 5 cm radius concentric circle centered at the starting point to the 25 cm radius circle. I calculated the displacement speed using the path length divided by the time spent traveling. In addition, an index of linearity (R. Caldwell, personal communication) was created using the linear length from the starting circle at 5 cm to the 25 cm radius circle (i.e. 20 cm) divided by the actual path length of the animal. Values close to 1 indicate linear movement and small values imply a convoluted path.

The responses of linearity and displacement speed on current and odor were compared using ANOVA. To analyze the effect of current and odor on the nudibranchs to move toward the stimuli (sponge or coral), I applied an ANOVA to the deviation from the position of the stimulus (when nudibranchs left the 25 cm radius circle centered on their starting points). Lastly to examine whether current had an effect on success of the nudibranch in finding prey, I performed a binomial test on the proportion that reached the prey in current compared with still water.

Photographic data were analyzed for path length and displacement angle using the software ImageJ provided by the National Institute of Health. Statistical tests were performed using the software Jump IN, except for the angular testing which utilized the software Oriana.

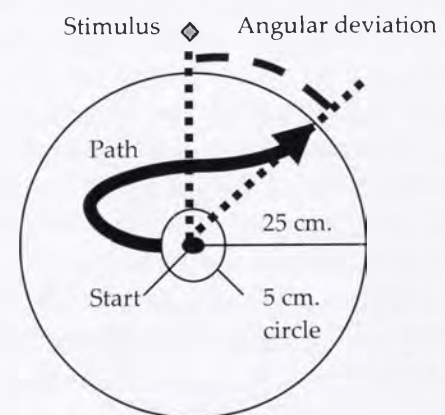
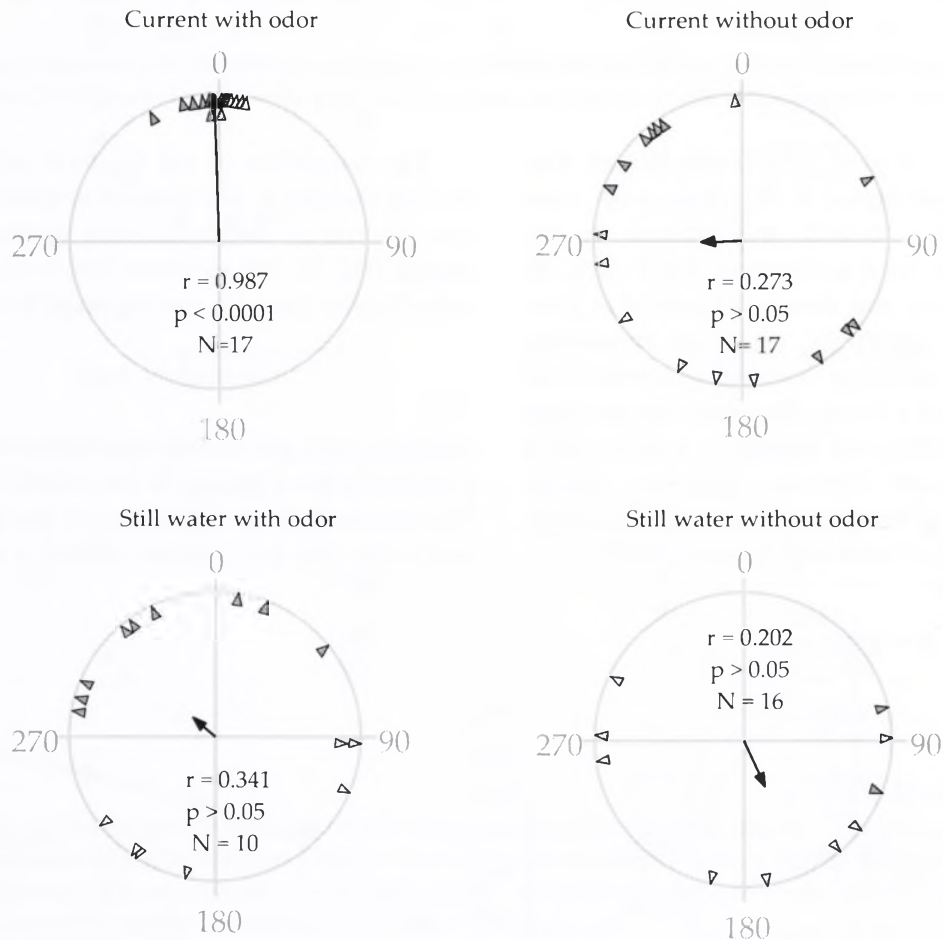


FIG. 3. Measurements from table experiments.

FIG. 4. Orientation of *G. cincta* in presence and absence of current and prey odor. Triangles represent the angles at which nudibranchs left a 25cm radius circle centered on their starting point and arrows represents the mean vector r , mean vector length. A significant orientation was only found in the treatment involving current and no prey odor (Rayleigh test).



RESULTS

Animals were found to be most active during daylight hours for both species. The greatest numbers of individuals of both species were active between the hours of 5 AM and 3PM. The preliminary experiments revealed that neither species exhibits a relative preference for aggregation. Additionally, there was no indication of trail following behavior.

Glossodoris cincta

G. cincta exhibited strong upstream orientation in the treatment with both current and prey odor

and 14 of the 17 animals tested successfully found the prey within the 10 min period (Fig. 4). The other 3 nudibranchs in this treatment also oriented towards the odor initially but seemingly lost the odor plume along the way. In contrast, there was no significant orientation for the treatment with a prey in still water and not one of the 16 *G. cincta* completing the trial located the prey. Also, there was no significant orientation in either treatment without prey odor whether in current or still water. Overall, the ability of *G. cincta* to locate prey was much better in current than still water (binomial test, $p < 0.001$). The ANOVA applied to the four treatments indicate that only displacement speed was significantly influenced

factor	Deviation from 0°				Linearity index				Displacement Speed			
	df	SS	F	p	df	SS	F	p	df	SS	F	p
Odor	1	48486.20	19.70	<0.01	1	0.01	2.797	<0.1	1	0.01	7.69	<0.01
Current	1	28399.50	10.12	<0.01	1	0.04	2.40	>0.1	1	0.00	0.04	>0.5
Current x odor	1	89894.78	51.45	<0.01	1	0.01	0.30	>0.5	1	0.01	8.93	<0.01
C. Total	59	191233.33			60	1.13			60	0.07		

Table 1. Results of ANOVA applied to angular deviation from the position of stimulus, displacement speed (cm/s), and linearity index investigating the effects of presence of current and prey odor on the behavior of *G. cincta*.

by the presence of prey odor (Table 1) such that animals averaged fastest in the absence of odor (0.11 cm/s, SE = 0.0056) and slowest in the presence of both odor and current (0.075 cm/s, SE = .0074). Presence and absence of current or prey odor had no significant effect on movement linearity. The deviation from the position of the stimulus showed a strong effect from the presence of odor ($p < 0.001$) and current ($p < 0.01$) and a strong interaction between the two factors corresponding to the high success rate of *G. cincta* in the presence of odor with current (Table 1).

The orientation of the *G. cincta* subjects that reached the prey in the presence of current became more precise as the nudibranchs approached the sponge (Fig. 5). Mean vector length (r) increased with distance from the starting point ($r = 0.69x^{0.25}$).

Chromodoris lochi

Although food preference observations confirmed a diet of the black sponge *H. erecta* for *C. lochi*, only the treatment involving current in the absence of prey odor did nudibranchs exhibit a significant

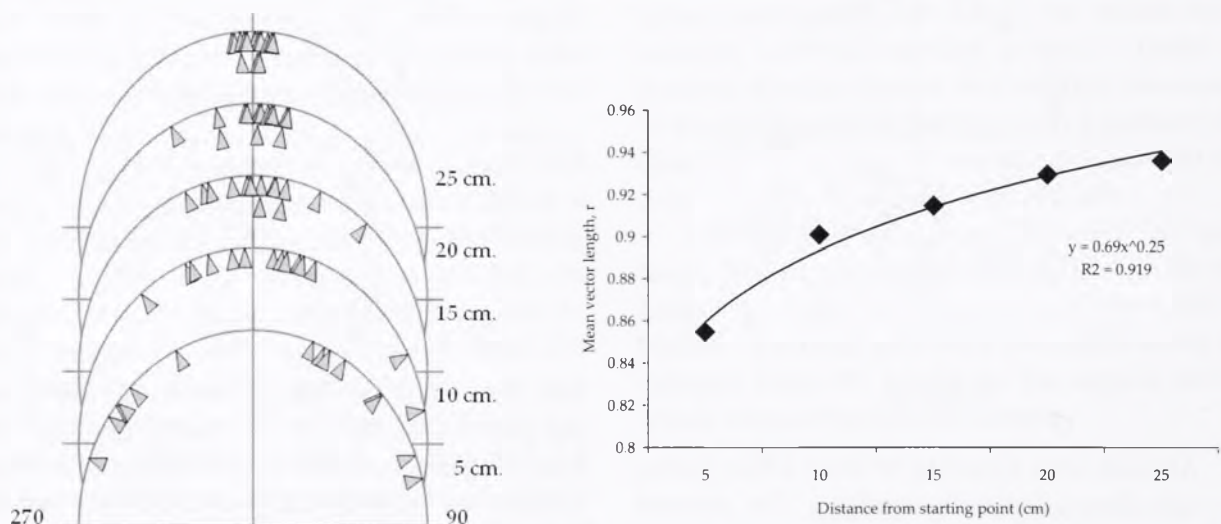


Fig. 5. Relation between precision of orientation and distance to prey for *G. cincta* that successfully reached prey. Left, semicircular plot showing angles at which individuals left concentric circles (radii 5, 10, 15, 20, and 25 cm). Right, plot of mean vector length against distance from starting point.

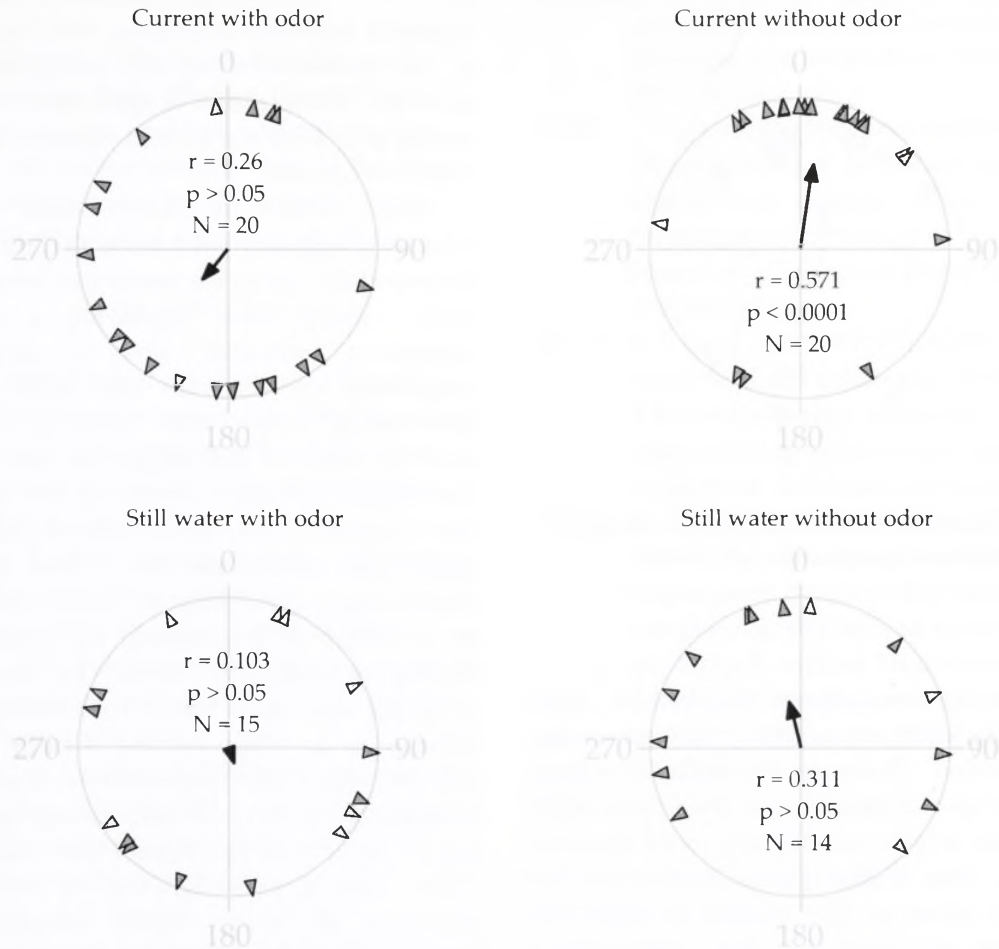


FIG. 6. Orientation of *C. lochi* in presence and absence of current and prey odor. Triangles represent the angles at which nudibranchs left a 25cm radius circle centered on their starting point and arrows represents the mean vector r , mean vector length. A significant orientation was only found in the treatment involving current and no prey odor (Rayleigh test).

factor	Deviation from 0°				Linearity index				Displacement Speed			
	df	SS	F	p	df	SS	F	p	df	SS	F	p
Odor	1	36880.35	13.68	<0.01	1	0.00	0.03	0.87	1	0.00	0.03	>0.1
Current	1	808.44	0.25	>0.1	1	0.02	1.10	0.30	1	0.00	1.96	>0.1
Current x odor	1	19123.36	6.46	>0.1	1	0.00	0.16	0.69	1	0.00	1.93	>0.1
C. Total	68	217546.688			68	1.19			68	0.13		

Table 2. Results of ANOVA applied to angular deviation from the position of stimulus, displacement speed (cm/s), and linearity index investigating the effects of presence of current and prey odor on the behavior of *C. lochi*.

orientation (Fig. 6). In the treatment with prey odor and current only 3 of 20 animals successfully located the prey. The ANOVA applied to the four treatments indicates that neither the presence of prey odor or current had any significant effect on displacement speed or the index of linearity (Table

2). Deviation from the stimulus did show a strong effect from the presence of odor but not current with no interaction between the two factors. A follow up treatment with *C. lochi* using 2 conspecifics on prey in the presence of current elicited a significant upstream orientation (Fig. 7)

towards prey, but only 7 of 19 animals located the stimulus.

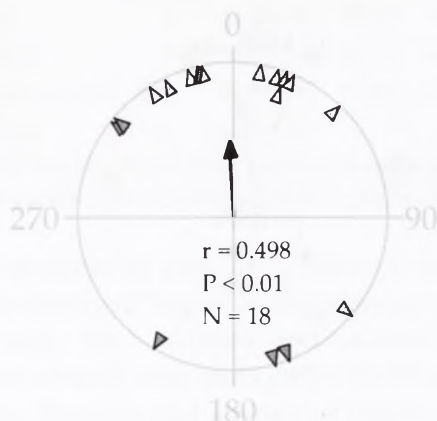


FIG. 7. Orientation of *C. lochi* in presence of prey odor, current, and conspecifics.

DISCUSSION

This study demonstrates that both *G. cincta* and *C. lochi* have the ability to navigate using chemodetection. However, this ability is entirely dependent upon hydrodynamic conditions as the nudibranchs only found the prey in the presence of current. Thus neither species seem to have the capacity to follow an odor gradient in still water. Nonetheless, data from this study does reveal a surprisingly strong difference in the movement behavior of these two closely related species.

The results clearly show that *G. cincta* is highly sensitive to molecules released by prey and is efficient in localizing upstream prey in the presence of current. Orientation for the nudibranch toward the prey was precise and became more precise as the distance from the prey decreased (Fig. 5). In addition, the nudibranch only moved upstream (positive rheotaxis) when moving toward prey odor. Further, the significant decrease in displacement speed in the presence of food odor suggests a possible kinetic effect (Avila 1998). This could indicate a model of search behavior typified by slower movement for more accurate chemodetection.

In contrast, *C. lochi* demonstrated positive rheotaxis without prey odor but unexpectedly did not exhibit the same behavior in the presence of prey. The follow up treatment using a stimulus of

prey and conspecifics elicited a strong but imprecise upstream orientation with less than 40% of the nudibranchs actually navigating to the stimulus. These results suggest that *C. lochi* uses neither prey odor nor current exclusively to guide their movements.

When tested in current alone *G. cincta* wandered aimlessly but I found that current plays an important role in the movement behavior of *C. lochi*. Aside from displaying a significant orientation upstream, when I calculate the orientation relative to the flow direction (considering only a 180° plot by taking the absolute value of the angles for that treatment from Figure 6), I obtain a value of 46°. This shows that not only do they detect current and use it to guide their movements but *C. lochi* specifically moves diagonally upstream. This is considered to be a means of maximizing the probability of finding an odor of interest by continuously sampling new areas rather than resampling the same area as when moving directly upstream (Chase 2002). *C. lochi* was observed in preliminary laboratory studies to eat other sponge species and likely has a more generalized diet relative to *G. cincta*. Also, *C. lochi* was observed in the field to aggregate at much higher frequencies and concentrations than *G. cincta*. It may be one or a combination of these factors that are responsible for the apparent lack of motivation to feed in *C. lochi* (Wyeth and Willows 2001, 2003). For instance it is possible that distance chemodetection plays only a minor role in locating prey because of the greater abundance of prey items compared to *G. cincta*. Alternatively, finding mates may be a dominant behavior of *C. lochi* that inhibits the motivation to feed or perhaps the presence of conspecifics is a requisite for prey localization.

This study shows the differences in the movement and orientation behavior of two closely related nudibranch species that share a habitat and prey. The behavioral models developed here are the beginning of a good framework for the interpretation of physiological experiments. Future laboratory and field studies of *G. cincta* and *C. lochi* would be useful to elucidate the chemo-orientation mechanisms and to examine their behavior in the presence of conspecifics.

ACKNOWLEDGEMENTS

I am grateful to Professors Roy Caldwell, Carole Hickman, Brent Mishler, George Roderick, Rosemary Gillespie, and James Bartolome for guidance and encouragement. Special thanks to the staff and friends of the UC Berkeley Gump Research Station for a wonderful experience.

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BIOLOGY OF *FAGRAEA BERTEROANA*: HABITAT SPECIALIZATION AND CONDITIONS FOR BIOLOGICAL SUCCESS OF AN ANOMALOUS POPULATION

SARA A. STEIDL

Integrative Biology or Environmental Science Policy and Management, University of California, Berkeley, California 94720 USA

Abstract: Habitat selection has normally been considered to be a term for only mobile organisms but has recently been applied to plants. *Fagraea berteriana* is an epiphytic plant that has demonstrated adaptive capabilities by establishing a population at a lower elevation. The indications as to why this may have occurred are found within the habitat at this particular location because they differ from where it is normally found in higher elevations. With the right dispersers this particular epiphyte has shown that it is capable of specializing in an environment that offers ideal neighbors, substrates and light exposure. Chi-square test's, ANOVA and t-test analysis on the population revealed that there were many links between all of these properties and not one factor could be attributed to the success of *F. berteriana* in this particular habitat.

Key words: *epiphytes; arboreal flora; Pua; habitat choice; habitat selection; Moorea, French Polynesia*

INTRODUCTION

For some plants, habitat selection is defined by evolutionary adjustments that have been made in response to changing environmental factors (Maina 2001). As this happens plants often become more flexible when living in various habitats (Bazzaz 1991). Habitats are chosen because of spatial and time reasons generally by those that have dispersal mechanisms. While habitat specialization is the result of a species functioning well in a given area. Certain plants can specialize in a habitat that has defined community dynamics in order to handle environmental changes.

The study of habitat selection in plants has become an area of interest more recently because of the human impacts on the environment. Humans have initiated a rapid change of global environments and which has led to many consequences within fragmented landscapes (Whitaker 1998). Researchers are now focusing on the stresses that effect populations under these changing conditions. Organisms with in different biotas often experience a variety of pressures such as limited available resources and spatial availability. On tropical islands, space is often an important factor that limits colonization creating more competition (Begon 1986).

Epiphytes should provide early indications of floristic response to changes throughout much of the tropics because of their influence on adjacent biotas and their sensitivity to global change. (Benzing 1998). As an integral part of tropical rainforest diversity, arboreal flora contribute to whole-system processes and play important roles in primary production, hydrology and nutrient production (Cushing 2002). Epiphytes are more responsive to surrounding ecosystem because they are dependent on what resources are available. Most will survive only under narrowly prescribed circumstances and therefore are co-dependent on each other (Benzig 1998).

Relationships often develop in order to better the living conditions and to help stabilize the habitat in response to environmental change. Epiphytic lichens and mosses have been shown to aid in establishing an ideal environment for other organisms (Raven, et. al 516). Lichens help to break down the substrate and make it more available for use while mosses help to maintain moisture and nutrient supplies. Vascular epiphytes can then colonize and establish relationships with the mosses and lichens, which can result in such ideal habitats that the biomass of epiphytes may exceed the foliage mass of the supporting trees (Benzig 1983).

The vascular epiphyte *Fagraea berteriana* A. Gray ex Benth., in the gentian family, is a native plant that has the capabilities of adjusting to environmental conditions in order to establish a viable population (Whistler 2005). More commonly known as Pua, is indigenous from New Guinea, Northern Australia to the Marianas, and eastward to the Marquesas. It occurs from sea level to as high as 1060 meters and in French Polynesia it is generally found from 300 to 600 meters in elevation, and can be found near sea level as a result of cultivation (Whistler 2004). Pua is known for its seasonal fragrant flowers, durable wood and medicinal properties. The large tubular fragrant flowers go from white to yellow and then fall off (M. Kellum, pers. com.). Bright orange fruits form a year after pollination and are eaten by birds (mynah's and ring eyed finches) and bats (Whistler 2005). The digested seeds are then deposited on the chance that they will land in a habitat that is ideal. Once the plant is in the adult stage it forms adventitious roots that find their way to soil to get more nutrients.

Pua has demonstrated the ability to specialize in a habitat that offers pollinators, dispersers, symbionts (or associates), and a supply of resources. By having the capability to spread seeds away from the parent tree into a suitable environment offers a much higher chance of seedling growth (Bazzaz 1991). Specializing in a habitat that contains symbionts or neighbors, while having the possibility of spatial selection also puts dispersers at a greater advantage.

Habitat choices for Pua plants are generally limited to higher elevations for reasons that are not well understood. However: the result of cultivated plants near sea level at 0-52 meters in elevation can lead to the establishment of a larger population. This is the case on the property of Marimari Kellum on Moorea, French Polynesia. One cultivated tree has lead to a population of over 200 individuals.

In this study I set out to find what properties of this habitat contributed to the success of the *F. berteriana* in this particular region. Substrate characteristics, epiphyte community and canopy density are the three main components that I examined. I also ran a germination study to try and create the

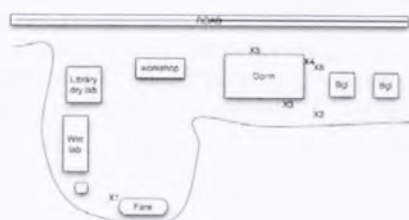
“choice” habitat for Pua seeds. I hypothesized that the germination of Pua seeds would occur in coconut mulch versus the monocot tree mulch. I expected to find more moss, lichen, and fern epiphytes growing in close vicinity to Pua saplings and juveniles. I also expected canopy cover to be correlated to plant size and height.

METHODS

Germination

Pilot surveys were conducted on Moorea, French Polynesia (17°30'S, 149° 50'W) on September 21 through 30, 2005. Sites of special interest that were known to contain Pua populations were Marimari Kellum's property, 3 coconuts trail summit, 3 Firs trail summit and the Cross Island trail summit (B. Mishler pers. com.).

Coconut mulch, monocot tree mulch, and sifted compost soil were used as substrates. The Tree mulch was sterilized at 16 P.S.I. for 36mins, the Coconut mulch was sterilized at 20 P.S.I. for 43 minutes and the soil was sterilized at 17 P.S.I. for 40 minutes. Fruits for the experiment were collected from a roadside tree (UTM 8061595), the seeds were removed and 142 groups of 20 were counted. In order to simulate bird scarification, 80 seeds were placed into a 5% HCl wash for 10mins and then were rinsed twice with water and another 80 seeds were filed. 108 pots consisted of 36 pots that were filled with each substrate. Substrates were assigned letters A (tree mulch), B(coconut mulch) and C(soil). The seeds and fruits were placed on top of the substrate. Full shade, full sun, partial shade and partial water treatments set up at various locations throughout the Gump Station (Fig. 1). Upon the exception of pots that were placed in area X5, all pots were watered enough to make the substrate moist. Area X5 was on an automatic watering system in which the plants were watered twice a day. Watering was started at 200 mL but was soon decreased to 100mL upon the discovery that the substrate was not drying. The pots that contained dirt were decreased to 80 mL in order to allow for the substrate to dry a little. Those pots in full shade treatments were watered everyday with 60mL. The pots were



Location	Elevation/Coordinates	Treatments
X1	10m /UTM8063936	Full sun Full Shade
X2	4m /UTM8063998	Full Sun Partial Shade Full Water Partial Water
X3	5M /UTM 8063995	Full Water Partial Water
X4	15M /UTM8063994	Full Water Partial Water
X5	12M /UTM8063991	Full water
X6	13M /UTM8063995	Full water
X7	10M /UTM8063978	Full water
X8	5M /UTM8063997	Full Shade Full Sun

Figure 1. This is a depiction of the Gump Station and detailed location as to where the different pot placement sites were located.

then rotated weekly. 20 Normal seeds, 20 acid washed seeds, 20 filed seeds and 1 whole fruit were placed onto moist filter paper in covered petri dishes.

Substrate characteristics

At the same time the quadrat was used to sample the epiphyte community, the substrate of each epiphytic Pua plant was recorded. The data from substrate availability was used to compare the substrate preferences of the Pua. The height of the plant from the ground was measured if it was less then 2 meters in order to demonstrate the range of habitat preference within the under story. The height, number of leaves and age of each plant was recorded as well.

Epiphyte associations

The epiphyte community that was surrounding each Pua plant sampled was surveyed using a 1meter by 1-meter quadrat made from a sheet of plastic. The quadrat was divided into 25, 10cm by 10cm squares. In order to examine the species richness, each square was examined in order to count the presence of the major groups of mosses, lichens, and vascular epiphytes. This helped to give an idea as to the nature of the conditions and what other epiphytes co-exists in the same habitat. The average total for each grouping was then divided by 25 and multiplied by 100 in order to give a percent cover of the area. These were then plotted on a bar graph, average percent cover versus arboreal groups.

Canopy cover

Canopy cover was measured by using a 1.75-inch densitometer (a girded convex mirror). This was done to provide a range of shade cover that Pua prefers. Average canopy cover for each age category was calculated and then graphed.

Data analysis

Chi-square tests were performed on the age category and substrate data as well as epiphyte presence on the different substrates. An ANOVA analysis of variance with factors of age, substrate and response variable height was performed. A t-test was used to determine whether canopy cover was significantly different between the two categories of substrate. Another a-nova test

with 2 factors for each epiphyte type, fern, moss lichens and vascular epiphyte was also performed.

RESULTS

Germination

During the 8 weeks of the germinating experiment many of the pots were observed to have ants crawling about the substrate. Final examination of the pots revealed that ants had colonized some pots. None of the seeds that were planted had germinated, 1 fruit was missing, and ants or other organisms had eaten 4 of the fruits out.

Substrate characteristics

Along with epiphyte coverage substrate was also recorded. Upon examination of the data collected 73% of the plants surveyed were found to be growing on coconut stands. While 26% of the time Pua was found to be growing on a variety of other substrates, such as Banyan trees, Brazilian plum trees and Avocado trees (Fig. 2).

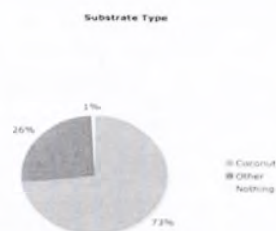


Figure 2. The survey of the population showed that a larger amount of Pua plants were living on coconut stands.

Epiphyte associations

Pua was found to be located in areas where other epiphytes were growing. On average epiphyte coverage was found to be highest for mosses and lichens (Figure 3). A comparison of epiphytes on two types of substrates revealed that frequency of epiphytes found was lower on other substrates (Fig. 4). But, by charting the average % epiphyte cover on the different substrates, one can see that the values are nearly the same. The study of the population also revealed that 66% of the individuals were

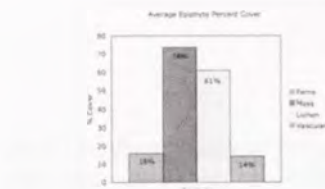


Figure 3. Depicts the average percent cover of each category of epiphyte for all of the substrates.

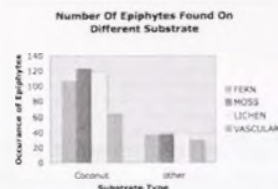


Figure 4. Depicts the frequency of epiphytes found on the different types of substrate

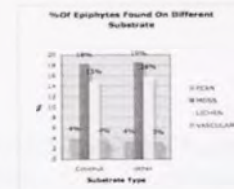


Figure 5. This shows the average % epiphyte coverage for the different substrates.

juveniles while the 30% were saplings and 4% of the population contained the fruiting adults (Fig. 5). Further analysis of whether or not there is a correlation between the epiphyte community and Pua plant population is needed.

Canopy Cover

A study of the canopy cover is important because of the effects of sunlight on both plants and the habitat. Percent cover on coconut stands ended up being higher at 73% versus a 26% percent canopy cover for the plants that were on other substrates (Fig. 6). Percent canopy cover for coconut stand epiphytes was on average 80. While those Pua plants that were found to be on other substrates had about 55 % canopy cover. Percent canopy cover also varies for juveniles versus saplings. Over all of the juveniles sampled the average percent canopy cover was 79, while the saplings had about 74 % canopy cover (Fig. 7).

Data Analysis

The data that was collected from each plant community surveyed was to be analyzed using chi-square tests, ANOVA tests and t-tests. The chi-square that was performed on the age group and substrate type data yielded a value of 8.25(df = 2 , $p < .016$). While the chi-square test for the epiphyte presence on the different substrates data resulted in 2.1(df = 3 , $p < .6$, not significant). The a-nova (output and data files attached.) tests resulted in an RSquare of 0.849 or a proportion of 85%. Of the factors, age was highly significant in explaining height ($p < 0.0001$). However: interaction between age and substrate were not significant. The t-test performed on the canopy cover and substrate data yielded t-statistic of 35.3 (df =1, $p < .0001$) and the mean

for coconut was 78.6 while the mean for the other substrate was 52.1. The t-test for epiphyte cover indicated that 3 of the epiphyte interactions (fern, lichen and moss) between substrate and age were significant ($p < .05$ or less). For vascular epiphytes, there was no interaction between substrate and age, but age alone was important in explaining the percent of the epiphyte.

DISCUSSION

Germination

There are several factors that could have been the reason as to why the germination experiment didn't work. Ants and other organisms changed the dynamics of seed germination by colonizing the substrates or by taking seeds. The seeds may have also needed proper scarification by being digested by a bird. Another factor could have been the fact that the non-vascular epiphyte community needed to be established in order for the Pua seeds to germinate. It is quite possible that the presence of arboreal flora is one of the properties that contribute to the selection of a habitat. Sun exposure could have been another element that might have prevented the seeds from germinating. The amount of light available is important because it is a major component of plant function is an important source of energy. There were may factors that could have been the reason as to why seeds did not germinate but there is not any data that can give a clear reason as to properties they may have been.

Substrate characteristics

It is quite clear that the woody substrate of coconut stand bases provides an ideal location for epiphyte colonization. The results had shown that there is a higher percentage of Pua

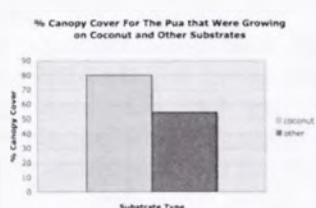


Figure 6. Gives the % canopy cover for the plants that were found on the different substrates.

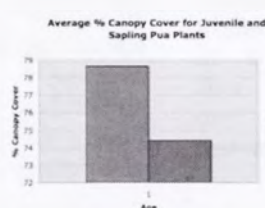


Figure 7. Shows the %canopy cover for the different age groups, juvenile and sapling.

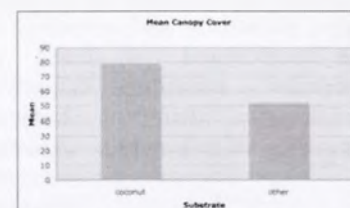


Figure 8. Depicts the mean canopy cover calculated from the t-test analysis

and epiphytes on coconut then on other substrates (Figure 6). This lead to further discovery that the community found on coconut palms differed from the other types of substrates. While substrate was found to not be a contributing factor to Pua plant height, it was clear that the age of the plant explained the height ($p < 0.0001$). The fact that the age of the plant and the height of the plant showed more of a relationship, as plants age they become larger and taller. Another conclusion that was made from the chi-square test was that the coconut and other substrate were clearly independent of each other (Chi-square 8.25). There are more individuals in communities that have established on coconut stands, which indicate that the environment provides enough resources. Selecting the right habitat is very important to success when space and nutrients are vital commodities in an area where competition is at its highest.

Epiphyte associations

The community of local flora is also another important factor for the habitat choice exhibited by the Pua plant. The epiphyte cover on coconut versus other substrates was significantly different. Colonization will generally occur where resources are available and environmental conditions are ideal. This may explain why communities found on coconut stands had larger populations. Other test results do indicate that Pua plant age was dependent on the other factors of epiphytes and substrate type. While the Pua was always found to be in the same place as mosses and lichens, the age of the plant depended on both percent of the epiphyte and the substrate. It is clear that Pua seeds germinate best under conditions where both the substrate and the epiphyte cover contribute to plant establishment and growth.

Since epiphytes are known to play major roles in community health by contributing to whole ecosystems and environments they are important factors when it comes to being successful in a habitat. Another important factor for growth and development is that epiphytes also play huge roles in nutrient cycling because of their large storage capacity (Benzig, 1998). Non-vascular epiphytes sometimes hold a key in making a habitat more hospitable to other flora and fauna, which seems to be an ideal situation for the Pua plant.

Canopy cover

In conjunction with the epiphyte community and the coconut substrate, light plays a vital role in habitat functions. Light is the source of energy for photosynthesis and too much light can cause the photosynthetic processes to actually decrease (Cronquist 1961). Light is the first vital component of photosynthesis and sugar production.

The canopy seemed to provide the Pua and other epiphytes with the right amount of shade and light. The canopy cover was significantly different between the two substrates and could possibly be the reason as to why there was a higher establishment of epiphytes on coconut stands. With mean canopy cover for coconut being 78 it is clear that this aspect of the habitat was more ideal and that a majority of the arboreal flora performed best under these conditions (Fig.8).

Pua plants also demonstrated flexibility in shade requirements because it was found to be growing in conditions where the canopy cover was nearly 50%. Since the mean canopy cover on other substrates was lower and Pua was found to be growing in these conditions this particular epiphyte demonstrates its evolution and that it can specialize in a variable habitat.

CONCLUSION

Habitat specialization in plants reveals a lot about the types of environments that form. Since epiphytes get their vital nutrients from their environment and not their host (or substrate) it is important to be successful in a habitat that provides enough resources for seed germination and growth. *F. berteriana* demonstrated that it has the capabilities to specialize a habitat that provides enough resources for its needs. It spreads quite easily because of the presence of proper disperser's, which allow for the plant to colonize areas where space is available. It grows epiphytically along with other epiphytes such as moss and lichens, which are known to contribute to local nutrient stores. Pua also has great flexibility when selecting a substrate to utilize because it was found to be growing on lava rock at higher elevations to dead Banyan trees and coconut stands at lower elevations. The amount of moisture, substrate availability and light are important properties that have allowed for the Pua plant to establish itself on Marimari Kellum's property.

However, there are certain factors that limit this plant from establishing large invasive populations. Is it the fact that when it

comes to spatial competition it can't establish itself? Is it a possibility that there is too much competition for vital nutrients and therefore it can't survive? What about epiphyte associates, are they really necessary to establish a population? These are just some of the vital questions that could be potentially explored.

ACKNOWLEDGEMENTS

I would like to thank Marimari Kellum for her contributions to preservation and also letting me explore her property for my study. I would also like to thank Brent Mishler for encouraging me to look into this unusual epiphyte. I am also very thankful to George Roderick, Carole Hickman and Rosie Gillespie for their help with everything! Thank you Ian Sanchez for being my germination buddy and cohort. Thank you Alicia Bihler for helping me with my tiny seeds and pot labeling. Sarika Cullis-Suzuki and Sarah Daniels were a source of tireless support and encouragement of which I really appreciated. I am also very thankful to all of the GSI's and students of the 2005 Moorea class for all of their help and support.

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PATTERNS OF COMMUNITY COMPOSITION: A PHYLOGENY OF THE HOLOTHURIANS OF MOOREA

Kristin M. Tremain

Department of Integrative Biology, University of California, Berkeley 94720

Abstract: Community species composition usually depends on the array of exploitable niches available. Holothurians are a large and diverse group of echinoderms that inhabit many different ocean environments of the world. The large diversity of sea cucumbers (Holothuria) and their species co-occurrence in multiple habitat types, presents them as ideal organisms to study the patterns of community species composition. In this study, a morphological phylogeny of sea cucumbers (Holothuria) was created using 27 characters and 11 species to help understand the evolutionary relationships between members of the Holothurian order Aspidochirotida. This phylogeny was used to observe patterns of species composition at two levels of community composition: island community and habitat. This study was conducted on the island of Moorea, French Polynesia. Given the apparent isolation of the Society Islands, I predicted that the sea cucumber species of Moorea would be phylogenetically clumped. After mapping ecological data onto the phylogeny, I observed patterns of phylogenetic clustering, overdispersion, evolutionary polarity and phylogenetic conservatism. Phylogenetic clustering appears to be the primary pattern of species composition on Moorea, however more evidence is required to support this. Until further phylogenetic and statistical analyses are conducted, this topic remains unresolved.

Key words: sea cucumber; morphology; phylogeny; colonization; overdispersion; phylogenetic clustering; evolutionary polarity; phylogenetic conservatism; niche; habitat; Moorea, French Polynesia

INTRODUCTION

Species composition of a community usually depends on the array of exploitable niches available. Currently there are two accepted patterns of community composition: environmental filtering and overdispersion (Cavender-Bares et. al, 2004). First, if closely related species share similar environmental constraints, such as isolation, then environmental filtering may occur. Only a select number of species will colonize and be successful; these species are closely related, as species which are not closely related are unsuccessful and 'filtered out'. This mechanism is referred to as 'phylogenetic clustering'. Second, limited resources could prevent the ability of closely related species to co-occur, due to competitive exclusion. This second pattern is termed 'overdispersion' and predicts that character similarities are a result of evolutionary convergence.

Within a community, patterns of species composition can be observed at many different levels. The co-occurrence of a particular group of individuals or species within a given habitat is often the result of multiple patterns of species composition being displayed concurrently. In this study the species composition of a given habitat is

explained through the ecological patterns of evolutionary polarity, phylogenetic conservatism, overdispersion, and phylogenetic clustering.

In this study, an ecological niche is defined as the set of conditions and resources that a species population requires and/or tolerates (Futuyma, 1998). A community is defined as an assembly of species co-inhabiting the same geographical area under similar environmental conditions. A habitat is the place where an organism lives (Futuyma, 1998).

Holothurians or sea cucumbers are a large and diverse group of echinoderms that inhabit the oceans of the world. More than 1400 described, extant species, assigned to more than 140 genera in 5 orders, occur in the world's oceans today (Smiley, 1994). This class of marine invertebrates has undergone a significant evolutionary radiation into different marine habitats, ranging from tropical inter-tidal pools to deep ocean and cold polar waters. Many Holothurians are sediment feeders that remove the organic coating from sediment grains and excrete this nutrient-depleted sand as fecal pellets on the ocean floor (Tran, 2003).

Ranging in length from less than a centimeter long to 5 meters (Mortenson, 1938), A recent estimate suggests that only 50% of sea cucumbers in the world's oceans are described and known (A.

Kerr, personal communication). The large amount of variability in Holothurians raises multiple questions about holothurian evolution.

Sea cucumbers are typically recognized by non-rigid, elongated bodies, with variation on a cylindrical body plan. Holothurians are unique in that, unlike other echinoderms, their external skeleton has been greatly reduced into microscopic ossicles that are embedded in the soft-tissue body wall. These ossicles serve as an excellent tool in phylogenetic analysis, because they are evolutionary baggage with a signal that has been preserved in their fossil record.

The island of Moorea is a member of the Society Island Archipelago, a remote volcanic island chain located in the South Pacific Ocean. It is a true oceanic island in that it has never been connected to the mainland (D'Artenay, 2004). Due to its location and short history, few species of Holothurians have colonized the waters surrounding island of Moorea.

Sea cucumbers are most diverse in the tropics (Kerr, 2001), cohabiting areas where coral gardens are found. In Moorea, French Polynesia, sea cucumbers are a conspicuous faunal element along the shore and on lagoonal reef flats. The diversity of morphology in these areas is large, and in some places more than 55 individuals have been recorded from a .25-meter area of sediment (Strong, 2003). Comparatively little is known about the distribution and full range of species of sea cucumbers on Moorea, and past studies (Filliatrault, 1996; Bruschi, 1999; Strong, 2003; Tran, 2003) have concentrated on single or few species at few localities. This paper provides a catalogue and distribution map of the 11 species located and identified in this study.

Morphology-based phylogenies have been constructed in the past (e.g. Clark, 1907; Gilliland, 1993; Kerr, 2001; Samyn 2005), with a large focus on the variation of microscopic ossicles embedded in the body wall. Here, I present a morphology-based phylogeny based on 26 characters that I establish and define. Characters are based on external and internal morphology differences among species, as well as variation in ossicle type.

The primary objective of this study was to generate a morphological phylogeny to help understand the evolutionary relationships between members of the Holothurian order Aspidochirota. The phylogeny was used to determine whether the diversity of Holothurians on Moorea is best explained by phylogenetic clumping, or overdispersion. Given the apparent isolation of the Society Islands, I predicted that the sea cucumber species of Moorea would be phylogenetically clumped.

I showed the evolutionary relationships between the members of the Holothurian order Aspidochirota. The maximum parsimony phylogeny that I created revealed four patterns of species composition on Moorea. Patterns observed include phylogenetic clustering, overdispersion, evolutionary polarity and phylogenetic conservatism. Phylogenetic clustering appeared to be the primary pattern of species composition on Moorea, however more evidence is required to support this.

MATERIALS AND METHODS

All work was conducted on the island of Moorea, French Polynesia (17°30' S, 149°50' W) in the South Pacific, during October and November of 2005. Holothurian individuals were collected from five habitat types in multiple locations on the northern portion of the island (see figure 1). Animals were collected from multiple shallow sub tidal localities ranging from shore to 5 meters deep in the lagoon.

Individual Collections

Individuals were collected from the northern portion of the island, from Haapiti in the west to Temae in the east (see figure 1). All observed individuals were identified and counted. A minimum of two representatives from each species were collected and held in the lab for observation and dissection.

Individuals were collected using Zip-Lock™ freezer bags, a dive bag, and bucket. Gloves were used in collecting to prevent skin burns. In case of

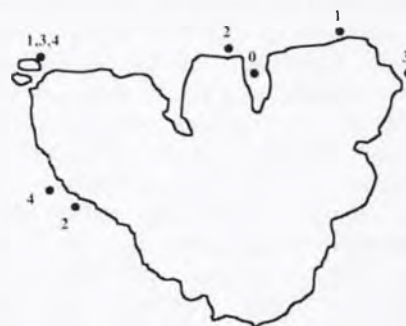


FIG. 1. Moorea, French Polynesia. Dots represent study sites. See figure 6 for number descriptions of habitat types.

cuvierian tubule expulsion by a *Bodaschia* species, a wooden stick was carried and used to remove tubules from skin and gloves. Individuals were maintained in tanks in an open seawater system.

Taxon Selection and Rooting

Representatives from a total of 11 species were included in this study and 9 are in the order Aspidochirotida. The two outgroup species, *Synapta maculata* and *Chirotida hawaiiensis*, are in the order Apodida. A representative of each of the eleven species was dissected. A minimum of two individuals from each of the eleven species were collected and scored for a total of 31 characters (27 were used in the phylogeny). Within the order Aspidochirotida, there are currently three families: Holothuriidae, Stichopodidae, and Synallactidae. This study focuses on the relationships within and among Holothuriidae and Stichopodidae. Of these eleven species, eight are within Holothuriidae and one is in Stichopodidae.

Character Definition and Scoring

A total of 31 post-larval morphological and ecological characters were defined and scored in adults of 11 holothurian species. Characters were based on external morphology, internal anatomy, and ossicle type. Characters designated in this phylogenetic study are adapted from those used in previous phylogeny studies (e.g. Clark, 1907; Gilliland, 1993; Kerr, 2001; Samyn 2005), but are defined from personal experience of variation in features that differ among species.

After analyses of independence, inheritability, and discreteness, four characters were discarded for not satisfying all three requirements. All characters used in this phylogeny were independent, inheritable, and discrete. Independence defines a character state as being unique and unlinked to any other character states used in the study. Inheritability requires that all characters are evolutionarily heritable to the offspring of the taxa studied. All characters in this study were discrete, signifying that the character states were distinguishable and finite.

External Morphology

Nine external characters were defined and scored on a total of 30 individuals. Individuals were removed from free flow tanks and placed in a shallow bucket for scoring. Animals were observed in bucket and closely examined to observe differences in external morphology. Characters, character states, and illustrations are detailed in the following section.

Internal Morphology

One representative from each taxon was dissected and examined for internal characters. Five internal anatomical characters were defined based on comparison among species. *Holothuria leucospilota*, *Holothuria hilla*, *Holothuria* (*Lessonothuria*) *pardalis*, and *Chiridota hawaiiensis* were dissected using a dissecting microscope magnified at 30x and 60x. Organisms were irrigated with 90% ethanol during dissection. Internal structures were identified using Lutz, 1986, and photographed with a digital camera. Characters, character states, and illustrations are detailed in the following section.

Ossicles

Ossicles were obtained from mid-dorsal and mid-ventral body wall regions. Seven ossicle characters were defined and scored. In order to isolate ossicles, a portion of body wall approximately 1-2 cm² was dissolved in a closed container of 100% household bleach at 7 degrees Celsius for 48 hours. The solution was then rinsed in tap water and the top layer of liquid was poured off, leaving the ossicles at the bottom of the container. Using a pipette, the ossicle-liquid solution was then placed on a slide and viewed under a compound microscope at a high light aperture. Ossicles were magnified to 100x and 420x, and photographed with a digital camera.

Phylogenetic Analysis

Heuristic search analysis of the data was performed using PAUP* for Macintosh.



FIG. 2. Microscopic ossicles examples from dissected sea cucumbers. Clockwise from upper left corner: rosette, web, barbed rod, cloud, table, wheel, rosette, anchor, cloud, and button. All ossicles were obtained by dissolving a piece of tissue in household bleach. Ossicles are magnified at 450x.

Phylogenies were created using maximum parsimony analyses, as the principle of parsimony in phylogenetic study proposes that we tentatively accept the simplest hypothesis that accounts for the data (Futuyama, 1998).

A significant amount of character data for individuals in the matrix was incomplete because dissections were performed on only one representative of each taxa. A strict consensus tree and maximum value consensus tree were inferred from characters scored on all individuals. If an individual was not used in dissection, it was scored with a '?' for those 11 characters. An exemplar tree was created from the consensus tree, using one representative from each taxa to represent the species.

Ecological Analysis

Changes of individual characters along branches and habitat ecology were examined using *MacClade*.

RESULTS

Character Analysis

24 morphological characters and 7 ecological characters were defined and scored. Morphological characters were divided into external morphology, internal morphology, and ossicle morphology. An example of an external morphology character used in this study was the *Overall body shape*. Three character states were observed: *vermiform and egg-shaped* = 0; *vermiform and elongated* = 1; *thin and rope-like* = 2. Vermiform body shape is defined as having an uncontracted body diameter that is circular in cross-section, with the diameter either constant along the length of the body or slightly decreasing posteriorly. Overall body shape is described as being vermiform and egg-shaped, vermiform and elongated, or super-elongated, thin, and rope-like (Kerr and Kim, 2001). Members of the genus *Bodaschia* and *T. ananas* were observed to have vermiform and egg-shaped bodies. Members of the *Holothuria* genus and *Chiridota hawaiiensis* had vermiform and elongated bodies. *Synapta maculata* had an elongated, thin and rope-like body shape.

A total of 6 Internal Morphology characters were described and scored. An example character is the *Appearance of cuvierian tubules*: *long, thick and white* = 0; *short, thin and translucent blue* = 1. Due to the large variation in cuvierian tubule morphology, a second cuvierian tubule character observed. Members of the genus *Bodaschia* have long, large, white cuvierian tubules that are expelled from the anus. *Holothuria leucospilota* was

observed to have very thin, iridescent blue cuvierian tubules. Both forms of cuvierian tubules are very adhesive and can cause skin burns (Samyn, 2005).

Observations of microscopic ossicles revealed 9 ossicle characters that were defined and scored. An example is the *Web ossicle*: *absent* = 0; *present* = 1. Web ossicles consist of a central bar with usually four curved arms rising out from the two ends of the central bar. The terminal end of each curved arm splits into two or three smaller branch ends, similar to the end of a tree branch. This character is probably the same as the *Psychropotid rods* described by Kerr and Kim. Kerr and Kim scored presence and absence of *Psychropotid rods*, and described them as possessing a unique ossicle type, a cross of usually four curved arms, each with a centrally arising branch (Kerr and Kim, 2001). Web ossicles were observed in *Synapta maculata* (an outgroup species) and *Thelenota ananas*.

Ecological traits were observed and 7 characters were defined and scored. An example of an ecology character is the *Period of activity*: *diurnal* = 0; *nocturnal* = 1; *both* = 2. Most observed individuals had a distinct period of activity during the day where found actively moving around (both in the tanks and in the ocean) and feeding or releasing a cloudy liquid into the water. Individuals of *Bodaschia argus* and *Thelenota ananas* were observed active during both the day and night.

Phylogenetic Analysis

The heuristic search identified over 500,000 most parsimonious trees (stopped randomly at 503,133), with a shortest length of 69 steps. The consistency index, or measure of the average fit of all the characters to the tree, was 0.58 (perfect fit = 1, worst fit = 0) (see Figures 3A and 3B). Although both outgroup species are members of the order Apodida, the strict consensus of these trees (Fig. 5) distinguished only the outgroup *Synapta maculata* from the order Aspidochirotida. A maximum parsimony 50% majority rule tree was also created (see Figure 4). The other outgroup species, *Chiridota hawaiiensis*, was not distinguished from members of the order Aspidochirotida. The four species in the genus *Bodaschia* were distinguished as a separate clade on the phylogeny. The strict consensus tree also separated out species *Holothuria atra* and the unknown *Holothuria* (Actinopyga) species.

Due to the result of over 500,000 equally parsimonious trees, a maximum parsimony 50% majority rule consensus tree was created, and produced a more resolved tree (Fig. 3A). Only one

polytomy between members of the genus *Bodaschia* is visible.

Ecological data in characters has been included as an additional line of evidence. Removing ecological data decreases the resolution of the tree.

Ecological Analysis

Habitat – Individuals from the 11 species were observed in five habitat types on Moorea. Habitat types observed on Moorea were located on the northern and western regions of the island. Seven different areas were visited. On the motu Tiahuru, three distinct habitat types were observed (See Figure 6).

DISCUSSION

Phylogenetic Discussion

Bodaschia spp.

A polytomy existed between species A and B (*Bodaschia argus*), J (*Bodaschia marmorata*), and species K (*Bodaschia unknown*), within the family Holothuriidae. This polytomy reinforced the idea that A and B are morphotypes of the same species, *Bodaschia argus*. Species J has been named *Bodaschia marmorata*, a closely related species (personal communication, A. Kerr).

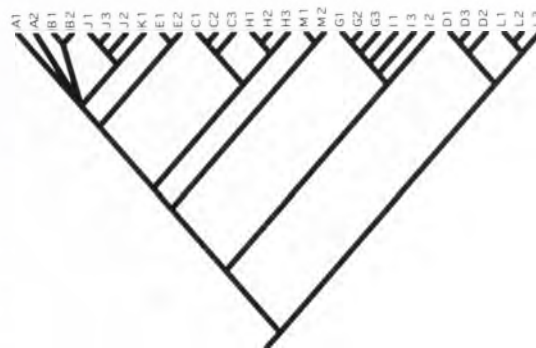


FIG. 3A. Maximum Parsimony tree generated from 21 morphological characters and 7 ecological characters. This is tree #1 generated by PAUP and represents over 500,000 equally parsimonious trees with a tree length of 69.

Key to Species Names:

- A1-2 *Bodaschia argus* (black color morph)
- B1-2 *Bodaschia argus* (white color morph)
- C1-3 *Holothuria atra*
- D1-3 *Synapta maculata*
- E1-2 *Thelenota ananas*
- G1-3 *Holothuria leucospilota*
- H1-3 *Holothuria (Actinopyga) spp.*
- I1-3 *Holothuria hilla*
- J1-3 *Bodaschia argus*
- K1 *Bodaschia unknown spp.*
- L1-3 *Chiridota hawaiiensis*
- M1-2 *Holothuria (Lessonothuria) spp.*
(possibly *H. pardalis*)

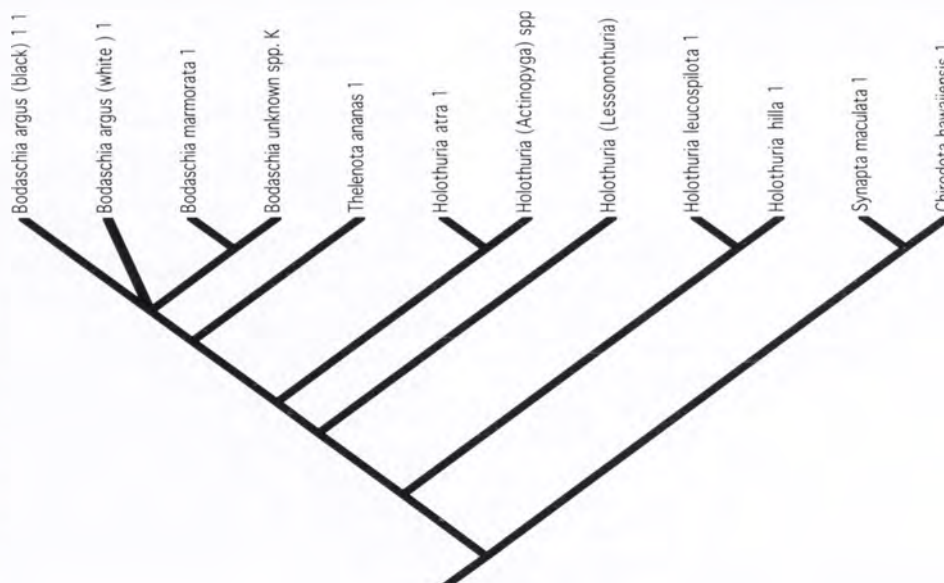


FIG. 3B. Maximum parsimony exemplar tree of eleven species, including both morphotypes of *Bodaschia argus*. Phylogeny was created using 27 morphological and ecological characters.



FIG. 4. Maximum Parsimony 50% majority consensus tree displays evolutionary relationships between individuals. Outgroups are distinguished as *Synapta maculata* (D) and *Chirodota hawaiiensis* (L).

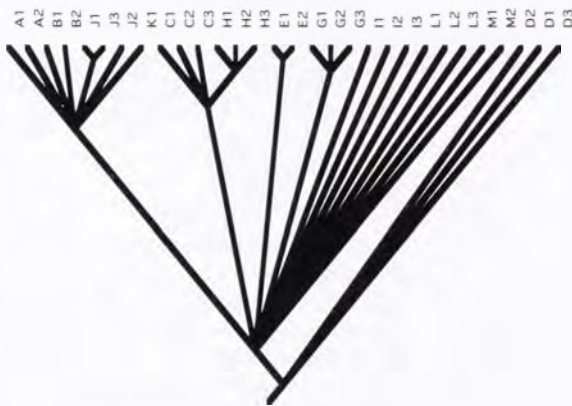


FIG. 5. Maximum Parsimony Strict Consensus Tree. The genus *Bodaschia* is distinguished from the other members of the Aspidochirotida family. Tree is highly unresolved

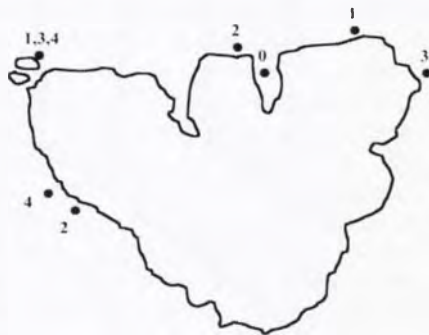


Figure 6. Habitat Types on Moorea: 0 = Water less than 2 meters deep, gradual slope, coral bottom; 1 = Water less than 2 meters deep, coral bottom, gradual slope, current; 2 = Water depth quickly varies from 0 to 4+ meters deep, rocky area, coral bottom; 3 = Strong current, greater than 2 metres deep, coral bottom; 4 = Water depth less than 1 meter deep, high sun exposure, little - no coral, sandy bottom

Bodaschia marmorata, as they display very similar morphological and ecological character states. All individuals had a thick cylindrical body shape and spots were present. Long, thick, white cuvierian tubules were present in all individuals of *Bodaschia marmorata* and species K. Equivalent microscopic ossicles were present in the form of clouds and smooth rods. Oral tentacles were of a 'plunger' shape. Further molecular analyses may further reveal the relationship between these individuals.

Bodaschia argus (both color morphs) was active during the day and night, whereas individuals of groups *Bodaschia marmorata* and unknown *Bodaschia* species K burrowed underneath the sand during the day and actively fed on top of the sand at night. The close similarity of life history traits in the individuals of these taxa suggest that these individuals are morphotypes of the same species. This pattern is also observed in the two color morphs of *Bodaschia argus*, and support that they too, are morphotypes of the same species.

Holothuria spp.

All members of the genus *Holothuria* were grouped together on the phylogeny generated in this study (see Figure 3A). The clustering of this group of individuals supports the species identification of these animals and that they are closely related individuals of the genus *Holothuria*. Species *Holothuria atra*, *Holothuria (Actinopyga) spp.*, *Holothuria (Lessonothuria) spp.*, *Holothuria leucospilota*, and *Holothuria hilla*, are classified within the family *Holothuriidae*. These five species are members of the genus *Holothuria* (Kerr, personal communication). The genus *Holothuria* appears to be basal to the genus *Bodaschia* in this phylogeny. This result supports a recently published molecular phylogeny (Kerr, 2005).

Previous morphology-based phylogenetic studies, using different members of the genus *Bodaschia*, display the genus *Bodaschia* as basal to the genus *Aspidochirotida* (Samyn, 2005). This discrepancy is possibly due to fewer, and new morphological characters that have not been used before. Past character descriptions are brief and in some instances poorly explained, thus although the characters appear similar, they may have been recorded and scored very differently.

Thelenota ananas

In this analysis, species E, *Thelenota ananas*, a member of the *Stichopodidae* family, is an intermediate between the genus *Bodaschia* and *Holothuriidae*. These results are inconsistent with previous *Holothuriidae* systematic studies.

Previous studies place the family Stichopodidae basal to the family Holothuriidae. Members of the Stichopodidae family are often used as an outgroup in phylogenetic studies of members of the Holothuriidae family (Kerr, 2001; Kerr, 2005; Samyn, 2005).

Thelenota ananas is the only member of the family Stichopodidae I found on Moorea. A previous study (Filiatrault, 1996) on Moorea observed members of the genus Stichopus on the reef flat. Species composition of a habitat changes over time and individuals of this species may no longer exist in the shallow sub-tidal waters surrounding Moorea. The family that is evolutionarily basal to the family Aspidochirotida according to Kerr (2005).

The evolutionary position of species E in this study may be due to a significantly larger amount of more defined morphological characters used in previous studies, particularly pertaining to the microscopic ossicles (101 ossicle characters used in Kerr, (2005) versus 11 ossicle characters in this study). Further analyses including molecular data would affect the results, and most likely modify the tree presented in this study, to more closely resemble current accepted Holothurian phylogenies.

Outgrouping

Chiridota hawaiiensis and *Synapta maculata* are basal to the family Aspidochirotida, and lie within the family Apodida. These two species were chosen as outgroups to root the tree, and they are commonly found on the island of Moorea.

Evolutionary Polarity and Phylogenetic Conservatism

Community assembly of a particular habitat is the combination of multiple ecological patterns displayed concurrently. In this study the co-occurrence of multiple species found in the five habitat types is related to the ecological patterns of evolutionary polarity, phylogenetic conservatism, overdispersion, and phylogenetic clustering. To infer if these patterns were present in sea cucumbers on Moorea, ecology data was mapped onto the maximum parsimony phylogeny.

One goal of this study was to try and determine if closely related species occupy the same habitats and whether the ability to inhabit a specific environment is derived or ancestral. The direction of evolutionary polarity was inferred in character states of closely related individuals in relation to their ability to occupy a specific habitat.

Evolutionary polarity is an inference about the directionality of evolutionary change. To infer which character state is plesiomorphic (ancestral)

and which state is apomorphic (derived) is to infer evolutionary polarity (Futuyma, 1998).

Phylogenetic conservatism is a term used to describe evolutionary characters that have a very low rate of evolutionary change (Futuyma, 1998). The traits required for a sea cucumber on Moorea to inhabit a specific environment appear to be phylogenetically conserved. Evidence supports that the degree of phylogenetic conservatism may be related to the ease of inhabiting a specific environment.

In my analysis of habitat types, I found that habitat type 1 is characterized by water less than 2 meters deep, a gradual slope, and a sand and coral bottom (Figure 7A - see appendix). In this habitat type, eight of the eleven species can be found. According to the tree, the ability to inhabit this environment is ancestral and was lost within the Holothuriidae family. The tree suggests that this trait was lost twice: once prior to *H. leucospilota* and *H. hilla*, and once before *H. (Actinopyga) spp.* However since three of the four members of this family lack the ability to live in this environment, the trait may have been lost in the ancestor to the Holothuriidae family and regained in *Holothuria atra*.

Habitat type 2 (figure 7b) is very similar to type 1, however it also contains a forcible current. The ability to inhabit this environment evolved independently three times: in *Holothuria atra*, *Holothuria hilla*, and in *Bodaschia argus* (both morphotypes) and only occurs in these three species.

In habitat type 3 (figure 7c), a steep slope quickly varies the water depth from 0 to 4+ meters deep. The area is rocky, and has a sand and coral bottom. This character appears to be ancestral, and was lost in *C. hawaiiensis*, *H. (Lessonothuria) spp.*, *H. hilla*, *B. marmorata*, and *B. unknown species K*. Three of the five members of the Holothuriidae family coexist on the rocky coral habitat (3). Over half of the holothurian species found on Moorea occupy this habitat type.

Habitat type 4 (figure 7d) has a strong current, and has a sand and coral bottom. The ability to inhabit this environment appears recent, and only *B. argus*, *H. atra*, and *H. (Lessonothuria) spp.* are found here.

In habitat type 5 (figure 7e), the water depth is less than 1 meter deep. There is high sun exposure, little to no coral, and a sandy bottom. Only species *H. atra*, *B. argus*, are found here.

High sun exposure (Habitat 5) and the presence of a forcible current (Habitats 2 and 4) are ecological components that appear to be difficult for Holothurians to adapt to. This explains the presence of over 54% and 72% of observed species on Moorea in Habitats 1 and 3

respectively, while the remaining habitats with high sunlight and a forcible current contained less than 28% of observed species on Moorea.

In future studies or if more time were allotted, community assembly of the Holothurians of Moorea would be statistically analyzed. Ecology data of the five habitat types observed on Moorea would be mapped onto the maximum parsimony phylogeny generated in this study in order to determine if closely related species are found more or less ecologically grouped than expected by random on Moorea.

In order to test this, a null hypothesis would be created which uses MacClade to randomly assign the character state (presence or absence to occupy a certain habitat) to the taxa in the phylogeny. This null model would state that there was no association between the character and the phylogeny; the distribution appears random. Character state (presence or absence of individual in a given habitat) of a specific habitat would then be mapped on the tree for each individual. The consistency indexes among the five habitat types would then be compared across the trees (one tree per habitat type). If the consistency index were greater than the null value, then evidence would support that the organisms are phylogenetically clustered in their habitats. If the consistency index were less than the null value, then evidence would support that the organisms are ecologically overdispersed across habitats. This data would reveal useful and interesting information about the community assembly of the Holothurians of Moorea.

Phylogenetic Clustering and Overdispersion

If closely related species share similar physiological limitations and exhibit evolutionary niche conservatism, then environmental filtering will tend to cause closely related species to co-occur. This pattern can be termed "phylogenetic clustering" (Cavender-Bares, 2004).

The contrary to phylogenetic clustering is the pattern of overdispersion. The competitive exclusion principle states that if two species occupy the same niche, and coexist in the same ecosystem, then one of these species will be excluded from the community due to intense competition (Gauss, 1934).

The pattern of overdispersion reveals that species occupying the same niche are not evolutionarily closely related. Instead, species appear similar visually due to trait convergence.

I predicted that pattern of phylogenetic clustering would be apparent in the species composition of sea cucumbers on Moorea. Moorea is an isolated volcanic hot-spot island, that was

never connected to a continent. Due to this extreme isolation and process of formation, I predicted that species of sea cucumber present on Moorea would appear similar and would be evolutionarily closely related.

Analysis of the relationships among taxa at the family level revealed that the families used in this study are closely related (see Figure 8). The ingroup families Stichopodidae and Aspidochirotididae are sister species on this morphological phylogeny (modified from Kerr, 2000). Of the 11 species used in this study, 7 are within the family Aspidochirotididae and 1 is in the family Stichopodidae. The two outgroup families are found within the family Synaptidae and the family Chiridotidae. These two families appear as sister taxa on this phylogeny as well. This data supports phylogenetic clustering as the pattern of species composition on Moorea. Due to the lack of phylogenetic data of the Holothurians at the species level, this issue remains unresolved.

Future phylogenetic study is required to determine if phylogenetic clustering is the pattern of species composition on the island of Moorea. With the organization of a super-tree, the location of Holothurian species found on Moorea within

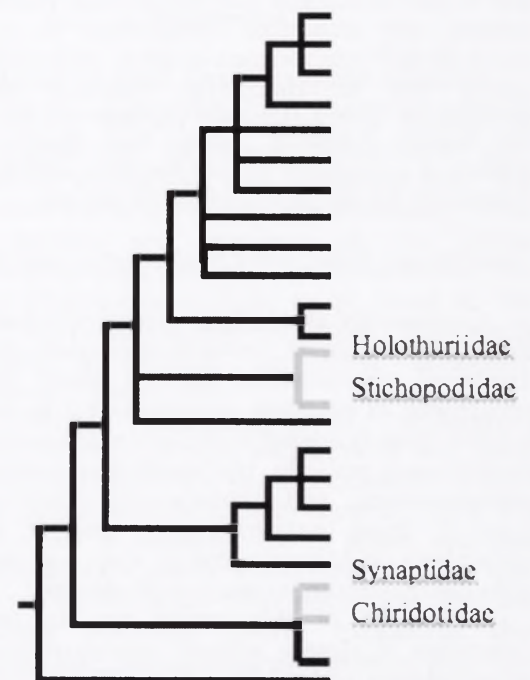


FIG. 8. Phylogeny of Holothurian families. Reveals the close evolutionary relationship between ingroup families Stichopodidae and Aspidochirotididae. The outgroup families Synaptidae and Chiridotidae also are evolutionarily closely related. This phylogeny supports possibly of Phylogenetic Clustering being an observable pattern of species composition on Moorea.

the larger phylogeny of sea cucumbers could be resolved. From this, the pattern of species composition on Moorea could be resolved. If the species found on Moorea appeared clustered on this super-tree, then phylogenetic clustering would be the pattern. If the species found on Moorea appeared very distantly related on the super-tree, then overdispersion would be the pattern of species composition.

Statistical analyses similar to those explained in the previous section on *Evolutionary Polarity and Phylogenetic Conservatism* could also help to resolve this issue. Establishment of a null hypothesis that stated that species composition was completely random could be compared to the actual species composition of the Holothurians of Moorea using the consistency index. If the results were greater than the random number generated by the null hypothesis, then phylogenetic clustering would be the pattern. If the results were less, then overdispersion would be the pattern of species composition of the Holothurians of Moorea.

The presence of phylogenetic clustering does not reject the possibility of phylogenetic overdispersion, for the lack of Holothuria (*Lessonothuria*) in this habitat may be explained by competitive exclusion. Phylogenetic Clustering appears to be the pattern of species composition on Moorea, however future phylogenetic and statistical studies are necessary.

Conclusion

Multiple patterns of community composition are contributing to the species composition of sea cucumbers on Moorea. These patterns can be observed at both the island community, and habitat levels. These patterns include phylogenetic clustering, overdispersion, evolutionary polarity and phylogenetic conservatism.

Phylogenetic clustering appears to be the primary pattern of species composition on Moorea, but more evidence is required to support this. Until further phylogenetic and statistical analyses are conducted, this topic remains unresolved.

ACKNOWLEDGEMENTS

Thank you to Brent Mishler, Rosie Gillespie, Carole Hickman and George Roderick, Roy Caldwell, and Jamie Bartolome for assisting me with project development and ideas. This project would not be complete without the assistance of Dr. Alexander Kerr on species identification and project development. A special thank you to Shane Geange for assistance with sea cucumber

dissections, ossicle identification, and digital photography of ossicles. Thank you to Joe Spagna for patience and assistance with computer programs PAUP and MacClade.

A big thank you to everyone on the program, including students and graduate students, for making this semester my best in college.

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APPENDIX A : CHARACTERS

EXTERNAL MORPHOLOGY

1. *Overall body shape: vermiform and egg-shaped = 0; vermiform and elongated = 1; thin and rope-like = 2.* Vermiform body shape is defined as having an uncontracted body diameter that is circular in cross-section, with the diameter either constant along the length of the body or slightly decreasing posteriorly. Overall body shape is described as being vermiform and egg-shaped, vermiform and elongated, or super-elongated, thin, and rope-like. Characters were taken primarily from Holothuriidae are usually ventrally flattened and (Kerr and Kim, 2001)
2. *Firmness of body wall (in response to touch): collapsed flat when touched = 0; firm to touch = 1.* When the individual sea cucumber is held out of water, the body collapses and is no longer circular in cross section, but instead flat and 2-dimensional, *collapsed flat when touched*, or the body remains 3-dimensional, and the body diameter remains circular in cross-section, *firm to touch*. Apodans usually have a very thin layer of connective tissue that causes them to easily collapse and appear transparent. This may facilitate gas exchange in the absence of respiratory trees. Most Holothurians possess well developed circular muscles and a relatively thick connective tissue layer (Kerr and Kim, 2005).
3. *Body segmentation: not ribbed = 0; ribbed and even = 1; ribbed and uneven = 2.* When the animal was touched and observed in the wet lab aquaria, differences were observed in body segmentation. Overall body segmentation varied greatly. A plain body that was not segmented visually or by touch, was considered *not ribbed*. A body type that had perfectly symmetrical, evenly spaced body segmentation that appeared like rings and caused the body to look ribbed, was *ribbed and even*. A body that was segmented irregularly and appeared to random folds and ribbing was *ribbed and uneven*.
4. *Rugosity of body wall: smooth = 0; rough textured = 1.* The texture of the body wall when observed in water can either be smooth to the touch and not course, *smooth*, or abrasive, bumpy, and course to the touch, *rough textured* (Kerr, 2005).
5. *Arrangement of Ventral Tubefeet: entire ventral surface = 0; two places on ventral surface = 1; three places on ventral surface = 2.* Arrangement of tube feet on ventral surface varies among species. In some organisms tube feet were arranged along the entire ventral surface of the body, *entire ventral surface*. In some animals tube feet were excluded from the central ventral section, *two places on ventral surface*, and in other sea cucumbers tube feet were absent from two portions of the ventral surface, *three places on ventral surface*. Based off of character used in Samyn 2005, however description of character states is unclear. **EXCLUDED:** character state is not discrete. The quantification was difficult and continuous, results vary by day.
6. *Position of Anus: terminal = 0; super terminal-dorsal = 1.* Although three character states were defined for this character in a previous study (Kerr, Kim 2001), in this study only two character states existed. The anus located was located posteriorly at the end of the body and not visible from above, *terminal*, or the anus was located super-terminal on the dorsal side of the body easily visible from above, *super terminal-dorsal* (Kerr, 2000).
7. *Enlarged dorsal papillae: none = 0; moderately enlarged = 1; greatly enlarged = 2.* Papillae are bumps located on dorsal side of body. Papillae size in some individuals were not seen with the naked eye, *none*, papillae were clearly visible but under 0.25 cm in length, *moderately enlarged*, or were larger than 0.5 cm

in length, *greatly enlarged*. Most members of Stichopodidae have greatly enlarged, thickened dorsal papillae (Kerr and Kim, 2001).

8. *Water exits anus when held: water does not exit = 0; water exits = 1*. When certain sea cucumbers were held out of water, a water-like liquid would spray forcefully from the anus. Local Tahitians informed me that if this liquid got in a person's eye, blindness is a possibility.

9. *Papillae Shape (as seen under dissecting microscope at 60x): simple peak = 0; stretched peak = 1; peak and cylinder = 2*. Simple peak is defined as a simple upside-down 'v' of skin. Stretched peak is a simple peak shape but the skin appears greatly stretched at base. Peak and rod describes species that display noth peaks, and cylindrical shaped papillae on the body wall surface. **EXCLUDED:** based on non-discreteness of character states. The states were too similar to tell apart for certain individuals.

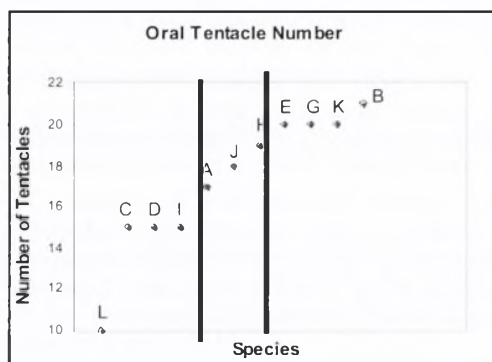
INTERNAL MORPHOLOGY

10. *Shape of oral tentacles: digitate = 0; peltate, indentations deep = 1; peltate, indentations shallow = 2; pinnate, indentations shallow = 3; pinnate, indentations deep = 4*. Digitate is defined as finger-like projections connected to the tentacle stalk at the base of the projections. Peltate is defined as leaf-like projections where the tentacle stalk is attached at the center of the leaf rather than at the base of the stalk. Shallow versus deep indentations refer to the profile image of the tentacle. In shallow, the tip of the tentacle is more separated than in deep indentations. Pinnate describes the tentacles as feather-like (Kerr and Kim, 2001; Kerr, 2001)

11. *Number of oral tentacles: 10 – 15 = 0; 16 – 19 = 1; 20 – 21 = 2*. Oral tentacle number varied in number from 10 to 21. Oral tentacle number was graphed and distinct character states were classified based on breaks in the graph at 15 and 20 oral tentacles. Although this character appears to be independent, inheritable, and discrete, I am unsure of the validity of oral tentacles as a good character. Inheritability appears to be operating, however no other morphological phylogenies use this character.

KEY to Oral Tentacle Graph

A	<i>Bodaschia argus</i> (black color morph)
B	<i>Bodaschia argus</i> (white color morph)
C	<i>Holothuria atra</i>
D	<i>Synapta maculata</i>
E	<i>Thelenota ananas</i>
G	<i>Holothuria leucospilota</i>
H	<i>Holothuria</i> (Actinopyga) spp.
I	<i>Holothuria hilla</i>
J	<i>Bodaschia argus</i>
K	<i>Bodaschia</i> unknown spp.
L	<i>Chiridota hawaiiensis</i>
M	<i>Holothuria</i> (Lessonothuria) spp.



12. *Presence/Absence of cuvierian tubules: absent = 0; present = 1*. Cuvierian tubules are very adhesive, thread-like tubes that are expelled from the anus as a form of defense. They are created adjacent to the respiratory tree near the anus and are quickly regenerated after expulsion. Only certain members of *Holothuria* possess cuvierian tubules (Kerr and Kim, 2001).

13. *Appearance of cuvierian tubules: long, thick and white = 0; short, thin and translucent blue = 1*. Due to the large variation in cuvierian tubule morphology, a second cuvierian tubule character observed. Members of the genus *Bodaschia* have long, large, white cuvierian tubules that are expelled from the anus. *Holothuria leucospilota* was observed to have very thin, iridescent blue cuvierian tubules. Both forms of cuvierian tubules are very adhesive and can cause skin burns (Samyn, 2005).

14. *Anal appendages: absent = 0; present = 1.* Anal appendages exist in the form of anal teeth and anal papillae. An anal appendage was observed in the unknown *Bodaschia* species K, are were believed to be anal papillae. No explanation of these character states could be found. No definition of anal teeth could be found, thus their shape and location are unknown, thus discreteness of character states could not be distinguished. Anal appendages are **EXCLUDED**: Identification of teeth was very difficult; not a discrete character (Samyn, 2005).

15. *Gonad color: white or near-white = 0; bright pink or purple = 1.* Different coloration of the gonads were observed in Holothurians during dissections performed at the Gump Station. Gonads are large, mop-like organs that are connected to the body by a single long tissue. In *Holothuria atra*, the gonads were bright pink in the two dissections performed on this species. The gonad color and overall internal body color of *Thelenota ananas*, was purple. This was interesting as all other Holothurians dissected had a clear or pink internal body color.

OSSICLE CHARACTERS

16. *Tables: absent = 0; present = 1.* This ossicle consists of a basal plate of few and often regularly arranged holes from which centrally arises a spire of two to four pillars linked by crossbeams. All table ossicles observed in this study contain four pillars (Kerr and Kim, 2001; Samyn, 2005).

17. *Webs: absent = 0; present = 1.* Consist of a central bar with usually four curved arms rising out from the two ends of the central bar. Terminal end of each curved arm splits into two or three smaller branch ends, similar to the end of a tree branch. This character is probably the same as the *Psychropotid* rods described by Kerr and Kim. Kerr and Kim scored presence and absence of *Psychropotid* rods, and described them as possessing a unique ossicle type, a cross of usually four curved arms, each with a centrally arising branch (Kerr and Kim, 2001).

18. *Wheels: absent = 0; present = 1.* Wheel ossicles are shaped like circular wagon wheels with spokes connecting them together at a central point. Wheel ossicles observed in *Chiridota hawaiiensis* had 6 spokes and teeth that line the inner rim of the wheel. They are present in adults of the Chirodotidae family and thus were only present in *Chirodota hawaiiensis*. This character was **EXCLUDED**, because it was only present in one species and thus could not be used to explain evolutionary relationships between organisms in this study (Kerr and Kim, 2001; Kerr, 2001).

19. *Smooth rods: absent = 0; present = 1.* Smooth rods with no knobs or barbs were observed in all members of the genus *Bodaschia* and in *Holothuria leucospilota*. Terminal ends of smooth rods were characterized as having branching ends that were not knobbed or rounded (Samyn, 2005).

20. *Knobbed or barbed rods: absent = 0; present = 1.* Rods that were not uniformly smooth contained knobs or barbs. Knobbed rods consisted of a rod that was knobbed at both terminal ends and contained one or more knobs in the center of the rod. Barbed rods were observed in *Thelenota ananas*. The rods of *Holothuria (Actinopyga) spp.* were not smooth, however the distinction between knobs and barbs in this species was unclear (Kerr, 2001, Samyn, 2005).

21. *Buttons (six-holed): absent = 0; present = 1.* Buttons contain two parallel rows of holes. Perimeter of button is oval shaped. Presence of these buttons is common in members of the family Holothuriidae (Kerr and Kim, 2001).

22. *Rosettes: absent = 0; present = 1.* Rosettes were observed as large rounded ossicles containing lots of perforations. Regularity of perforations appears waffle-like (Samyn, 2005).

23. *Anchors: absent = 0; present = 1.* Large, anchor-shaped ossicles were only found present in the outgroup *Synapta maculata*. **EXCLUDED**: was only observed in one species and thus is an uninformative character.

24. *Clouds: absent = 0; present = 1.* Cloud-like ossicles occurred in members of the genus *Bodaschia* and in the outgroup *Synapta maculata*. Clouds are described as having a central axis with numerous bubble-like rounded projections splaying off of central axis to give the appearance of a cloud. Central axis

is often curved. No description of this ossicle type was observed in previous studies. This character may have been recorded under a different name in the past. Some characters are not explained in previous papers and only listed under a name that the author decided on (may be included under *other ossicles*, in Samyn, 2005). This may be a result of that, as cloud ossicles are an excellent morphological character.

ECOLOGY CHARACTERS

25. *Body color: black = 0; very dark brown = 1; gray = 2; tan = 3; brown = 4; maroon = 5; white = 6; bronze and gold = 7; green-brown = 8; pink = 9.* Body color varied greatly among individuals used in this study. Body colors observed ranged from white to black, with many variations in between.

26. *Body spots: present = 0; absent = 1.* Body spots were present in all members of the genus *Bodaschia*, as well as in certain members of the genus *Holothuria*. Body spots were present in both outgroup species, *Synapta maculata* and *Chirodota hawaiiensis*. Presence of body spots may be related to dorsal papillae and ossicle location.

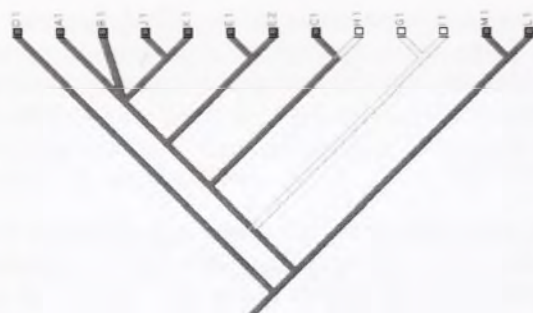
27. *Adhesiveness to tank: easy to remove, does not stick = 0; difficult to remove from tank wall, sticks = 1; very difficult to remove from tank wall, tube feet tear off body when pulled from tank wall = 2.* Individuals scored in this study had different levels of adhesiveness to the aquarium walls. Individuals of *Holothuria* (*Actinopyga*) spp. always held very strongly to the tank walls and were very difficult to remove from the tank. When removed, tube feet were always torn from the body and remained stuck to the tank wall. Members of the genus *Bodaschia* held tightly to the walls of the tank, but were much easier to remove and tube feet were not usually torn off from the body in the process. *Chirodota hawaiiensis*, *Holothuria leucospilota*, and *Holothuria* (*Lessonothuria*) were never observed stuck to the tank wall and were easily removed from the tank.

28. *Burrows under sand: no = 0; yes = 1.* Burrowing under the sand was observed in multiple individuals in this study. After sifting through the top 5 centimeters of sand, certain individuals would be found. Two members of the genus *Bodaschia* were found burrowed in the sand daily. *Chirodota hawaiiensis* is an infaunal sea cucumber, and was never observed on or above the surface of the sand.

29. *Daily behavior pattern: fully submerged under sand during day, fully exposed on top of sand during night: does not display behavior pattern = 0; displays behavior pattern = 1.* *Bodaschia marmorata* and *Bodaschia* unknown species K were observed to be fully submerged under the sand during the day light hours. During the night, they emerge from underneath onto the surface of the sand and actively feed.

30. *Found under rocks: no = 0; yes = 1.* Individuals of species *Holothuria hilla* and *Holothuria* (*Lessonothuria*) were usually found hidden between the bottom of a coral rubble piece and the sand, or found hidden inside the coral rubble.

31. *Period of activity: diurnal = 0; nocturnal = 1; both = 2.* Most observed individuals had a distinct period of activity during the day where found actively moving around (both in the tanks and in the ocean) and feeding or releasing a cloudy liquid into the water. Individuals of *Bodaschia argus* and *Thelenota ananas* were observed active during both the day and night.

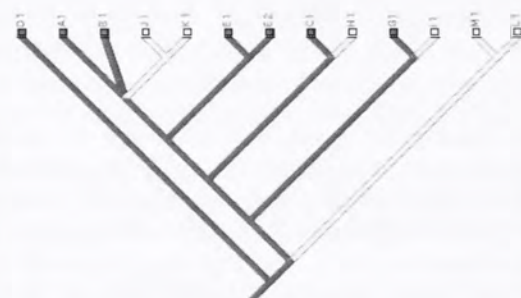


■ Species is present
 □ Species is absent

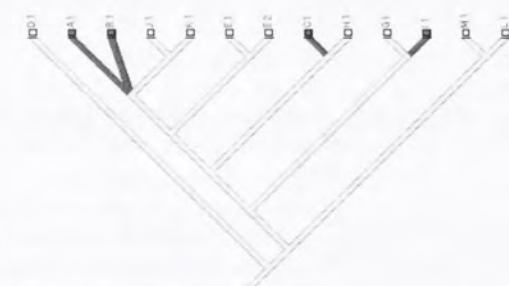
7A.) Habitat 1: Water less than 2 meters deep, gradual slope, coral bottom



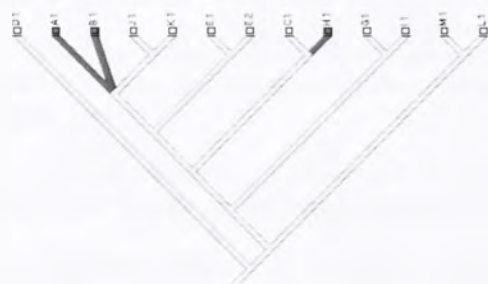
7B.) Habitat 2: Water less than 2 meters deep, coral bottom, gradual slope, current



7C.) Habitat 3: Water depth quickly varies from 0 to 4+ meters deep, rocky area, coral bottom



7D.) Habitat 4: Strong current, coral bottom



7E.) Habitat 5: High sun exposure, water less than 1 meter deep, no coral, sand bottom

FIG. 7A-D. Habitat Preference. The five habitat types observed on Moorea have been mapped onto the phylogenies below. These are maximum parsimony trees of treelength 69, generated by PAUP in this study.

THE ETHNOBOTANY AND ECOLOGY OF *GEOPHILA REPENS* AND *CENTELLA ASIATICA* ON MOOREA, FRENCH POLYNESIA

ALANNA R. TROTTER

Department of Integrative Biology, University of California, Berkeley 94720

Abstract. Two plants used in traditional Polynesian medicine, *Geophila repens* and *Centella asiatica* were selected for a study of their ethnobotany and ecology. Ethnobotanical data was collected via interviews with healers or other knowledgeable persons on the island of Moorea. Bioactivity was assessed using *Artemia salina* for cytotoxicity assays and *Saccharomyces cereviae* for antifungal assays. Cytotoxicity tests showed that an aqueous extract of *Centella asiatica* significantly enhanced shrimp survivorship; an ethanol extract of positive control *Cantharanthus roseus* resulted in the lowest survivorship. The antifungal assay design was inadequate to produce significant results because the positive control failed. Transects of the forest environment in which *Geophila repens* grows were conducted and those species that were part of its community were documented and analyzed for community associations. A small germination study was performed but no seeds germinated. Ordination showed a distinct community assemblage at one of the three main ecological study sites. This study suggests that *Centella asiatica* is a positive factor in brine shrimp longevity although the reason for this remains to be determined.

Key words: *Geophila repens*; *Centella asiatica*; ethnobotany; community ecology; bioassays; French Polynesia

INTRODUCTION

The range in which a plant species occurs depends upon a variety of factors, including how that plant disperses or is dispersed. On islands, plants arrive by means of wind or water, or they are brought, intentionally or unintentionally by humans. Once they arrive, the success of the plant depends on the conditions of the new habitat, niche availability, the presence or absence of other organisms, and the adaptability of the plant. Some of the most widespread plants are generalists which can live in a wide range of environments under varied conditions.

Over 25% of prescription medicines contain ingredients from plant sources (Farnsworth 1985). Medical ethnobotany is the study of how different cultures use plants medicinally. Even with the advent of modern medicines and synthesized drugs, ethnobotany can be extremely important as a way of maximizing the efficiency with which new plants are chosen for bioactivity screenings. The likelihood of finding useful plants from among those that traditional healers have used for generations is much

higher than the likelihood of finding useful plants in a random screening. Unfortunately, much traditional knowledge has been lost over time and with the "development" of undeveloped areas.

In French Polynesia, speaking the Maohi language and practicing traditional medicine were forbidden until the 1980s. Healers, always protective of the knowledge passed down to them through the generations or gained through their own experimentation, grew even more secretive. Even today, healers do not typically share knowledge outside of the family. Nevertheless, even basic ethnobotanical data combined with screening for bioactivity can be enormously effective in identifying new plants that are useful to humans (Trotter 1983, Farnsworth 1985).

There are sufficient bioassay techniques available that prescreening for almost any type of biological activity can be done without using intact animals (Farnsworth 1985). Trotter (1983) asserted that a simple bioassay for pharmacological activity using *Artemia salina* (brine shrimp) is not only

inexpensive and possible to do in the field but actually makes obsolete the former method of ethnopharmacological research which entailed simply documenting the uses of the remedy and collecting voucher specimens. Trotter (1983) conjectured that data from basic bioassays can prove or disprove traditional knowledge and "rapidly provide a source of alternate pharmaceutical resources for physicians and other health officials in areas where economic conditions and/or the cultural environment make the use of manufactured pharmaceuticals more difficult."

Bioassays serve as preliminary screens for more extensive bioactivity testing. Of 119 plant-derived drugs, 74% were discovered because of further research being done on plants used in traditional medicine (Farnsworth 1985). *Catharanthus roseus* (L.) G. Don (1837), the source of antineoplastics vinblastine and vincristine, was screened only because it was used as a hypoglycemic in traditional Asian medicine (Mann 2002). The

that retain the floral lobes and contain two tan-colored pyrenes.

In the Maohi language of French Polynesia, *Geophila repens* is called 'tohetupou' and is used medicinally. *Centella asiatica* (L.) Urban (1879), family Apiaceae, has the same Maohi name of 'tohetupou' due to similar gross morphology (see Fig. 1,2) and is sometimes used interchangeably as *Geophila* in traditional Tahitian medicine (Whistler 1992).

Generally, bioactive compounds run in genealogical families. As plants evolve, the manufacture of certain secondary compounds might be selected for in order to deal with the stresses of the environment. These compounds may be conserved throughout speciation and consequently the presence of bioactive compounds would tend to parallel evolutionary plant families. Given this, we would not predict *Geophila* and *Centella* to have similar properties, so it would be interesting to see if the same name was given to the two plants because they have similar



FIG. 1. Morphology of *Geophila repens*.



FIG. 2. Morphology of *Centella asiatica*.

antineoplastic activities of Taxol, a compound from the bark of *Taxus brevifolia*, were found during the National Cancer Institute's 1962 screening for antitumor agents.

Geophila repens (L.) I.M. Johnson (1949), in the family Rubiaceae, occurs throughout the tropics (Verdcourt 1989) but is restricted to moist, shady valleys (Verdcourt 1989). It is a prostrate creeper with rounded reniform leaves, solitary or rarely clustered five or rarely six-petalled white flowers and globose bright orange or red glossy berries

medicinal properties as well as similar gross morphologies.

Screening Paraguayan plants for antifungal properties indicated that *Geophila repens* displays broad-spectrum antifungal activity (Portillo 2001). A leaf dichloromethane extract of *G. repens* showed activity against six strains of fungi: *Cladosporium cladosporioides*, *Microsporum gypseum*, *Neurospora crassa*, *Saccharomyces cerevisiae* and particularly *Cryptococcus neoformans* and *Trichophyton mentagrophytes*; a methanol extract was active against *C.*

neoformans and *N. crassa*, and aqueous extract exhibited activity against *N. crassa* (Portillo 2001).

Centella asiatica is commonly used medicinally in Asian and African cultures and is generally quite well known under the names gotu kola or Asiatic pennywort (Brinkhaus 2000, Oyedeji 2005, GRIN Taxonomy). Both crude extracts and partially purified fractions of *Centella asiatica* have retarded the development of tumors and increased the lifespans of tumor bearing mice (Babu 1995). Ethanolic extracts of *Centella asiatica* have been found to elicit an increase in nerve regeneration with oral administration (Soumyanath 2005). Significant increases in the phagocytic index and total white blood cell count were observed in laboratory mice treated with *Centella asiatica* extract (Jayathitha 2004). Oyedeji (2005) found that an essential oil extract exhibited a broad spectrum of antibacterial activities against gram-positive (*Bacillus subtilis*, *Staphylococcus aureus*) and gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*, *Shigella sonnei*) organisms.

In this study, I investigate the ethnobotany of *Geophila repens* and *Centella asiatica* by conducting interviews with knowledgeable persons on Moorea. I perform bioactivity assays for both cytotoxic and antifungal properties. While the ethnobotany and bioactivity of medicinal plants are of obvious significance to humans, the importance of understanding plant ecology may be less so. Understanding ecology is important because it helps with conservation efforts. If a plant is found to be useful, it faces the danger of over harvesting. If we understand the plant's habitat requirements and how it acts as part of a community, we can protect it from this danger. Additionally, ecological knowledge is beneficial for purposes of cultivation. In this study, I examine the forest understory community in which *Geophila repens* lives and conduct a germination study. I exclude *Centella asiatica* from these parts of my study because it is a weedy plant with a large range from Ohio to Malawi (Verdcourt 1989).

METHODS

Collection of Ethnobotanical information

Ethnobotanical information was collected by interviewing knowledgeable persons on the island of Moorea. Interviewees were asked whether they knew *Geophila* and *Centella* and whether they used them medicinally. If they did use the plants, they were asked what the plants were used for, and how they were prepared. Two interviews were conducted with the aid of a translator, Valentine Brotherson, who is fluent in Maohi (Tahitian), French and English. Two interviews were conducted at the Gump station and two were conducted at the homes of the interviewees.

Bioassays: Preparation of Extracts

Plant extracts were made using a kitchen blender and scissors to chop up plant stems, leaves and runners into pieces 1/4cm² or less in area. 3.0g of each plant, *Geophila repens*, *Centella asiatica*, and *Catharanthus roseus*, were combined with 10mL of filtered tap water or 10mL of 95% EtOH in a capped plastic tube. The tubes were left at room temperature (~80°F) for 24 hours and then refrigerated at ~10°C for 48 hours. Then the extracts were filtered through filter paper using a syringe to press extra liquid from macerated plant material. The filtered extracts were stored in capped tubes in the refrigerator until use. Voucher specimens of *Geophila repens* and *Centella asiatica* were deposited at the Jepson Herbarium of the University of California, Berkeley.

Bioassays: Cytotoxicity test

A cytotoxicity test was selected because it is a simple test that often generates provocative results (Trotter 1983). Brine shrimp were hatched using a bubbler in sea water that had been run through the wet lab filter system. A pilot study was conducted in which fifty shrimp were transferred by dropper to each of 8 capped vials with tapered tips. Five drops of extract or filtered tap water (for the positive control) were added to each vial. In addition to *Geophila* and *Centella*, both water and 95% ethanol extracts of *Cantharanthus roseus*, a plant with known cytotoxicity (Mann 2002, Raskin 2002), were tested for a negative control. Shrimp

THE CHARACTERISTICS OF HERMIT CRAB POPULATIONS AND SHELL AVAILABILITY ON MOOREA, FRENCH POLYNESIA

JULIA VO

Integrative Biology, University of California, Berkeley, California 94720 USA

Abstract. Resource availability is the driving force behind many ecological phenomena. I examined the relationship between shell availability and the population characteristics of three *Coenobita rugosus* populations on Moorea, French Polynesia. The results indicate that shell availability can influence the crab mass distribution and crab-mass-to-shell-volume variance within a population. The data also suggests that factors other than shell availability play a role in the population characteristics of *C. rugosus*.

Key words: *Coenobita rugosus*, resource availability, Ideal Free Distribution, population structure

INTRODUCTION

The effect of resource availability on organisms, communities, and ecosystems is a primary topic in the study of biology. Resource availability has been shown to influence growth and development, spatial distribution, community composition, and many other aspects of ecology (e.g. Allombert et al. 2005, Anderson and Sabado 1995, Winsome et al. 2005). It is also the primary factor in many broad, well-used theories, such as Tilman's Resource Ratio Hypothesis (1986).

The Ideal Free Distribution theory predicts that at equilibrium, all occupied habitats are equally suitable for an outside individual. Assuming that 'suitability' decreases with increased density, the distribution of a species, at equilibrium, will reflect the distribution of resources between habitats (Fretwell 1972). The distributions of different resources that may be essential to the same organisms, however, are not necessarily related. A habitat abundant in one resource isn't necessarily abundant in others. What are the specific population characteristics that are affected by the accessibility of a single resource and *how* are these characteristics affected?

Hermit crabs are unique for their obligate existence in empty gastropod shells. Empty shells are an important resource for hermit crabs because they are used to protect the hermit crabs' soft, vulnerable abdomens. Such dependence on a resource has the potential to be a major constraint on hermit crab populations. Shells that are too small can inhibit an individual's growth even

though other resources may be plentiful (Fotheringham 2003) and 'improper' shells have been shown to limit reproductive success (Hazlett 1989).

Coenobita rugosus is a terrestrial hermit crab species that occurs on the shores of many Pacific islands, including the tropical Moorea, French Polynesia. By comparing three *C. rugosus* populations on Moorea, this study aims to test three hypotheses regarding the relationship between population structure and shell availability: populations with access to fewer empty shells will exhibit...

1. a larger variance in crab-mass-to-shell-volume ratios.
2. an increase in the diversity of shells that are used.
3. a different crab-mass distribution from populations with access to many shells.

METHODS AND MATERIALS

Study sites

Three sites on the coast of Moorea were selected for hermit crab population comparisons. A public beach in Papetoai, Temae Public Beach, and a shore adjacent to the University of California, Berkeley's Gump Research Station were chosen based on a visual assessment of their shell availabilities (Figure 1).

The Papetoai site is a sandy beach scattered with coconut trees growing approximately 3m apart. A *C. rugosus* population is found at the southern end of the beach, spanning about 50 meters (0197250, 8063785utm). The crabs are

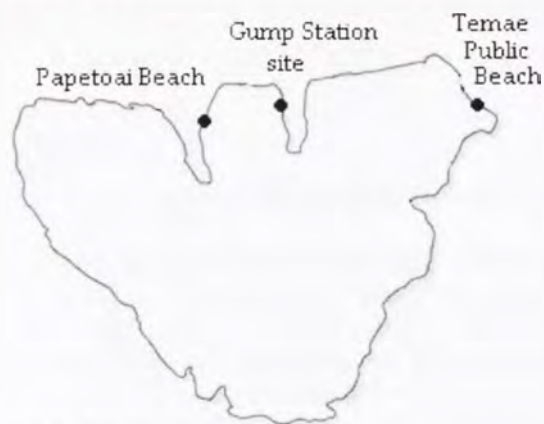


FIG. 1. Site locations on Moorea..

mainly found beneath the roots of coconut trees where the ocean waves have eroded the sand and exposed the roots. Papetoai Public Beach is regularly raked for large debris and the number of empty shells that are found there is relatively low.

The Temae Public Beach site and the shore south of the Gump Station are both coral rubble beaches with high numbers of unoccupied shells. The area studied at Temae is limited to the coral rubble section north of the sandy beach (020785, 8063268utm). This beach is commercially developed and has few trees. The coral rubble at the Gump Station site extends a meter into land before being replaced by a lawn of foreign grasses. Several tree species, including coconut palms, are distributed about 5m apart on the beach and grass.

All three sites have an intertidal range of about one meter and are exposed to weak to medium wave action.

Data collection

In order to assess shell availability, two 50m transects, one starting at low tide and the other at high tide, were run at the water's edge at each site. Tide times were determined using the University of South Carolina's Tide Predictor website. Empty shells were collected within a 1-meter square at every other meter. Bivalve and significantly damaged gastropod shells were not collected. The gastropod families of the shells were identified by comparing the shells to photographs in Salvat and Rives's *Shells of Tahiti* (1984). Shell volumes were measured by weighing the empty shells, filling them with

water, and weighing them again. All shells were measured to the nearest 0.01ml.

Hermit crabs were collected at all sites between October and mid-November of 2005. *C. rugosus* was primarily found in the exposed roots of coconut trees (Papetoai) or under large, fallen foliage (Temae and Gump). All crabs were kept in shaded, flow-through aquaria, each with rocks above the water level.

In order to remove the hermit crabs from their shells, heat was applied to the shell using a 60-watt light bulb. Naked crabs were then weighed to the nearest 0.01g and their shell volumes were measured with the same technique used on the shells that were collected from the transects. Crabs were held in captivity for no more than 3 days before they were weighed. The families of the hermit crab shells were also identified using *Shells of Tahiti* (Salvat and Rives 1984).

Statistical analyses

Significant differences in the shell abundances between sites were determined using t-tests. The number of shells in each quadrat was treated as an individual sample.

In order to examine the diversity levels of the occupied and unoccupied shells, Shannon-Weiner diversity indices were calculated for the shells that were collected from the transects and those that were found on crabs. Several shell families with similar shapes and aperture structures were merged for the statistical analyses: bursid shells were placed into the cymatiid group, colubrariidae and cerithiidae shells were combined in the 'cerithiidae' group, littorinidae and vanikoridae were included in the 'turbinidae' group, and costellaridae shells were included in the 'mitridae' group. In addition, conidae and cypraeidae shells were combined into the 'cypraeidae' group because none of the sampled crabs were found in these two shell types.

The "Unequal Variance" test in JMP was performed on the crab-mass-to-shell-volume ratios to discover significant differences between the variances of the mass-to-volume ratios (JMP). This test was also executed exclusively on the three most abundant shell groups. In order to account for the 'smallness' of the data values, mass-to-volume ratios were divided by the average mass for each population. This adjustment adds weight to deviations in the data that are perceived as 'small' simply because the

data values are small. Crabs whose shells had a volume of less than 0.01ml were not included in these tests.

Crab mass distributions were compared using JMP. An ANOVA was used to find significant differences in the average crab masses between sites. A test for unequal variance was used to find differences in the weight distributions between sites (JMP). Because the crab mass distributions were skewed at every site, the ANOVA was run on log-normalized data.

Crab masses that were less than 0.01g and shell volumes that were less than 0.01ml were recorded as 0's for the statistical analyses. The mass and shell volume of egg-carrying females were not included in the statistical analyses.

RESULTS

T-tests run on the transect data showed that the shell abundance at the Papetoai site was significantly lower than that of the Gump and Temae sites ($p < .001$ for both comparisons), but no significant difference was found between Gump and Temae ($p = 0.303$). The available shells collected from the Papetoai site also showed a very low level of diversity. Empty shells from the Temae and Gump sites had Shannon-Weiner diversity indices of about 0.63 while the Papetoai site's diversity index was 0 – cone shells

TABLE 1. Shannon-Weiner diversity indices calculated from the shell families of inhabited shells and empty shells.

Site	Occupied	Available
Temae	0.8323	0.6375
Gump	0.9560	0.6291
Papetoai	0.7160	0.0000

TABLE 2. p-values from the "Unequal Variance" tests for mass-to-volume ratios in JMP (JMP). Insignificant p-values ($p > 0.05$) are shown in bold.

Comparison	all shells	cerithiidae	neritidae	muricidae
All	<0.0001	<0.0001	0.0109	<0.0001
T-G	0.1054	0.2287	0.7069	0.8816
G-P	<0.0001	<0.0001	0.0182	<0.0001
T-P	<0.0001	<0.0001	0.019	<0.0001

TABLE 3. Results of the ANOVA on the log-normalized crab masses.

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Location	2	60.29	30.14	17.69	7.36E-08
Error	223	379.90	1.70		
C. Total	225	440.19			

and cowry shells were the only ones that were present in the quadrats. The diversity level of used shells at Papetoai was the lowest of the 3 while the Gump site had the highest diversity index for occupied shells (Table 1).

The "unequal variance" test in JMP showed that there was some significant difference in the variances of the mass-to-volume ratios between the Temae, Gump, and Papetoai sites (Table 2). Pair-wise comparisons revealed that this difference occurred between Papetoai and the other two sites. The tests that were performed on the individual shell groups produced the same results (Table 2). Scatter plots of the crab masses and shell volumes are shown in Figure 2 (JMP).

Log-normalizing the crab mass data enabled it to 'pass' all tests for normality in JMP (JMP). The results of the ANOVA indicate that the average crab masses between the three sites are significantly different (Table 3). A Tukey-Kramer "Honestly Significantly Different" test

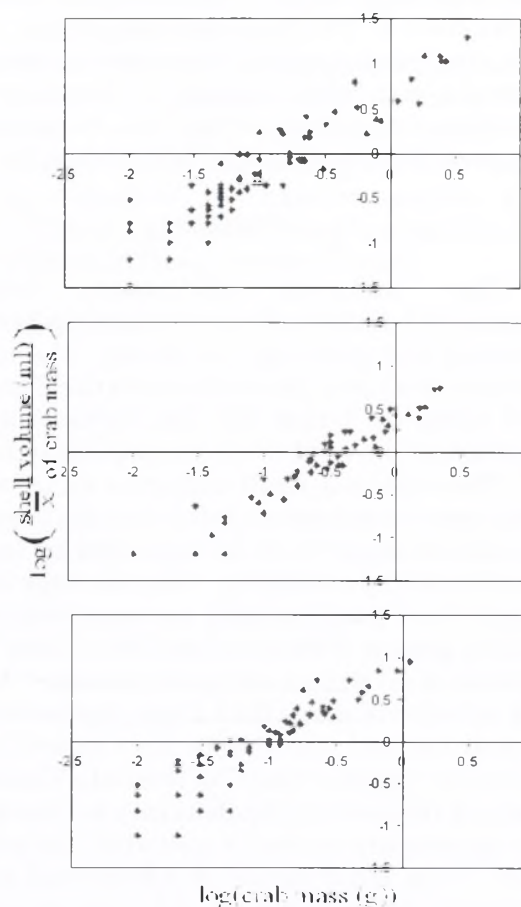


FIG. 2. Plots of log-transformed crab masses and shell volumes. Egg-carrying females and crabs with shells that were <0.01 are excluded.

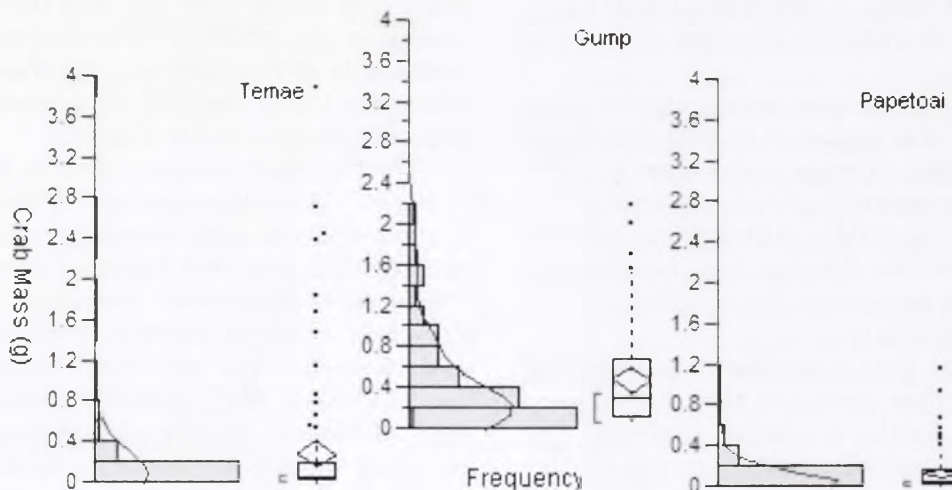


FIG. 3. Mass distributions of *C. rugosus* at the three study sites (JMP).

showed that the mean crab mass at the Gump site was significantly higher than those at the Papetoai or Temae sites. The test for equal variances indicates that the weight distributions of the crabs at the three sites are not the same. Pair-wise comparisons of the variances revealed that the crab mass variance at Papetoai was significantly smaller than the variances at Temae or the Gump Station (JMP) (Figure 3).

DISCUSSION

The measured differences between populations demonstrate a relationship between resources and population attributes. The results indicate that *some* factors between the Papetoai and Gump or Temae site are influencing the qualities of the local *C. rugosus* populations.

The relatively small crab-mass variance and large mass-to-volume variance that the Papetoai population exhibits can be explained by its low level of shell availability. Because large shells require more energy to carry and small shells can inhibit growth (Fotheringham 2003), there is an optimal shell size for any given crab size. When the selection of shells that a crab can choose from is more limited, the chance that he will come across his 'optimal shell' is reduced. Therefore, many of the crabs at Papetoai may not have had the opportunity to inhabit a shell of the optimal size. This inhabitation of sub-optimal shells increases the variance of the mass-to-volume ratios.

The smaller mass variance at Papetoai is related to the volume distribution of shells. Small shells are much more common than large

shells. Assuming that the shell-volume distribution is uniform across all sites, a site with fewer shells will inherently have fewer large shells, which are required for large hermit crabs. This explanation is confirmed by the observation that the main difference between the three mass distributions lies in the lengths of the tails located at the higher ends of the graphs (Figure 3).

The calculated diversity indices for occupied shells did not follow the expected trend. The hypothesis that stated that occupied shell diversities would increase with decreased shell availability was made under the assumption that the overall shell diversity at each site was relatively equal. One explanation for why the diversity indices were unexpected could be that this assumption was untrue. The diversity of shells used by hermit crabs is limited by the overall shell diversity at that site. If the overall shell diversity at Papetoai was significantly low, then the diversity of shells used by the Papetoai crabs must also be low.

Out of 261 hermit crabs, not a single one was found in a cowry or cone shell, even though both types made up a large portion of the empty shells collected from the three study sites. The observation that all of the shells collected from Papetoai were either cone or cowry shells may indicate the presence of a nearby niche boundary for *C. rugosus*. This observation also implies that the number of suitable shells at the Papetoai site is the primary limiting factor for the *size* of the Papetoai population.

Based on the Ideal Free Distribution theory, the significant difference in the shell selections

and availabilities between the three sites implies the existence of other essential resources for *C. rugosus* (Fretwell 1972). The significantly large average crab mass at the Gump site but not the Temae site shows that these other resources are also capable affecting the characteristics of the *C. rugosus* populations.

The population is a useful unit. It focuses on a target species but includes multiple individuals that share a common environment. These traits make it inherently useful for conservation groups, who are usually required to identify and work on improving specifically defined groups of organisms. It is no surprise that resource availability can influence population characteristics when research has already shown that it has a great effect on individuals and ecosystems (e.g. Anderson and Sabado 1995). The affect of a resource on a population, however, is much more specific and likely less difficult to predict through logic than the affect of that resource on an ecosystem. If general trends between population characteristics and the limitation of specific resources can be uncovered through further research, these trends could be used to efficiently identify the problems that encumber a dying population. The study of resources and populations, a unit that is small enough to comprehend but large enough to significantly affect an ecosystem, is a topic that has tremendous potential for providing information that would be invaluable to biological and conservational research.

ACKNOWLEDGEMENTS

I thank the six professors who taught Biology and Geomorphology of Tropical Islands, 2005 for their expert and well-considered input: Jaime Bartolome, Rosemary Gillespie, George Roderick, Carole Hickman, Brent Mishler, and Roy Caldwell. I especially thank the three Graduate Student Instructors who were helpful throughout the project: Scott Fay, Michele Weber, and Matt Medieros.

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FACTORS INFLUENCING THE ECOLOGICAL DISTRIBUTION OF TWO SEA URCHINS, *DIADEMA SAVIGNYI* AND *ECHINOMETRA MATHAEI* TYPE A, IN MOOREA, FRENCH POLYNESIA

CARMEN YEUNG

*Department of Integrative Biology, University of California,
Berkeley, California 94720 USA*

Abstract. Niche separation is the concept that two species are able to coexist in the same community if they fulfill different ecological roles. To test this concept, I examined the distribution of two distinct sea urchins, *Diadema savignyi* and *Echinometra mathaei* Type A, in Moorea, French Polynesia. In addition to assessing their distribution, I analyzed their association to different substrate materials and varying flow rates. Results showed that the distribution of *E. mathaei* and *D. savignyi* were different. Interestingly, both species had the highest density in the lagoon. Upon further investigations, both species were associated to the presence of coral substrate and the lack of sandy substrate. They were also correlated to high flow. Despite these similarities, their use of coral as a source of protection differed, their feeding habits, and their methods of dealing with wave activity differed. These findings suggest that the two urchin species, *D. savignyi* and *E. mathaei*, occupy ecologically distinct niches, allowing them to both coexist in the lagoon.

Key words: niche separation, reef complex, distribution, substrate, flow, sea urchins, *Diadema savignyi*, *Echinometra mathaei* Type A, Moorea, French Polynesia

INTRODUCTION

Echinoderms are hemispherical in shape and encircled by a solid casing composed of calcareous plates that are armed with numerous sharp spines (Bagnis 1985). Despite these basic physical similarities, echinoids, one of the five major classes of living echinoderms which includes sea urchins, possess diverse morphological features and behaviors (McClanahan 1998). The variation in morphologies implies variation in the organisms' defensive capabilities from predation, interaction with substrate materials, and robustness from wave action (Coppard and Campbell 2005). This enables different echinoid species to exploit the reef while partitioning resources (Coppard and Campbell 2005). The concept of niche separation is illustrated by echinoids.

A niche refers to the role an organism plays in the ecosystem (Elton 1927). If their niches are identical, two species cannot coexist in the same community. However if there are one or more significant differences in their niches, ecologically similar species can coexist in a community (Elton, 1927). The separation of niches can depend on the utilization of different microhabitats within the community, feeding methods, and an organism's level of activity.

To test this idea of sea urchin morphology influencing niche separation on the Moorean reef complex, I examined the distribution of two morphologically distinct sea urchins, *Echinometra mathaei* Type A and *Diadema savignyi* (Michelin), and compared their responses to various substrates, flow rates, and predation. *E. mathaei* (Blainville), encompassing four discrete morphotypes, is widely distributed in shallow reef

environments throughout the tropical Pacific and Indian oceans, including French Polynesia (Gosliner 1996). On the tropical island Moorea, French Polynesia, two types of *E. mathaei* appear. *E. mathaei* Type A inhabits the reef complex while *E. mathaei* Type B is found within the fore reef (Stephen R. Palumbi, personal communication). Due to the inaccessibility of the fore reef, *E. mathaei* Type B was not included within this study. An adult *E. mathaei* Type A has a body size (includes test and spines) of eight centimeters with short, sturdy sharp white-tipped spines, a definite bright milled ring, and dark skin on the peristome (Arakaki et al. 1998). This specific species used various micro-habitats such as crevices, burrows, undersurface of corals, and bare smooth rock surface (Tsuchiya and Nishihira 1984). Burrows, which act as home cavities, were eroded by using both their spines and mouth containing five teeth (Bagnis 1985). Burrows and even crevices collect drift algae, and provide protection from predation and intense waves. *E. mathaei* is dependent on drift algae for their food supply (Khamala 1971, Ogden et al. 1989) and occasionally forages at night (Coppard and Campbell 2005). According to Tsuchiya and Nishihira, *E. mathaei* was seen mainly in the back reef but not on the seaward side of the algal ridge.

D. savignyi possesses a body size of 20 cm with many elongated, delicate black venomous spines protruding from its test and is also found within French Polynesia (Gosliner 1996). Also, a bright blue web-like pigment and a dark anal sac defines the aboral surface. *D. savignyi* exerts some bioerosion effects, but do not excavate burrows as extensively as *E. mathaei* (Lessios et al. 2001). *D. savignyi* seeks protection from predators and wave action by occupying large crevices under coral heads or aggregating in large groups in open water over sandy and rubble substrate (Pearse 1998). They actively forage at night for food and can cover distances of 40

meters in one night (Gosliner 1996). They typically reside in locations marked by calm water such as lagoons (Pearse 1998).

I analyzed the distribution of *E. mathaei* and *D. savignyi* on the reef complex, which is composed of an algal ridge, back reef, lagoon, inner lagoon, and fringing reef. From Tsuchiya and Nishihira, it was suggested that *D. savignyi* occupies the lagoon and *E. mathaei* tend to dwell in the back reef. My objective was to test the hypothesis that *E. mathaei* is found more abundantly within the back reef and the fringing reef while *D. savignyi* predominantly inhabits the lagoon on the Moorean reef. This would mean that these two echinoid species possess different niches within the reef complex. Substrate types and relative flow were possible ecological factors influencing the distribution of these two urchin species and therefore their niche separation. Since *E. mathaei* erodes coral to build burrows and lives in coral crevices, I hypothesized that *E. mathaei* is associated with coral. I expect *D. savignyi* to display no substrate preference, because of their wide range of living habitats. I hypothesize that *E. mathaei* will live in areas with higher flow rates in comparison to *D. savignyi* since they have short sturdy spines. I also anticipate *D. savignyi* to be in areas with low flow rates because of their delicate spines. Therefore, the possible presence of *D. savignyi* in the lagoon could be due to a slow flow rate in this portion of the reef. The hypothesized presence of *E. mathaei* at the back reef and the fringing reef could be linked to the high availability of coral. Also, I anticipate that fish will feed upon both *E. mathaei* and *D. savignyi*, which could be another factor influencing their distribution in the reef (McClanahan et al. 1994).

MATERIALS AND METHODS

Study sites

The three study sites in Moorea, French Polynesia were Temae Airport, located between Pointe Aroa and Pointe Faaupo (S 17° 28' 24.1" W 149° 46' 30.2"), Maharepa (S 17° 28' 82.2" W 149° 47' 64.2"), and Temae Public Beach (S 17° 29' 56.4" W 149° 45' 51.6") (Fig. 1). These sites were characterized by having a shallow reef complex, where the maximum depth was no greater than three meters. The reef complex encompassed five different zones: algal ridge, back reef, lagoon, inner lagoon, and fringing reef, which is nearest to shore (Fig. 2). A mixture of sandy sediment, large living and dead coral heads, and coral rubble characterizes the reef complex. The dominant corals included species of *Acropora*, *Montipora*, *Porites*, and *Pocillipora*. Each study site extended from the algal ridge to shore. This distance varied between each study site: Temae Airport (110 meters), Maharepa (725 meters), and Temae Public Beach (370 meters). The fore reef was not included in the study.

FIG. 1. Map of Moorea, French Polynesia (goaway.com, 2005).



Distribution

To determine whether *D. savignyi* lived predominantly in the lagoon and *E. mathaei*

at the back reef and fringing reef, two transect studies were conducted at each of the three sites. Data was collected from 6 October to 27 October 2005. Transects began at the algal ridge and ended at shore. For each transect, the data was divided into the five zones: algal ridge, back reef, lagoon, inner lagoon, and fringing reef (See Fig. 2). The location of each transect was chosen randomly. A 1 m² quadrat was placed multiple times along the transect. In each quadrat, the number of urchin species, the zone of the reef complex, algal species, depth, distance between quadrats, and the substrate types were recorded. The distance between quadrats varied among different transects. This depended on whether the features of the surrounding environment was constantly changing or remaining consistent. If the environment was constantly changing from one quadrat to the next, the distance between quadrats was shorter. If the features were consistent for three quadrats in a row, then the distance between the quadrats increased. Due to strong wave action, a few transects were not started precisely at the formation of the algal ridge.

To reject the null hypothesis that the distribution of *D. savignyi* and *E. mathaei* in the reef complex are the same, the chi-squared test of independence between two or more samples was applied to the total number of urchin species in each zone, using the data from the study sites, Temae Airport and Maharepa, which resulted in a total of 10 data entries.

FIG. 2. The 5 zones of the reef complex.



Substrate types

In each quadrat, I recorded the substrate types as the percentage of coral, included both dead and alive, sand, and rubble. Data was collected from 6 October to 27 October 2005.

To test the hypothesis that *E. mathaei* is linked to the presence of coral, the coral data from Maharepa and Temae airport were combined. This data, containing the number of *E. mathaei* and coral percentages per quadrat, were divided into two categories: quadrats containing at least one *E. mathaei* and quadrats containing zero *E. mathaei*. From each of the two categories, 15 quadrats were randomly selected. The t-test was conducted using the percentage of coral from the randomly selected quadrats. To see if *E. mathaei* was linked to rubble or sand, the same process and statistical test were used. The hypothesis that *D. savignyi* displays no substrate preference was tested using the above process. A total of six t-tests were conducted.

For the substrates with a rejected null using the t-tests, a regression analysis was conducted between the percentage of one substrate (x-axis) and the number of a specific urchin species (y-axis). This tested if a substrate percentage could predict the number of sea urchins in a quadrat. For example, is a high coral percentage correlated to a high number of *E. mathaei* in a quadrat?

Relative flow

To document rates of relative water flow in each zone, clod cards were employed. A clod card is a circular plaster of Paris, four centimeters in diameter and three centimeters thick circular, that was attached to a nine centimeters in diameter plastic cup lid. The plaster was glued to the cup lid using silicone adhesive. A hole was made in the lid where rope was placed through and later used to anchor the card to a substrate

material. When comparing different zones of the reef complex, the zone with the most dissolved plaster has the highest flow rate. Two clod cards were placed into each of the five reef zones at each study site. A total of 30 clod cards were made. Large plastic bottles acted as buoys to mark the location of the clod card. The cards were left at each site for 24 hours. The test of relative flow was conducted from 8 November to 16 November 2005. The weight of the plaster before and after its placement into the site yielded the amount of dissolved plaster on each reef zone. Therefore, this indicated the relative flow levels for each zone.

To observe if flow levels are linked to the number of *D. savignyi* or *E. mathaei*, a regression was conducted between the amount of dissolved plaster in a 24 hour period on the x-axis and the number of urchin species on the y-axis.

Predation

Cages were used to test predation as a factor influencing the distribution of *D. savignyi* and *E. mathaei*. The treatments comprised of a 5 sided 35 cm by 35 cm metal wire cage with an inwardly curved barrier and a fishing line perimeter, which was placed approximately half an inch from the barrier. The urchins in the treatment were tethered to the cage with a 30 cm long fishing line. The metal wire used the construct all the cages had a .65 cm square grind. The controls consisted of an urchin within a completely enclosed 30 cm by 30 cm metal wire cage with a chicken wire and green plastic squared bottom. The cage sizes for the control and treatment differed due to the cost of the materials. For each urchin species, there are 3 replicates of the control and treatment, leading to a total of 12 cages in this study. To prevent the cages from moving, a 10 cm by 10 cm brick was zip tied to the bottom of each cage.

RESULTS

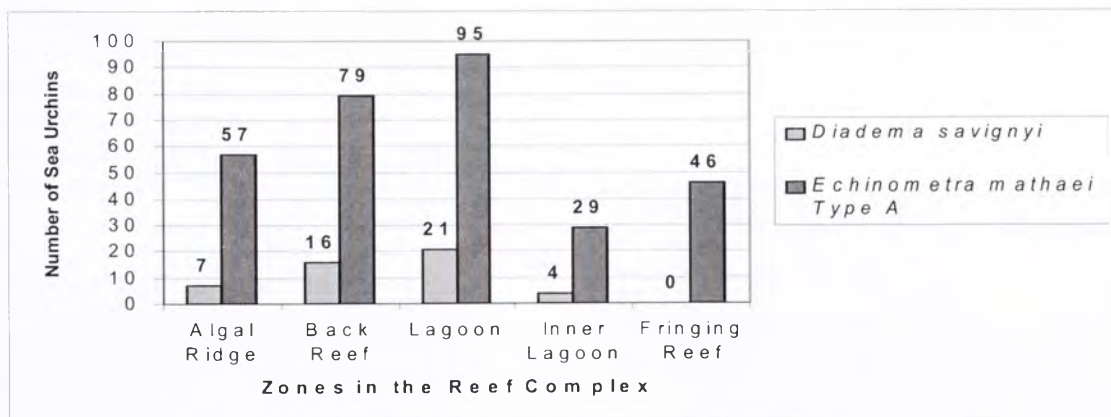
Distribution

The data from Temae Public Beach was excluded from all my analysis due to the absence of a fringing reef and the abnormally high density of *D. savignyi*.

When the distribution of *D. savignyi* was compared to the distribution of *E. mathaei* in the reef complex (Fig. 3), a significant difference was detected using the chi-squared test of independence between two or more samples. The null stating that the distribution of *E. mathaei* and *D. savignyi* are

equal in the reef complex was rejected. Both *D. savignyi* and *E. mathaei* were observed to have the highest densities on the lagoon. However, the lowest density for *D. savignyi* was at the fringing reef. The lowest density for *E. mathaei* was at the inner lagoon. This illustrates that the densities between the two urchin species varied a great deal throughout the reef complex (See Fig. 3).

FIG. 3. The number of sea urchins in the reef complex. The calculated P-value is 0.032, which means I was able to reject the null stating the distribution of *D. savignyi* is equal to the distribution of *E. mathaei*.



Substrate types

The amounts of substrate varied throughout the reef complex. At the algal ridge, I observed the highest percentage of coral coverage (60.5%). The lagoon was the second location with the highest percentage of coral (46.2%). The back reef contained the most rubble (61.27%). Sand (54.4%) is the most dominant feature in the inner lagoon (Fig. 4).

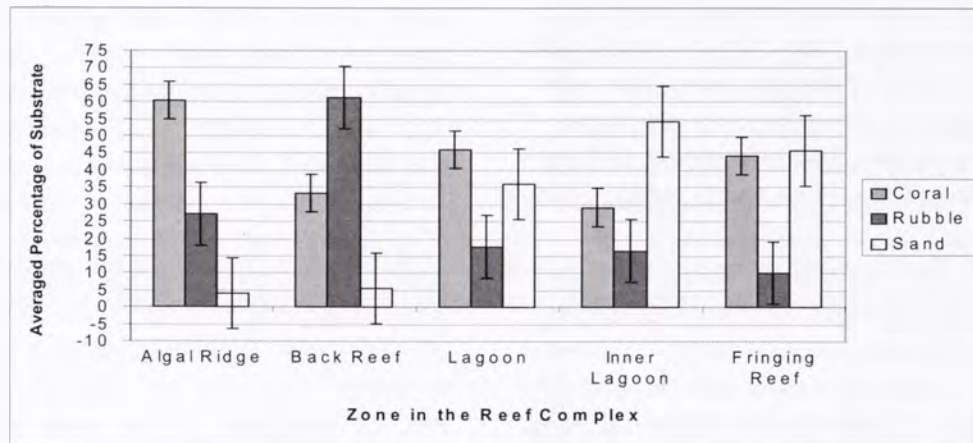
A t-test comparing the mean percentage of coral from two categories, quadrats containing one or more *E. mathaei* and quadrats with zero *E. mathaei*, showed a significant difference. Another t-test displayed a significant difference between

the mean percentages of sand from the same two categories (Table 1). This suggested that presence of *E. mathaei* was associated with the availability of coral and the lack of sand in a quadrat (Fig. 5). In a regression analysis, the percentage of coral present could not predict the number of *E. mathaei* (Fig. 6). For instance, a high percentage of coral did not indicate a large number of *E. mathaei*. The percentage of sand in a quadrat could not be used to predict the number of *E. mathaei* (Fig. 7). This meant that a low sand percentage in a quadrat did not correlate to a large quantity of *E. mathaei*. Although the amount of coral and sand could not predict the number of *E. mathaei*, the availability of coral and the lack of sand

were associated to *E. mathaei*. The t-test comparing the mean percentage of rubble from quadrats containing one or more *E. mathaei* and quadrats with zero *E. mathaei* yielded insignificant results (See Table 1).

This suggests that *E. mathaei* lacks an association to rubble (See Fig. 5).

FIG. 4. Average percentage of substrate types in the reef complex. The percentage of each substrate type from the total number of quadrats within each zone was averaged. Error bars are standard.

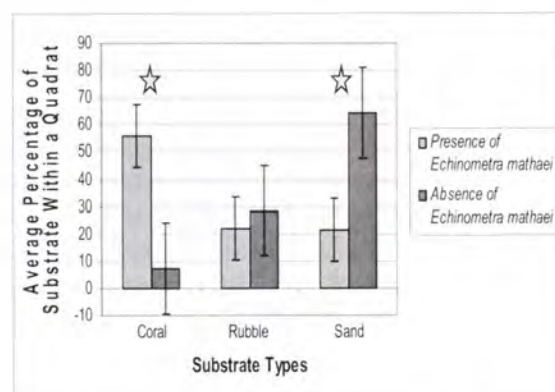


	Coral (%)	Rubble (%)	Sand (%)
Algal Ridge	60.5	27.1	4.1
Back Reef	33.2	61.3	5.5
Lagoon	46.2	17.8	36.0
Inner Lagoon	29.1	16.5	54.4
Fringing Reef	44.1	10.1	45.8

TABLE 1. Results from t-tests comparing the mean percentages of different substrate types. The mean percentages came from 30 randomly selected quadrats. 15 selected quadrats came from quadrats with one or more *E. mathaei* and the other 15 came from quadrats with zero *E. mathaei*.

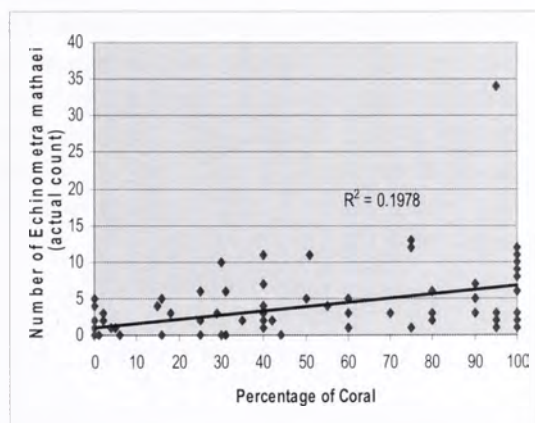
		Mean	Variance	df	P-value
Percentage of Coral	In quadrats one or more <i>E. mathaei</i>	51.1	1316	28	0.0002
	In quadrats zero <i>E. mathaei</i>	4.9	112.1		
Percentage of Rubble	In quadrats one or more <i>E. mathaei</i>	33.4	1426	28	0.532
	In quadrats zero <i>E. mathaei</i>	25.5	886		
Percentage of Sand	In quadrats one or more <i>E. mathaei</i>	28.1	1094	28	0.022
	In quadrats zero <i>E. mathaei</i>	58.4	1236		

FIG. 5. Comparing the presence and absence of *E. mathaei* to the availability of substrate materials in a quadrat. The data from Temae Airport and Maharepa were divided into two categories: quadrats marked by the presence of *E. mathaei* and quadrats marked by the absence of *E. mathaei*. From these two categories, the percentages of each substrate were averaged. Error bars are standard. Stars indicate that the P-values from the t-tests were significant.



Similar to the results for *E. mathaei*, a t-test of the mean percentage of coral from the quadrats containing at least one *D. savignyi* and the quadrats with zero *D. savignyi* yielded significant results. A significant difference was also recorded from the t-test

FIG. 6. A regression illustrating the relationship between the percentage of coral substrate in a quadrat and number of *E. mathaei*. Data from Temae Airport and Maharepa were used here. The line represents the best fit-line. ($R^2=0.1978$, $y=0.0558x+1.0853$)



between the mean percentages of sand from the two categories (Table 2). *D. savignyi* is also linked to the presence of coral and the absence of sand (Fig. 8). A regression demonstrated that there was no predictability between the percentage of

FIG. 7. A regression between the percentage of sand and the number of *E. mathaei*. ($R^2=0.1424$, $y=-0.0513x+5.1046$)

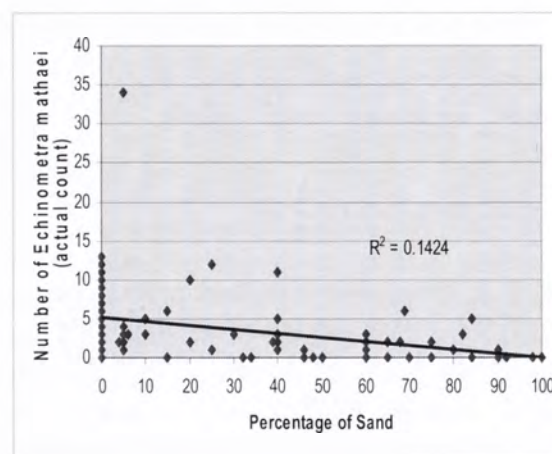
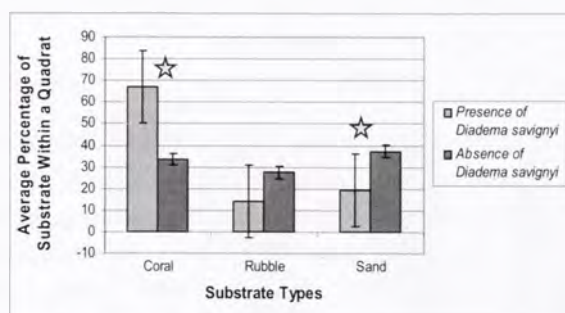


TABLE 2. Results from t-tests comparing the percentages of different substrate types from 15

randomly selected quadrats containing and lacking *D. savignyi*

		Mean	Variance	df	P-value
Percentage of Coral	In quadrats with <i>D. savignyi</i>	60.9	1160	28	0.010
	In quadrats without <i>D. savignyi</i>	26.0	1237		
Percentage of Rubble	In quadrats with <i>D. savignyi</i>	17.5	460	28	0.394
	In quadrats without <i>D. savignyi</i>	18.7	823		
Percentage of Sand	In quadrats with <i>D. savignyi</i>	10.5	376	28	0.037
	In quadrats without <i>D. savignyi</i>	36.8	1702		

FIG. 8. Comparing the presence and absence of *D. savignyi* to the availability of substrate materials. These percentages are derived from the total data from Temae Airport and Maharepa. Error bars are standard. The star indicates that the t-tests yield significant results.



coral or sand to the number of *D. savignyi* within a quadrat (Fig. 9 and Fig. 10). Although the regression was not significant for coral and sand, the t-tests showed that there was a significant association between *D. savignyi*, the presence of coral, and the absence of sand. The t-test between the mean percentages of rubble from the two quadrat categories offered no significant difference (See Table 2).

FIG. 9. A regression analysis displays the lack of correlation between the percentage of coral and the number of *D. savignyi*. ($R^2=0.1345$, $y=0.012x + 0.044$)

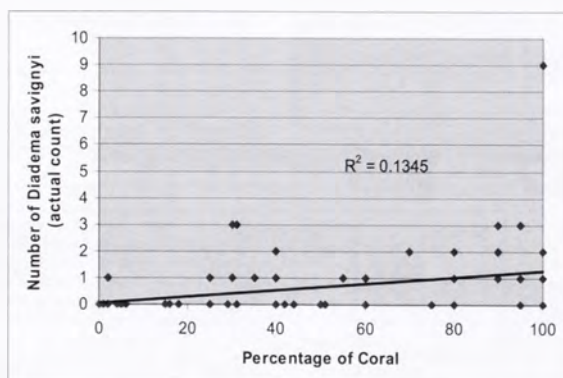
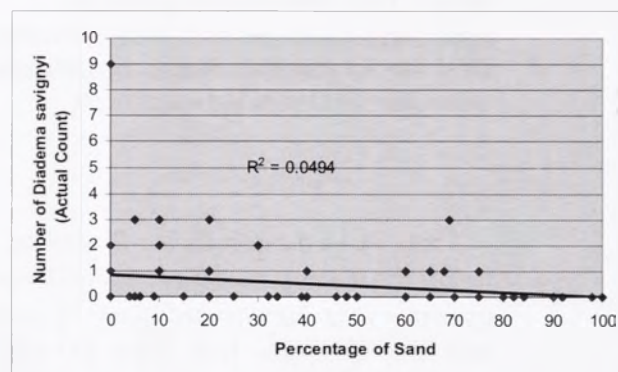


FIG. 10. A regression showing the relationship between the percentage of sand and the number of *D. savignyi*. The data from Temae Airport and Maharepa was used here. The line represents the best fit line. ($R^2=0.0494$, $y=-0.008x + 0.8218$)



Relative flow

Due to the length of the reef complex and the strong wave action at Maharepa, the clod cards were not retrieved. As a result, only the averaged amounts of dissolved plaster from Temae Airport were used. The clod cards in the lagoon were subjected to the highest currents as evident by significant loss of plaster mass (Fig.11). The amount of dissolved plaster was well correlated with both the number of *D. savignyi* and *E. mathaei* (Fig. 12).

Predation

I attempted to test whether the distribution of two urchin species was influenced by the threat of predation. The use of aluminum foil, duct tape, a simple five sided cage, tethering with fishing line, rubber bands as leashes, and various combinations of a curved barrier and a fishing line perimeter failed to contain *D. savignyi* within the treatment. To better understand the movement of *D. savignyi*, laboratory observations were conducted at night. However, *D. savignyi* continued to

escape the treatment. As a result, this portion of my experiment was halted.

FIG. 11. Amount of dissolved plaster in 24 hours (grams) at different zones of the Temae Airport reef complex. This is total plaster dissolved for 10 samples.

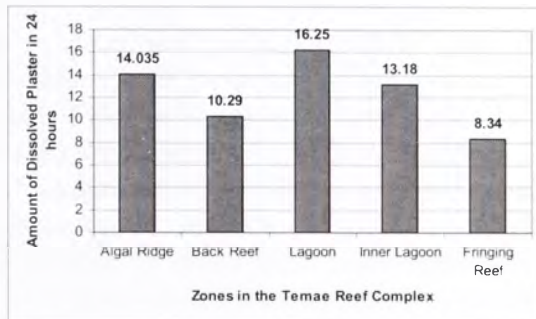
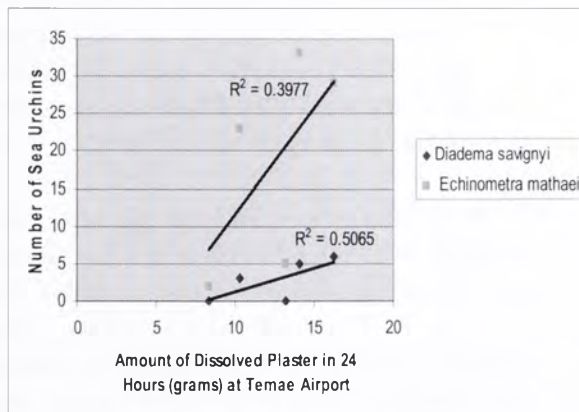


FIG. 12. A regression showing the relationship between the amount of dissolved plaster (grams) in 24 hours at Temae Airport and the number of each species of sea urchins. Each point represents one of the five zones of the reef complex. (*D. savignyi*: $R^2=0.5065$, $y=0.6233x - 5.051$; *E. mathaei*: $R^2=0.3977$, $y=2.8461x - 16.946$)



DISCUSSION

Distribution

The high abundance of *D. savignyi* in the lagoon suggested that to be the preferred living habitat, supporting my hypothesis. However, the abundance of *E. mathaei* within the lagoon was a surprising result. I

expected large numbers of individuals to be at the extremities of the reef complex: the algal ridge and the fringing reef. This could potentially be explained by the prevalence of algal vegetation and thus food supply on the lagoon (Perez-Castaneda and Defeo 2001), but further observations of algal distribution would be required to test this hypothesis. Upon further examination of substrate associations and relative flow, *E. mathaei* and *D. savignyi* occupied ecologically separate niches despite having the highest densities in the lagoon.

Substrate Types

The association of *E. mathaei* and coral supported my hypothesis. Sea urchins, especially *E. mathaei*, grind coral to create elaborate home cavities. Overtime, this significantly alters the reef morphology by transforming coral into large amounts of sandy sediment (Khamala 1971). Other urchins, such as *D. savignyi*, occupy crevices under large coral heads. Both home cavities and coral heads offer protection from predators and intense wave action, which explains *E. mathaei*'s and *D. savignyi*'s association with coral. Since *D. savignyi* and *E. mathaei* use the coral in two distinct manners, they possess different ecological niches. Initial field observations suggested that when both species inhabited the same coral head, they would partition this resource, allowing for niche separation to occur. *D. savignyi* would often be found at the bottom of the coral and *E. mathaei* would be occupying the top to mid-portion of the coral. Further studies are required to confirm these observations. Although there is a lack of correlation between the number of urchin species and the percentage of coral, an interesting future study would be examining the possible association of *E. mathaei* to a specific coral species. For example, *E. mathaei* and *Acropora* species could be linked together.

Previous studies have shown that areas with a higher amount of coral often contain a low percentage of sand (Alldredge and King 1977). As a result, both urchin species are unexpectedly linked to the lack of sand.

The insignificant result between rubble and *E. mathaei* might be explained by the lack of surface area on rubble. This might hinder the creation of sufficient burrows but future studies concerning whether there is an optimal burrow that *E. mathaei* Type A prefers and the types of substrate that *E. mathaei* prefers to use to make burrows are needed.

Relative Flow

The highest rate of water flow and the largest number of both urchin species were observed in the lagoon. This did not support my hypothesis that *D. savignyi* would prefer to live in the lagoon due to the low flow rates. However, large aggregations of *D. savignyi* could balance the threat of strong wave action breaking their spines. The lagoon was also found to have the second highest percentage of coral substrate. The coral could potentially offer a sufficient amount of protection from the waves. This would explain why both urchin species have the highest densities in this zone.

To live in a lagoon with strong flow, *D. savignyi* could be smaller than the average urchin of the same species, which would act as a measure of protecting their delicate spines. This future study would require the accurate measurement of *D. savignyi* spines and test size. Due to the small size and the sturdy spines of *E. mathaei*, coping with increased wave action does not appear to be as much of a threat. This result supported my expectation of *E. mathaei* inhabiting areas with strong water flow.

If the vegetation in this area was abundant, then the urchin species would not have to venture far in search of a food source. This short forging distance would

also minimize the threat of the intense waves.

Conclusions

In conclusion, *D. savignyi* and *E. mathaei* are both found more frequently in the lagoon portion of the reef complex. Both species are associated with the presence of coral, the absence of sand, and correlated with high flow. Despite these similarities, their partitioning of coral heads allows these two species to coexist within the lagoon. *E. mathaei* is usually found near the top portion of a large coral head while *D. savignyi* is found at the bottom of the same coral.

Also their forging habits differ, further allowing the co-inhabitation of these two urchins. Since *E. mathaei* exerts a great deal of time effort to build these homes, they are unwilling to leave it in order to actively forge. This would risk losing their protection (Nishihira et al. 1991). As a result, they use their spines in order to catch free floating pieces of algae (Ogden et al. 1989). In contrast, *D. savignyi* actively forges at night since they don't typically create extensive home cavities (Ogden et al. 1989). They seek protection under corals or in groups.

E. mathaei and *D. savignyi* are important organisms in the reef communities. These sea urchins particularly *E. mathaei* affect the bioerosion of the reef. Over time, the gradual grinding of coral alters the entire reef complex, which in turn causes the population of other marine organisms to change (Carreiro-Silva and McClanahan 2001). Since fish such as parrotfish and triggers are urchin predators, urchin populations indicate when fish groups are endangered (Peyrot-Clausade et al. 2000). Over fishing is signaled by increased numbers in the sea urchin population. Furthermore, sea urchins possess a commercial value. Their gonads are sought after for Japanese cuisines. By studying the distribution and niche separation of sea

urchins, their effect on the bioerosion of the reef, role as population indicators and conservation efforts to prevent over harvesting of urchins can be better understood.

ACKNOWLEDGEMENTS

I would like to thank George Roderick, Brent Mishler, Carole Hickman, Roy Caldwell, and Rosie Gillespie for their brainstorming efforts and their feedback on my paper. Jamie Bartolome assisted in my data analysis process. Ali Kattan, Ginevra Ryman, Michele Weber, Julia Vo, and Courtney Hart were essential in the data collection process. Stephen Hatosy photographed my sea urchins in the field. Natalie Reeder helped recover clod cards. Michele guided me through confusing emails, statistical analysis, and offered a different perspective. Thank you all for the amazing memories.

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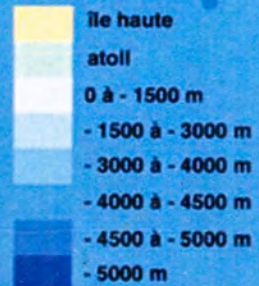
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