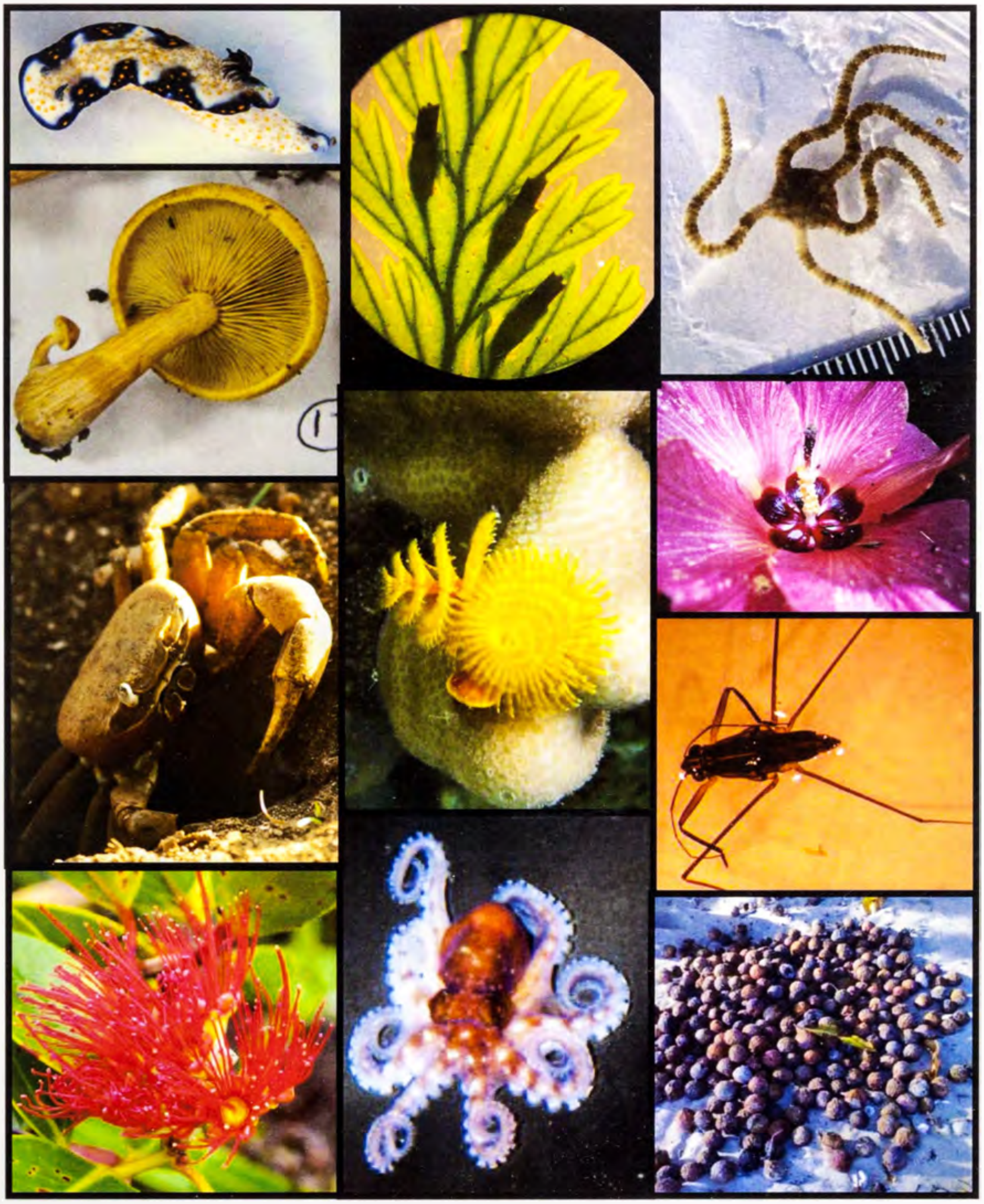
Biology and Geology of Tropical Islands
Research Papers - Fall 2006



University of California, Berkeley
Richard B. Gump Pacific Biological Research Station
Moorea, French Polynesia

Biology and Geology of Tropical Islands

Moorea Research Papers

Volume 15

Fall 2006

Environmental Science, Policy and Management 107; Integrative Biology 158

Front Cover: Some of the organisms studied by students in the Fall 2006 Moorea Class. Left to right and top to bottom: Nudibranch, filmy fern, brittle star, mushroom, Christmas tree worm, Hibiscus, burrowing crab, water strider, Metrosideros blossom, Octopus, and Tamanu seeds. See papers for details.

Back Cover: From top left to bottom right: Great crested tern, Moorea Motus, Noni, sea urchin, Inocarpus forest, dolphin, Black-tipped reef shark, hermit crab, Blue-footed Booby, booby chick, sea cucumber, landscapes on Moorea

Front and Back Cover photo credits: J.Castillo, S. Chinn, A. Dobbs, S. Ghazali, J. Himes, V. Howell, J. Lipps, J. Nitta, M. Riley, D. Song, E. Spotswood, and F. Wheaton.

Racing towards Tetiaroa (J. H. Lipps image)

The Moorea 2006 Class

From Left to Right, Back to Front: Professor Jere H. Lipps, MelissaRiley, Maggie Groff, Tom Bell, Dan Song, GSI Liz Perotti, GSI Alison Purcell, Valerie Howell, Zach Hanna, Professor Jamie Bartolome, Professor Brent Mishler, Sarah Chinn, Tom Hata, Felicia Wheaton, Joel Nitta, Sigi Hawkins, Lori LaRue, Sameen Ghazali, Julie Himes, Maya Almaraz, Carol Chang, (kneeling) GSI Erica Spotswood, Andrew Greene, Natalie Valenica, Aimee Ellison, April Dobbs, Jessica Castillo, and Erin Prado.

The Moorea Class of 2006 dedicates this book to Professor Jere H. Lipps for his hard work and his dedication to the course.

Jere discusses an ancient fire pit (right) with students along the shore during a field excursion to Motu Tiahura (NW tip of Moorea).


POLYNÉSIE FRANÇAISE
ARCHIPEL DE LA SOCIÉTÉ
ÎLES DU VENT
MOOREA

Gump Station facilities on the shore of Cook's Bay, Moorea.
Photograph by Jere Lipps, 2006.

The Moorea Class 2006

Introduction

The Moorea Class, "Biology and Geomorphology of Tropical Islands" (Environmental Sciences and Policy Management 107 and Integrative Biology 158), is a unique undergraduate experience. The class lasts an entire semester, nine weeks of which is spent on the Island of Moorea, just 18 kilometers from Tahiti in French Polynesia. Moorea is a wonderful place for a field science course; it's a tropical paradise with much geology, oceanography, anthropology, and biology all around. We take the Berkeley undergraduates from lecture hall into the world of professional scientists through the creation, design, and completion of their own original research project. Like any research expedition, the course includes planning, supply, logistics, field reconnaissance, field research, and oral and written presentation of the final results. After three weeks at Berkeley preparing and equipping ourselves, we go to Moorea, where a week or so is spent exploring all the many environments and research opportunities on Moorea and nearby Tahiti and Tetiaroa. Students explore the reefs, rivers, mangrove and marsh swamps, highlands, and shorelines to find their own special research interest. 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 come away with great insights into scientific subjects, as well as themselves. Back in Berkeley during the last two weeks of the semester, the students finalize their work and deliver their final presentations. Their scientific papers are published in this series of books on the "Biology and Geomorphology of Tropical Islands", which are distributed widely to libraries and personnel at Berkeley, on Moorea and Tahiti, as well as elsewhere.

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 benefited significantly from the Gordon and Betty Moore family and Foundation. The students and instructors are grateful to these far-sighted people who recognized the importance of exposing students and researchers to Moorea.

Faculty members and Graduate Student Instructors (from IB and ESPM) with expertise in terrestrial biology, botany, geology, and marine and freshwater biology 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 time on Moorea.

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 then. Demand for the course is always high. As the living and research facilities expanded, the class grew larger too, so now 22 students take part. Over 200 students have done research in the course on their own original projects. Many students published their research international scientific journals. Many have also gone on to graduate school and careers as professional scientists.

The 2006 course was also immensely successful. Twenty-two students conducted careful, original research on plants, animals and physical processes on Moorea. This book presents the written research results produced by the class of fall 2006.

Acknowledgments

The Moorea field 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 2006 class successful. We all appreciate and remember with wonderful feelings the help, good times, camaraderie, and excitement we shared with them. Those of us in the field with the students also learned a good deal from each of them, as they developed and explored their own research.

At Berkeley, other faculty and staff presented lectures and workshops on a variety of topics, including George Roderick (Director, Gump South Pacific Research Station), Tom Carlson (Integrative Biology), Roy Caldwell (Integrative Biology), Pat Kirch (Integrative Biology), Jim Hayward (UCB Diving Safety Officer), Norma Kobzina and Beth Weil (Bioscience and Natural Resources Library), and Maggie Kelly, Abe Hendricks, and Karin Tuxen from Geospatial Informatics & Imaging Facility, ESPM.

We especially thank Steve Owen (Integrative Biology), and Teresa Trice (ESPM) who kept the course going administratively. The staff of the University and Jepson Herbaria assisted with the workshop on plant vouchers and curation, David Hassl of the Museum of Paleontology assisted with the workshop on invertebrate vouchers and curation and the Visualization Center provided assistance with and use of their resources.

On Moorea, we are grateful to many people, especially the staff of the Gump Station (George Roderick, Director, Neil Davies, Executive Director, Frank Murphy), without whom none of this would have been possible, and also to Hinano Murphy (Atitia Center), Mari Mari Kellum, and many other Mooreans, who provided so much assistance and interaction. Rene Galzan, Director, and staff of the French research station (CRIOBE) were generous with access to their library and collections. We are also grateful to Priscille Frogier and Jean-Yves Meyer of Délégation à la Recherche, Gouvernement de Polynésie française for their continuing interest in and support of this class. Finally, to Gordon and Betty Moore who have helped to make the Gump Station one of the finest field laboratories in the tropics.

Our class commonly relies on the assistance of experts at many other institutions for advice and information on various groups of organisms or subjects. These experts are individually acknowledged in the student papers, and we thank them for aiding our students.

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

Jere H. Lipps, Professor, Integrative Biology
Course Coordinator.
James Bartolome, Professor, ESPM
Carole Hickman, Professor, Integrative Biology
Brent Mishler, Professor, Integrative Biology
Vincent Resh, Professor, ESPM
Alison Purcell, GSI, ESPM
Erica Spotswood, GSI, ESPM
Liz Perotti, GSI, Integrative Biology

Biology and Geology of Tropical Islands

Volume 15

Contents

INTERSPECIFIC INSECT INTERACTIONS AND MUTUALISM ON THE UNDERSIDE OF GUAVA *PSIDIUM GUAJAVA* LEAVES, MO'OREA, FRENCH POLYNESIA

MAYA ALMARAZ

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

Abstract. Interspecific mutualisms between ant and scale insect species have been well documented as symbiotic relationships that merit resource acquisition in exchange for protection. In this study, insect removal experiments were preformed on the branches of *Psidium guajava* in order to measure the effects that insect populations have on one another. Five ant removal, five scale removal and five control trees were treated. Data was taken daily over the course of 18 days. Ant activity was measured using a one-minute, unidirectional, point count and the number of scales per branch was determined by photographing a sample of five leaves per branch. Wilcoxon rank-sum tests found that ant populations differ significantly to that of controls in the absence of scale insects. Scale insect populations were not significantly affected by the removal of ants. Observational ant behavior data was also collected that supports possible tending behavior.

Key words: Ants (Hymenoptera, Formicidae); scale insects; mealy bug; Nipaecoccus nipae*; guava;* Psidium guajava*; insects; arthropods; mutualism; tending; Moorea, French Polynesia;*

INTRODUCTION

Mutualisms are commonly found in arthropod communities. Social insects, such as ants (Hymenoptera: Formicidae), are well recognized as a component with a great amount of influence over arthropod communities (Morrison 1996a, Krushelynycky 2001). In tropical rainforests ants have been found to dominate canopy arthropod fauna (Davidson 1997). Ant herbivory of insect exudates is believed to be a leading cause for their overwhelming abundance. The ability to feed off insect exudates allows ants to benefit from the highly productive canopy foliage, (Davidson 2006).

There are roughly 7000 scale insect species worldwide, most of which have mutualisms with ants, (Eastwood 2004). Through this mutualism, ant tending provides scale insects with protection from predation, parasitoidism and reduces exudate associated fungal growth by removal of honeydew, overall enhancing scale insect survival rates. In turn, the honeydew of scale insects provides the ants with a stable source of energy, (Eastwood, 2004). Little nitrogen is acquired by ants through scavenging and predation, but rather through the processing of large amounts of carbohydrate rich, nitrogen poor insect exudates (Davidson 1997, Davidson et al. 2003).

Adaptations of particular ant species to feed on scale insect exudates lead to the dominance of few ant species in the canopy. High densities of exudate-feeding ants in the canopy can sustain large populations of the scale insects they tend (O'Dowd 2003). Those ant species that adapt to specialize for exudate-feeding have privileged access to resources and an advantage over competing species, improving their success in the environment (Heckroth et al. 1998). Such specialization has the potential to threaten faunal heterogeneity (Wilson and Taylor 1967). The relationships that ants form with scale insects attract ants to form closer relationships with plants. These relationships play an important role to the evolution of myrmecophytes (Heckroth et al. 1998). Mechanisms relating to interspecific mutualisms are key to understanding the organization of community dynamics, evolution and the greater ecological system.

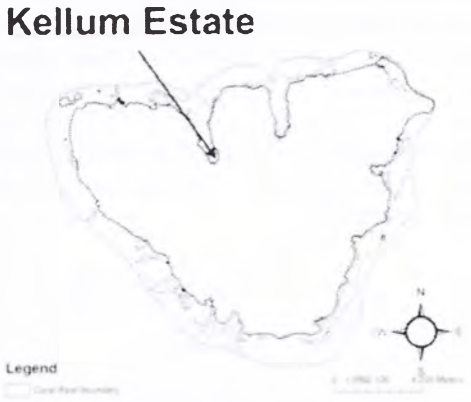


FIG. 1. Kellum Estate, Mo'orea, French Polynesia.

French Polynesia is hot spot whose biodiversity is being threatened due partially to human population and biological invasions. The ant fauna of French Polynesia are primarily dominated by tramp species whose increasing immigration, eased by modern day technology, has increased Moorea's ant species diversity (Morrison 1996b, Gillespie and Roderick 2002, Wetterer 2002). In 1907, Moorea was found to have only 13 species of Formicidae (Wheeler 1908). Today there exists at least 39 naturalized species of Formicidae (Krushelynycky 2001, Ledoux et al. 2003). Despite the significance of scale insects to ecological systems, ant-plant studies have thus far paid little attention to associations with scale insects (Heckroth et al. 1998).

The objective of this study is to collect evidence relating to interspecific mutualism and tending behavior on the tropical island environment of Moorea, French Polynesia. Populations of the scale insect *Nipaecoccus nipae*? Maskell, 1983, in the family Pseudococcidae will be measured in their response to removals of an unidentified species in the family Formicidae. Scale insect removal experiments will also be performed to investigate the effect *N. nipae* populations have on associated Formicidae. In order to establish results, experimental data will then be compared to data gathered from control trees where no insect removals were performed. In order to draw conclusions about tending behavior, an observational study will examine the behavior and movement of Formicidae in the presence and absence of *N. nipae*. The interaction of interest will be studied exclusively on the underside of leaves from guava *Psidium guajava* Linn in the family Myrtaceae.

METHODS

Study site

The study took place at the Kellum Estate on the island of Moorea, in French Polynesia (149° 50' West longitude, 17° 32' South latitude). The Kellum Estate is located approximately 15 meters above sea level (Fig. 1), and is adjacent to Mo'orea's main highway. The study site was located approximately 100 yards from the ocean. This site was selected because of the high prevalence of *P. guajava* that existed in a single area of relatively uniform elevation and habitat. The study site was once used as grazing land and is now partially cultivated (Marimari Kellum, personal communication). The study took place over the course of 18 days, during the months of October and November in 2006. A total of 15 trees were selected for treatment based on the criteria that a) the entirety of the tree was easily accessible (mainly determined by height), b) the interaction of interest between Formicidae and *N. nipae* was present on at least one branch of the tree, and c) the interaction took place on a branch that was isolated from adjacent branches, for the purpose of accomplishing complete insect removal without interference.

Experimental design

In order to investigate the effects that Formicidae and *N. nipae* populations have on each other, insect removal experiments were performed. Ants were removed from a single branch on each of five trees. *Nipaecoccus nipae* and larvae were removed from a single branch on each of five trees. In addition, five trees were treated as controls from which no insects were removed.

Ants were removed from branches by applying a Tanglefoot equivalent to the base of each branch (Eastwood 2004). Tanglefoot is a sticky, paste barrier designed to prevent crawling insects from reaching the tops of trees, and is traditionally used for pest management. Within one to two days ants were found to be entirely absent from the branch. *Nipaecoccus nipae* and larvae were removed from each leaf by hand with the use of a small paintbrush. Control trees were treated by using a paintbrush to brush each leaf with the intention of not removing insects. The control treatment performed was chosen in order to compensate for possible confounding factors that leaf brushing might have on ant prevalence, by either indirectly influencing ants to leave or by removing natural plant produced chemicals that attract the ants.

Sampling

In order to determine ant activity in the absence of *N. nipae*, ant activity, unidirectional, point measurements were performed. On each scale removal tree a single point at the base of each treated branch was chosen. Ants walking on to the branch were counted over the course of one minute. Ants were counted moving in only one direction for consistency and in order to avoid multiple counting of Formicidae individuals.

In order to measure *N. nipae* presence on leaves where ants were no longer present, five leaves from each ant removal branch were selected randomly and photographed. Scale insects were later counted using Photoshop. Control trees were measured for both ant activity and scale presence. All measurements were taken on a daily basis.

Observational study design

In order to investigate the possible presence of ant-scale tending behavior, an observational component was included in this study that examined ant behavior on leaves of *P. guajava*. Two leaves were selected from each study tree, one of which had both Formicidae and *N. nipae* interacting and the other of which had only Formicidae present in the absence of *N. nipae*, for a total sample size of 30 leaves. No leaves occurring on treated branches were included in the observational study.

Data were recorded once every minute for a total of five minutes at each leaf. At the start of every minute each Formicidae individual was recorded as doing one of six activities, 1) sitting on scales (SS), 2) running towards the edge of the leaf (RE), 3) running towards the branch of the leaf (RB), 4) sitting on the leaf where there was no scale (SL), 5) interacting with another ant (I), or 6) performing another activity (other). Ants whose behaviors were categorized as "other" were generally engaging in rapid movement without discernible direction and without leaving the leaf.

Leaves where ants occurred without the presence of scale insects were rare. Methods for their observation were altered slightly so that the observation time began the moment an ant was found, regardless of the duration of its presence on the leaf.

Statistical analysis

To test for differences in the number of insect individuals according to experimental treatment, the total numbers of insect individuals were calculated. Because the data found were not normally distributed, the data was used as the dependent variables in Wilcoxon rank-sum tests, conducted using JMP statistical analysis software. For all observational data, numbers of Formicidae individuals performing each behavior over five minutes intervals were converted into averages, as were the total number of Formicidae individuals, in order to summarize ant behavior. These values were used as the dependent variables in Wilcoxon rank-sum tests.

RESULTS

Experimental data

1) Influence of ant numbers on scale insect populations

Scale insect populations on branches where ants were removed were found not to differ from those where ants were not removed (Fig. 2), indicating that scale presence is likely not dependent on ant presence.

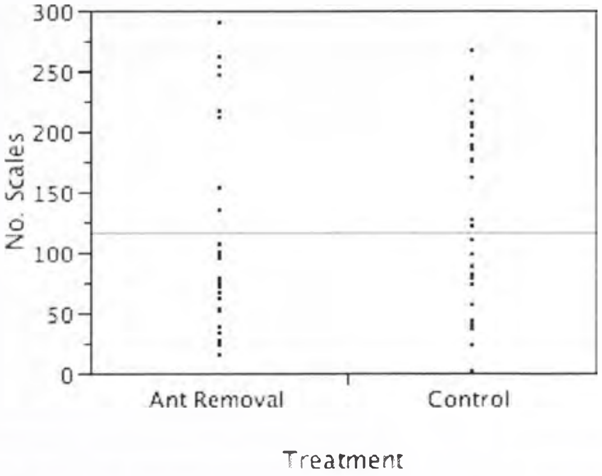


Fig. 2 Wilcoxon rank-sum test. Control showed no significant difference of number of scales from ant removal. P= 0.1966.

2) Influence of scale insect numbers on ant populations

Ant populations on branches where scales were removed were found to differ significantly from those where scales were not removed (Fig. 3). Fewer ants were found on branches where scale insects were removed, indicating that there is a high degree of probability that ant presence is dependent on scale presence

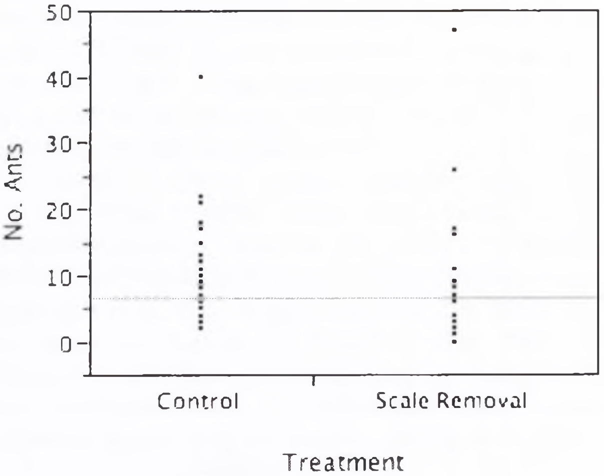


Fig. 3 Wilcoxon rank-sum test. Control showed significantly more ants than ant removal. P< 0.001.

Observational data

1) Influence of scale insects on ant populations

Total number of ants on leaves where scales were present was found to differ significantly from those where scales were not present. More ants were present on those leaves where scales were present. Very few ants were found on leaves without scales (Fig. 5). Upon multiple inspections, no control leaf could be found for tree 3, and the sample was excluded from analysis.

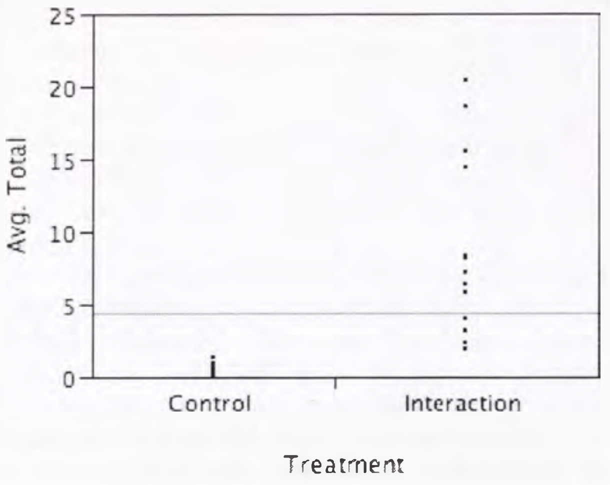


Fig. 5 Wilcoxon rank-sum test. Control showed significantly less ants than interaction. P< 0.001.

2) Influence of scale insects on ant movement

Ant sitting behavior differs significantly on leaves with scales than from those without. Ants are found to move more on leaves without scales and to remain stationary more on leaves with scales (Fig. 6).

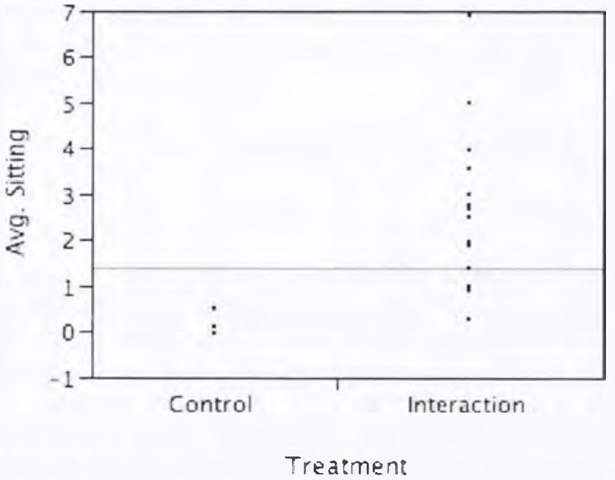


Fig. 6 Wilcoxon rank-sum test. Control showed significantly less sitting than interaction. P< 0.001.

3) Ant Behavior

Ant behavior was divided into six categories. Ant behavior was found to differ dramatically by treatment. The primary behavior that ants displayed on leaves with scales were to sit on those scales, the second most common behavior being to perform another movement activity that did not result in their leaving the leaf (Fig. 7).

Ant behaviors found on leaves without scales appeared to be more evenly distributed, with the dominant behavior being running towards the edge of the leaf (Fig. 8). Tree 13 was rare in that it was a control that was found with five ants on it that remained stationary for the entire five minutes of observation.

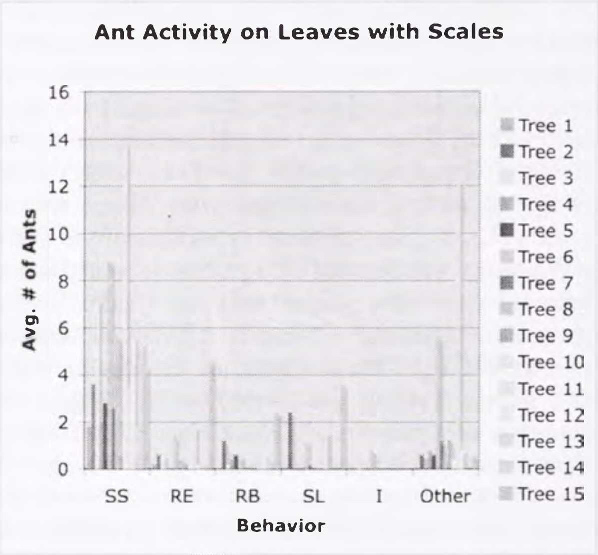


Fig. 7 Ant behavior for interaction leaves

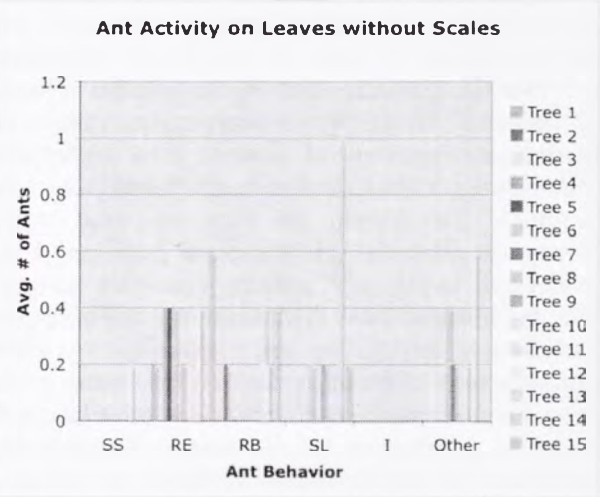


Fig. 8 Ant behavior for control leaves

DISCUSSION

Results support that while Formicidae populations on leaves show a dependence on scale presence, *N. nipae* populations do not fluctuate significantly according to ant presence. Evidence found in this study supports the occurrence of commensalisms between ant and scale insects, where ants benefit from the resources provided by scale insects while having no effect on scale insect populations.

Experimental data

1) Influence of ant numbers on scale insect populations

By removing ants from the branches of leaves where scales existed, one is able to see whether *N. nipae* populations were dependent on Formicidae. Results showed that *N. nipae* populations were not significantly altered after the removal of ants.
The degree of this dependence unknown but these results provide evidence that the scale's relationship to the ant this instance appears to be facultative rather than an obligate one, as the scale numbers are not affected by the absence of ants (Heckroth et al. 1998, Eastwood 2004). However, the brevity of the experiment may have had an effect on this result seeing as how it may take longer than 18 days to see changes in herbivory, parasitoidism or fungal growth.

These ant exclusion experiments also suggest that ants do not have an effect on scale survival rates, therefore lessening the likelihood of a mutualism. Studies researching protection behavior would be of use in order to further explore this possibility.

Seasonal changes relating to breeding may also influence ant effects on scale survival rates. Ant-aphid mutualisms have shown attendant ants to provide aphid eggs with protection, grooming and transport to nests, all shown to increase their rates of survival (Matsuura and Toshihisa, 2006).

2) Influence of scale insect numbers on ant populations

The numbers of ants found on leaves were found to be highly dependent on the presence of scale insects. Ants did not have an attraction to leaves where scales were not present, suggesting that scales serve as a resource to the ants, evidence also supported by the observance of tending behavior.

Observational data

1) Influence of scale insects on ant populations

Observational studies counted more Formicidae individuals overall on leaves where they interacted *N. nipae*. When ants were removed from tree branches they succeeded in building bridges made of dirt across the tangle foot on trees 6 and 14. The benefit of these the building of the bridges can be assumed to be worth the energy they require. These observations provide evidence that the ants present on *P. guajava* leaves are interested in obtaining resources from *N. nipae*.

2) Influence of scale insects on ant movement

Sitting behavior for ants (both on scales and on leaf without scales) were combined and those numbers were averaged for both control and interaction trees. When compared, it was found that those leaves where the interaction occurred had significantly less movement overall. This high occurrence of Formicidae sitting still on those leaves with scales, when they were other wise moving on those leaves without scales, provides behavioral evidence that tending behavior is occurring.

3) Ant Behavior

The individual behaviors that took place on both classes of leaves differed greatly. On leaves where the interspecific interaction took place, ants were mainly observed sitting on scale insects or placed in the category "other". "Other" ants were mostly observed moving quickly, with a constant change of direction, occasionally pausing before resuming movement. These ants were typically found to stop moving once they found a scale insect on which to sit. The "other" is most likely a category for ants that are in the process of selecting a scale on which to sit.

Ants on leaves where scale insects were not present were mostly observed running towards the edge, eventually leaving the leaf. One explanation for this had to do with the sampling technique that was used. Control leaves with ants and no scales were exceptionally hard to find. As a result, methods for observing control leaves were changed. Because control leaves were difficult to find, upon the immediate discovery of an ant on a leaf without scales the timed observation began regardless of the amount of time the ant was present. This mainly resulted in ants being caught during a quick visit to a leaf where nothing was of interest, and then retreating to the edge of the leaf.

Sample trees were selected randomly. Coincidently, control trees were found to be disproportionately located in areas of partial cultivation where there was a greater degree of sun exposure (Fig. 9). Whether or not this factor had an affect on the results found in this study is unknown. Further research related to interspecific insect interactions in relation to cultivation or sun exposure would of great interest and value to many.

Tree Location

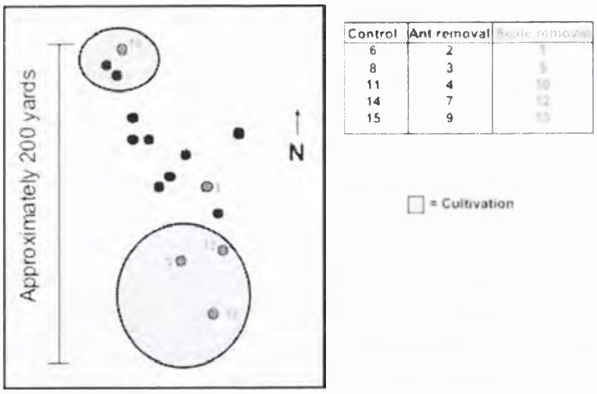


FIG. 9. Tree location of control trees in relations to areas of cultivation.

CONCLUSION

The sitting behavior on leaves where scales were present, high abundance of ants on the same leaves and the high degree of difficulty finding leaves where ants existed without scale insects are all factors that strongly support tending behavior of ants of scale insects on the leaves of *P. guajava* in French Polynesia.

A linear regression analysis of ant and scale insects in their natural populations would likely reveal a positive correlation, seeing as how leaves with Formicidae and without *N. nipae* were exceptionally hard to find. A positive correlation would provide evidence supporting the occurrence of a symbiotic relationship between Formicidae and *N. nipae*. Evidence provided from this study specifies that the likelihood of that relationship would be one of commensalism rather than mutualism.

ACKNOWLEDGMENTS

I thank The Gump Field Station management and staff. I thank Marimari Kellum for allowing me to perform my research on her property. I thank the IB 158 class of 2006 for their assistance in the field. I thank the course faculty for their guidance and encouragement, and the graduate student instructors for the countless hours of consultation they provided their students. I thank the University of California, Berkeley, the Department of Integrative Biology, and the Department of Environmental Sciences, Policy and Management.

LITERATURE CITED

Brenton, L. M., and J. F. Addicott. 1992. Density-dependent mutualism in an aphid-ant interaction. Ecology **73**:2175-2180.

Davidson, D. W., S. C. Cook, R. R. Snelling and T. H. Chua. 2003. Explaining the abundance of ants in lowland tropical rainforest canopies. Science Magazine **300**:969-972.

Davidson, D. W. 1997. The role of resource imbalances in the evolutionary ecology of tropical arboreal ants. Biological Journal of the Linnean Society **61**:153-181.

Eastwood, R. 2004. Successive replacement of tending ant species at aggregations of scale insects (Hemiptera: Margarodidae and Eriococcidae) on *Eucalyptus* in south-east Queensland. Australian Journal of Entomology **43**:1-4.

Gillespie, R. G., and G. K. Roderick. 2002. Arthropods on islands: colonization, speciation, and conservation. Annual Review of Entomology **47**:595-632.

Heckroth, H. P., B. Fiala,, P. J. Gullan, A. H. J. Idris and U. Maschwitz. 1998. The soft scale (Coccidae) associates of Malaysian ant-plants. Journal of Tropical Ecology **14**:427-443.

Krushelnycky, P. 2001. A Distributional survey of ants of Moorea, Tahiti and Raiatea.

Ledoux, S., Y. Allouche, J. Y. Meyer, R. Putoa and H. Jourdan. 2003. An update of the ant fauna of Tahiti and Moorea (French Polynesia).

Matsuura, K. and T. Yahiro. 2006. Aphid egg protection by ants: a novel aspect of the mutualism between the tree-feeding aphid *Stomaphis hirukawai*and and its attendant ant *Lasius productus*. Naturwissenschaften **93**:506-510.

Morrison, L. W. 1996a. Community organization in a recently assembled fauna: the case of Polynesian ants. Oecologia (1996) **107**:243-256.

Morrison, L. W. 1996b. The ants (Hymenoptera: Formicidae) of Polynesia revisited: species numbers and the importance of sampling intensity. Ecography **19**: 73-84.

O'Dowd, D., P. T. Green and P. S. Lake. 2003. Invasional 'meltdown' on an oceanic island. Ecology Letters **6**:812-817.

Wheeler, M. W. 1908. Ants from Moorea, Society Islands. Bulletin American Museum of Natural History **24**:165-167.

Wetterer, J. K. 2002. Ants of Tonga. Pacific Science **56**:125-135.

Wilson, E. O. and R. W. Taylor. 1967. An estimate of the potential evolutionary increase in species density in the Polynesian ant fauna. Evolution **21**:1-10.

MORPHOLOGICAL AND CHEMICAL DIFFERENCES AMONG POPULATIONS OF HIBISCUS TILIACEUS ALONG AN ELEVATIONAL GRADIENT IN MOOREA, FRENCH POLYNESIA

THOMAS W. BELL

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

Abstract. Environmental variables change over elevational gradients and can isolate plant populations. Three varieties of *Hibiscus tiliaceus L.* exist on an elevational gradient in Moorea, French Polynesia. These variety's morphological and chemical characteristics are associated with the differences between their environments. Leaf and flower morphological data were collected and analyzed and found significant differences in petal width and length, anther count, burgundy center color, and leaf width and length between the varieties, particularly between the coastal and mountain types. The increased rainfall and lower temperatures of the high mountains lowered net primary production for the mountain variety as compared to the coastal and mid-mountain varieties. The mid-mountain variety was found to have greater competition for light than the other varieties. These can be linked to the significant size differences in leaves and inflorescence. Chemical differences were analyzed using anti-microbial and anti-cancer bioscreens. Significant differences were found in the anti-microbial bioscreen between the mid-mountain variety, which showed little activity, and both the coastal and mountain varieties. The anti-cancer screen showed increased activity from the coastal and mountain leaves. Chemical differences are influenced by increased insolation and chemical protection from microbes in wet conditions. Anthropological uses of the varieties are linked to the greater size of the coastal type. The plant populations studied are associated with the differences in their environments.

Key words: elevation; environmental pressures; bioscreen; Moorea, French Polynesia; flower; leaf

INTRODUCTION

Ecological variables that inhibit the transfer of genetic information can lead to differentiation between members of the same species (Wright 1943). The biological species concept explains that species are interbreeding groups that are reproductively isolated from other groups. They may be separated by prezygotic isolating factors, which can be factors such as physical environmental barriers and pollination by different insects (Coyne 1992). Different environmental pressures that occur over a variety of habitats can impede seed and pollen dispersal, which may isolate plant populations and drive them apart genetically. These environmental pressures can include differences in wind intensity, insect presence, and proximity to bodies of water (Kudoh and Whigham 1997). Environmental pressures change over elevational gradients and can affect the rate of pollen dispersal (Alonso 2005). The differences in pollen dispersal rates over elevational gradients isolate plant populations at different elevations (Heywood 1991). For example, studies have shown differences in taxa of pollinating insects over elevational gradients (Kearns 1992). Use of different insects for pollination would inhibit transfer of genetic information between different elevational populations. In this paper I discuss the differences in flower morphology, leaf morphology, medicinal properties, and anthropological uses in *Hibiscus tiliaceus* L.

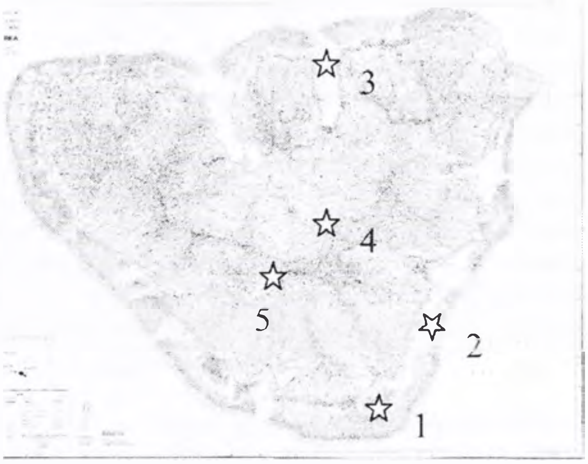


Fig. 1. Sites sampled in this study.

and how these differences are related to the differentiated varieties that have arisen due to elevational isolation. I chose to study *H. tiliaceus* for several reasons.

Hibiscus tiliaceus is a tree that grows in a variety of habitats, from the coastline and along streams up to 800-meter high mountain peaks in the South Pacific. It is a medium size tree up to 15 meters high and is common secondary forest tree. The flowers are 5-merous, with a yellow corolla, a burgundy center at the base of each petal, and staminal column joined to the base of the ovary (Petard 1986, WHO 1998). *Hibiscus tiliaceus* is reported to have a variety of medicinal properties that are used by many peoples in the South Pacific. Medicinal chemicals in *H. tiliaceus* have positive effects on fractured bones, sprained muscles, gonorrhea, skin diseases, eye infections, and stomachaches. The peoples of over 10 South Pacific island groups use the tree medicinally (WHO 1998).

Hibiscus tiliaceus occurs in three wild varieties on the island of Moorea, French Polynesia. These varieties occur over an elevational gradient with *H. tiliaceus* var. *henryanus* occurring along the coastline and low-mountain streams, *H. tiliaceus* var. *sterilis – Paritium trilobatum* occurring in the mid-mountain ranges, and *H. tiliaceus* var. *sterilis* occurring on the higher peaks of the island (Franc 2001). The differences in environmental pressures along an elevational gradient have affected the morphology of these varieties (Petard 1986). Differences in environments along this elevation gradient are associated with these three varieties. The following questions are addressed in this paper: Are populations of *H. tiliaceus* along an elevation gradient differentiated in: (1) flower morphology, (2) medicinal properties, (3) leaf morphology, and (4) anthropological uses between the different varieties.

METHODS

Five study sites were used for this study. The first three are sample sites for *H. tiliaceus* var. *henryanus*, the coastal and stream variety (Fig. 1). The first is adjacent to the Gump Station on the coast. The second is on the Viaroro Stream 300 meters from the coast. The third is 1000 meters north of the Viaroro Stream at the intersection of a small stream and the coast. The fourth is the sample site for *H. tiliaceus* var. *sterilis – Paritium trilobatum*, the mid-mountain range variety, adjacent to Marae Tetiiroa near the Belvedere lookout at an elevation of 100 – 180 meters. The fifth is the sample site for *H. tiliaceus* var. *sterilis*, the high-mountain variety, at the top of Three Coconut trail at an elevation of 394 meters.

Flower morphology

Flowers were collected from each of the five study sites between the hours of 11AM and 4PM over the course of multiple days. Approximately 15 trees were sampled of each variety except for *H. tiliaceus* var. *sterilis – Paritium trilobatum*, in which 10 trees were sampled due to the difficulty of finding this variety. 25 flowers were collected from each species by hand picking, using a noose attached to PVC piping, or collecting after dropping from treetops. The petals were removed from each flower and maximum petal length and petal width was recorded. The maximum length and maximum width were recorded for the burgundy center area on each petal. The direction of petal curve was recorded for each petal. The anthers were removed from the staminal column and counted for each flower. The separation or closure of the five stigma parts was also recorded for each flower.

Data for each aspect of the flower were analyzed with JMP software and a one-way ANOVA and student's t-test was used to determine means, standard deviation, standard error, and p values.

Leaf morphology

Leaves were collected from each of the five study sites between the hours 9AM and 2PM over the time period of a week. 50 leaves were collected from each variety using at least 10 trees from each variety. No more than 5 leaves came from any one single tree. Leaves were all exposed to full sunlight and were at similar stages of mature development. Maximum length and width measurements were taken from each leaf.

Data for each aspect of the leaf were analyzed with JMP software and a one-way ANOVA and student's t-test was used to determine means, standard deviation, standard error, and p values.

Medicinal properties

Extract preparation. Leaf and flower samples were collected from each of the three varieties of *H. tiliaceus*. All plant material was air-dried and then ground into a fine powder.

The leaf and flower powder was each weighed and extracted with 70% ethanol to attain a .1g/mL concentration of plant extract (Vlietinck 1995). The leaf and flower suspensions from each variety were each stored at 4°C for the period of 3 days.

Anti-cancer bioscreen. Brine shrimp were hatched and aged 48 hours. 10mL of salt-water and 20 brine shrimp were added to 9, 100mm Petri dishes. 0.2 mL of the leaf and flower extracts of each variety were added to different Petri dishes. 0.2 mL of 70% ethanol was run as a negative control and 0.2mL of *Catharanthus roseus* extract, a proven anti-cancer plant prepared in the same fashion as the leaf and flower extracts, was run as a positive control. After a period of 24 hours the number of brine shrimp killed in each Petri dish was recorded. This test was replicated 6 times.

Anti-microbial bioscreen. 20 mL of sterilized liquid peptone agar was added to each of 24, 100 mm Petri dishes and stored at 4°C. The Petri dishes were inoculated with a sterile suspension of yeast. 5mm filter paper disks were inoculated with 0.2 ml of each of the 6 plant extracts or control substances. The plant extract disks from each variety were placed on different dishes inoculated with yeast (Dimayuga and Garcia 1991). A disk of evaporated ethanol was used as a negative control and a disk of Ciprofloxacin was used as a positive control. All plates were incubated at 37°C for 24 hours. The zones of inhibition around the disk were measured at the end of the period. This test was replicated 9 times.

Data for each aspect of the medicinal bioscreens were analyzed with JMP software and a one-way ANOVA and student's t-test was used to determine means, standard deviation, standard error, and p values.

Environmental data

Environmental data were collected over a 3-week period from late October to mid-November. Sites 1, 4, and 5 were sampled for the three varieties in the form of light (lux), wind speed (m/s), soil temperature (degrees Celsius), and air temperature (degrees Celsius). Data were collected over the period of five different days between the hours of 10AM and 3PM. Three data collections were competed each day with at least 2 minutes separating the samples. Maximums and minimums were recorded for each light and wind sample. JMP software was used to determine means and standard deviations.

Anthropological uses

Local healers were consulted to determine whether the Polynesians used each of the three varieties of *H. tiliaceus* in different ways or for different medicinal purposes.

RESULTS

Flower morphology

The average petal width ranged between 5.4 cm and 7.5 cm (Fig. 2). Petal width is significantly different at $p < .01$ between the coastal, mid-mountain, and mountain varieties. The average petal length ranged between 6.5 cm and 8.0 cm (Fig. 2). The mountain petal length is significantly different at $p < .01$ from the coastal and mid-mountain varieties, which are not significantly different from each other.

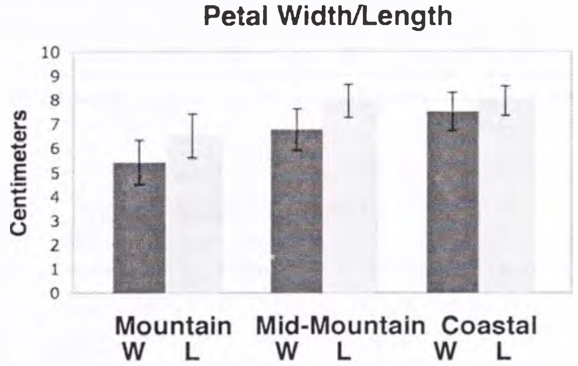


Fig. 2. Average petal width decreases as population site elevation increases, all varieties are significantly different. Mountain petal length is significantly smaller than the mid-mountain and mountain varieties, which are not significantly different.

The average burgundy center width ranged from 14 mm to 18 mm (Fig. 3). The mountain burgundy center width is significantly different at $p < .01$ from the coastal and mid-mountain varieties, which are not significantly different from each other. The average burgundy center length ranged from 17 mm to 23 mm (Fig. 3). Burgundy center length is significantly different at $p < .01$ between the coastal, mid-mountain, and mountain varieties.

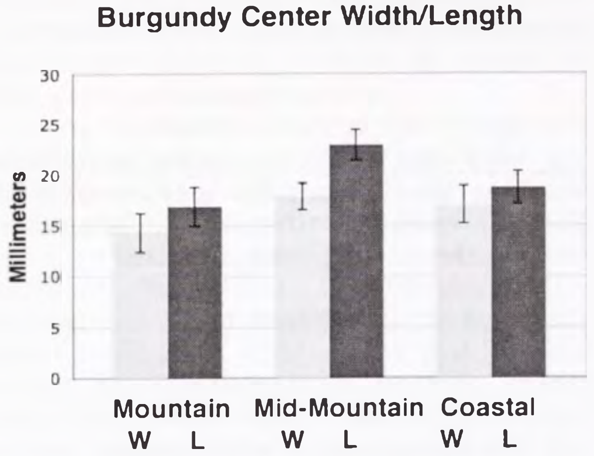


Fig. 3. Burgundy center width and length are largest in the mid-mountain variety. The width in the mountain variety is significantly smaller than the mid-mountain and coastal varieties. The lengths of all varieties are significantly different.

The average anther count ranges from 82 anthers to 92 anthers (Fig. 4). The coastal and mountain varieties are significantly different at $p < .01$, while the coastal and mid-mountain varieties are at $p < .05$. The mid-mountain and mountain varieties are not significantly different.

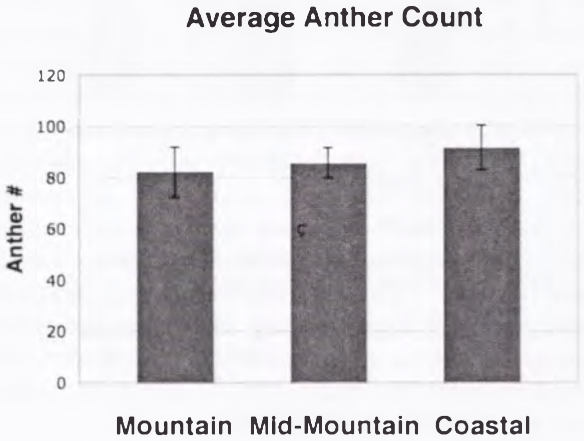


Fig. 4. Average anther count decreases as population elevation increases. The coastal variety average anther count is significantly different from the mountain and mid-mountain varieties, which are not significantly different from each other.

The open stigma parts average ranges from 4% to 96% (Fig. 5). The coastal variety is significantly different at $p < .01$ from both the mountain and mid-mountain varieties, which are not significantly different from each other.

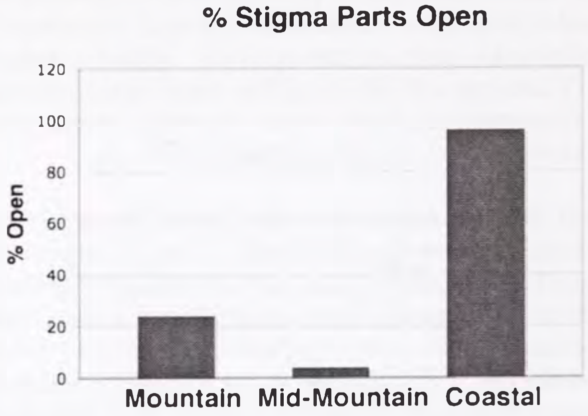


Fig. 5. The coastal percentile of stigma parts open is significantly different from both the mountain and mid-mountain varieties, which are not significantly different from each other.

The rightward petal direction percentage ranges from 40% to 64% (Fig. 6). The varieties are not significantly different.

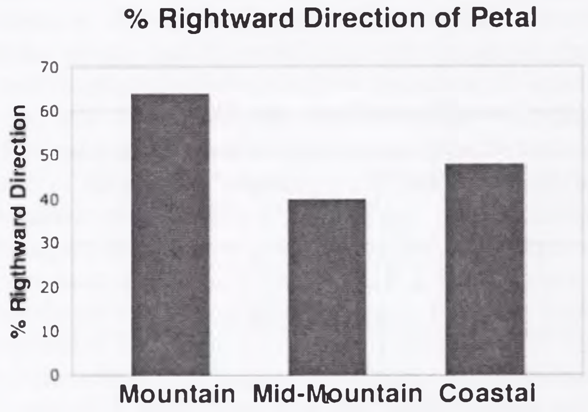


Fig. 6. The percentile of rightward direction of petal curve is not significantly different between the varieties.

Leaf morphology

The average leaf width ranges from 16.2 cm to 22.0 cm (Fig. 7). The mountain variety is significantly different at $p < .01$ from both the coastal and mid-mountain varieties, which are not significantly different from each other. The average leaf length ranges from 16.5 cm to 21.9 cm (Fig. 7). The mountain variety is significantly different at $p < .01$ from both the coastal and mid-mountain varieties, which are not significantly different from each other.

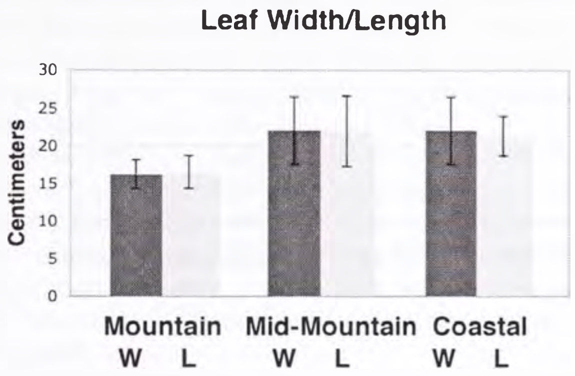


Fig. 7. The width and length of the mountain variety are significantly smaller than both the mid-mountain and coastal varieties, whose widths and lengths are not significantly different from each other.

Medicinal properties

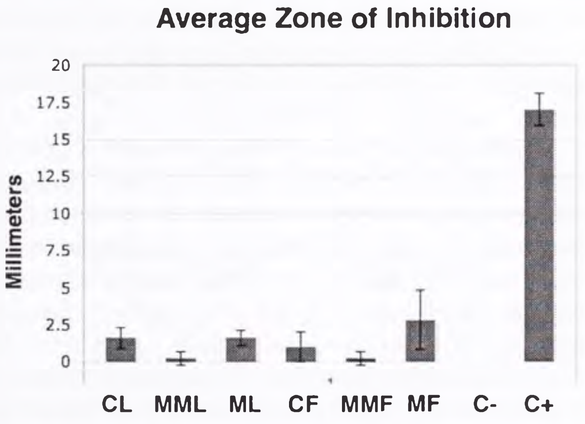


Fig. 8. Average zone of inhibition for the mid-mountain leaf was significantly less than the coastal and mountain varieties, which were not significantly different. Average zone of inhibition for the mountain flower was significantly greater than both the mid-mountain and coastal varieties, which were not significantly different from each other.

Anti-microbial bioscreen. The average radius of the zone of inhibition for the flower varieties ranges from .22 mm to 2.8 mm (Fig. 8). The mountain variety flower effectiveness is significantly different from the mid-mountain variety at p < .01 and coastal at p < .05. The coastal and mid-mountain varieties are not significantly different. The average radius of the zone of inhibition for the leaf varieties ranges from .22 mm to 1.6 mm (Fig. 8). The coastal and mountain varieties leaf effectiveness is significantly different from the mid-mountain variety at p < .01. The mountain and coastal varieties are not significantly different.

Anti-cancer bioscreen. The average percent brine shrimp killed for the flower varieties ranged from 53% to 67% (Fig. 9). The varieties are not significantly different. The average percent brine shrimp killed for the leaf varieties ranged from 60% to 87% (Fig. 9). The mountain variety is significantly different from the mid-mountain variety at p < .01, and the coastal variety at p < .05. The mid-mountain and coastal varieties are not significantly different.

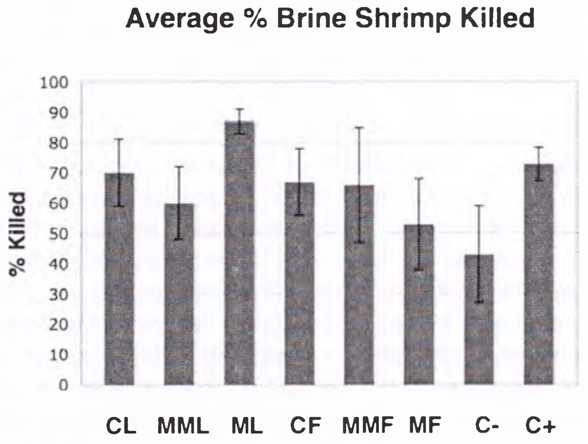


Fig. 9. Average percent brine shrimp killed for the flower varieties was not significantly different for any variety. Average percent brine shrimp killed for the mountain leaf variety was significantly different than the mid-mountain and coastal varieties, which were not significantly different from each other.

Environmental data

The average and standard deviation for light (lux) measurements for the coastal site is 542 lux (318), mid-mountain 290 lux (241), mountain 559 lux (360). The average wind speed (m/s) and standard deviation for the coastal site is 1.2 m/s (1.0), mid-mountain .59 m/s (.70), mountain 1.1 m/s (1.8). The average soil temperature (^{O}C) and standard deviation for the coastal site is 27.6 ^{O}C (2.0), mid-mountain 23.8 ^{O}C (1.4), mountain 23.2 ^{O}C (1.7). The average temperature (^{O}C) and standard deviation for the coastal site is 31.1 ^{O}C (5.0), mid-mountain 29.0 ^{O}C (2.9), mountain 29.2 ^{O}C (2.5).

Anthropological studies

Two interviews were conducted in Moorea on the different medicinal and anthropological uses of *H. tiliaceus* varieties. The coastal variety is most used by the native peoples due to its large size. The large amounts of wood are best for boat building and the large leaves can serve purposes for cooking, eating, and use as bandages. The smaller mountain flowers were not used for these purposes due to their small size and remote comparative location. The coastal flowers can be used to make a boiling water infusion, which is cooled until warm and drank for a period of three days. This can be used as a remedy for menstrual abnormalities. The mid-mountain variety bark can be used to make rope (Papa Mita, personal communication, Nov. 4, 2006). The coastal variety roots can be pulverized and used as a salve for eye infections. Coastal flowers can also be boiled and the resulting gel can be applied to the skin to treat itching, infections, and jellyfish stings. The varieties are recognized by the native peoples as being different types of hibiscus, however the cuttings are used rather than seeds due to the frequency of the tree not inheriting the morphology of the parent tree (Hinano Murphy, personal communication, Nov. 10, 2006).

DISCUSSION

The differentiated varieties of *H. tiliaceus* on Moorea show many statistically significant differences in morphology and chemical effectiveness. The environmental conditions that change from the coastline to the mountaintops has had a large influence on plant characteristics (Korner 1989).

The leaf averages of length and width show coastal and mid-mountain leaves both significantly larger than the mountain variety. Leaf size has been shown to decrease with increasing elevation (Velízquez-Rosas, Meave, and Vízquez-Santana 2002). Net primary productivity decreases as mean annual precipitation increases in tropical islands (Schurr and Matson 2001). The average rainfall of the mountaintops in Moorea is 4.7 times greater than at the coast (800 cm/year mountains, 170 cm/year coast) (Mueller-Dombois and Fosberg 1998). This would explain why the mountain variety has smaller overall leaf size. Mountain conditions are usually more extreme over the course of a year including lower overall temperatures and greater rainfall. The environmental data collected showed that average temperature and soil temperature were lower by 2 degrees and 4 degrees Celsius respectively. Light data was similar but yearlong data shows less light over the course of the year rather than a 3 week period (Mueller-Dombois and Fosberg 1998). This means that overall the mountain peaks present a cooler environment that stresses the plant with heavy rain and low light levels. This would explain why the mountain variety has significantly smaller leaves than the other varieties.

The mid-mountain leaves are similar in width and length to the coastal leaves but occur at a higher elevation. The coastal variety is one of the tallest trees on the coastal habitat while the mid-mountain variety faces greater competition for sunlight from taller trees such as the Tahitian Chestnut. It has been shown that with decreasing insolation leaf size increases, and the mid-mountain variety has large leaves in order to be exposed to as much sunlight as possible (Ackerly, Knight, Weiss, Buron, and Starmer 2002).

Overall the average lengths and widths of the petals can determine flower size. The petal width was shown to decrease as elevation increases and all three varieties were significantly different. Petal length showed similar measurements for the coastal and mid-mountain varieties with significantly smaller measurements for the mountain flowers. Again, since the mountain conditions are stressful and primary productivity is less, smaller flower size can be anticipated. Mid-mountain flowers are overall smaller than the coastal flowers because the greater competition experienced at the mid-mountain ranges leaves less energy for floral size. The coastal variety has more energy to devote to reproductive organs. Anther count closely mirrors trends seen in petal width and overall flower size. The petals protect and house the staminal column. The size of the staminal column is therefore a result of flower size and the number of anthers that can fit on a smaller staminal column is less than the amount that can fit on a larger one. Anther counts on a smaller flower will be less than that of a larger flower. The rightward or leftward curve of the petals in a flower is the same for all the petals in a specific flower. The overall percentage of rightward or leftward curve in the petals of a variety are not significantly different from one another. This is a random effect that does not seem to be influenced by environmental factors.

The yeast and brine shrimp bioscreens show increased toxicity for the coastal and mountain variety leaves and flowers. Statistically the coastal and mountain leaves showed the greatest degree of significant separation of the bioscreens when measuring the effectiveness against yeast. Mountain and coastal leaves also showed increased toxicity in the brine shrimp bioscreen. The mountain and coastal varieties have little competition and therefore the leaves receive a large amount of direct UV-B light in periods of low cloud cover (Sullivan, Teramura, and Ziska 1992). Leaves may expel salts and toxins from the interior of the leaf to protect the inner tissue from extreme insolation (Karimi and Ungar 1989). This leads to higher overall toxicity in leaves exposed to increased sunlight.

The extract of the mountain flower showed significantly increased anti-microbial activity. Anti-oxidants such as DPPH have been found in the flowers of *Hibiscus sabdariffa L.* (Tseng, Kao, Chu, Chou, Lin Wu, and Wang 1997). This antioxidant has been shown to have anti-microbial effects (Yildirim, Mavi, and Kara 2003). The ethanol extracts may have attracted non-polar phenolic compounds from the flower material, which could have also played a role in the anti-microbial bioscreen. The mountain flower in particular could have contained more of these anti-oxidants and phenolic compounds in order for increased disease resistance due to the heavy rainfall and stressful conditions of the mountain ridges (Nicholson and Hammerschmitt 1992).

The anthropological uses of the different varieties seem to be limited to the location of the people. The great majority of Moorean people live near the coast, and almost all of the uses of the tree are limited to the coastal variety. The coastal tree is larger and can provide better uses for cooking and boatbuilding than the smaller mountain variety. The coastal variety can grow larger due to the increase in primary productivity (Schurr and Matson 2001). The shoots of the mid-mountain variety are straight and have smooth bark, which is ideal to strip and make rope. These shoots may exist to climb straight up towards the sky in an effort to collect more sunlight due to competition. Medicinally the use of the coastal variety could be because the local knowledge of the mountain variety was lost after the population moved to the coast, or the other varieties do not present greater medicinal value for the afflictions treated.

The use of cuttings instead of seeds may present insights into whether the varieties are genetically different or merely ecophenotypes, in which all the trees have the same genotype but differ phenotypically due to the environment. Further studies should be conducted to investigate.

The variation in morphology and medicinal effects of *H. tiliaceus* across an elevational gradient in Moorea can provide insights into gene flow and perhaps plant plasticity. The different environmental pressures placed on genetically isolated populations eventually lead to adaptations and differences between them. In the case of this species, sea-dispersed seeds have populated the tropical regions of the world with *Hibiscus* species that have differentiated from each other after gene flow between the populations have ceased (Takayama, Kajita, Murata, and Tateishi 2006).

In conclusion, this is a classic case of observing differences between isolated populations due to differing environmental pressures. It is interesting because the three microhabitats, which forced this single species into three distinct varieties, can all be accessed and studied in a single day. Further studies in plasticity, and perhaps self-pollination in the varieties presenting significant stigma parts closure should be investigated.

ACKNOWLEDGMENTS

I would like to thank the professors and graduate student instructors of IB 158. I would also like to thank Sameen Ghazali, Maggie Groff, Zachary Hanna, Valerie Howell, Melissa K. Riley, and Felicia Wheaton for assistance in data collection. Special thanks to Hinano Murphy and Papa Mita for their generous nature in explaining the traditional uses of *H. tiliaceus*.

LITERATURE CITED

Ackerly, D.D., C.A. Knight, S.B. Weiss, K. Burton, and K.P. Starmer. 2002. Leaf size, specific leaf area and microhabitat distribution of chaparral woody plants: contrasting patterns in species level and community level analysis. Oecologia **130**: 449-457

(Anonymous). 1998. Medicinal plants of the south pacific, information on 102 commonly used medicinal plants in the south pacific. WHO Regional Publications, Manila

Conchita, A. 2005. Pollination success across an elevational and sex ratio gradient in Gynodioecious *Daphne laureola*. American Journal of Botany **92**(8): 1264-1269

Coyne, J.A. 1992. Genetics and speciation. Nature **355**: 511-515

Dimayuga, R.E. and S.K. Garcia. 1991. Antimicrobial screening of medicinal plants from baja california-sur, mexico. Journal of Ethnopharmacology **31**(2): 181-192.

Franc, M. 2001. Reproduction and distribution of *Hibiscus tiliaceus* from the coast to the mountains on moorea, french polynesia. Biology and Geomorphology of Tropical Islands **10**: 120-134

Heywood, J.S. 1991. Spatial analysis of genetic variation in plant populations. Annual Review of Ecology and Systematics **22**: 335-355

Karimi, S. and I. Ungar. 1989. Development of epidermal salt hairs in *Atriplex triangularis Willd.* in response to salinity, light intensity, and aeration. Botanical Gazette **150**(1): 68-71

Kearns, C.A. 1992. Anthophilous fly distribution across an elevational gradient. American Midland Naturalist **127**: 172-182

Korner, Ch. 1989. The nutritional status of plants from high altitudes. Oecologia **81**: 379-391

Kudoh, H. and D.F. Whigham. 1997. Microgeographic genetic structure and gene flow in *Hibiscus moscheutos* populations. American Journal of Botany **84**(9): 1285-1293

Mueller-Dombois, V. and F.R. Fosberg. 1998. Vegetation of the tropical pacific islands. Ecological Studies **132**; 1-675

Nicholson, R. and R. Hammerschmitt. 1992. Phenolic compounds and their role in disease resistance. Annual Review of Phytopathology **30**: 369-389

Petard, Paul. 1986. Quelques Plantes Utiles de Polynesie Francaise de Rae Tahiti. Editions Here Po No Tahiti

Schuur, E. and P. Matson. 2001. Net primary productivity and nutrient cycling across a mesic to wet precipitation gradient in Hawaiian montane forest. Oecologia **128**: 431-442

Sullivan, J., A. Teramura, and L. Ziska. 1992. Variation in uv-b sensitivity in plants from a 3,000-m elevational gradient in hawaii. American Journal of Botany **79**(7): 737-743

Takayama, K. T. Kajita, J. Murata, and Y. Tateishi. 2006. Phylogeography and genetic structure of *Hibiscus tiliaceus* – speciation of a pantropical plant with sea-drifted seeds. Molecular Ecology **15**: 2871-2881

Tseng, T.H., E.S. Kao, C.Y. Chu, F.P. Chou, H.W. Lin Wu, and C.J. Wang. 1997. Protective effects of dried flower extracts of *Hibiscus sabdariffa L.* against oxidative stress in rat primary hepatocytes. Food and Chemical Toxicology **35**(12): 1159-64

Velízquez-Rosas, N., J. Meave, and S. Vízquez-Santana. 2002. Elevational variation of leaf traits in montane rain forest species at la chinantla, southern mexico. Biotropica **34**(4): 534-542

Vlietinck, A.J. 1995. Screening of 100 rwandese medicinal plants for antimicrobial and antiviral properties. Journal of Enthnopharmacology **46**(1): 31-47

Wright, S. 1943. Isolation by distance. Genetics **28**: 114-138

Yildirim, A., A. Mavi, and A. Kara. 2003. Antioxidant and antimicrobial activities of *Polygonum cognatum* meissn extracts. Journal of the Science of Food and Agriculture **83**: 64-69

PREDATOR DEFENSE MECHANISMS IN SHALLOW WATER SEA CUCUMBERS (HOLOTHUROIDEA)

JESSICA A. CASTILLO

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

Abstract. The various predator defense mechanisms possessed by shallow water sea cucumbers were surveyed in twelve different species and morphs. While many defense mechanisms such as the presence of Cuverian tubules, toxic secretions, and unpalatability have been identified in holothurians, I hypothesized that the possession of these traits as well as the degree to which they are utilized varies from species to species. The observed defense mechanisms were compared against a previously-derived phylogeny of the sea cucumbers of Moorea. Furthermore, I hypothesized that while the presence of such structures is most likely a result of the species' placement on a phylogenetic tree, the degree to which they utilize such structures and their physical behavior are influenced by their individual ecologies. The presence of a red liquid secretion was restricted to individuals of the genus *Holothuria* (Linnaeus 1767) however not all members of the genus exhibited this trait. With the exception of *H. leucospilota*, which possessed both Cuverian tubules and a red secretion, Cuverian tubules were observed in members of the genus *Bohadschia* (Ostergren 1896). In accordance with the hypothesis, both the phylogenetics and individual ecology appear to influence predator defense mechanisms. However, even closely related species of similar ecology may differ considerably.

Key words: holothurians; defense; toxicity; Cuverian tubules; Moorea, French Polynesia

INTRODUCTION

Sea cucumbers belong to the phylum Echinodermata and the class Holothuroidea. Echinoderms are distinguished by having a coelomic, water vascular system and pentaradial symmetry. Holothurians in turn are characterized by having feeding tentacles that extend from a circumoral ring. Individuals can range in size from 10 to 2000mm in length (Lawrence, 1897). Holothurians come in many shapes and sizes and inhabit a wide variety of habitats from the intertidal zone to the deep sea (Anonymous, 2003).

Holothurians are important ecologically as well as economically and culturally. Sea cucumbers have been collected and utilized as a food item, especially in Southeast Asia, for generations. Food products include dried gonads, dried body meat and longitudinal muscles, and salted fermented intestine (Sakthivel et. Al, 1994). Approximately 20 species in two families and five genera, including *Holothuria*, *Bohadschia*, and *Thenelota*, are traded internationally (Anonymous, 2003). The topic of overhavesting and sustainable aquaculture has become increasing important in recent years.

Studies have noted that predation plays a major role in influencing community structure as well as spatial and temporal distribution of marine invertebrates (Bingham, 1986; Francour, 1997). Predators of holothurians include fish, crustaceans, and most importantly sea stars (Francour, 1997). Because of the shape and structure of sea cucumbers, either a portion of the sea cucumber must be bitten off by the predator, or the individual must be ingested whole (Lawrence, 1987). In response to predation pressures, sea cucumbers possess a wide variety of defense mechiansms. Such defenses include toxicity, thickened body wall, evisceration, autotomy cryptic behavior, unpalatability, and swimming or other active avoidance of the predator (Bingham, 1986).

A toxic secretion has been identified in species belonging to the genus *Holothuria*, particularly *H. atra*. This secretion is burgundy in color and is thought to be a toxin that was used by inhabitants of the Pacific to poison fish, thus it is most likely utilized as a defense mechanism by the sea cucumber (Delia, 1977).

Possibly the most studied defense mechanism is the expulsion of Cuverian tubules. Cuverian tubules are found in several species belonging exclusively to the family Holothuriidae (Flammang et. al, 2005). The tubules are white, intracoelomic caeca attached to the basal part of the left respiratory tree (Van Den Spiegel, 1987). When physically stimulated, the sea cucumbers expel a few tubules through the anus which become adhesive as they elongate and stick to almost any surface (Flammang, 2003). The expulsion is directed toward the point of stimulus and the number of tubules expelled is proportional to the intensity of stimulation (Hamel, 2000). The tubules can elongate to up to 30 times their original length, or approximately 60cm depending on the species, and can maintain both their strength and adhesiveness for several days (Van Den Spiegel, 1995).

Cuverian tubules are particularly effective against fish as the fish become entangled and immobilized in the tubules (DeMoor, et. al, 2003). Furthermore, it is thought that the presence of Cuverian tubules is correlated with a higher degree of fish predation observed at lower latitudes (Lawrence, 1987).

This study includes a survey of the sea cucumbers in shallow waters surrounding Moorea, as well as an assessment of their predator defense mechanisms. I investigated the possible relationships between defense mechanisms and habitat and life histories as well as phylogeny and relatedness of species. While an attempt was made to qualify all defense machanisms exhibited, I focused on the following: presence of Cuverian tubules and or a liquid secretion, toxicity, body structure and texture, coloration, and behavior.

I expect that individuals will exhibit multiple defense mechanisms and that not all mechanisms will be expressed to the same extent. Additionally, I expect to see some similarities among closely related species, but it is likely that the individuals' life histories will also influence the way they respond to potential predators.

METHODS

Collection and initial observations

I surveyed shallow water sites on the northern and western sides of Moorea for sea cucumbers. Sites were reached either by swimming, snorkeling, or boat. The collection sites include Temae (1), Cook's Bay directly off of the Gump Station (2), Cook's Bay near Pihaena Point (3), a reef just offshore between PK 14 and PK 15 (4), three sites at the Motu on the northwest side of the island (5), and the Mangrove Marsh near Haapiti (6) (Figure 1). I also observed and collected sea cucumbers on Tetearoa; an atoll approximately 59km to the North of Papeete, Tahiti. I picked up the individual sea cucumbers gently with rubber coated gloves and placed them in a plastic bin while still underwater so as to disturb them as little as possible. They were kept in the bins until I returned to the Gump Station, where they were placed in a large concrete tank (8'x4'x2') with flowing sea water. Ecological factors such as depth, substrate, distance from shore, and proximity to coral and other organisms were noted for each species observed.

Individuals were identified to species whenever possible using Guille, 1986 and Clark and Rowe, 1971. When identifications could not be made based on superficial characteristics alone, ossicles were extracted from the body wall. This was done by soaking an approximately 2cm^2 segment of body wall in sodium hypochlorite for 48 hours at approximately 7 degrees celcius. The ossicles were collected from the bottom of the small jar, slide mounted, and photographed for identification.

FIG. 1. Study Sites.

Individual and behavioral observations

I observed the sea cucumbers individually in a small, glass aquarium (approximately 25x50x25cm) with flowing sea water. Once placed in the tank, I left the sea cucumbers alone for approximately 15 minutes to acclimate. At this time I took photographs for identification and voucher purposes, and I took physical measurement in an undisturbed state. I measured length and width of the sea cucumbers using a measuring tape and I measured volume using a large graduated plastic container.

I simulated a predator attack by mechanically stimulating the sea cucumbers using large, blunt-tipped, metal forceps to pinch the dorsal integument. I described and recorded the response as well as the effort it took to elicit a response. If Cuverian tubules were expelled, I removed the tubules and placed them in a plastic cup using forceps and while wearing inverted latex gloves (the adhesive tubules stuck to most surfaces, but I was able to remove them most easily from the smooth latex). I counted the number of times tubules were expelled as well as the approximate number of tubules expelled each time. If tubules were not expelled as a result of pinching with forceps, I turned the sea cucumbers over and poked their ventral side either with forceps or my fingers. I then picked up the individuals and gently constricted along the body with my hands. Lastly, I removed the cucumbers from the water and held them momentarily. For the individuals that secreted a colored liquid when stimulated, I held them above a plastic cup and squeezed gently in a peristaltic motion to collect the liquid. Lastly, I placed the individuals in plastic freezer bags and placed them in the freezer for a minimum of six hours to sacrifice them before dissection.

Toxicity assessment

I removed the sea cucumbers from the freezer and allowed them to thaw for a few minutes before I dissected them using scissors and a razor blade. I noted the presence or absence of Cuverian tubules within the body cavity. I then separated the external tegument and flesh from the internal organs, muscles, and connective tissue. If a liquid secretion was present in the plastic bag, I collected it for the toxicity assessment either by pouring it into a graduated cylinder or by removing it from the bag with a syringe.

To test toxicity I performed a series of brine shrimp bioassays. I raised brine shrimp using a salt water solution of 2 tablespoons sea rock salt and 1L of tap water. I measured 0.5mL of either Cuverian tubules, liquid secretion, external body, or internal body parts by filling a graduated cylinder with 10mL salt water and adding the desired part until the water line read 10.5mL. The salt water solution used was of the same concentration as the brine shrimp were raised in. I then poured the contents of the graduated cylinder into a Petri dish and added 25 live brine shrimp. I recorded the number of live brine shrimp in each Petri dish after one hour and at multiple hours for a 24 hour period.

Analysis

The data collected from the bioassays were analyzed in a number of ways. First I looked at mortality after one hour by subtracting the number of live brine shrimp in each test from the number of live brine shrimp in the control to get the number of brine shrimp that died as a result of the test substance. I then did three one-way Anova analyses comparing the various genera, species, and sea cucumber parts to level of mortality. Additionally, I did a regression analysis comparing the volume of the individual ea cucumbers with mortality after one hour.

To determine the estimated median lethal time (LT50), I extrapolated when mortality equals 50% for each assay. I repeated the same three one-way Anova analyses for LT50 as with mortality after one hour.

Additional Anova analysis and regression were done with *Holothuria atra* comparing volume and toxicity.

Finally, I used a phylogeny of Moorean sea cucumbers created by K. Tremain, 2005 and adapted it for the species included in this study. I overlaid the presence of Cuverian tubules, a secretion, and various other physical characteristics onto the tree using *MacClade* software.

RESULTS

Collection and initial observations

I surveyed eight sites with varying habitats and found 15 different species and morphs of sea cucumbers in three different families (Appendix 1). Twelve species and morphs are included in this study. The most abundant species encountered was *Holothuria (Halodeima) atra* Jaeger, 1833. I found *H. atra* at every site and in almost every habitat type. A previous survey of the island conducted by J. Pearse in 1989 encountered this species and noted that it was "probably the most abundant and common echinoderm in French Polynesia" (Pearse, 1989). These small to medium sized sea cucumbers are almost black in color and are often seen with sand stuck to their bodies. When touched, most large individuals secreted a burgundy liquid through their skin at the place of contact. The individuals ranged in size from seven cm to 27cm in length. I split the species into two groups according to size in order to investigate the possible relationship between size and predator defence.

Two other species of *Holothuria* were observed: *Holothuria leucospilota* Clark, 1920 and *Holothuria fuscogilva* Cherbonnier, 1980. I only encountered *Holothuria leucospilota* in the very shallow calcium carbonate composite of Temae. Individuals of this species are almost black with soft spikes all over. They are thin and can reach a length of over 50cm. Every individual I observed was almost entirely hidden under rock and when I tried to pick them up they became wedged under so tightly that it was impossible to collect them except when I was able to pick up the entire rock itself. Pearse observed *H. leucospilota* at four locations around Moorea; always completely or partially under either rubble or rock.

Two individuals of *Holothuria fuscogilva* were found; one light grayish and the other brownish purple. The lighter one was found on coral sand between coral heads and the darker one was found partially under an overhang of coral. Both were difficult to see as they blended in with their surroundings. Pearse does not list this species, but another study conducted in the Opunohu Bay of Moorea did note its presence (Parmentier, 2005).

The second most abundant species encountered was *Bohadschia argus* (Jaeger 1833). I found three color morphs of this species, but only included two in this study: a light purple with dark spots and a dark burgundy with orange spots. The third color morph was marbled brown with dark brown spots. This species was found in all but two sites. It was also found in all but two habitats which had shallow water less than 0.5m deep with no live coral. Pearse observed the two color morphs included in this study and noted that while both were abundant, the darker morph was encountered more often. During collection I observed many individuals expelling Cuverian tubules when disturbed either purposely or accidentally by me. About one third to half of the total individuals observed were partially obscured either by sand or by coral.

I also collected individuals of *Bohadschia marmorata?*, *Bohadschia vitiensis marmorata*, and *Bohadschia spp.*. Pearse describes the species I am referring to as *B. vitiensis marmorata* as *B. vitiensis* but notes that it is also referred to as *B. marmorata* in Connon & Silver, 1986. It is possible that these two species are in fact the same species, but for the purposes of this study I consider them two different species. All species of Bohadschia were found in areas with sandy substrate and scattered coral heads. Some individuals were found in areas of predominantly coral and coral rubble and a few were found in shallow water with sandy substrate and with no nearby coral heads. Also, while collecting I observed individuals from each species expell Cuverian tubules when provoked. All of the above species of *Holothuria* and *Bohadschia* belong to the family Holothuriidae.

Two genera of the family Synaptidae were encountered: *Chiridota* and *Synapta*. I collected *Chiridota sp.*, tiny pink sea cucumbers 0.5 to five centimeters long, by sifting through the fine coral sand with my fingers. They were buried two to six centimeters in the sand. After collecting them my fingers were pink where I had touched them. Pearse describes *C. rigida* from two locations around Moorea, however a study conducted in 2005 describes *C. hawaiiensis* from the same location I encountered them (Tremain, 2005). I believe that the same species, *Chiridota spp.*, was observed in the two previous studies.

The second synaptid encountered is *Synapta maculata*. Thos species is long, snakelike, and very active. Their integument feels and acts like Velcro hooks when touched; clinging even to latex gloves. In contrast to *Chiridota*, this species was observed to reach lengths of over 130 centimeters.

Thenelota ananas is the largest of the species I encountered and the only member of the family Stichopodidae. They are grayish beige in color with many forked spikes that, as the specific name suggests, resemble a pineapple. They also have a soft, bright pinkish-orange underside.

Individual and behavioral observations

When physically disturbed, large individuals of *H. atra* pulled in their tentacles used for feeding, erected spikes all over their bodies, stuck to the glass of the aquarium, and secreted a burgundy liquid. Some individuals were active and attempted to escape, however most showed little or no movement. Small individuals of *H. atra* also erected spikes all over their bodies and stuck to the glass. They were slightly more active than the larger individuals. I did not observe a liquid being secreted, but when wiped with tissue paper the paper became burgundy. After being placed in a plastic bag and frozen, a small amount of burgundy liquid could be obtained from the plastic bag.

Like *H. atra*, *H. leucospilota* erected spikes all over and pulled in their tentacles. They became slightly shorter and more robust. One individual was very active and tried to escape, but the other three showed little or no movement when being physically stimulated. A single individual expelled a few very thin Cuverian tubules when first disturbed. I removed the tubules but could not get it to expel again. I did not observe a secretion during my observations, but after being frozen a secretion as observed in the plastic bag.

I did not observe any of the behaviors exhibited by *H. atra* or *H. leucospilota* in *H. fuscogilva*; except clinging to the substrate. The two individuals of *H. fuscogilva* were extremely hard and I could not deform their shape in any way. During the dissection, I could not cut the tegument with scissors at all and it was very difficult to do so with a razor blade. Their texture was also rough like coarse sandpaper.

All the individuals belonging to the genus *Bohadschia* erected small spikes all over their bodies. These spikes were in the center of their spots, except in *B. vitiensis marmorata* which does not have spots. *Bohadschia sp.* remained soft as it erected its spikes while all others became more rigid. All but one individual expelled Cuverian tubules when physically stimulated. *Bohadschia argus* (dark), *B. vitiensis marmorata,* and *B. marmorata?* curved their anal end back in an upright "C" position and expelled Cuverian tubules toward the point where the physical stimulation was being exerted. One individual of *B. argus* (light) made a slight motion and *B. sp.* made no motion while expelling tubules. All individuals pushed water through their anal opening as they were expelling tubules which made the tubules move and appear greater in number.

When disturbed with forceps in a Petri dish full of water and no sand, individuals of *Chiridota sp.* changed their shape slightly and move away from the disturbance, but did not appear to do anything else. In their natural setting they were observed to burrow under the sand.

Synapta maculata were extremely active and move in a snake-like manner away from the disturbance. When touched they clung to my gloves and often left tiny, hook-like structures behind. I did not observe any other defense mechanisms.

KEY TO SPECIES NAMES

1	*Bohadschia argus* (dark)
2	*Bohadschia argus* (light)
3	*Bohadschia marmorata?*
4	*Bohadschia vitiensis marmorata*
5	*Bohadschia* spp.
6	*Thenelota ananas*
7a	*Holothuria atra* (small)
7b	*Holothuria atra* (large)
7	*Holothuria leucospilota*
8	*Holothuria fuscogilva*
9	*Synapta maculata*

Thenelota ananas do not appear to respond to physical stimulation other than by clinging to the substrate and becoming slightly more rigid. However, when the stimulation ceased they moved away from the location where it took place relatively quickly. Fish, belonging to the family Carapidae, were found in every individual of *T. ananas* dissected. One individual had two carapid fish inside it; one of which was 22 cm long, or almost 40% of the total body length.

Toxicity assessment

After one hour, the highest mortality was observed in *T. ananas,* followed by *B. marmorata,* and *S. maculata* and the least mortality was observed in *H. atra* (small) (Figure 2). The highest mortality of all the individual parts was observed in the external body parts of *T. ananas* and internal body parts of *S. maculata.*

The oneway anova analyses for variance showed that there is a significant difference in mortality after one hour at both the genus and species level (p is <0.0001 for both) (Figures 3 and 4). However when comparing only the genera *Bohadschia* and *Holothuria,* there is no significant difference in mortality after one hour (p=0.32).

A regression analysis showed that volume of the individual is correlated with mortality after one hour (p<0.0001). Additionally, a oneway Anova showed that individual volume and species are highly correlated (p<0.0001) as well.

According to the calculated median lethal times (LT50), *H. atra* (large) displayed the highest mortality rate followed by *T. ananas* and *B. marmorata,* and the lowest was displayed by *H. atra* (small). The oneway anova analyses showed a significant difference between LT50s at the species level (p<0.0001), a slight difference at the genus level(p=0.049), and very little difference among parts independent of taxa (p=0.25). Again, there was no significant difference between the genera *Bohadschia* and *Holothuria.*

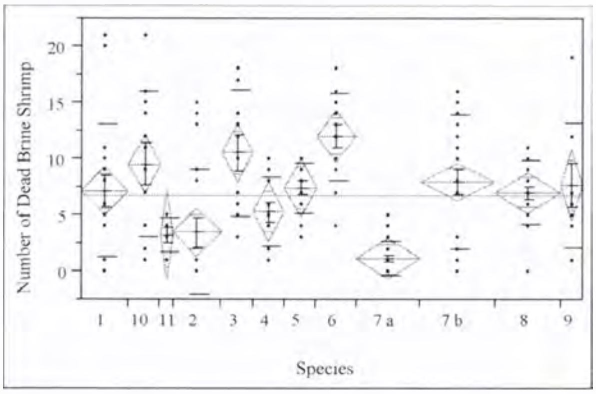


Figure 3: Comparing mortality after 1 hour between species.

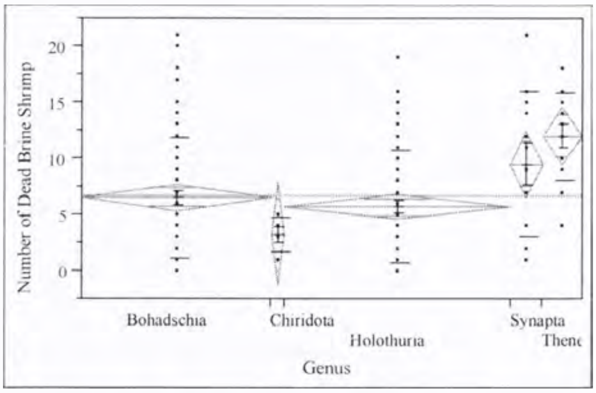


Figure 2: Comparing mortality after 1 hour between genera.

There is a significant difference in the toxicity between large and small individuals of *Holothuria atra* observed in both the mortality after 1 hour and LT50 (p<0.0001 for both) with larger individuals being much more toxic. A regression analysis showed a

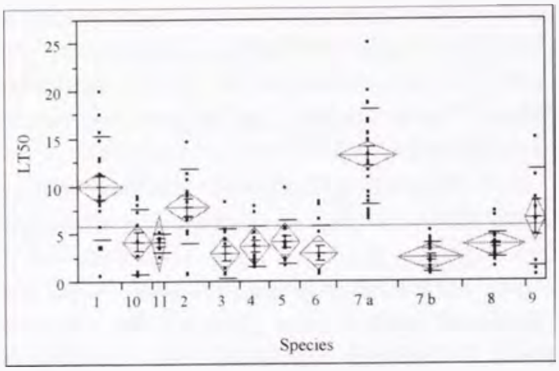


Figure 4: Comparing LT50 between species.

strong correlation ($p<0.0001$) between size and mortality after one hour. Anova analyses for large individuals showed no significant difference in mortality after one hour between secretions collected off of live individuals and from the bag after freezing ($p=0.46$). Thus being frozen did not have an effect on toxicity. No significant difference was observed in mortality after one hour between the secretions of individuals characterized as either large or small ($p=0.16$).

Phylogeny

The presence of Cuverian tubules, a red liquid secretion, an active flight response, and a thickened tegument were mapped onto the phylogeny adapted from Tremain 2005. The phylogeny was originally created using 27 morphological and ecological characteristics (Tremain, 2005). The placement of *Thenelota ananas* between *Bohadschia* and *Holothuria* is somewhat problematic because *Thenelota* belongs to the family Stichopodidae while the other two belong to the family Holothuriidae. However, for the purposes of this study the phylogeny is still very useful.

Eight of the twelve species in Tremain's study were present in this one. I omitted one species from the phylogeny and substituted a few of the species in this study for closely related species. For instance, I replaced *Holothuria* (*Lessonothuria*) with *Holothuria fuscogilva*, and *Bohadschia* unknown spp. K with *Bohadschia* spp.

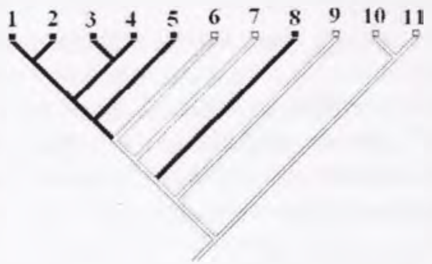


FIG. 5. Presence of Cuverian tubules.

FIG. 6. Presence of a red, liquid secretion.

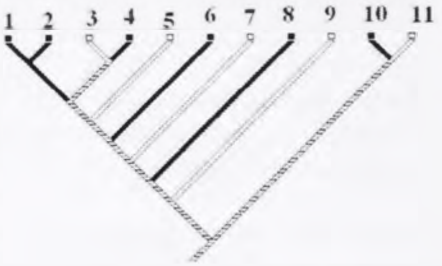


FIG. 7. Presence of an active flight

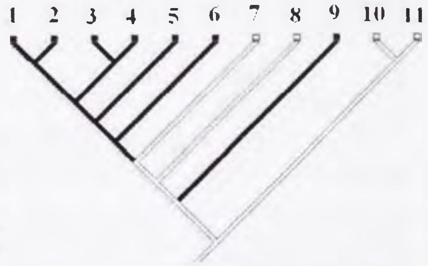


FIG. 8. Presence of a thickened tegument.

DISCUSSION

Sea cucumbers as a group have a wide variety of predator defense mechanisms and most species utilize one or more (Bingham, 1986). I observed that individual species not only possess multiple mechanisms, but they exhibit these mechanisms to varying degrees. The most striking of the defense mechanisms observed was the expulsion of Cuverian tubules. All members of the genus *Bohasdchia* that I observed expelled tubules, but not all the tubules produced the same mortality in the bioassays. For instance tubules from *B. argus* (light) produced relatively high levels of mortality after one hour while tubules from *B. argus* (dark) produced relatively low levels. It is possible that the toxicity of the tubules themselves may not be very important for their function in predator deterrence. It has been noted that while the tubules do possess toxins (Parmentier, 2005), they function primarily by entangling and immobilizing potential predators (DeMoor et. al, 2003).

In addition to Cuverian tubules, the coloration of *B. argus* may also play a role in predator avoidance. Depending on the substrate, the light or dark color morphs of *Bohadschia argus* may be cryptic. The lighter color morph blends in better with the calcium carbonate sand and light colored corals, while the darker color morph blends in more with darker corals. However both morphs were found out in the open, near all types of coral, and partially buried in the sand. In addition to color, the spots may serve to disrupt the overall shape of the cucumber and make it even more cryptic; or the spots may be aposematic, warning potential predators to stay away. The two are considered different color morphs of the same species and a further look into how color is determined could provide more insight into the relationship between color and predator defense.

Bohadschia vitiensis marmorata did not produce particularly high mortality rates in the toxicity assessment, however it expelled Cuverian tubules readily and in large quantity. One individual even eviscerated its entire digestive tract when I agitated it after having removed all the Cuverian tubules. I only observed this behavior in one individual, but the regeneration of the intestinal tract is one of the most studied in regeration studies of holothurians (Van Den Spiegel, 2000). Immediately after evisceration, the sea cucumber rapidly crawled away from its intestines. This is an extremely effective defense behavior especially for predators that prefer to only eat part of the cucumber, such as fish or crustaceans (Francour, 1997). The predator is distracted by the intestines and the sea cucumber has a chance to escape, however this is an extremely high energy cost to the cucumber and that is probably why I only observed it after persistent agitation and removal of all Cuverian tubules.

Holothuria leucospilota, the only species other than members of *Bohadschia* possessing Cuverian tubules, possessed multiple defense mechanisms yet did not respond very much to agitation. Both Cuverian tubules and a liquid secretion were present in this species, but neither were utilized to a great extent during this study. A previous study of *H. leucospilota* observed evidence of expulsion of Cuverian tubules in 2.3-6.1% of individuals in the field (Hamel, 2000), showing that they do in fact utilize this mechanism in the wild. Toxicity of this species is moderate compared to the other species in this study. *Holothuria leucospilota* was observed to wedge itself under rocks to avoid predation. This is most likely a very successful behavior for avoiding larger predators and the moderate toxicity may be enough to deter smaller predators.

A significant difference in the toxicity between large and small individuals of *Holothuria atra* was observed in both the mortality after 1 hour and LT50. This suggests that there is an increase in toxicity as the individual ages. Individuals of all sizes were observed out in the open and were usually covered in sand in the wild. Further study into the ontogeny of the species and any behavioral differences might provide more insight into why larger individuals are more toxic.

Holothuria fuscogilva is the most cryptically colored of all the species observed in this study. I only encountered two, but it is likely that there were more because it was found in a relatively common habitat type. The bioassays showed that it is only moderately toxic, however it is unlikely that many predators can penetrate its extremely rough, thick integument.

Thenelota ananas, the most toxic of the species studies, exhibited relatively passive forms of predator deterrence by becoming slightly more rigid and clinging to the substrate. It is possible that the spikey appearance suggests to predators that, although it is cryptically colored, *T. ananas* is unpalatable. Another explanation for the spikey texture, in conjunction with a thickened integument, is to make it difficult for potential predators to penetrate into the body. The bright underside may also serve in predator defense by being aposematic, however I did not observe any behaviors that might support this.

Thenelota ananas is also the largest of the species encountered and while a regression analysis showed a strong positive correlation between volume of the individual and toxicity, volume and species are also strongly correlated. Therefore, it is uncertain whether individual size plays a role in toxicity, or if both size and toxicity can be attributed to the species independent of the other.

The four carapid fish found in three specimens of *T. ananas* is probably related to the large size and absence of Cuverian tubules. A study of carapid fish in the Opunohu Bay of Moorea found fish in 81% of *T. ananas* examined. The fish are generally found in the respiratory tree where they take advantage of the water flow and if the diameter of the respiratory tree is too small the fish cannot penetrate into it. *Thenelota ananas* was observed in this study to have a relatively high level of toxicity, however the carapid fish are particularly resistant to sea cucumber toxins (Parmentier, 2005). Even Cuverian tubules prove to be an ineffective defense mechanism against these well adapted fish as they are covered in mucus which keeps the tubules from adhering to them (Parmentier, 2005). One small fish was found in a specimen of *Holothuria leucospilota* as well.

Comparing the presence and absence of Cuverian tubules to a phylogenetic study performed in 2005 suggests that Cuverian tubules either evolved separately in *Bohadschia* and *H. leucospilota*, or more likely that they were present in *Holothuria* and then were subsequently lost by multiple species or lineages. A previous phylogenetic study of the family Holothuriidae which included eight species and five genera suggests that Cuverian tubules were present before the initial radiation of the family and then lost by various members (Kerr et. al, 2005). The results of this study support Kerr et. al's findings. Kerr et. al also state that because a basal lineage of the family possesses Cuverian tubules which are small, not adhesive, and rarely expelled, Cuverian tubules may have evolved for some other function than defense (Kerr et. al, 2005).

The red secretion was only observed in the species *Holothuria atra* and *Holothuria leucospilota*. According to the phylogeny examined, this specific secretion probably only evolved once. Thickened skin appears to be characteristic of *Bohadschia* and has evolved multiple times in other species as well. Having an active flight response, on the other hand appears to not necessarily be related to a species' phylogenetic history, but rather it varies from species to species and is most likely a result of the particular ecology and life history of the animal.

A total of 12 species and morphotypes were examined in this study and different defense mechanisms and levels of toxicity were observed in each. Inheritance and phylogenetic relationships appear to play a large role in determining what mechanisms are present. However the expression of the traits and utilization of certain mechanisms is also influenced by the individual's ecology; thus there appears to be a degree of phenotypic plasticity among the species.

Future studies including introducing potential predators such as asteroids, crustaceans, and gastropods into a controlled setting may provide additional knowledge of holothurian defense behavior. For example, a particular species may respond differently to different predators and thus the presence of multiple defense mechanisms within a single species may be explained. Also, many of the phylogenetic relationships within Holothuroidea remain unresolved. This study serves as a baseline for further study into sea cucumber defense as well their ecology in general.

ACKNOWLEDGMENTS

I thank Jacques and Tony of the UC Berkeley Gump station for taking me out on the boat to my study sites. I thank Zachary Hanna for all his help finding cryptic sea cucumbers that I would have otherwise missed and for diving down to get all the ones I could not reach. Also, I thank Jaime Bartolome, Brent Mishler, Jere Lipps, Carole Hickman, and Vincent Resh for all their help and support. And of course I thank all the GSIs that made this class function.

LITERATURE CITED

[Anonymous]. 2003. Holothurians resources and its management. Oseana **28**:9-10.

Bingham B. L., L. F. Braithwaite. 1986. Defense adaptations of the dendrochirote holothurian Psolus chitonoides Clark. Journal of experimental marine biology and ecology **98**:311-322.

Clark, A. M., and F. W. Rowe. Monograph of shallow-water Indo-west Pacific echinoderms. Trustees of the British Museum (Natural History). London. 1971.

Delia T. J., L. W. Hertel, and L. W. Wittle. 1977. Characterization of the aglycones of the toxic principle of the sea cucumber Holothuria atra. Toxicon **15**:461-462.

DeMoor S., H. J. Waite, M. J. Jangoux, and P. J. Flammang. 2003. Characterization of the Adhesive from Cuvierian Tubules of the Sea Cucumber Holothuria forskali (Echinodermata, Holothuroidea). Marine Biotechnology **5**:45-57.

Flammang P. 2003. The glue of sea cucumber Cuvierian tubules: a novel marine bioadhesive. Ifremer, Plouzane (France).

Flamming, P. R. Santos, and D. Haesaerts. Echinoderm adhesive secretions: from experimental characterization to biotechnological applications. Progress in Molecular and Subcellular Biology Subseries Marine Molecular Biotechnology. V. Matranga (Ed.), Echinodermata. Springer-Verlag Berlin Heidelberg. 2005.

Francour P. 1997. Predation on Holothurians: A Literature Review. Invertebrate Biology **116**:52-60.

Guille, A. Guide des etoiles de mer, oursins et autres echinoderms du lagon de Nouvelle-Caledonie. Collection Faune tropicale, no 25. Paris. 1986.

Hamel J. -., A. Mercier. 2000. Cuvierian tubules in tropical holothurians: usefulness and efficiency as a defence mechanism. Marine and Freshwater Behaviour and Physiology **33**:115-139.

Kerr A. M., D. A. Janies, R. M. Clouse, Y. Samyn, J. Kuszak, and J. Kim. 2005. Molecular Phylogeny of Coral-Reef Sea Cucumbers (Holothuriidae: Aspidochirotida) Based on 16S Mitochondrial Ribosomal DNA Sequence. Marine Biotechnology **7**:53-60.

Lawrence, J.M. A functional biology of echinoderms. Johns Hopkins University Press. Baltimore. 1987.

Parmentier E, P. Vandewalle. 2005. Further insight on carapid-holothuroid relationships. Marine Biology 146: 455-465.

Pearse, J. S. 1989. Holothuroids, echinoids and asteroids seen on Moorea, French Polynesia, during a working visit to the Richard B. Gump South Pacific Biological Research Station of the University of California at Berkeley. 2 January-4 February 1989. Institute of Marine Sciences University of California, Santa Cruz, California 95064. Unpublished work.

Sakthivel M., P. Swamy, K. Rengarajan, and D. e. James. 1994. International trade in sea-cucumbers. CMFRI, Cochin (India).

Tremain, K. 2005. Patterns of Community Composition: a phylogeny of the holothurians of Moorea. Biology and Geomorphology of Tropical Islands, Student Research Papers. University of California, Berkeley.

Van Den Spiegel D. 1995. Sea cucumber: The cotton spinner. OCEANORAMA.no.25

Van Den Spiegel D., M. Jangoux. 1987. Cuvierian tubules of the holothuroid Holothuria forskali (Echinodermata): A morphofunctional study. Marine biology. Berlin, Heidelberg **96**:263-275.

Van Den Spiegel D., M. Jangoux, and P. Flammang. 2000. Maintaining the Line of Defense: Regeneration of Cuvierian Tubules in the Sea Cucumber Holothuria forskali (Echinodermata, Holothuroidea). Biological Bulletin **198**:34-49

APPENDICES

APPENDIX A: Study sites and species encountered.

Genus	**Species**	**Cook's Bay at Gump Station**	**Cook's Bay Reef Crest**	**Mangrove Marsh near Haapiti**	**North side of Motu**	**East side of Motu Tiahura**	**Temae**	**Tetearoa**	**West of Sheraton b/w PK14 & PK15**
Bohadschia	vitiensis marmorata				x			x	
Holothuria	leucospilota						x		
Holothuria	atra	x	x	x	x	x	x	x	x
Thenelota	ananas				x				x
Synapta	maculata	x					x		
Chiridota	spp.					x		x	
Bohadschia	argus (dark)	x	x		x	x	x	x	x
Bohadschia	argus (light)	x	x		x	x	x	x	x
Bohadschia	marmorata				x			x	
Bohadschia	spp.				x			x	
Holothuria	fuscogilva				x				

1 Temae: Composite CaCO3 near shore, predominantly coral with some sandy bottom further.

2 Cook's Bay at Gump Station: Sand bottom with coral rubble and scattered coral heads.

3 Cooks Bay near Pihaena Point: Sand bottom with coral rubble and scattered coral heads

4 West of Sheraton between PK14 & 15: Composite CaCO3 near shore, predominantly coral with some sandy bottom further.

5 a: East side of Motu Tiahura: Fine coral sand.
b,c: North side of Motu: Sand and coral rubble bottom with scattered, large coral heads. Deep water ~1.5m

6 Mangrove marsh near Haapiti: Fine sand and sediment bottom. Scattered mangrove trees and occassional coral

7 Tetearoa: Fine sand and sediment bottom. Scattered mangrove trees and occassional coral

APPENDIX B: Physical and behavioral characteristics observed by species.

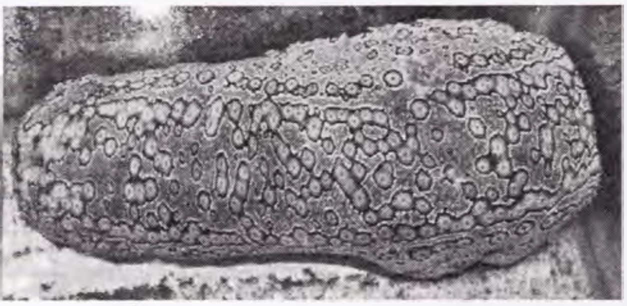
Species	Cuverian Tubules Expelled	Secretion Present	Thickened body wall	Active Escape Behavior	Change in Shape/ Texture	Retractable mouthparts	Burrowing/ Hiding under rock behavior	Volume >500mL	Volume >1000mL
Bohadschia argus (dark)	1	0	1	1	1	1	1	1	1
Bohadschia argus (light)	1	0	1	1	1	1	1	1	1
Bohadschia marmorata	1	0	1	0	1	1	1	1	1
Bohadschia spp.	1	0	1	0	1	1	1	1	0
Bohadschia vitiensis marmorata	1	0	1	1	1	1	1	1	0
Chiridota spp.	0	1	0	1	0	1	1	0	0
Holothuria atra (large)	0	1	0	0	1	1	1	1	0
Holothuria atra (small)	0	1	0	0	1	1	0	0	0
Holothuria fuscogilva	0	0	1	0	0	1	0	1	1
Holothuria leucospilota	1	1	0	1	1	1	1	0	0
Synapta maculata	0	0	0	1	1	1	0	0	0
Thenelota ananas	0	0	1	1	0	1	0	1	1

APPENDIX C: Characteristics of habitats inhabited by species of holothurians.

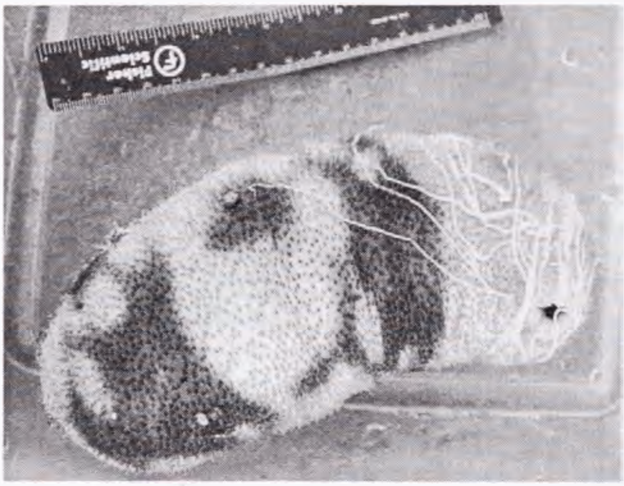
Species	Shallow (<0.5m) CaCO3 Composite	Shallow (<0.5m) coral sand substrate	~1m predominately coral heads and rubble w/ little sand	~1m coral sand substrate w/ few coral heads	1-2m sand/coral rubble w/ scattered coral heads	4-6 m sandy substrate w/ few coral heads
Bohadschia argus (dark)	0	0	1	1	1	1
Bohadschia argus (light)	0	0	1	1	1	1
Bohadschia marmorata	0	0	0	1	1	0
Bohadschia spp.	0	0	0	1	1	0
Bohadschia vitiensis marmorata	0	0	0	1	1	0
Chiridota spp.	0	1	0	0	0	0
Holothuria atra (large)	1	1	1	1	1	0
Holothuria atra (small)	1	1	1	1	1	0
Holothuria fuscogilva	0	0	0	0	1	0
Holothuria leucospilota	1	1	0	0	0	0
Synapta maculata	1	0	1	1	0	0
Thenelota ananas	0	0	0	0	0	1

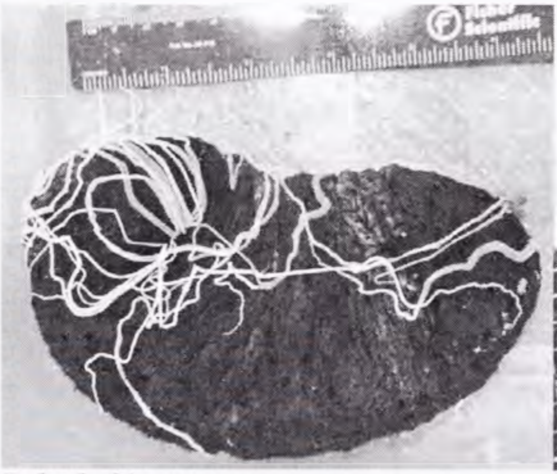
APPENDIX D: Species List and Photos


Bohadschia argus (dark)


Bohadschia argus (light)

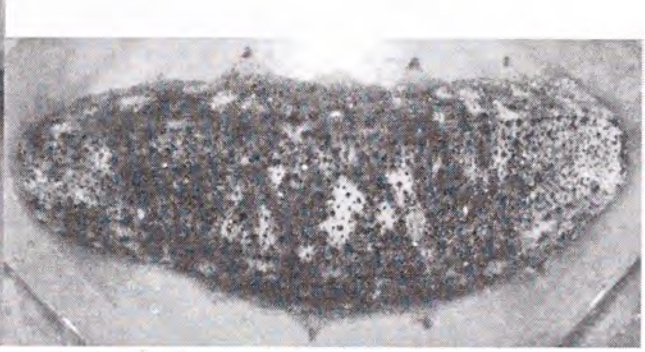

Bohadschia marmorata?


Bohadschia vitiensis marmorata

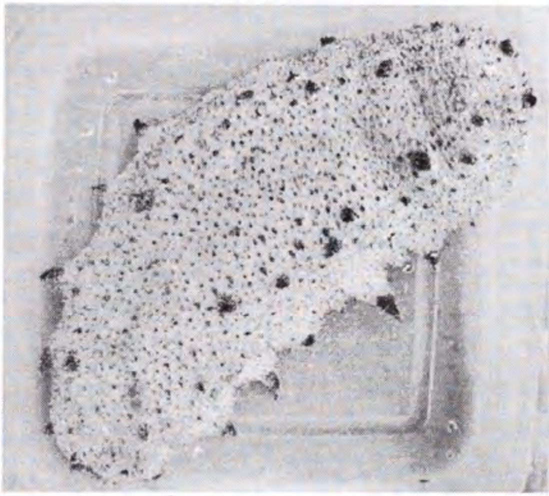

Bohadschia spp.


Chiridota spp.

Holothuria atra



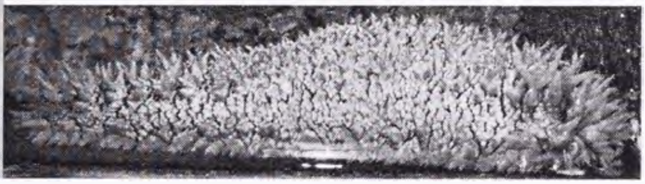
Holothuria fuscogilva



Holothuria fuscogilva

Holothuria leucospilota

Synapta maculata



Thenelota ananas

ANCIENT LAWNS IN A MODERN DAY WORLD: DISTRIBUTION AND CHARACTERIZATION OF MARINE MICROBIAL MATS AT TEMAE BEACH, MOOREA, FRENCH POLYNESIA

CAROL CHANG

Molecular & Cell Biology, University of California, Berkeley, California 94720 USA

Abstract. The study of microorganisms and filamentous blue-green algae holds great ecological and geological importance; such microbial communities to be on of the first forms of life as well as the initial source of atmospheric oxygen. In Moorea, an island in the South Pacific, the microbial communities found on the intertidal mudflats have been widely studied and characterized. However, little is known about the marine mats that are consistently covered with seawater. This study surveyed the algal communities present in the shallow lagoon formed at Temae Beach, a public sand beach located on the northeast tip of Moorea. Seven morphologically different mats were observed and their distributions determined and mapped throughout two regions: the littoral (intertidal and sub-tidal) and back reef. The majority of mats were found within the sub-tidal while none were seen within the intertidal and very few observed within the back reef region. A common trend observed within this distribution found specific morphological types consistently located within the same general areas. Additional observations of fish herbivory as well as shear stress and current flow over these mats were also briefly investigation. Overall, while this study provides a brief characterization of what is found in the lagoon, further studies of these marine mats are needed to strengthen these initial findings and, in particular, to investigate specific factors attributing to why the mats disperse as they do.

Key words: microbial mats; blue-green algae; cyanobacteria; distribution; Moorea, French Polynesia; marine mats

INTRODUCTION

The study of microorganisms and blue-green algae, and other microbial communities holds great ecological and geological importance; cyanobacteria has been said to be the key to understanding the evolution of life as it is believed to be one of the first life forms on Earth and the initial source of atmospheric oxygen (Golubic et al 1993, Kühl 2000). With fossil records dating back 3500 Ma, the earliest forms of cyanobacteria have evolved to become modern day cyanobacteria or have diversified into other eukaryotic phototrophic systems (Golubic et al 1993). Modern-day blue-green algae, the simplest of today's algal groups, are still considered to be mostly unchanged from its ancient forms, to be at an "evolutionary standpoint" so to speak (Abrams 1996). Therefore, by studying how blue-green algae and other microbial communities in the present day, we can begin to understand more about how it is that life came about.

Today's microbial communities have been studied in a wide range of habitats including brackish lakes in Antarctica (Taton et al 2003), grasslands and marshes in Belize (Rejmánková et al 2005), tidal flats in Southern California (Armitage et al 2004), and the hypersaline ponds in France (Fourçans et al 2004). Modern-day microbial mats have been found to have distinct layers of cyanobacteria and anoxygenic photosynthetic bacteria (Kühl 2000) that aide in sediment binding and stabilization (Wieland 2003). The success of today's cyanobacteria has been attributed to its nitrogen-fixing abilities (Golubic 1999); indeed, microbial mats in tropical marine environments have a primary role as nitrogen fixers (Hoffmann 1999).

Microbial mat investigations in the South Pacific have mainly been limited to the oceanic atolls of the Tuamotu Archipelago (Rougeaux et al 2001, Mao Che et al 2001, Sprachta et al 2001, Andréfouët et al 2003, Abed 2003). These studies have found that the composition and structure of mats at different locations are extremely similar: stratified layers of cyanobacteria and purple and green phototrophic bacteria (Mao Che et al 2001). Additional studies on the structure, composition, and environmental parameters making up and shaping the microbial communities on the intertidal and supralittoral mudflats of Moorea have also found similar findings (Poetker 2000, Bauer 2003, Magudia 2003). While the intertidal and terrestrial mats have been well studied, much less is known about marine mats consistently covered by seawater that are located in the sub-tidal zones and beyond.

In this study, the algal communities located within the lagoon at Temae Beach in Moorea, French Polynesia were investigated. Specifically, the distribution of microbial communities across the sandy floor was observed and the presence of substrates within each mat was characterized according to phenotype: color, texture, shape, etc. Ideas for future studies including tests on mat herbivory and dispersal mechanisms are also briefly considered, although more data is necessary in all cases. Because these marine mats have not yet been described in detail, overall, this study aims to provide a basis for future research on more specific aspects of these mats.

METHODS

Study Site

The study was conducted between October 2006 and November 2006 on the island of Moorea (17°29'24"S latitude, 149°45'43"W longitude), a part of the Society Archipelago in the South Pacific (French Polynesia). Moorea is situated 17 kilometers northwest of Tahiti. The actual study site was located within the lagoon at Temae Beach, a public sand beach found on the northeast end of the island. At this location, the barrier reef is situated close to the shoreline effectively becoming a fringing reef; this results in a shallow lagoon.

The layout of the lagoon between the shore and the reef crest was broken up into two distinct zones for the purpose of this study: the littoral and the back reef. The littoral can be further divided into the intertidal, defined by the region along the shore that is covered with seawater during high tide and exposed during low tide, and the sub-tidal, defined by the region immediately after the intertidal that is consistently covered with seawater. The back reef is defined as the region immediately after the sub-tidal and before the reef crest.

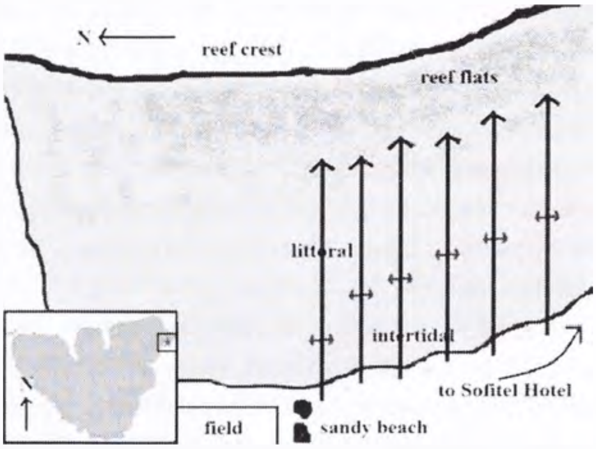


FIG. 1. Temae Public Beach located on the northeast tip of Moorea. Aerial view of study site depicting belt transects run perpendicular to the shore.

Mapping Distribution

To determine the distribution of microbial mats over the lagoon floor, a transect tape was first run along the shoreline starting from the southern edge of the soccer pit out towards the Sofitel hotel (Fig. 1). Then, a belt transect perpendicular to the initial line transect was run every 25-meters from the shore towards the back reef; each belt was 2-meters wide (1-meter to each side) and ended when the percent coral coverage was approximately 80% of the 2-meter width. A total of 6 belt transects were run.

To determine the area of mat coverage, a minimum of 4 GPS coordinates were taken, 1 at each extreme edge, for each mat encountered along this belt transect. These GPS waypoints were later used to develop a map of the distribution of mats along the lagoon floor. This was done by plotting each point on a sheet of graph paper and marking the mat boundaries.

Mat Collection & Analysis

To determine which mats were the same between each belt transect, the general morphology for each mat encountered in the field and its overall habitat were briefly described. Mat samples were collected and stored in a zip-lock bag for transport back to the lab for further analysis including observations of color, strand size and characteristics, extend of substrate binding, and/or texture using a Spencer dissecting microscope and a Tokyo Olympus light microscope.

Small samples of each collection were placed in a Petri dish and observed under the dissecting microscope. Slides were prepared using samples fixed in a solution of 5% formalin in seawater for at least 24 hours; samples were mounted on microscope slides in filtered seawater (made by running seawater through a coffee filter) using a cover slip sealed on 3 sides with clear nail polish.

Current Flow Study & Analysis

Four mats of different thickness and densities were chosen to test the rate of current flow by determining the speed at which fluorescence dye was carried 2-meters over the center of each mat. Approximately 2.5mL of fluorescence (diluted in seawater) was released at mat level 0.5-meter in front of the 0-meter marker. Timing started when the head of the cloud of dye crossed over the 0-meter marker and stopped when the head of the cloud of dye reached the 2-meter mark. This was repeated 4 times in the same spot for each mat tested. The times for each mat location were analyzed by one-way ANOVA using JMP 5.1; results were considered significant for a p-value less than or equal to 0.05 and with an F-ratio $_{0.5, 3, 15}$ that was greater than or equal to 3.287.

RESULTS

Mapping Distribution, Collection, & Analysis

The distribution of microbial mats throughout the northern half of the lagoon was mapped using a series of GPS waypoints (Fig. 2). Results show that mats were observed as close as approximately 30-meters from the shoreline and as far out as 200+ meters out. Several general trends were noted relating to mat types and their subsequent distributions. Most notably, algal mats were found only within the sub-tidal zone and the back reef. However, as the percent coral coverage increased, the frequency of mats decreased. In fact, the majority of mats were found within the sub-tidal regions.

Each mat recorded was grouped into one of 7 phenotypes named Species 1 through Species 7. Observations in the lab revealed that all mats were made up of filamentous algae for the most part with a few diatoms present as well. Some mats had more diatoms than others, but the frequency was not determined. These phenotypes were determined through observations made considering color, textures, morphology, and other such qualitative characteristics. A common trend observed within this distribution found specific phenotypes consistently located within the same general area on the distribution map. In addition to this, there were also trends in the overall shape of different mat types: those located closer to shore were long expanses whereas mats farther out were characteristically more patchy and broken up.

The phenotype closest to shore was Species 1. This mat was relatively continuous in coverage, was a dark yellow-green, almost brown color, and arranged itself with short, spike-y strands. Its binding ability was moderate in that I was able to pick it up, but the edges would readily crumble into small bits; the dissecting microscope revealed that the algal strands formed a net over the sediment resulting in a thin, but shallow (did not penetrate deeply) layer. Species 1 was situated in a wide-open area and ran in a continuous, long strip parallel to shore.

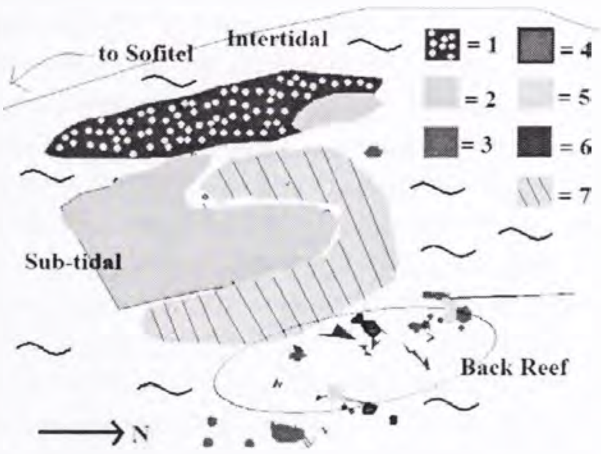


FIG. 2. Approximate distribution of 7 mat phenotypes. Areas marked indicate boundaries within which mats were found; it does not indicate density.

The next type out was Species 2, located almost adjacent to Species 1 in some areas. This mat was made up of gray-brown tufts of long fibers that wove deeply into tight, thick patches. However, patches were situated close enough together to maintain a mostly continuous coverage (resulting in a high density) over the sandy sediment. Species 2 was also mostly found in wide-open areas, away from any major coral heads, and situated in moderately long strips parallel to shore and in wide patches in open areas farther out.

Species 3 had very sparse coverage and poor sediment binding ability. It was light green in color and took on an almost film-y appearance over the sandy floor. These mats were almost exclusively found in somewhat close proximity to coral heads and were only located near the far sub-tidal and back reef regions. Additionally, Species 3 was usually found in shapeless patches ranging from very small (0.25-meters) to 4-5-meters in diameter.

Species 4 was also usually found near coral areas. One particular mat was observed situated next to a bed of Staghorn coral (*Acropora*). Its appearance was gray-brown and crumbly. The mat layer formed shapeless patches and was very shallow with poor sediment binding overall. There appeared to be less sand immediately below these mats. Instead, rockier sediment was observed.

Within the sub-tidal-back reef boundary was Species 5. Intermixed in opener areas as well as locations heavily surrounded by coral heads, Species 5 formed small, discrete rust-red islands ranging from a few centimeters to a few meters in diameter. There were also air-filled sacs that floated above the mats attached by thin filaments. Although the general distribution of Species 5 was in patches, the mat coverage within each patch was continuous with shallow penetration of the mat into the sediment. Species 5 was also observed on some low-lying coral.

Species 6 was often found immediately adjacent at the boundaries of Species 5 lawns. It had a crusty appearance, tan coloration, and moderate sediment binding ability. Under the dissecting microscope, Species 6 featured 2 colors; olive/army green and black. The algal strands were randomly interwoven with sediment, but both colored strands were intermixed with one another. Microscopic analysis also showed a few segmented (possibly calcified) branches also present.

In the wide-open, sandy region between the edges of Species 2 and the start of appearance of patchier-shapeless mats, Species 7 was found covering an extremely large swath of sandy bottom. Light beige in color, mats of Species 7 were almost indistinguishable and easily overlooked. Sediment binding was moderately poor with substrates loosely held together (would crumble easily into small clumps too large to simply be sediment particles).

Current Flow Study

The rates of current flow were relatively determined between mats of different textures. Mat IDs, mat depth, and ranges of current speed at mat level are summarized in Table 1. Mat A had the slowest average speed at 36.75 seconds/meter; it was also the thickest and had the densest algal coverage. Mat D was considered the sparsest of the mats tested; it had an average current flow of 22.625 seconds/meter. In fact, the general trend shows that as the texture of the mat increases (along with thickness and/or density), the calculated speed of water flow increases as well.

The ANOVA results give an F-ratio of 3.7822 and a p-value of 0.0404 ($F \geq 3.287$, $p \leq 0.05$) (Fig. 3). Therefore, these differences in speed between different mat textures are statistically significant; however, because the ANOVA results are so close to the borderline values, results are only weakly significant. This means that the differences in speed between different mats of varying textures are most likely due to an outside cause; the null hypothesis that the average speeds between mats of different textures are equal is rejected.

DISCUSSION

Composition & Mapping Distribution

Previous mat studies on Moorea found the microbial mats of the supra-littoral and intertidal mudflats were composed mostly of cyanobacteria stratified into distinct layers changing with different environmental parameters. These results were supported by past studies on mats in similar environments. The marine mats of this study, however, are much less complex. Of all 7 phenotypes observed, 6 were composed of a single layer made up of a single species of filamentous cyanobacteria; the seventh, Species 6, remains undetermined whether the two colors signify 2 species of algae. This also supports past research: sublittoral benthic mats are formed by miniblooms of filamentous cyanobacteria spread out over the sand and arranged by linear trichomes that move by a gliding mechanism (Abed et al 2003). Such mats generally show no vertical zonations and are often made up of a single filamentous species, almost opposite from their intertidal or

TABLE 1. Summarized data for mats of varying densities for current flow study.

Mat ID	Density Rank*	Depth (m)	Rate of Flow Range (sec/m)	Avg. Rate (sec/m)	Description
A	1	2.05	22 - 50.5	36.75	brown, tufty, dense
B	2	1.35	25 - 27.5	26.625	dark green, patchy
C	3	2.15	19.5 - 27.5	23.875	brown, tufty
D	4	1.97	16.5 - 26.5	22.625	light green, filmy

*1 = thinnest, 4 = thickest

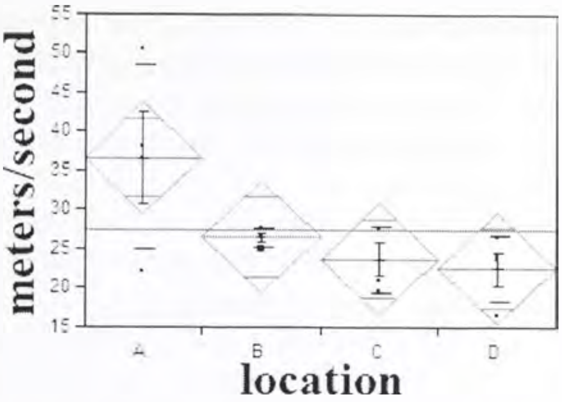


FIG. 3. One-way ANOVA results for rates of flow by location. p = 0.0404 (F = 3.7822, ≤ 0.05); therefore, the differences between rates of flow are overall statistically significant

supralittoral counterparts (Hoffmann 1999).

There were, however, additional trends suggested from the results. The first comes from the mapping – mats of the same phenotype are observed grouped together in the same general habitats. But the reason behind this is uncertain – is it the mats themselves that are separating themselves or is it an outside factor that is grooming the boundaries of the mat, so to speak, and keeping it from spreading about. If the blue-greens making up these mats can be identified and their functions determined, then it may be possible to infer that each mat's function may be a factor behind why it colonized where it was observed. And thus it should follow that other identical mats colonize in suit. For example, the success of today's cyanobacteria has been attributed to its nitrogen-fixing abilities (Golubic 1999); indeed, microbial mats in tropical marine environments have a primary role as nitrogen fixers (Hoffmann 1999). Should this be the case, if certain cyanobacteria species are better nitrogen fixers than others, then perhaps this may explain why one species is found more often closer to coral habitats than the other (having a better nitrogen-fixer around is more beneficial to the marine life living in such a habitat). Or, if one species is a better at sediment binding than another, the first can out compete the latter in areas of high current flow. Knowing the function of each mat is important to help determine why this grouping trend was observed.

A second trend reported is that of distribution: mats near the shore cover a greater area and are seemingly thicker than mats out towards the reef; these further mats are sparse, shapeless, and overall, spotty. Temae is a popular public beach on Moorea; Species 1 and a handful of mats of Species 2 (which both fall into the thicker mat category) are located in a shallow enough area so that swimmers can stand on the lagoon floor. When this happens, there is always the possibility of kicking up the mats and disturbing its situation. Does the disturbed mat get carried along and resettles further down the shore? Did Species 1 originate as a shapeless patch of a mat only to evolve into a long parallel strip as more and more visitors disturbed it? In other words, do these mats distribute themselves as a result of human disturbance or simply due to natural disturbances (i.e. current)?

Current Flow Study

In the current flow study, mats A, B, and C were all located in a somewhat open area, relatively close to shore. There were, however, differences in thickness of the mats between all 3 of them. If these mats distributed this way as a result of human activity, we should not see a change in flow rates between test sites. However, the fact that there was a difference in the average flow rate suggests that human disturbance does not affect mat distribution after all, but the cause is most likely simply different current speeds.

The rate of water flow can affect the ability of a mat to settle and effectively colonize. In areas with fast current, there may not be enough time to allow for mats to settle resulting in thinner, sparser mats. On the other hand, areas with slower current leave plenty of time for the algae to colonize properly and build up, producing a robust, dense coverage over the sediment.

The ANOVA test showed weekly significant results. The null hypothesis of identical average speeds was rejected, but just barely. There are 2 possibilities for this: 1) the sample size was not large enough to produce significant results (this was really just a brief test to see what would happen – further colonization studies are needed to support this finding) or 2) it is not the current that is the leading factor behind mat dispersal.

Herbivory

Herbivory of the mats is another aspect that needs to be explored in relation to distribution of the mats. Cyanobacteria in reef habitats are subject to high rates of herbivory and lack structural defenses (Hoffmann 1999); this may help explain why the majority of mats were observed within the sub-tidal instead of the fore reef. However, when a preliminary survey of fish herbivory was conducted, fish were observed swimming right past the mats. When bits of mat were suspended in the water column, fish would nibble at the pieces, but then spit it right back out. Is there something chemically unpalatable to prevent fish from eating these mats? Is it a defense mechanism?

Microbial mats have been found to perform optimally in "light exposed habitats, where bioturbation and grazing are minimal or even absent" (Kühl et al 2000); preliminary observations appear to rule out fish, but what about other organisms living within the coral habitat? Echinoderms? Burrowers?

CONCLUSION

The marine mats located at Temae Public Beach are very different from the intertidal mats that used to be found on the Temae mudflats. For the most part, the marine mats are much simpler, composed only of 1 or 2 filamentous species in a single layer. There is no vertical stratification of layers nor are there extreme changes in environmental parameters from one mat to the next. There are, however, 2 distinct trends that emerged from mapping the 7 phenotypes determined to be present:

1) mats of similar phenotype tend to be grouped together in similar habitats and regions.
2) different types of mat dispersal are apparent in that mats close to shore are long, spanning many square meters where mats near reef habitats cover a small area and are generally discontinuous and patch-like

This study provides a brief description and characterization of the marine mats in the lagoon off shore at Temae. Further studies are needed to strengthen and find support for these initial findings and observations. More specifically, each mat needs to be identified so one can explore function versus how it behaves in the field. Additionally, studies on rate of water flow over marine mats and herbivory of mats are especially needed to further explore how mats disperse as they do and potentially even wage a prediction on how the might behave in the future.

ACKNOWLEDGMENTS

I would like to thank Jere Lipps for piquing my interest in microbial communities and for providing much appreciated support and encouragement throughout; and to Vince Resh for his (neon yellow) hands-on assistance in the field. Thank you to Jaime Bartolome, Carole Hickman, and Brent Mishler for their continuous advice and enthusiasm; to our Awesome GSIs Liz Perotti, Alison Purcell, and Erica Spotswood. Special thanks to Daniel Song for endless early mornings and long hours adrift in the sea kayak (best buddy ever!). And the rest of the Moorea 2006 Class – thanks for filling up all the time in between!

LITERATURE CITED

Abed RMM, Golubic S, Garcia-Pichel F, Camoin GF, Sprachta S (2003) Characterization of microbialite-forming cyanobacteria in a tropical lagoon:

Tikehau atoll, Tuamotu, French Polynesia. J of Phycology 39: 862-873

Abrams Karl J (1996) Algae to the Rescue! Logan House Publications, Studio City, CA

Andréfouët S, Hochberg EJ, Payri C, Atkinson MJ, Muller-Karger FE, Ripley H (2003) Multi-scale remote sending of microbial mats in an atoll environment. Int J of Remote Sensing 24(13): 2661-2682

Armitage AR, Fong P (2004) Upward cascading effects of nutrients: shifts in a benthic microalgal community and a negative herbivore response. Oecologia 139: 560-567

Bauer, EE (2003) Faunal distribution and abundance of a saline microbial mat in Moorea, French Polynesia. The Biology and Geomorphology of Tropical Islands 1: 109-116

Fourçans A, Garcia de Oteyza T, Wieland A, Solé A, Diestra E, van Bleijswijk J, Grimalt JO, Kühl M, Esteve I, Muyzer G, Caumette P, Duran R (2004) Characterization of functional bacterial groups in a hypersaline microbial mat community. FEMS Microbial Ecology 51: 55-70

Golubic S, Seoung-Joo L (1999) Early cyanobacterial fossil record: preservation, palaeoenvironments and identification. European J of Phycology 34: 339-348

Hoffmann L (1999) Marine cyanobacteria in tropical regions: diversity and ecology. European J of Phycology 34: 371-379

Kühl M, Fenchel T (2000) Bio-optical characteristics and the vertical distribution of photosynthetic pigments and photosynthesis in an artificial cyanobacterial mat. Microbial Ecology 40: 94-103

Mao Che L, Andréfouët S, Bothorel V, Guezennec M, Rougeaux H, Guezennec J, Deslandes E, Trichet J, Matheron R, Le Campion T, Payri C, Caumette P (2001) Physical, chemical, and microbiological characteristics of microbial mats (KOPARA) in the South Pacific atolls of French Polynesia. Canadian J of Microbiology 47: 994-1012

Magudia K (2003) Temporal and spatial variation in physical factors at the Temae intertidal and supralittoral microbial mats in Moorea, French Polynesia. The Biology and Geomorphology of Tropical Islands 1: 90-108

Poetker S (2000) Intertidal and supra-littoral microbial mats of the Temae mudflat on Moorea, French Polynesia. The Biology and Geomorphology of Tropical Islands 1: 167-184

Rejmánková E, Komárková J (2005) Response of cyanobacterial mats to nutrient and salinity changes. Aquatic Botany 83: 87-107

Rougeaux H, Guezennec M, Mao Che L, Claude P, Deslandes E, Guezennec J (2001) Microbial communities and exopolysaccharides from Polynesian mats. Marine Biotechnology 3: 181-187

Sprachta S, Camoin G, Golubic S, Le Campion T (2001) Microbialites in a modern lagoonal environment: nature and distribution, Tikehau atoll (French Polynesia). Palaeogeography, Palaeoclimatology, Palaeoecology 175: 103-124

Taton A, Grubisic S, Brambilla E, de Wit R, Wilmotte A (2003) Cyanobacterial diversity in natural and artificial microbial mats of Lake Fryxell (McMurdo Dry Valleys, Antarctica): a morphological and molecular approach. Applied and Environmental Microbiology 69(9): 5157-5169

Wieland A, Kühl M, McGowan L, Fourçans A, Duran R, Caumette P, García de Oteyza T, Grimalt JO, Solé A, Diestra E, Esteve I, Herbert RA (2003) Microbial mats on the Orkney Islands revisited: Microenvironment and microbial community composition. Microbial Ecology 46: 371-390

HABITAT DISTRIBUTION AND COMPARISON OF BRITTLE STAR (ECHINODERMATA: OPHIUROIDEA) ARM REGENERATION ON MOOREA, FRENCH POLYNESIA

SARAH CHINN

Department of Integrative Biology, University of California, Berkeley 94720 USA
sarahchinn@gmail.com

Abstract. Autotomy and regeneration are widespread in many groups of invertebrates and vertebrates, such as annelids, crustaceans, amphibians, and reptiles. Regeneration is common in all classes of Echinodermata and prevalent in ophiuroid brittle stars. Moorea, French Polynesia was surveyed for species of brittle stars living on coastal areas of the island in different habitats. Ophiuroid populations were sampled in habitats such as a mangrove marsh, a sandy beach with coral rubble and a jetty with coral rubble and conglomerate coral to determine percentages with regenerating arms. *Macrophiothix longipeda* (Lamarck 1816) from the mangrove marsh and two populations of *Ophiocoma scolopendrina* (Lamarck 1816) from the beach and jetty were studied to determine if there were differences in experimental rates of arm regeneration after induced autotomization. Each habitat was colonized by distict ophiuriod assemblages and had different percentages of regenerating individuals; *M. longipeda* was found to be regenerating multiple arms simultaneously and had the highest rate of regeneration. Regeneration rates differed by species; mostly likely influenced by habitat, ecology and biology of each species.

Key words: echinoderm, ophiuroid, brittle star, autotomy, regeneration, intact arms, regenerating arms, Macrophiothrix longipeda, Ophiocoma scolopendrina

INTRODUCTION

Autotomy is an effective strategy evolved by many invertebrates and vertebrates to avoid lethal predation. Autotomy results in the loss of biomass, which likely affects an individual's energy metabolism and allocation during regeneration of the lost tissue. Species that differ in their ecology, physiology, and phylogeny may autotomize and regenerate at different rates and in response to different stimuli such as predation, environmental factors such as water flow, asexual reproduction (fission), or conspecific interactions.

Loss of biomass can be caused by physical perturbations (Ball et al., 1967; Woodley, 1980; Tilmant et al., 1994); interspecific and intraspecific fighting (Berzin and Caldwell, 1983; Harris, 1989; Smith and Hines, 1991); and partial predation (Vlas, 1979a; Turner et al., 1982; Bowmer and Keegan, 1983; Clavier, 1984; Woodin, 1984; Bergman et al., 1988). In sublethal predation the affected individual regenerates the missing structure, thereby escaping death (Pomory and Lawrence, 2001). Regeneration after injury allows the individual to survive and contribute reproductively to the population. However, regeneration requires additional energy in addition to normal energetic processes. Pomory and Lawrence (2001) suggest that regeneration can affect the fitness of an individual by using energy that would otherwise be distributed to basal energetics, reproduction, and growth.

Many marine organisms, particularly echinoderms, autotomize appendages. Regeneration occurs in all five classes of Echinodermata to replace external and internal organs like arms, appendages (spines and pedicellariae), viscera (digestive tube, gonads) (Carnevali and Bonasoro, 2001). Carnevali and Bonasoro (2001) observed that regeneration is frequent in crinoids and ophiuroids as both classes have long, fragile arms that are often amputated voluntarily or via trauma followed by total regrowth of the lost structures. Regeneration is so common that individuals assessed in nature usually have regenerating arms at many different growth stages. Additionally, lost body fragments can live for a period of time after being separated, and can sometimes undergo independent partial or total regeneration (Carnevali and Bonasoro, 2001). Also, some asteroids, ophiuroids, and holothuroids asexually reproduce via fission. Adult individuals split into two or three parts, regenerating all parts into complete, independent individuals (Emson and Wilkie, 1980). The commonality of regeneration across the phylum suggests that regeneration is an essential aspect of their life-cycle (Carnevali and Bonasoro, 2001).

Brittle stars are capable of losing arms or parts of arms, and sometimes the aboral portion of the central disk and viscera to predation, autotomy, and asexual reproduction (Wilkie, 1978). Wilkie (1978) observed that a significant proportion (20-100%) of the ophiuroid population may be regenerating lost structures. Emson and Wilkie (1980) provided evidence that a majority of noneuryalid ophiuroids (i.e. brittle stars) have the ability to autotomize their arms at any intersegment. Arm breakage is common and may be caused by waves, intraspecific confrontations and predations (Emson and Wilkie, 1980). It is considered an adaptation in which the sacrificed structure allows for the survival of the individual, even though the amount of energy for regeneration is considerable (Bowmer and Keegan, 1983). The family Amphiuridae can release the whole aboral disc, which holds the stomach, gonads, and genital bursae (Emson and Wilkie, 1980). *Ophiocomina nigra* drops arm spines, most likely a form of opportunistic self-detachment (Wilkie, unpub. obs.). Studies involving many species suggest that sublethal damage is a heavy burden and there is prominent variation between conspecific populations and within populations over time (Sides, 1987; Skold and Rosenberg, 1996).

The primary goals of this study were to describe the brittle star diversity and distribution in Moorea and determine if arm regeneration rates differ between species from various habitats and with different ecologies. Specifically the objectives were: (1) describe locations where brittle star species were observed to provide useful ecological context; (2) measure the relative abundance of these species in different habitats; (3) assess the proportion of the populations with fully intact arms; and, (4) measure and compare the rate of regeneration of two species, *Macrophiothrix longipeda* (Lamarck 1816) and *Ophiocoma scolopendrina* (Lamarck 1816), collected in different habitats. *Macrophiothrix longipeda* I expected species richness to be different at each location due to environmental factors and ecologies of each species. Percent of individuals with fully intact arms were expected to differ by species and location because of the specifics of each habitat and characteristics of each species, such as feeding mode and escape response to predators. Rate of arm regeneration was anticipated to differ between *O. scolopendrina* and *M. longipeda* dependent on ecology and physiology, as with rate of autotomization.

METHODS AND MATERIALS

Site descriptions

Moorea (17° 30' S, 149° 50' W) is a high volcanic island of the Society Archipelago, French Polynesia, in the Pacific Ocean. Four coastal study sites around Moorea were assessed from September to November, 2006 for brittle stars (Fig. 1).

The marsh (17° 33.382' S, 149° 52.457' W) near Haapiti is an area lined by mangrove trees on the banks. This location was assessed via snorkeling in < 2 m of water by turning over rocks and coral rubble, and digging into the sand.

Motu Tiahura is located off the northwest tip of Moorea (17° 29.241' S, 149° 54.632' W). The survey location was characterized by a sandy beach with shallow water and dispersed coral rubble and rocks on the eastern side of the island. Brittle stars were assessed by turning over rocks and rubble.

The Vaipahu barrier reef crest (17° 28.562' S, 149° 49.299' W) located outside of Cook's Bay, North of the Richard B. Gump Research Station, on the northeastern side of the island. It was characterized by several species of coral and coral rubble. Various collections of brittle stars took place in September and October via snorkeling, collecting coral rubble, and allowing the ophiuroids to drop out of the rubble onto the water table due to stress and lack of water.

The jetty (17° 29.110' S, 149° 49.893' W) between Opunohu and Cook's Bay, beyond the Vaipahu barrier reef crest was assessed on two separate days for brittle stars. The jetty consisted of concrete and rock rubble protruding 30 m from the beach into the ocean. Brittle stars were observed under rocks and in crevices.

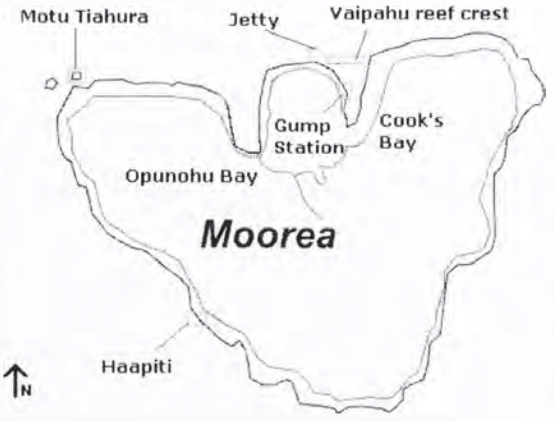


Fig. 1. Map of Moorea, with study sites boxed.

Collection techniques

Brittle stars with all intact arms were collected at the four sites to assess differences in species abundance, diversity between different locations, and to observe arm regeneration in the laboratory. However, the ophiuroids from the mangrove marsh were mostly not fully intact and already in the process of regenerating arms.

Brittle stars from the mangrove marsh in Haapiti were collected via snorkeling and turning over various sizes of rocks and coral rubble. Ophiuroids were handled gently as they were fragile, often losing arms or parts or arms when contacted. Five individuals of *M. longipeda* were collected 23 September, 2006 and 18 individuals of the same species were collected 3 October, 2006. Also on 11 October, 2006 a single *Ophiocoma erinaceus*? Muller and Troshel 1842 individual was collected under coral rubble.

Ophiuroids on Motu Tiahura were collected by turning over rocks and coral rubble in shallow water < 1m deep. *O. scolopendrina* was the only species found. Eleven individuals were collected on 25 September, 2006 and seven individuals were collected on 9 October, 2006.

Collections at the barrier reef crest were more haphazard, as a majority of the rubble brought back to the Gump Station was collected for potential octopus dens. Brittle stars were collected only after they emerged from the rubble because of lack of water. Therefore, a majority of the animals collected died and thus were not included in the arm regeneration study.

Two species of brittle stars were observed and one was collected at the jetty. Ten *O. scolopendrina* were collected on 8 October, 2006 and 14 individuals were collected on 18 October, 2006 under rock and within crevices of coral and conglomerate rock.

Natural history

The marsh, motu and jetty habitats were surveyed to determine distribution of species around Moorea, population density, percent of the population with arms fully intact, and percent regenerating. However, the barrier reef crest was not surveyed because all the ophiuroids were hiding in rocks and coral crevices. Therefore, it was impossible to determine these parameters in the field.

The habitats were surveyed for 1.5 hours in an area 29 m by 24 m. An equal and thorough search was conducted by turning over all rocks and rubble that were large enough to provide shelter for ophiuroids and small enough for one person to turn over. Every individual was counted and all were visually assessed as having all complete arms or as incomplete because the arms were not fully intact, and how many of those arms were regenerating. Percents of fully intact individuals and those regenerating arms were calculated.

Experimental design

Individuals captured to assess arm regeneration in situ were housed in individual containers with 2 mm mesh to allow for constant water flow and aeration. Each individual was provided with a thin layer of sand and coral rubble for shelter. They were fed a small piece of turkey meat every other day and fresh sand was provided once a week. The sea water temperature ranged from 26-29 ° C.

Fifteen *O. scolopendrina* from Motu Tiahura, 19 *M. longipeda* from the marsh and 18 *O. scolopendrina* individuals from the Vaipahu jetty served as study animals for the regeneration experiment. Three individuals from the motu, four from the marsh and seven from the jetty served as controls. A majority of the *M. longipeda* ophiuroids from the marsh were in the process of regenerating arms; therefore, they were simultaneously regenerating arm A (which was experimentally autotomized) as well as the other arms.

On 19 October, 2006, designated day 0, each of the study individuals were pinched mid-arm to induce autotomize of the arm opposite the madreporite, designated arm A. The animal was provoked so that it would voluntarily release its arm or part of its arm under these artificial sublethal predatory conditions. The number of segments away that the arm was autotomized from the provoked point, and the time elapsed between provocation and release were recorded. Central disc diameter measurements were taken then and also again on day 25, the last day of measurement to establish an average body size proxy. Measurements of growth were taken every other day with verneir calipers, beginning on day 2 and lasting until day 25.

Three other species from the Vaipahu reef crest were collected, though not in significant numbers. However, they were assessed similarly for arm regeneration rates. Two regenerating individuals and one control of *Ophiocoma sp.*, and two regenerating *Ophiarthrum elegans* Peters 1851 were supplemented into the experiment on 23 October, 2006. One *Ophiocoma sp.* individual was already missing a tip of an arm that had yet to begin regenerating, which was used for the regeneration data. The other individual was induced to autotomize arm A. One of the *O. elegans* individual was already missing the tip of an arm, which was designated A and measured. The other individual was regenerating two missing tips; both were measured for regeneration. One ophiodermatid brittle star was added to the experiment on 27 October, 2006, which was provoked to autotmize part of its arm.

Controls were handled similarly to the study individuals; however, they were not induced to autotomize and left with fully intact arms. Arm A was measured every other day beginning on day 2 to assess for baseline growth. All *M. longipeda* individuals were simultaneously regenerating multiple arms; therefore, regeneration data was also collected for the other four arms.

Statistical analyses

The means of the disc diameters, individual rates of regeneration, and total regeneration over the duration of the experiment for the marsh, motu and jetty ophiuroids were compared via a one-way ANOVA (or Kruskal-Wallis for non-normally distributed data) analysis using JMP IN 5.1 (SAS Institute 2004), with an $\alpha < 0.05$. ANOVA analysis was also used to compare the time elapsed between provocation and autotomization, and the number of segments away the ophiuroid autotomized from the disturbed point between the marsh, motu and jetty individuals. Additionally, *M. longipeda* was regenerating multiple arms in addition to the experimental regenerating arm. Therefore, ANOVA analysis was performed to determine differences in rate of regeneration among the other four arms. Tukey-Kramer pair tests, with the Bonferroni correction for multiple pair tests, were also performed to assess which species were significantly different from each other. Individual regeneration was normalized for body size by standardizing over disc diameter.

RESULTS

Natural history

One dominant ophiuroid species was found at each of the marsh, motu and jetty locations. *M. longipeda* was found under large rocks and coral rubble at the mangrove marsh. When collecting, *M. longipeda* would burrow into the coral rubble and sand to escape. Often times, only arms were recovered. Arms were readily lost while collecting; therefore, arms were most likely broken by handling prior to the beginning of the regeneration experiment. *Ophiocoma scolopendrina* was the only species living at Motu Tiahura. Individuals were found living under rock and coral rubble in shallow water. Several species of brittle stars were collected within the coral rubble: an ophiodermatid species; *Ophiocoma brevipes*? Peters 1851; *Ophiarthrum elegans* Peters 1851; *Ophiocoma sp.*; *Ophiocomella sexradia* (Duncan 1887); and, another small 6-armed species. Individuals emerged from the rubble due to stress when exposed to the dry environment for an extended period of time; therefore, they died due to extreme stress. *O. scolopendrina* was also the major species living at the Vaipahu jetty. However, one *M. longipeda* individual was observed under a rock and subsequently burrowed into the sand.

The jetty habitat had the highest abundance of ophiuroids, followed by the mangrove marsh and Motu Tiahura (Table 1). The brittle stars at the motu had the highest population percentage of regenerating arms. The highest population percentage of intact arms was found at the motu (Table 3).

LOC	N	NI	NR	N Unk
M.T.	40	16	23	1
M.M	28	3	15	10
V.J.	126	34	56	36

Table 1. Abundance, number of individuals with all intact arms and regenerating arms. LOC = Location; M.T. = Motu Tiahura; M.M = Mangrove marsh, Haapiti; V.J. = Vaipahu jetty; N = total number of individuals; NI = number of individuals with all intact arms; NR = number of individuals with regenerating arms; N Unk = unknown regeneration.

Individuals observed to be regenerating one or two arms were prominent at the jetty and motu, whereas only one individual from the mangrove marsh was regenerating either one or two arms (Table 2). Ophiuroids regenerating three arms were observed at a higher population percentage at the marsh, followed by the motu and jetty (Table 2). Few individuals from Motu Tiahura or the Vaipahu jetty were found to be regenerating four or five arms. However, the majority of the population sampled at the mangrove marsh was regenerating four or five appendages (Table 2).

LOC	1 R	2 R	3 R	4 R	5 R
M.T.	12	7	3	1	0
M.M	1	1	4	4	5
V.J.	32	10	11	2	1

Table 2. Number of individuals regenerating one or more arms. Locations are the same as Table 1. 1R = regenerating one arm; 2R = regenerating 2 arms; 3R = regenerating 3 arms; 4R = regenerating 4 arms; 5R = regenerating 5 arms

LOC	% I	% R	% UNK
M.T.	40%	58%	2%
M.M	11%	53%	36%
V.J.	27%	44%	29%

Table 3. Population percentages of intact, regenerating and unknown individuals. Locations are the same as Tables 1 & 2. % I = percent of the population with all intact arms; % R = percent regenerating; % UNK = percent with unknown regeneration

O. sexradia and the other species of 6-armed brittle star were very small, disc diameters ranged from 2-5mm. After a few days of captivity, they were observed to either be cannibalizing each other, or dividing by fission. Small ophiuroids commonly reproduce asexually by autotomizing half of their body, or part of the visceral disc.

Arm regeneration experiment

Average regeneration of the autotomized arm per day and the total length regenerated over the duration of the experiment significantly differed by species and their habitat, $p < .0001$ (Table 4). The average rate of arm regeneration per day was significantly different between *M. longipeda* and both *O. scolopendrina* populations. Similarly, the total regeneration was significantly different between *M. longipeda* and *O. scolopendrina*, but the jetty and motu populations were not significantly different. *M. longipeda* had the highest regeneration rate, followed by *O. scolopendrina* (Fig. 2).

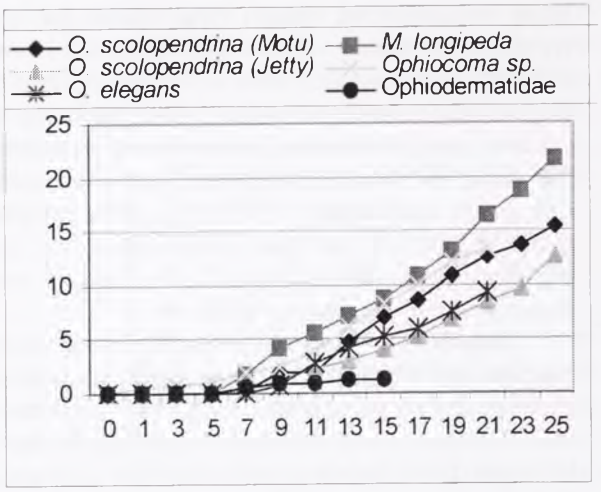


Fig. 2. Total rate of arm regeneration (mm) for the duration of the experiment for all species.

The average regeneration rate of the three ophiuroid groups had a strong positive relationship ($R^2 = 0.64$) for the duration of the experiment, implying that the regeneration rate increased with each subsequent day (Fig. 3).

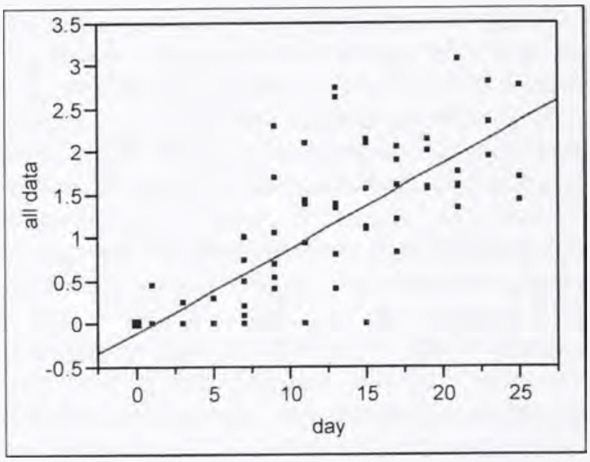


Fig. 3. Average regeneration rate (mm) of motu, marsh and jetty ophiuroids over the duration of the experiment.

Average body size, or disc diameter, was significantly different within the three groups of ophiuroids, $p < .0001$ (Table 4). Furthermore, disc diameter was significantly varied between the ophiuroids from the marsh, motu and jetty habitats (Tukey-Kramer test). As disc diameter increased, arm regeneration decreased, as shown by Fig. 4. The average regeneration rates of all the individuals were weakly, negatively correlated to disc diameter ($R^2 = 0.08$). Similarly, the total regeneration of all individuals was also weakly, negatively correlated to disc diameter, $R^2 = 0.11$, as seen in Fig. 5.

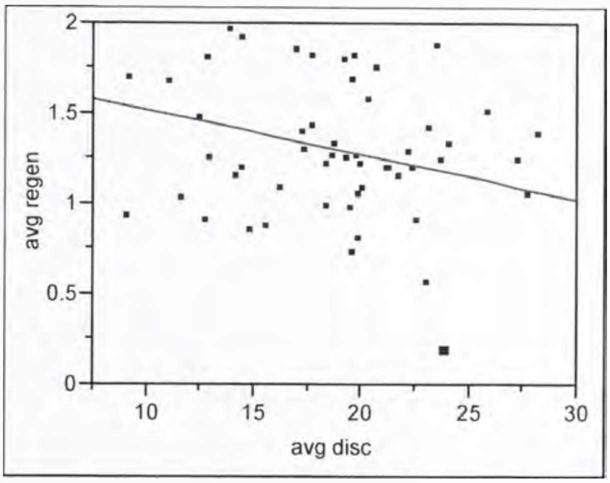


Fig. 4. Average regeneration rate (mm) in relation to disc size (mm) for motu, marsh and jetty ophiuroids.

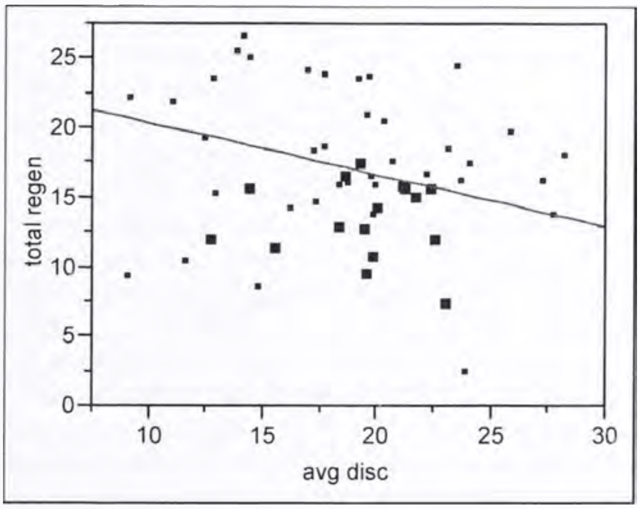


Fig. 5. Total regenerated growth (mm) in relation to disc size (mm) for motu, marsh and jetty ophiuroids.

The time elapsed between provocation and autotomization of the arm was also significantly different among the marsh, motu and jetty brittle stars (Table 4). Specifically, there were significant differences between the times it took for the jetty ophiuroids to release their arms compared to those from the motu or mangrove marsh. However, there was no significant difference between brittle stars from Motu Tiahura or the marsh in the time leading up to autotomization. *M. longipeda* had the shortest elapsed time before releasing the arm (Fig. 6).

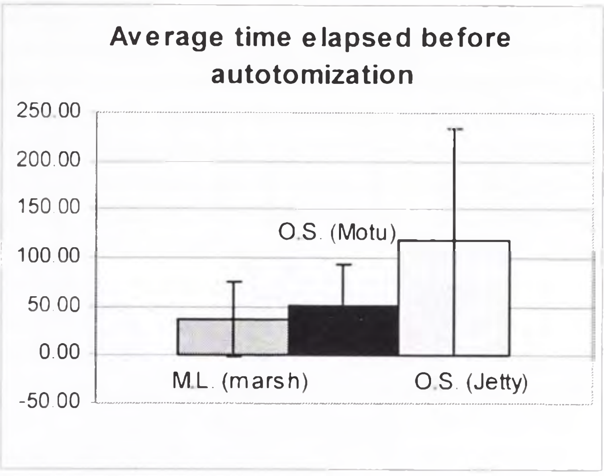


Fig. 6. Average time elapsed (s) between provocation and autotomization for motu, marsh and jetty populations. M.L. = *M. longipeda*; O.S. = *O. scolopendrina*.

The number of segments away from the point of disturbance where brittle stars autotomize is thought to be directly related to type of stress it experiences. The same force and effort was applied to each ophiuroid to provoke autotomization. There were no significant differences between the marsh, motu, or jetty populations in terms of how many segments away from the point of stress it released it arm, $p > 0.2000$ (Table 4).

M. longipeda was simultaneously regenerating multiple arms while also regenerating arm A. There were significant differences in the rates of regeneration of arms B-E, $p > 0.04$ using a one-way Kruskal-Wallis test. However the rates between pairings of each arm using the Tukey-Kramer test showed no significant differences between arm B-E and no difference when paired with the regeneration rate of arm A (Table 4).

The average baseline growth of the intact arm A in the controls was higher in comparison to the average regeneration rate of experimental organisms in the three groups of ophiuroids (Fig. 7).

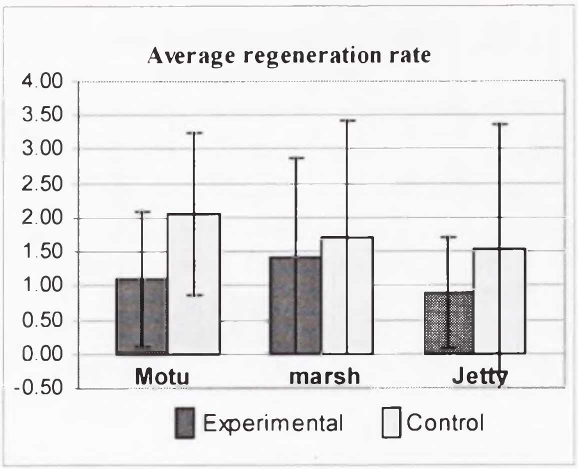


Fig. 7. Average regeneration rate (mm) of study and control individuals

The numbers of ophiuroids collected from the Vaipahu barrier reef crest were not significantly adequate for statistical analyses. However, the preliminary data showed that *Ophiocoma sp.* had the highest total regeneration and rate of regeneration among the species from the reef, followed by *O. elegans* and the ophiodermatid brittle star (Fig. 2 and 8).

Data	Test	p-value	T-K test
Avg disc D	ANOVA	<0.0001*	*M.L.* A *O.S.*† B *O.S.*Φ C
T disturb. and auto.	Kruskal-Wallace	>0.0071*	*M.L.* A *O.S.*† A *O.S.*Φ B
# seg away auto.	Kruskal-Wallace	>0.2000	*M.L.* A *O.S.*† A *O.S.*Φ A
Avg R/day	ANOVA	<0.0001*	*M.L.* A *O.S.*† B *O.S.*Φ B
Tot. R	ANOVA	<0.0001*	*M.L.* A *O.S.*† B *O.S.*Φ B
M. L. avg R	Kruskal-Wallace	>0.0432*	no arms sig. different

Table 4. Summary of ANOVA and Kruskal-Wallis tests for difference between groups and Tukey-Kramer tests for differences between pairs. * = significant. D = diameter; T = time; auto = autotomization; R = regeneration; *M.L.* = *M. longipeda*; *O.S.* = *O. scolopendrina*; † = motu; Φ = jetty

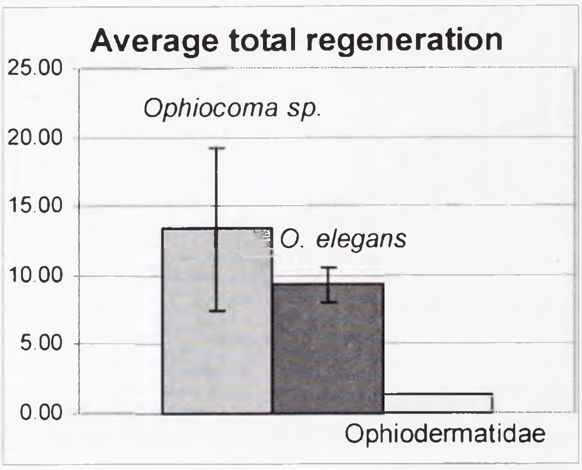


Fig. 8. Average total regeneration (mm) for reef crest species.

DISCUSSION

Natural history

Unique brittle star assemblages were observed in the mangrove marsh in Haapiti, the intertidal area of Motu Tiahura, Vaipahu jetty, and Vaipahu barrier reef crest. Some species, such as *M. longipeda* were only found in one location, suggesting habitat preference. Other species, *scolopendrina*, *O. elegans*, *Ophiocoma sp.*, *O. brevipes*?, *O. erinaceus*?, *O. sexradia* and the ophiodermatid, occurred at different habitats. Distinct characteristics of these habitats, such as water flow, substrate, nutrient resources, and predation most likely influenced the abundance and distribution of these species in Moorea and the motu. For example, *M.* longipeda, only found in the fine sediment at the marsh, probably utilized this habitat because it is a deposit-feeder (pers. obs.) and the fine sand grains had higher nutrient value.

Abundance and density in each habitat could also be mediated by interspecific and intraspecific factors unique to each location, such as predation and territoriality. Hydrodynamics could mediate where ophiuroids were located and what nutrients were available.

The occurrence of *O. scolopendrina* at Motu Tiahura and the jetty could be attributed to the locations having similar habitat. Although the brittle stars at the motu were found under rocks and rubble on the sandy substrate and those at the jetty buried within conglomerate rock and rubble, both locations were very shallow and the water was calm. *O. scolopendrina* is a suspension feeder, waving its arms in the water column, and a deposit-feeder, like many other ophiuroids (Oak and Scheibling, 2006). The hydrodynamics at the motu and jetty could have been similar, carrying similar nutrients in the water column. The flow rate in combination with the shallow water could have been more damaging to ophiuroids with longer, more fragile arms, for example *M. longipeda*, but benign those with more robust appendages, such as *O. scolopendrina*.

Varying proportions of the population with intact and regenerating arms between habitats could have been influenced by many factors. Burrowing ophiuroids tended to lose arms more readily than epibenthic species (Emson and Wilkie, 1980). *M. longipeda* was observed to be regenerating multiple arms simultaneously. This species was also observed burrowing into rubble and sand. *O. scolopendrina* was not regenerating many arms simultaneously and was observed under rocks or in crevices, and not burrowing. These observations supported Emson and Wilkie's idea that burrowing species more readily lost arms.

Predation and abundance of predators could have affected the number of individuals experiencing sublethal predation and autotomizing appendages. Palatability could have also influenced the portion of the population that is regenerating appendages (Soong et al., 1997).

Behavior such as feeding could correlate to how much exposure a species experiences. Feeding modes could be dependent on habitat and water flow. Therefore, a species that suspension feeds by waving its arms into the water column was more exposed to predators than a species that deposit fed under coral rubble. *O. scolopendrina* is a suspension feeder and deposit feeder and *M. longipeda* is predominantly a deposit feeder. However, *M. longipeda* more readily autotomized. Arms were not as important in deposit feeding as they were in suspension feeding; therefore, releasing arms might not hinder *M. longipeda* feeding.

Arm regeneration experiment

Total and rate of arm regeneration differed by species. *M. longipeda* had a higher rate and total regeneration, while *O. scolopendrina* from the motu and jetty were comparable, as expected. These differences could be attributed to the ecology of each species.

M. longipeda could have had a higher rate and total regeneration because it lost arms more readily. This species' arms autotomized frequently while in the field and had the fastest time between stimulus to autotomization in the laboratory. Arms might not be vital to its ecology; therefore it more readily lost arms to predators or disturbances. Since it lost arms more often and with less severe stress than *O. scolopendrina*, it could be compensating by regenerating at a faster rate; thereby growing a longer new appendage for the duration of the experiment. *M. longipeda* was slower moving than *O. scolopendrina* and burrowed into the substrate as a defense (pers. obs.). Therefore, its best defense against predation could be autotomization. It may have evolved to more readily release it arms to a predator because it could not out-run a predator. In order for the species to maintain its fitness, it also evolved to regenerate that tissue more rapidly. Also, because arms might not be vital in burrowing, arms are often lost as a way to confuse predators and prevent them from burrowing after the brittle star.

O. scolopendrina less readily lost arms and also had a lower total and rate of regeneration. In the field, *O. scolopendrina* was observed to predominately suspension feed. This mode of feeding could largely depend on having intact arms. Therefore, *O. scolopendrina* was more reluctant to autotomize arms. Also, since arms were not lost as readily, fast regeneration would not be as important, as regeneration did not seem to be as prevalent compared to *M. longipeda*. *O. scolopendrina* was also observed to crawl away very rapidly from a predator. Arms were used in locomotion; therefore, they were important in escaping predators and not as easily released (Woodley, 1975).

Rate of regeneration is thought to be dependent on temperature or seasonality and gonad development (Pomroy and Lawrence, 2001). Decreased or no regeneration was observed in other studies during times of colder temperatures (Stancyk et al., 1994; Dobson, 1988). Temperature could be refuted as a cause of the differences in rates of regeneration between species for the duration of this experiment, as the ambient water temperatures were constant between species' enclosures.

Regeneration is also likely food dependent (Skold and Rosenberg, 1996; Stancyk et al., 1994). If there were sufficient nutrients, the brittle stars would not have to use stored reserves to regenerate. Feeding was constant between species; therefore, food availability was most likely not the reason for the differences in regeneration between species. However, dominant feeding modes differed between species. For example, *O. scolopendrina* was often observed suspension feeding, while *M. longipeda* was predominantly observed to deposit-feed.

Simultaneous regeneration of multiple arms also affected the rate at which growth occurs (Soong et al., 1997). Arms were designated according to their position to the madreporite. Therefore, position differentiation would lead to functional differentiation among the arms. Some arms could be designated for feeding while others are used for gripping the substrate. The feeding arms could be more important to the brittle stars' fitness; therefore they grew back faster. Regeneration could also have been affected my neighboring arms. *M. longipeda* was regenerating multiple arms at the same time at different stages, including the forced autotomization of arm A. There were no significant differences between the growth rates of any of the arms. It was possible that *M. longipeda* did not have differentiation between the five arms. If they all served the same or equal functions, there would be no benefit to regenerate one faster than the others. Also, when multiple arms are regenerating, more energy is required. Therefore, it is logical that the more arms a brittle star is regenerating, the slower the growth will be, due to increased energy expenditure. However, Zeleny (1903) stated that when multiple arms were regenerating, the energy put into replacement of the lost appendages was greatly increased. Accordingly, the more arms regenerating, the greater is the rate of regeneration of each arm.

Regeneration rate increased over time in all species. This observation could be attributed to the undifferentiated stem cells. After the initial healing of the autotomized area, new undifferentiated cells divided and regenerated. As more and more stem cells were formed by division of the generation before, regeneration increased exponentially (Carnevali and Bonasoro, 2001).

Disc diameter was significantly differed between the groups of ophiuroids. The biology and physiology of the *M. longipeda* could be different than that of *O. scolopendrina* in terms of size. Also, because there was a disc size disparity among the groups, regeneration rates were normalized for body size.

Regeneration rate decreased with body size when marsh, motu and jetty ophiuroids were analyzed. This correlation suggested that smaller individuals had a greater regeneration rate. This could be an adaptation for survival. Smaller individuals might be pressured to grow large faster in order to compete. Or, because smaller individuals did not have as much biomass to sustain, more energy was put into regeneration. This relationship was contrary to other studies. Zeleny (1903) concluded that there was a relationship between the size of the brittle star and the rate of arm regeneration. The maximum rate was achieved by medium sized individuals and lower for both small and large individuals. Manner et al. (1960) stated that size had no effect on regeneration in salamanders. There was no relationship within each species of brittle star.

Autotomization occured when the arm separated from any intersegmental joint proximal to the stressed area (Wilkie, 1978). No significant differences were observed in the number of segments away autotmization occurred from the point of disturbance for the marsh, motu or jetty ophiuroids. Autotomization was likely dependent on the type of stress. Since the provocation of autotomization was constant throughout the populations, it could explain why there was no difference between them. Therefore, this characteristic might not have been species specific or dependent on habitat within these selected populations. However, other brittle stars like amphiurids, tended to autotomize significantly farther away from the stimulus (Wilkie, 1978).

As expected, baseline growth in the controls was greater than the regeneration rate of the autotomized arm for all the groups of brittle stars. The control animals did not undergo the stress of losing an arm nor have to recuperate. They had all five functioning appendages and did not have to put in extra energy or siphon energy from other processes to regenerate an arm.

CONCLUSION

This study collected nine different species from four locations in Moorea and its motu. Undoubtedly they were more ophiuroids inhabiting the island within the surveyed habitats and those not in this survey.

In the field, different species of brittle stars commonly had between 20-100% of the population with regenerating individuals (Wilkie, 1978). The *M. longipeda* population at the mangrove marsh in Haapiti and the *O. scolopendrina* population at Motu Tiahura had over half of the individuals regenerating at least one arm. *O. scolopendrina* at Vaipahu jetty was found to have 44% of the population regenerating at least one arm. *M. longipeda* was commonly regenerating multiple arms, mostly with 4 or 5 regenerating simultaneously. Multiple regenerating arms were less common in both *O. scolopendrina* populations. The differences in regenerating proportions of each population were likely due to habitat characteristics and ecology of each species.

The experimental regeneration study yielded data that supported the hypothesis that regeneration rates differ between species. *M. longipeda* had a faster regeneration rate and a greater total regeneration than *O. scolopendrina* from both Motu Tiahura and the Vaipahu jetty. However, the two populations of *O. scolopendrina* did not have significantly different regeneration rates.

Differences in regeneration rate and length of regeneration were most likely due to ecological, physiological and biological factors of each species. Habitat, feeding mode, predation, available nutrient resources, body size, and the simultaneous regeneration of multiple arms have been suggested as influences on regeneration.

ACKNOWLEDGEMENTS

I thank Professors Jere Lipps, Carole Hickman, Vince Resh, Brent Mishler, and James Bartolome for their support, motivation, and knowledge throughout this study. Thanks to the graduate student instructors Liz Perotti, Erica Spotswood and Alison Purcell-O'Dowd for their insight, help, and ideas. Thank you to the Richard B. Gump station and staff for their hospitality and this amazing opportunity. I extend my appreciation to Gordon Hendler for his help in identifying organisms. I am particularly grateful to all the students that helped me collect organisms and survey habitats: Maya Almarez, Carol Chang, Julie Himes, Joel Nitta, Daniel Song, and Natalie Valencia. I thank Aimee Ellison, Erin Prado, and Tom Hata for helping me record my many measurements. I am forever indebted to Melissa Riley for her hours of recording.

LITERATURE CITED

Aronson, R.B. 1989. Brittlestar beds: low-predation anachronisms in the British Isles. *Ecology* 70: 856-865.

Aronson, R.B. 1991. Predation, physical disturbance, and sublethal arms damage in ophiuroids: a Jurassic-Recent comparison. *Mar Ecol. Prog. Ser.* 74: 91-97.

Ball, M.M, E.A. Shinn, K.W. Stockman. 1967. The geologic effects of hurricane Donna on south Florida. *J. Geol.* 75: 583-597

Bergman, M.J., H.W Van der Veer, L. Karczmarski. 1988. Impact of tail-nipping on mortality, growth and reproduction of *Arenicola marina*. *Neth. J. Sea res.* 22: 83-90.

Berzins, I.K. and R.L. Caldwell. 1983. The effect of injury on the agonistic behaviour of the stomatopod, *Gonodactylus bredini* (Manning). *Mar. Behav. Physiol.* 10: 83-96.

Bowmer, T., B.F. Keegan. 1983. Field survey of the occurrence and significance of regeneration in *Amphiura filiformis* (Echinodermata: Ophiuroidea) from Galway Bay, west coast of Ireland. *Mar. Biol.* 74: 65-71.

Carnevali M.D. Candia and F. Bonasoro. 2001. Introduction to the biology of regeneration in Echinoderms. *Micro. Res. Tech.* 55: 365-368.

Clavier, J. 1984. Production due to regeneration by *Euclymene oerstedi* (Claparede) (Polychaeta: Maldanidae) in maritime basin of the Rance (northern Britanny). *J. Exp. Mar. Biol. Ecol.* 75: 97-106.

Dobson, W.E. 1988. Studies of the process, mechanism and limiting factors affecting tissue regeneration after autotomy in ophiuroid echinoderm *Macrophiophiolis gracillima*. Ph.D dissertation. University of S. Carolina, Columbia, S. Carolina.

Emson, R.H. and I.C. Wilkie. 1980. Fission and autotomy in Echinoderms. *Oceanogr. Mar. Biol. Ann. Rev.* 18:155-250.

Harris, R.N. 1989. Nonlethal injury to organisms as a mechanism of population regulation. *Am. Nat.* 134: 835-847.

Manner, H.W., J.A. Vallee, W.F. Zapisek. 1960. The effect of body size on rate of salamander limb regeneration. *The Anatomical Record.* 137: 278.

Oak, T. and R.E. Scheibling. 2006. Tidal activity pattern and feeding behaviour of the ophiuroid *Ophiocoma scolopendrina* on a Kenyan reef flat. *Coral Reefs* 25: 213-222.

Pomory C.M., and J.M. Lawrence. 2001. Arm regeneration in the field in *Ophiocoma echinata* (Echinodermata: Ophiuroidea): effects on body composition and its potential role in a reef food web. *Marine Biology* 139: 661-670.

Reichman, O.J.. 1984. Evolution of regeneration capabilities. *American Naturalist*. 123: 752.

Sides, E.M. 1987. An experimental study of use of arm regeneration in estimating rates of sublethal injury on brittle stars. J. Exp. Mar. Biol. Ecol. 106: 1-16.

Skold, M. and R. Rosenberg. 1996. Arm regeneration frequency in eight species of Ophiuroidea (Echinodermata) from European sea areas. *J. of Sea Res*. 35: 353-362.

Smith L.D. and A.H. Hines. 1991. Autotomy in blue crab (*Callinectes sapidus* Rathbun) populations: geographic, temporal, and ontogenetic variation. *Biol. Bull.* (Woods Hole) 180: 416-431.

Soong, K., Y. Shen, S. Tseng, C. Chen. 1997. Regeneration and potential functional differentiation of arms in the brittlestar, *Ophiocoma scolopendrina* (Lamarck) (Echinodermata: Ophiuroidea). *Zoological Studies* 36(2): 90-97.

Stancyk, S.E., H.M. Golde, P.A. Pape-Lindstrom, W.E. Dobson. 1994. Born to lose. I. Measures of tissue loss and regeneration by the brittlestar *Mircophiopholis gracillima* (Echinodermata: Ophiuroidea). *Mar. Biol.* 118: 451-462.

Tilmant J.T., R.W. Curry, R. Jones, Szmant, C.C. Zieman, M. Flora, M.B. Robblee, D. Smith, R.W. Snow, H. Wanless. 1994. Hurricane Andrew's effects on marine resources. *Bioscience* 4: 230-237.

Turner, R.L., D.W. Heatwole, S.E. Stancyk. 1982. Ophiuroid discs in stingray stomachs: evasive autotomy or partial consumption of prey? In: Lawrence, J.M. (ed) Echinoderms. Proceedings of the International Conference, Tampa Bay, Fla. Balkema, Rotterdam, pp 331-335.

Wilkie, I.C. 1978. Arm autonomy in brittle stars (Echinodermata: Ophiuroidea). *J. Zool. Lond.* 186: 311-330.

Woodin, S.A. 1984. Effects of browsing predators: activity changes in infauna following tissue loss. *Biol. Bull.* (Woods Hole) 166: 558-573.

Woodley, J.D. 1975. The behavior of some amphiurid brittle-stars. *J.Exp. Mar. Biol.* 18: 29-46.

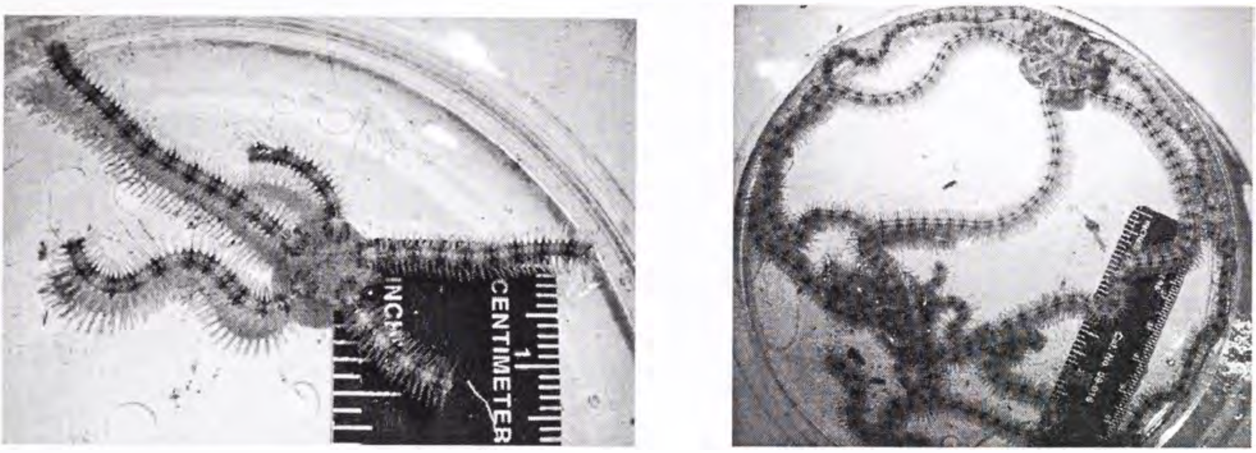
Woodley, J.D. 1980. Hurricane Allen destroys Jamaican coral reefs. *Nature* 287: 387.

Vlas J. de. 1979a. Secondary production by tail regeneration in a tidal flat population of lugworms (*Arenicola marina*), cropped by flatfish. *Neth. J. Sea Res*. 13 : 362-393.

Zeleny, C. 1903. A study of the rate of regeneration of the arms in the brittle-star *Ophioglypha lacertosa. Biol. Bull.* 6: 12.

BRITTLE STAR APPENDIX

SARAH CHINN

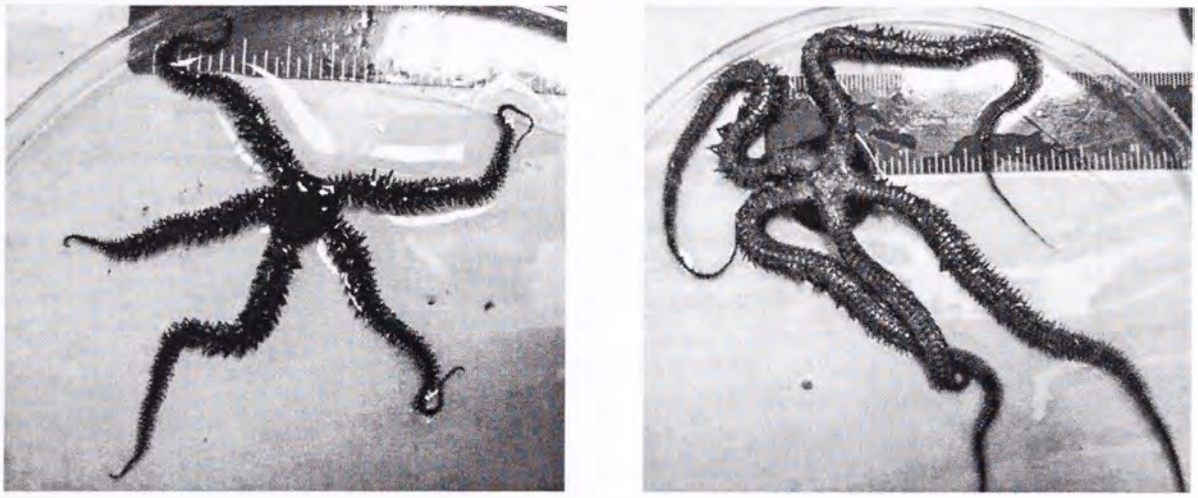


Macrophiothrix longipeda (Lamarck 1816)

Description: Body size varies (25-120 mm); central disc color varies (gray, pink, blue); silky aboral disc; very long, fragile arms; arms banded with long brittle spines.

Habitat: Under rocks and coral rubble; in crevices or coral rubble in depths >1-2 m. Haapiti mangrove marsh.

Behavior: Burrow into substrate; readily autotomize arms as escape response; deposit feeder.

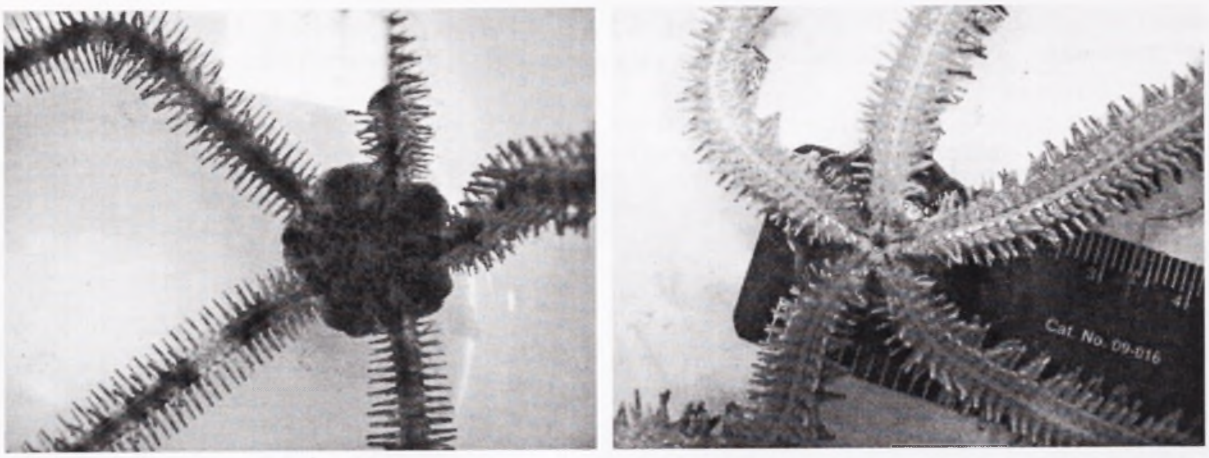


Ophiocoma erinaceus? Muller and Troshel 1842

Description: Dark aboral and oral surfaces; prominent spines on arms; red or black tube feet.

Habitat: Under rocks or coral rubble in shallow depths (0-1 m); in coral rubble; living in coral (Porites sp.). Cook's Bay.

Behavior: Fast moving; suspension feeder and detritivore.

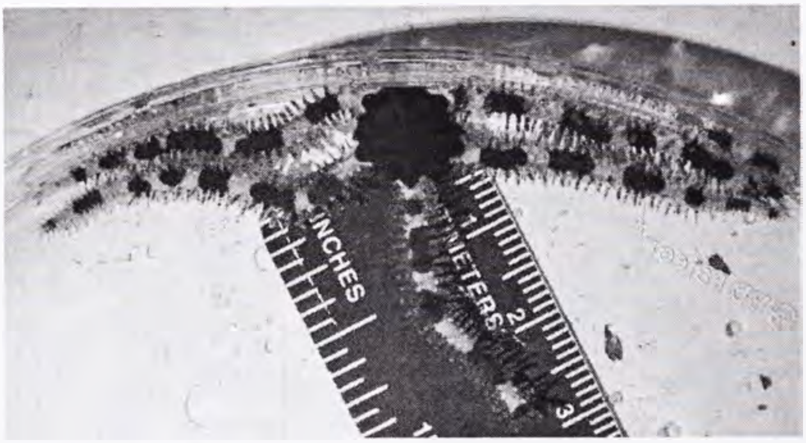


Ophiocoma scolopendrina (Lamarck 1816)

Description: Dark brown/gray aboral disc; banded light and dark arms; light oral surface; prominent spines.

Habitat: Under rocks and coral rubble at shallow depths (0-1 m); central disc embedded in conglomerate rock/rubble with arms protruding. Cook's Bay; widespread.

Behavior: Suspension feeder by waving arms; color changes due to light/time of day from dark in the daytime to light gray at night.



Ophiarthrum elegans Peters 1851

Description: Red central disc; arms banded red and beige; prominent spines banded beige and black.

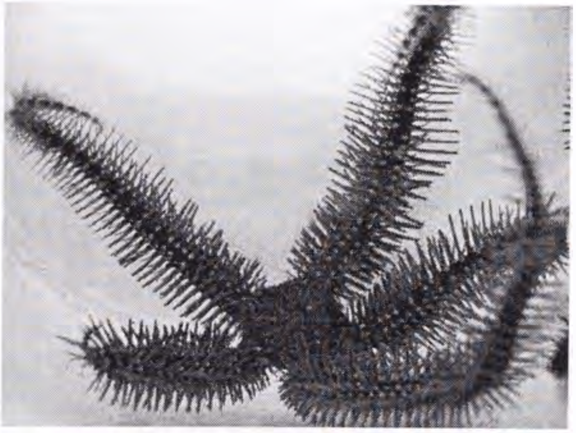
Habitat: In coral rubble crevices; living in coral (Porites spp.). Vaipahu barrier reef crest.

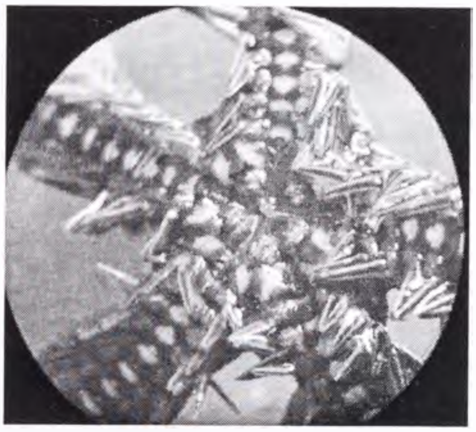
Behavior: Minimal suspension feeding or other feeding.

Ophiocoma brevipes? Peters 1851

Description: Small; gray-green color; speckled central disc; banded arms.

Habitat: Crevices of coral rubble. Vaipahu barrier reef crest.



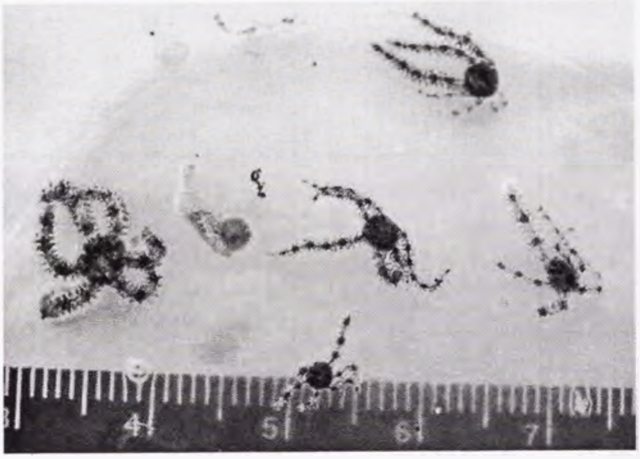


Ophiocoma sp.

Description: Black oral and aboral surface; yellow at base of spines; prominent, robust spines; yellow bands on oral surface of arms; bright red tube feet.

Habitat: Crevices of coral rubble; living in live *Porites* sp.. Vaipahu barrier reef crest.

Behavior: suspension feeding with tips of arms; hiding in coral.

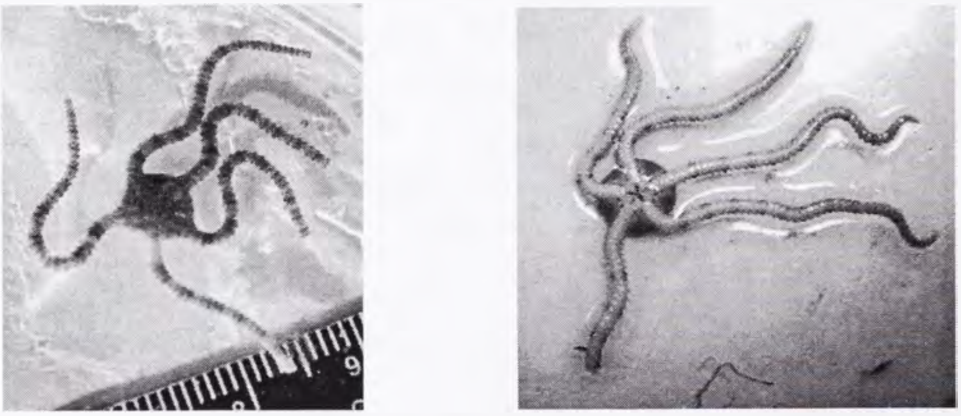


Ophiocomella sexradia (Duncan 1887)

Description: Very small; six arms; varies in color; banded arms.

Habitat: Crevices of coral rubble. Vaipahu barrier reef crest.

Behavior: Asexual reproduction by fission; possible cannibalism.



Family Ophiodermatidae

Description: Small; smooth; green color aboral surface; beige oral surface; V-shaped arm plates on aboral surface.

Habitat: Crevices of coral rubble. Vaipahu barrier reef crest.

Behavior: Fast moving; hiding in rubble or under any other protective covering.

FACTORS INFLUENCING EPIPHYTE HABITAT PREFERENCE IN MOOREA, FRENCH POLYNESIA

April M. Dobbs

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

Abstract. Epiphytes are important in forest ecosystems because they contribute to species diversity and aid in nutrient cycling. Despite this, the ecology of tropical epiphytes, particularly nonvascular species, is not well understood. This study compared epiphyte richness, cover, diversity, and species distributions to host tree diameter, canopy cover, aspect, and height on the trunk. Thirty-two *Metrosideros collina* trees were sampled for epiphyte species every 0.5 centimeters along circumferential transects at 0, 0.75, and 1.5 meters off the ground. Host tree diameter at 0.5 meters was measured, as well as canopy cover at North, South, East, and West. Epiphyte cover was lowest at the bottom of the trunk and highest at 1.5 meters. Richness correlated positively with diameter and canopy cover, but it did not vary significantly with height or aspect, according to the Wilcoxon and Tukey tests. Epiphyte cover correlated positively with canopy cover, but it did not vary significantly with diameter or aspect. Moss and fern cover increased significantly with diameter but did not vary with height. Liverwort and lichen cover were not correlated with diameter, but they were lowest at the bottom of the trunk and highest at 1.5 meters. Diversity, calculated with the Shannon-Wiener index, correlated positively with epiphyte cover and tree diameter but did not vary significantly with height. Overall, epiphytes preferred habitats in the upper trunk region on trees with high canopy cover, and their distributions were correlated with every variable except aspect.

Key words: *epiphytes; habitat preference;* Metrosideros collina; *Moorea, French Polynesia;*

INTRODUCTION

Epiphytes are plants that grow on trees or shrubs non-parasitically (Hietz 1998), although most "benefit substantially" from their hosts (Callaway *et al.* 2002). They can be mosses, liverworts, ferns, angiosperms, and lichens (which fungal/algal symbioses often treated as plants) (Caldiz 2005). Vascular epiphytes, which include ferns and angiosperms, alone make up 10% of the world's flora (Benzing 2001). Although little is known about nonvascular epiphytes, which include mosses, liverworts, and lichens, they contribute substantially to forest biomass and biodiversity (Holz 2003). Because epiphytes absorb much of their water and nutrients from the atmosphere, they are good indicators of air quality (Benzing 2001). They also play in important role in nutrient cycling (Nadkarni 1984).

Epiphytes have been shown to segregate along environmental gradients, especially in substrate and atmospheric moisture (Benzing 2001, Heitz 1998). For example, nonvascular epiphytes prefer mid-montane forests with daily mist or clouds, whereas vascular epiphytes are most common at lower montane forests with less moisture (Benzing 2001). Bryophytes and lichens are particularly sensitive to moisture levels because they exhibit poikilohydry, or rapid equilibration of their internal water levels with the environment (Benzing 2001). They also require moist air to photosynthesize. Vascular epiphytes, being less poikilohydrous, can photosynthesize in drier environments. Although vascular and nonvascular epiphytes can be desiccation tolerant, or able to revive from a desiccated state, they usually thrive in moist environments (Benzing 2001). Epiphyte distribution can also vary within a constant height, due to aspect and inclination, but this is most pronounced in areas with substantial variation in light and wind levels (Kenkel et al. 1981).

Habitat preference in epiphytes of French Polynesia is particularly interesting because of the environmental gradients unique to islands. On Tahiti, epiphytes are common in montane cloud forests, in which 70% of the island's endemic trees live (Meyer and Florence 1996). Such cloud forests exist on Moorea, an island near Tahiti with similar elevations. On Moorea, one montane tree species, *Metrosideros collina* Wilkes 1854, hosts more epiphyte species and has greater epiphyte cover than other trees in the same habitat (Cushing 2002). This type of environment is thought to be "among the most sensitive and vulnerable in the world to climate change" due to the specialization and endemism of its inhabitants, as well as its "steep environmental gradients" (Loope and Giambelluca 1998). Montane cloud forests have persistent and seasonal, wind-driven clouds, a high net precipitation, and increased interception of water by the canopy. These factors probably contribute to moisture and sunlight gradients by elevation and within individual trees, which causes variation in epiphyte cover.

The purpose of this study is to determine whether changes in epiphyte distributions on *M. collina* correlate with canopy cover, aspect, host tree diameter, and height on the trunk. I predict that a) epiphyte species richness and abundance will vary significantly with tree age, canopy cover, height within the tree, and aspect, b) older trees will more epiphyte cover and more species due to increased time and opportunities for succession, c) trees with the most canopy cover will have the greatest richness and cover because many epiphytes prefer moist, shady environments, and d) Richness will be lowest at the bottom of the tree due to the moisture-trapping ferns on the ground. I hypothesize that any differences due to aspect will be due to the desiccating north-to-south trade winds (Kuhlmann 1983), rather than the angle of the sun because the sun is usually overhead (Osborne 2000). My null hypotheses are a) epiphyte richness and cover will not vary significantly with tree age, canopy cover, height within the tree, and aspect, b) older trees will not have more richness and cover than younger trees, c) trees with the most canopy cover will not have the greatest richness and cover, d) richness will not decrease with increasing height.

METHODS

Site and Study Organism

All data were collected in a mid-elevation tropical forest (approximately 300 meters above sea level) called The Belvedere, in Moorea, French Polynesia (UTM coordinates S 17° 32.434' W 149° 49.602'). My study site was a 280 meter trail connecting two North-facing lookout platforms. My study organism was *M. collina* (Myrtaceae), a tree endemic to Fiji, Samoa, Rarotonga, the Austral Islands, and Tahiti (Wright et al. 2001). One of the dominant trees at the Belvedere, it grew as a tree or shrub from 1 to 20 meters tall and had red compound inflorescences. It grew from elevations of 100 meters to mountaintops (Wickland 1999).

Experimental Design

I sampled every tree within fifteen meters of the trail, except those that forked into more than five trunks below a height of 0.5 meters. My sample size was 32 trees. Rather than measuring the diameter at the conventional 1.3 meters, I measured it at 0.5 meters because most of the trees forked just above this height. If the tree forked into two trunks below 0.5 meters, I sampled each trunk separately and combined the diameters at 0.5 meters. If a tree forked into three, four, or five trunks below 0.5 meters, I sampled the two thickest trunks and combined the diameters of all of the trunks at 0.5 meters. If a tree forked above 0.5 meters, I sampled the two thickest trunks above every fork.

I performed circumferential transects on each tree at heights of 0, 0.75, and 1.5 meters, perpendicular to the sides of the trunk. If the tree grew on a slope, I took all height measurements from the highest side of the slope. For each transect, I recorded the epiphyte species that touched the top of the measuring tape every 0.5 centimeters and estimated the directional aspect at North, South, East, and West with a compass. I estimated canopy cover at each tree by holding a densiometer (convex mirror with quarter inch grid) at arm's length (arm at 90 degree angle), facing away from the tree at its North, South, East, and West faces.

I identified the liverworts and lichens to genus and the mosses and ferns to species using identification keys by Whittier (1976), McCune and Geiser (2000), Gradstein (1989), D'Artenay et al. (in press), Piippo (1990), and Murdock and Hinkle (1999). Voucher specimens were submitted to the University of California and Jepson Herbaria, and a photograph appendix of nonvascular epiphytes was included at the end of this paper.

Statistical Analysis

I used the non-parametric Wilcoxon and Tukey Tests to compare richness, diversity, and cover with aspect, and height within the tree because my data could not be normalized, using JMP 5.1 (©2003). To test for correlations between richness, diversity, epiphyte cover, diameter, and canopy cover, I used Spearman's rho, a nonparametric version of the linear regression, also using JMP 5.1. I used Spearman's rho rather than linear regression because my data could not be normalized. To determine whether certain species preferred similar habitats, I plotted the average canopy cover and host tree diameter for each species. I calculated percent cover as the percent of occupied intervals on the transect tape divided by the total possible.

To calculate diversity, I calculated the Shannon-Wiener Index for each transect, which accounted for species richness and evenness. The Shannon-Wiener index (Rousseu, et al. 1998) was calculated as follows:

$$H = \sum_{i=1}^{s} [(p_i)(\log_{10} p_i)]$$

where p_i = proportion of total cover species i, and s = number of species.

To compare the four aspects, I calculated percent cover over a four centimeter band at due North, South, East, and West.

RESULTS

There were twenty-four epiphyte species total, including twelve lichen, five liverwort, four moss, and three fern species. Liverworts were on average most abundant, followed by mosses, lichens, and ferns (Fig. 1, Appendix A, B).

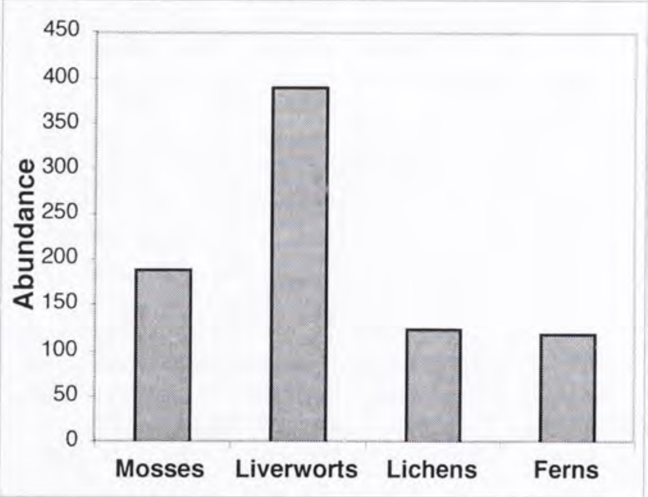


FIG. 1. Average abundance by species type

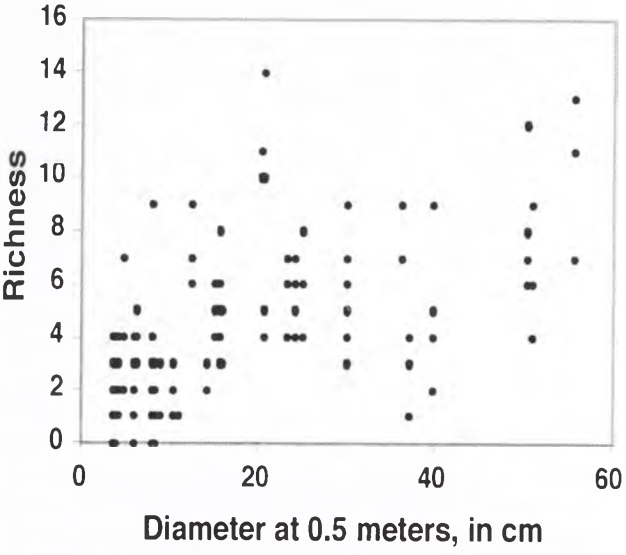


FIG. 2. Epiphyte richness by host tree

When epiphyte richness was compared among the three height groups, the three heights were not significantly different (Wilcoxon, p=0.6861). Richness did not vary significantly with aspect (Wilcoxon, p=0.8540). However, richness was positively correlated with diameter at 0.5 meters (Spearman's rho=0.5561, p<0.0001, Fig. 2, Table 1). Richness was also positively correlated with canopy cover, although loosely (Spearman's rho=0.1933, p=0.0013, Fig. 3, Table 1). Canopy cover was not significantly different among the four aspects.

Epiphyte cover was significantly different among the three heights (Wilcoxon, p<0.0001, Fig. 4, Table 2, 3). Cover was greatest 1.5 meters and least at 0 meters and was significantly different between each height (Fig. 4). It did not, however, vary with diameter (Spearman's rho=0.1135, p=0.2041), but was positively correlated with canopy cover, although loosely (Spearman's rho=0.2133, p=0.0004, Table 1). There was no significant difference in canopy cover among the four aspects (Wilcoxon, p=0.4899).

Moss cover increased significantly with diameter (Spearman's rho=0.4113, p<0.0001, Table 1) but did not vary significantly with height (Wilcoxon, p=0.4389). Liverwort cover was loosely correlated with diameter (Spearman's rho=0.2083, p=0.0188, Table 1) and increased with height (Wilcoxon, p=0.0071, Fig. 5, Table 2, 3). Liverwort cover was greatest at 1.5 meters and lowest at 0 meters. Lichen cover did not vary significantly with diameter (Spearman's rho=-0.0703, p=0.4324) but increased significantly

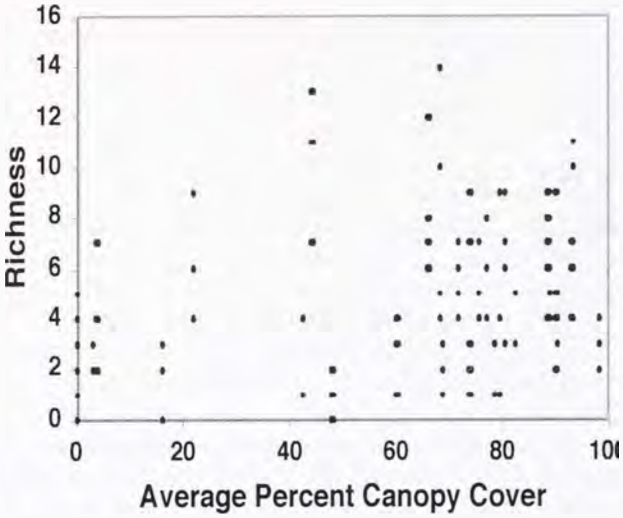


FIG. 3. Richness by average canopy cover. Although the data are not normal, an increase in richness with canopy cover is still visible.

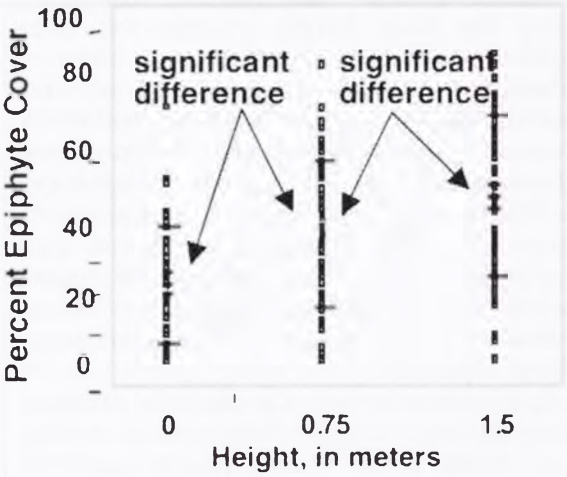


FIG. 4. Percent epiphyte cover at three heights, with means and standard deviations

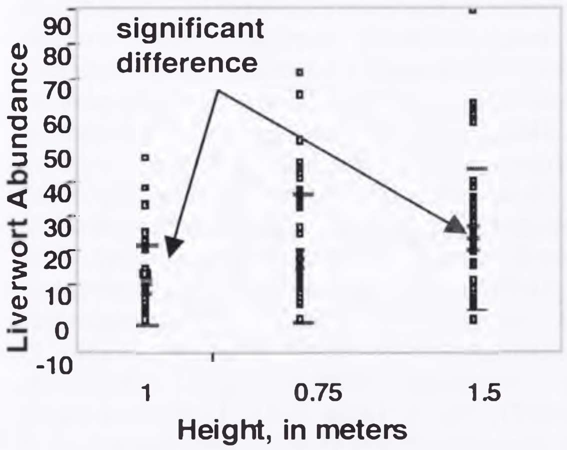


FIG. 5. Liverwort abundance at three heights, with means and standard deviations. Significant differences determined by the Tukey Test

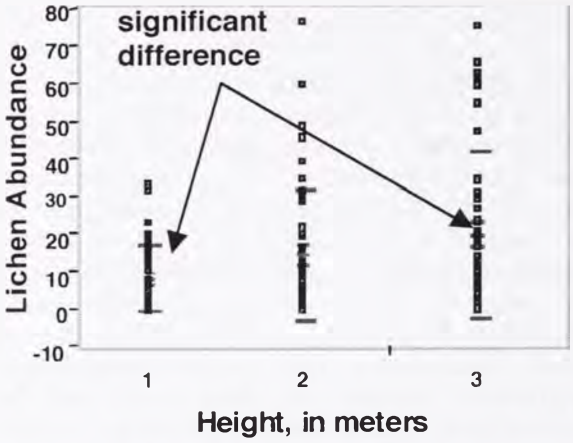


FIG. 6. Lichen abundance three heights, with means and standard deviations. Significant differences determined by the Tukey test

Variable 1	Variable 2	Spearman Rho	P-value
Richness	Diameter	0.5561	<0.0001
Richness	Canopy	0.1933	0.0013
Cover	Canopy	0.2133	0.0004
Moss cover	Diameter	0.4113	<0.0001
Liver.cover	Diameter	0.2083	0.0188
Fern cover	Diameter	0.5897	<0.0001
Diversity	% cover	0.5897	<0.0001
Diversity	Diameter	0.4678	<0.0001

TABLE 1. Spearman's rho constants and P-values for significant correlations

Variable 1	Variable 2	P
Richness	Height	0.0686
Cover	Height	<0.0001
Liverwort cover	Height	0.0071
Lichen cover	Height	0.0071

TABLE 2. Wilcoxon P-values for significant correlations

H 1	H 2	Total Cover	Liverwort Cover	Lichen Cover
0.75 m	0 m	Yes	No	No
1.5 m	0.75 m	Yes	No	No
1.5 m	0 m	Yes	Yes	Yes

TABLE 3. Tukey Test pairwise comparisons of total epiphyte cover, liverwort cover, and lichen cover among the three heights within the tree. Yes=significantly different

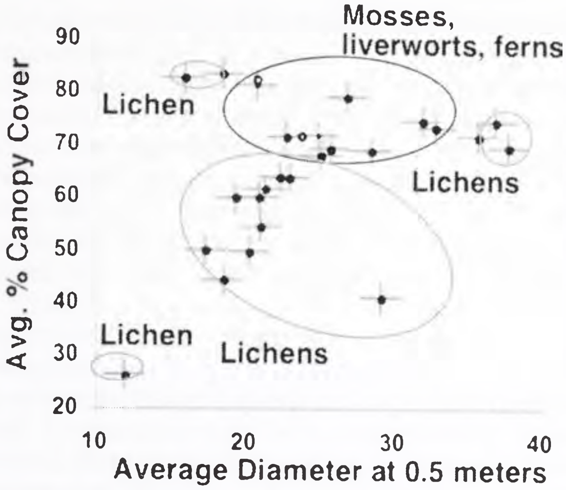


FIG. 7. Average tree diameter and canopy cover for each species, with species types circled.

with height (Wilcoxon, p=0.0071, Fig. 6, Table 2, 3). Lichen cover was greatest at 1.5 meters and least at 0 meters. Fern cover increased significantly with diameter (Spearman's rho=0.5897, p<0.0001, Table 1) but did not vary significantly with height (Wilcoxon, p=0.508).

Epiphyte diversity, quantified by H-values, was positively correlated with total cover (Spearman's rho=0.3729, p<0.0001, Table 1) and diameter at 0.5 meters (Spearman's rho=0.4678, p<0.0001, Table 1). Diversity did not vary significantly with height (Wilcoxon, p=0.3372), which was expected because richness did not vary significantly with height.

According to the scatterplot of species distribution by average canopy cover and average tree diameter, *Lopholejeunia sp. B* and *Leucobryum tahitense* grew at the highest canopy cover levels of the liverworts and mosses, respectively, but the other liverwort and moss species grew at similar canopy cover levels (Fig. 7). All of the lichens except Lichen E, A, B, and G grew on trees with lower average canopy cover than the other species types (Fig. 7). The species were not present at statistically significant average canopy covers (Wilcoxon, p=0.1939), but Lichen C was at a canopy cover significant lower than the other species (Tukey Test)

DISCUSSION

The lack of variation in species richness with height within the tree supported the null hypothesis that richness would not vary with height and disproved the hypothesis that richness would be greatest at 0 meters. It was predicted that richness would decrease with height because the *Dicranopteris* ferns covering the bottom of the tree would trap more moisture in the bark and in the atmosphere, creating an environment habitable for more epiphyte species. Based on observation, the bark under the ferns was considerably drier than the rest of the tree, presumably because the ferns intercepted rainfall and absorbed moisture from the ground. They also appeared to block most of the sunlight, creating a less habitable environment for epiphytes. Although species richness did not vary significantly with height, the drier environment at the base of the tree appeared to be inhabited more by lichens than by other species types. Because the species composition was able to change drastically without altering species richness, species composition (discussed later) was more informative about epiphyte responses to vertical environmental gradients.

The increase in richness with tree diameter supported the hypothesis that richness would vary with girth but did not necessarily support the hypothesis that older trees would have more species. Although the diameter at 0.5 meters was meant to be an index of tree age, older trees were not necessarily thicker than younger ones. Many of the thinner, shorter trees were growing in sunny areas that had been cleared for tourism. They appeared withered and unhealthy and may have had lower epiphyte richness due to other factors besides size and age. The age proxy was not reliable because of environmental variation and the fact that *Metrosideros* development in relation to diameter had not been documented. Due to the lack of growth rings in tropical trees, one would need to study multiple *Metrosideros* trees over several years to determine a precise age proxy.

Richness increased significantly with canopy cover, which supported the hypothesis that the two variables would be correlated. It also supported the hypothesis that epiphytes would prefer shadier environments rather than direct sunlight. The trees growing in sunnier areas had far less moisture in the bark (and, presumably, in the atmosphere) due to evaporation. The richness was lower on these trees probably because fewer species could tolerate the drier bark and the transpiration from their leaves. Because most of the epiphytes found were nonvascular and relied heavily on atmospheric moisture, more species were able to tolerate shady environments due to the increased moisture and decreased transpiration. Although this conclusion is plausible, two confounding factors are a) the sunnier areas were also more disturbed, and b) the trees growing in sunnier areas seemed unhealthy or partially dead.

The total epiphyte cover was lowest at the ground and highest at 1.5 meters, which suggested a positive correlation between height and cover and supported the hypothesis that cover would vary with height. There were fewest epiphytes at the bottom of the tree, probably due to the drier environment created by *Dicranopteris.* The increase in total cover with height was primarily due to increases in lichen and liverwort abundance because there was little increase in the mosses and ferns. It is unclear whether the liverworts and lichens increased more noticeably because they were more sensitive to *Dicranopteris,* or due to another variable, is unclear. To differentiate between the two causes, one would have to study trees with and without *Dicranopteris* (controlling other variables) to see if it caused the increase in liverwort and lichen cover or magnified it. The lack of variation in fern cover was probably because ferns grew up each tree on a single rhizome with few branches. Because fern cover was recorded as basal cover, rather than foliar, the rhizomes would have had to branch considerably to cause a significant increase.

The lack of variation in total epiphyte cover with tree girth supported the null hypothesis that cover would not correlated with tree diameter. The results suggested each tree had a maximum epiphyte load within a single height that did not increase as the trees increased in girth. As each tree thickened, the spread of existing epiphytes and the introduction of new populations probably fluctuated around a carrying capacity. Because there was always some unoccupied space on the tree, the carrying capacity was probably based on moisture in the bark, rather than on surface area. Had the carrying capacity been based mostly on available surface area, the epiphytes would have covered as much of the tree as possible.

Epiphyte cover increased significantly with canopy cover, which supported the hypothesis and could be explained with similar logic as the increase in richness. As canopy cover increased, not only could more species inhabit the environment, but they could spread more quickly. While the total cover did not change within a given height level, the richness varied within that height according to canopy cover. One problem with measuring percent cover in both wet and dry conditions was that the epiphytes were usually shriveled when dry and expanded when wet. They may have covered less surface area on trees in sunnier places because they were less hydrated, not because there were more individuals.

The scatter plot of species by mean diameter and mean canopy cover suggests segregation of some species types by canopy cover preference. The fact that all of the lichens except four grew on trees with lower average canopy cover than the other species types suggests that lichens can survive better in sunnier environments than mosses, liverworts, and ferns. The habitat preference of lichens may have less to do with desiccation tolerance than with access to sunlight, since the photosynthetic algae lived under a crusty top layer of cortex. One of the lichens appeared to prefer the least canopy cover of all of the species, which suggests that it is the most desiccation tolerant species or that it requires the most sunlight. In general, the mosses, liverworts, and ferns preferred shadier, thicker trees. The lichens appeared to grow in a wider variety of shade levels but were most likely of the four species types to inhabit sunny areas. The scatter plot, however, does not necessarily suggest epiphyte preference for tree size or shade level because all of the trees growing in sunny areas tended to be smaller than the others. In order to determine whether canopy cover, tree girth, or both caused the segregation, one would need a larger sample size that included thin trees in shady areas and thick trees in sunny areas.

The lack of variation in richness and cover with aspect supported the null hypotheses that either variable would correlate with aspect. Because canopy cover did not vary with aspect, any variation among the four aspects would have to be due to another factor besides shade level. This also explained why richness did not vary with aspect yet increased with canopy cover. The result disproved the hypothesis that the North-to-South trade winds would create a drier atmosphere on the North side of each tree. The trade winds probably had little effect on the epiphytes because most of the trees analyzed grew in densely-populated areas and were buffered from strong winds. Any trees growing in open areas, where trade winds would have been more noticeable, were so small in diameter (less than ten centimeters) that they were not used for the aspect analysis. One problem with the part of the study was that there was a large standard deviation because the sampling area at each aspect was only four centimeters wide, and the epiphytes were sampled every 0.5 centimeters. There was also a high chance of error in determining the exact aspect with the compass. While the small sample area allowed for a larger sample size, it was not appropriate for the study.

As total epiphyte cover increased, so did the Shannon-Wiener diversity index for each transect. This was expected because richness, which comprised a major part of the diversity index, also increased with epiphyte cover. The result implied that as a tree acquired more epiphyte cover due to the spread of existing species, new species were also colonizing it. If the increase in epiphyte cover had been due mostly to the spread of existing colonies, the diversity index would not have increased at as great a rate. Diversity also increased with diameter, which coincided with the increase of richness. Just as richness did not vary with height, neither did diversity.

The epiphytes studied on *M. collina* appeared to respond most strongly to moisture, which supports the conclusions from other literature that substrate and atmospheric moisture are the greatest determining factors in epiphyte distribution. This coincided with the facts that a) epiphytes lacked true roots, and b) nonvascular epiphytes could not regulate their internal water levels as well as other plants. The most common habitat for epiphytes was the upper trunk region in thick trees with high canopy cover, which was the wettest habitat on the trunk. While this study did not measure moisture levels directly, the results suggest that moist habitats support the greatest epiphyte diversity and total cover.

Although the epiphytes on Moorea showed mostly predictable habitat preferences, they probably differed physiologically from mainland epiphytes due to their unusual environment. For example, epiphytes in cloud forests probably adapted to greater moisture fluctuations than epiphytes in temperate forests. Whether the epiphytes on Moorea and other tropical islands radiated from a few ancestors or evolved within each species, they had to adapt to new environmental pressures unique to islands. Comparative studies of habitat preferences of mainland versus island epiphytes would illuminate differences in niche differentiation and adaptations, as well as convergent evolution among species and species types.

Conclusion

Liverworts, mosses, and ferns preferred shadier habitats and thicker trees than did lichens, which grew in sunnier areas but appeared to tolerate a range of shade levels. Lichens and liverworts preferred to grow higher on the trunk, but mosses and ferns did not show elevation preferences. Mosses and ferns were most abundant on larger trees, but lichen and liverwort abundances were not correlated to tree size. Epiphytes were most abundant high in the trunk and in high shade, yet were most diverse on thicker trees. Overall, nonvascular epiphyte distributions were correlated with height within the tree, canopy cover, and diameter, but were not correlated with aspect. Vascular epiphyte distributions were loosely correlated with height but were unaffected by other environmental factors. Future research should compare epiphyte populations on *M. collina* at different elevations to test wider variation in these environmental factors and determine whether epiphyte zonation by elevation occurs in remote tropical islands.

Acknowledgments

I thank Dr. Brent Mishler for his help with statistics and plant identification, and Liz Perotti, Alison Purcell, and Erica Spotswood for statistics help. I also thank Sigi Hawkins, Valerie Howell, Melissa Riley, Felicia Wheaton for their help in the field.

Literature Cited

Benzing, D.H. 1998. Vulnerabilities of tropical forests to climate change: the significance of resident epiphytes. Climatic Change **39**:519-540.

Caldiz, Mayra S. 2005. Diversity and growth of epiphytic macrolichens in northwestern Patagonian Nothofagus forests. Doctoral thesis. Swedish University of Agrocultural Sciences.

Callaway, R.M., Reinhart, K.O., Moorea, G.W., Moorea, D.J., Pennings, S.C. 2002. Epiphyte host preferences and host traits:

mechanisms for species-specific interactions. Oecologia **132**:221-230.

Cushing, L. 2002. Epiphyte community composition on three non-native and three indigenous tree hosts. Biology and Geomorphology of Tropical Islands **11**:15-22.

D'Artenay, T., Norris, D.H., Mishler, B.D. 2006. Studies on the moss flora of Moorea, French Polynesia. In press.

Gradstein, S.R. 1989. A key to the Hepaticae and Anthocerotae of Puerto Rico and the Virgin Islands. Reprinted from The Bryologist **92**:329-348.

Gradstein, S.R., Griffin, D., Morales, M.I., Nadkarni, N.M. 2001. Diversity and habitat differentiation of mosses and liverworts in the cloud forest of Monteverde, Costa Rica. Caldasia **23**: 203-212.

Harris, G.P. 1971. The ecology of corticolous lichens: 1. The zonation on oak and birth in South Devon. The Journal of Ecology **59**:431-439.

Hietz, P. 1998. Diversity and conservation of epiphytes in a changing environment. Pure and Applied Chemistry **70**:2114-2125.

Holz, I. 2003. Diversity and ecology of bryophytes and macrolichens in primary and secondary montane *Quercus* forests, Cordillera de Talamanca, Costa Rica. Doctoral thesis. Georg-August University, Gottingen

Kenkel, N.C., Bradfield, G.E. 1981. Ordination of epiphytic bryophyte communities in a wet-temperate coniferous forest, South-Coastal British Columbia. Vegetatio **45**:147-154.

Kuhlmann, D.H.H. 1983. Composition and ecology of deep-water coral associations. Helgoland Marine Research **36**:13-204.

Lloyd, L.L. and Giambelluca, T.W. 1998. Vulnerability of island tropical montane cloud forests to climate change, with special reference to east Maui, Hawaii. Climate Change **39**:503-517.

McCune, B., Geiser, L. 2000. Macrolichens of the Pacific Northwest. Oregon State University Press, Corvallis, OR.

Meyer, J., Florence, J. 1996. Tahiti's native flora endangered by the invasion of Miconia calvescens DC. (Melastomataceae). Journal of Biogeography **23**:775-781.

Murdock, A., Hinkle, A. 1999. Moorea Digital Flora Project. Online database accessed November 2006. http://ucjeps.berkeley.edu/moorea/index.html

Nadkarni, N.M. 1984. Epiphyte biomass and nutrient capital of a neotropical elfin forest. Biotropica **16**:249-256.

Osborne, P.L. 2000. Tropical Ecosystems and Ecological Concepts. Cambridge University Press, Cambridge, UK.

Piippo, S. 1990. Bryophyte flora of the Huon Peninsula, Papua New Guinea. XXXIV. Annales Botanici Fennici **27**:13-18.

Rousseau, R., van Hecke, P., Nijssen, D., Bogaert, J. 1998. The relationship between diversity profiles, evenness and species richness based on partial ordering. Environmental and Ecological Statistics **6**:211-223.

Whittier, H.O. 1976. Mosses of the Society Islands. University Presses of Florida, Gainesville, FL.

Wickland, A. 1999. Reproductive ecology of *Metrosideros collina* (Myrtaceae) of Moorea, French Polynesia. Biology and Geomorphology of Tropical Islands **8**:172-182.

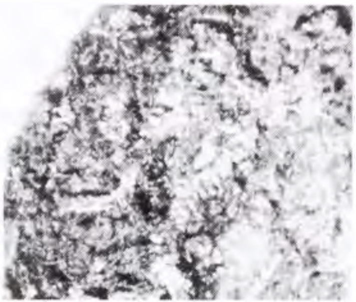
Wright, S.D., Yong, C.G., Wichman, S.R., Dawson, J.W., Gardner, R.C. 2001. Stepping stones to Hawaii: a trans-equatorial dispersal pathway for *Metrosideros* (Myrtaceae) inferred from nrDNA (ITS+ETS). Journal of Biogeography **28**:769-774.

APPENDIX A

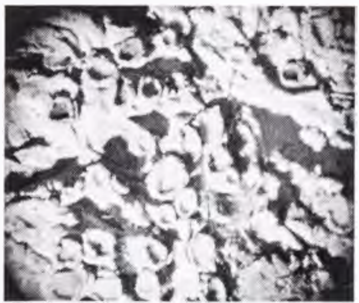
Lichens found on *Metrosideros collina* in Moorea, French Polynesia

Ahtiana sp.

Ahtiana sp.



Unknown A

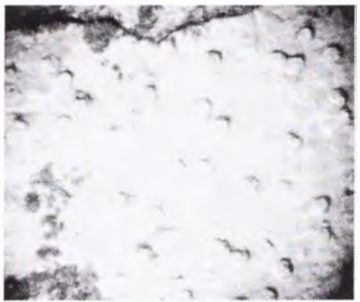


Melanelia sp.

Cavernularia sp.

Punctelia sp.

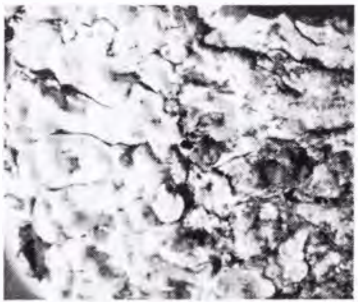
Unknown B



Punctelia sp.

Cavernularia sp.

Unknown C



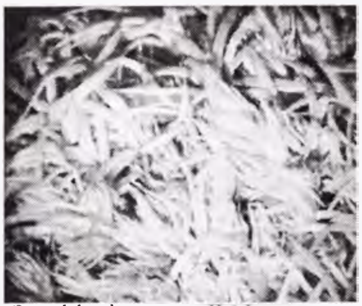
Parmelia sp.

Cavernularia sp.

APPENDIX B

Mosses and liverworts found on *Metrosideros collina*, in Moorea, French Polynesia

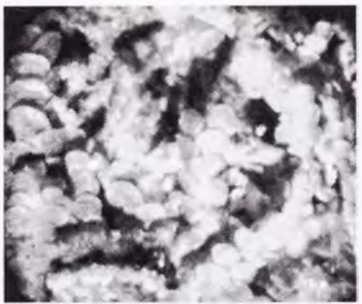
Syrrhopodon banksii



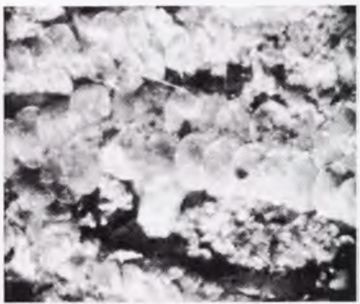
Octoblepharum albidum

Mitthrydium obtusifolium

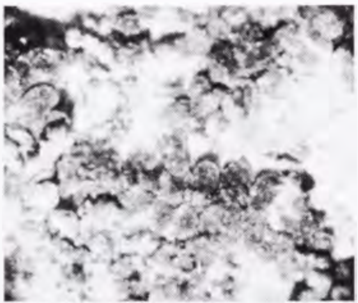
Leucobryum tahitense



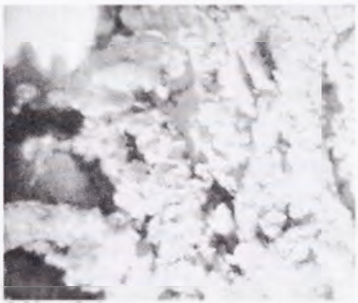
Cheilolejeunea sp.



Cheilolejeunea sp.



Species unknown



Microlejeunea sp.

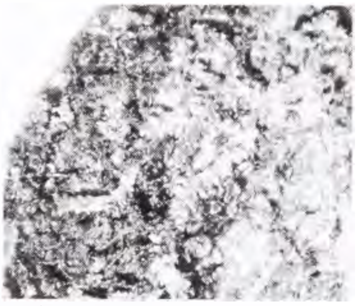
Lopholejeunea sp.

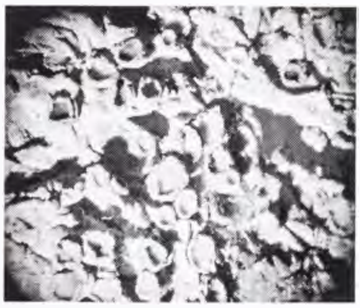
APPENDIX A

Lichens found on *Metrosideros collina* in Moorea, French Polynesia


Ahtiana sp.


Ahtiana sp.

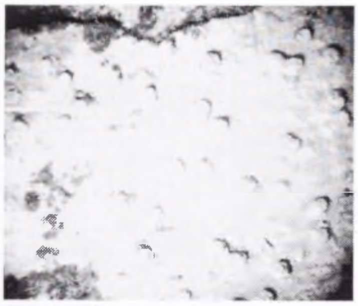

Unknown A

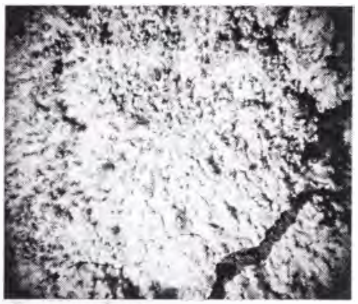

Melanelia sp.


Cavernularia sp.

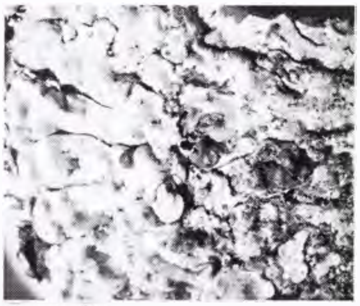

Punctelia sp.

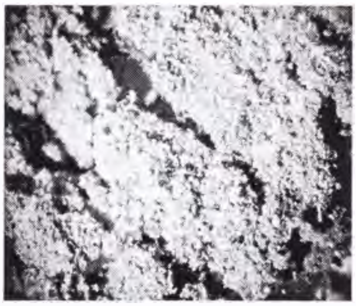

Unknown B


Punctelia sp.


Cavernularia sp.


Unknown C


Parmelia sp.


Cavernularia sp.

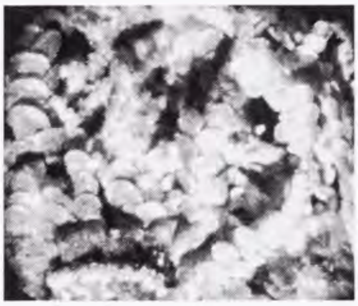
APPENDIX B
Mosses and liverworts found on *Metrosideros collina*, in Moorea, French Polynesia

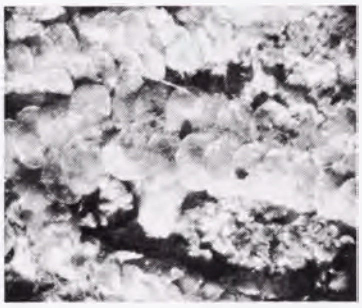

Syrrhopodon banksii

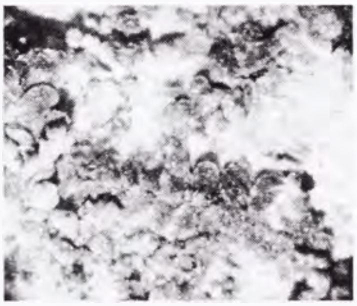

Octoblepharum albidum


Mitthrydium obtusifolium


Leucobryum tahitense


Cheilolejeunea sp.


Cheilolejeunea sp.


Species unknown


Microlejeunea sp.


Lopholejeunea sp.

HABITAT AND BLEACHING IN THE FORAMINIFERAN *PENEROPLIS PERTUSUS*

AIMEE ELLISON

Integrative Biology, University of California, Berkeley, California 94710 USA

Abstract. The effects of human activities on the earth's environment have gained increasing attention in recent years. With coral reefs declining worldwide, efficient tools for assessing reef health are more important than ever. The species of larger foraminifera known as *Peneroplis pertusus* share key characteristics with reef building corals. By examining the populations' natural distribution along with the abiotic factors affecting bleaching, a better understanding of reef systems as a whole is achieved. In this study, *P. pertusus* was collected from ten different sites on a fringing reef in Moorea, French Polynesia. Collected from coral rubble at one, two, and three meters depths, they were analyzed for abundance, size, and extent of bleaching. Light experiments were used in the laboratory to determine response to increased solar radiation. One-way statistical analysis, along with the Wilcoxon test found no strong correlation between depth and percent bleaching. A difference between individual size and percent bleaching was found and a natural population dynamics are presumed to occur on Moorea. Light experiments found increased bleaching in *P. pertusus* showing increased solar radiation to be a factor in bleaching.

Key Words. Larger foraminifera, Peneroplis pertusus, bleaching, distribution, Mo'orea, French Polynesia

INTRODUCTION

The coral reefs found in tropical and subtropical waters play a vital role in human society. More than 50% of the world's population lives within 100km of the ocean (Vitousek *et al.* 1997) and many of those people depend on reef ecosystems for their livelihood. Coral reefs are a valuable economic resource, providing income through tourism and fishing as well as lending protection to coastal habitats. Known as highly diverse and productive environments, coral reefs provide a place of continuing scientific discovery in the form new drugs and biochemicals (Hoegh-Guldberg 2006). Unfortunately coral reef habitats are in decline worldwide (Talge 2003). While susceptible to natural stressors such as disease and hurricanes, the increasing rate at which they are deteriorating has gained interest in recent years.

The impact of human activities on the earth's oceans has been well documented. Alterations to the earth's carbon and nitrogen cycles affect ocean chemistry and ecology. Primarily through fertilizer production, humans have doubled the annual input of biologically available nitrogen to terrestrial ecosytstems (Hallock 2000). Nutrification of estuaries and coastal waters can occur when a significant percentage of fixed nitrogen washes into the ocean (Vitousek *et al.* 1997). The increase of CO_2 in the earth's atmosphere is the best-documented global change (Vitousek *et al.* 1994). Achieved primarily through the burning of fossil fuels, this increase will be responsible for more than half the expected global warming in the next century (Vitousek 1994). Concentrations of greenhouse gases are expected to double over pre-Industrial Revolution concentrations in the early 21st century (Hallock 2000) and ocean temperatures have risen roughly 1°C in the past 100 years (Hoegh-Guldberg 2006). Depletion of ozone in the atmosphere is linked to an increase of more damaging ultraviolet radiation to reaching earth's surface (Kerr *et al.* 1993). All of these changes can have a profound effect on fragile coral reef communities that are already living at the extremes of their habitat requirements (Hoegh-Guldberg). With these trends from human impact showing no signs of slowing (Hallock 2000, Hallock *et al.* 2003), efficient tools for assessing reef condition are gaining more interest.

Larger benthic foraminifera have been found to be good indicators of reef health (Renema 2006). Foraminifera require many of the same environmental factors to survive as coral (Renema 2006). Larger foraminifera share three distinct traits with reef building corals. Both are producers of calcium carbonate, are reliant on algal endosymbionts for growth and test formation, and both are experienced bleaching events in the past decades (Hallock *et al. 2006).* A main difference between the groups is the apparent mechanism of bleaching. Coral bleaching occurs through the expulsion of symbionts and has been shown to most strongly correlate to elevated water temperature (Hallock *et al.* 2006). Bleaching in foraminiferan happens when damaged symbionts are digested and is induced through high intensity solar radiation (Hallock *et al.* 2006).

Hallock *et al.* (2003) points to the key characteristics that give foraminifera value as a bioindicators: 1) Their comparatively short life spans compared with corals, allows for comparisons between long and short-term trends 2) They are relatively small and abundant which allows for inexpensive collection and significant sample size 3) Collection and sampling has minimal impact on the reef environment.

Sensitivity to change is an important attribute of a bioindicator. Studies have shown that symbiont bearing foraminifera respond to photo-oxidative stress by bleaching within hours or days (Hallock *et al.* 2006), allowing for significant finding in short term studies. On a larger scale, nutrient influx causes a shift in assemblages from larger symbiont bearing foraminifera to smaller fast growing heterotrophic taxa (Cockey *et al.* 1996).

Peneroplis pertusus is a species of larger foraminifera that can be found abundance on the fringing reef of Mo'orea French Polynesia. It hosts the red algal symbiont *Porphyridium* (Hawkins *et al.* 1990). Most studies on distribution have been done on empty tests and habitats of only a few genera have been investigated in detail (Hohenneger *et al.* 1998). This study examines the distribution of *P. pertusus* along a three-meter depth gradient. The investigation of distribution by water depth represents a "typical ecological complex-gradient" (Hohenegger *et al.* 1995). Factors included in this gradient are topography, light penetration, hydrodynamics, and temperature (Hohenegger *et al.* 1995)

It is important to understand parameters effecting distribution in order to relate it to reef conditions as a whole. The identification of patterns in diversity and assemblage require extensive data (Tappan et al 1998) and this study hopes to contribute to that. The objective is to gather data on the abiotic factors that are effecting the populations of *Peneroplis pertusus*. Examination of distribution, habitat, and the extent of bleaching in the population's natural environment along with experimental manipulations were used to gain a better picture of their role in Mo'orea's reef system.

METHODS

Study Site

Mo'orea is a high volcanic island encircled by a barrier reef. Enclosed in the barrier reef is a shallow back reef between 500 and 100 meters in width (Venec-Peyre 1990). A fringing reef encircles much of the islands coastline including Cook's Bay. The site is located in Cook's Bay, Mo'orea at the University of California's Gump Station (Figure 1). It was chosen for the accessibility it provides to a suitable fringing reef. The coastline also offers a basic topography that allowed for straightforward site mapping and individual site distinction.

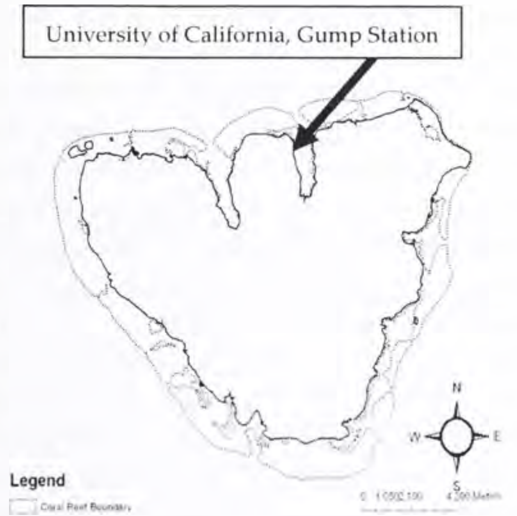


Figure 1. Map of Mo'orea showing Gump Station located in Cook's Bay.

The station's 81-meter coastline provides two distinct fringing reef topographies. The southern 27 meters of property consists of a reef with a sharp depth gradient, reaching three meters between 3-6 meters offshore. The remaining northern 54 meters has a shallow sloping reef reaching three meters in depth approximately 50 meters offshore.

Distribution and bleaching in the field

A pilot study was done to determine the best substrate for collection. Snorkeling along the reef, samples were taken from coral rubble, dead coral heads, sand and the green alga *Halimeda* at depths between one and two meters. Samples from the dead coral heads were taken by scraping the surface into a plastic bag while the other substrates were taken whole in plastic bags. The coral rubble was placed in a colander that sat on a #100 sieve. The overhead seawater hoses in the wet laboratory were used to provide running water through the set up while the coral was scrubbed with a stiff brush to dislodge any organisms. The *Halimeda* was washed using the same technique. The collections obtained in the sieve, as well as the sand and coral head samples were placed in separate petri dish and examined under a microscope for 15 minutes each. Total abundance of *Peneroplis* from each substrate was used to determine the suitable habitat for collection.

Once coral rubble was chosen, the coastline was mapped by hand starting at the southern most accessible site. Starting at sea water outlet for the wet laboratory, the coastline was measured and markers were placed at three-meter intervals. Stopping at the northern property boundary gave a total of 81 meters of coast and 37 individual sites. The sites were assigned numbers on a map (0-9 were on the south reef, 10-37 on the north reef) and a random number table was used to select ten study sites for transects. A collection of coral rubble at each site was done at one, two and three meters of water depth. A PVC pipe cut to three meters and marked at one-meter intervals was used to measure water depth. Collections of coral rubble were made by snorkeling perpendicular to the shore at the marked site. As soon as the water depth obtained one, two, and three meters, a collection of rubble was taken. An initial temperature reading was also taken at each depth. One-gallon plastic bags were used in each collection and rubble was taken until the bags became full. All samples were washed for ten minutes using the previously describe design. After washing was completed, the sieve was shaken to obtain even distribution and the sample was divided into four pie shaped slices. A random number table was used to determine which slice was chosen and the sample was put in a petri dish. Using a 10x microscope, the sample was examined 15-minutes to find any *P. pertusus*. Each organism found was removed place in another dish to be categorized after the observation time had ended. The specimens were then looked at under 3x magnification to determine size and percent bleaching. Size was evaluated by measuring the test at its widest part and was categorized as less than one mm (small), equal to one mm (medium) and greater than one mm (large). A few specimens were found to be two and three millimeters and were categorized as xlarge and xxlarge respectively. Bleaching was visually assessed through loss of purple pigment. This can typically be seen in two ways 1) color loss starting at the most recently added chamber and moving inward to varying degrees or 2) white spots appearing within the pigmented area. Percent bleaching was based on proportion of color loss in the whole organism and comparison to other specimens obtained.

To determine intrasample variability, a pilot study was done that compared abundance of *Peneroplis pertusus* in each of the four sample slices in the sieve. A pilot study into washing methods was completed to establish that a high enough proportion of the organism was obtained within ten minutes. This included two separate tests, done by washing the same rubble sample for ten minutes three consecutive times. To make a comparison with greater depths, a collection of rubble was made at nine meters by SCUBA divers certified in scientific diving by University of California Berkeley. Two one-gallon samples of rubble were taken, one each from the differing north and south sides of the reef.

Light manipulation study

Twenty individuals were gathered from coral rubble using previously described techniques. Each individual was placed in the plastic lid of small glass vials containing seawater. Each lid was numbered and the corresponding organism's degree of bleaching was recorded. The 20 organisms were randomly divided into two groups and placed in petri dishes also filled with seawater. One dish was placed directly under a full spectrum coral grow lamp (*get data for wattage and spectrum*) while the other was left on the laboratory countertop. At twenty-four hour intervals for three days, the organisms were observed under a 3x microscope. Percent bleaching was recorded.

Statistical Analysis

For the field study in distribution and bleaching, the percent bleaching data was turned into a proportion and a distributional analysis was preformed. Arcsine and square root transformations were preformed to try and obtain normal distribution. One way statistical analysis was used to compare site, depth, abundance and percent bleaching in individuals. The non-parametric Wilcoxon test was done on all pairs. If found to be significant in Wilcoxon, the Tukey-Kramer test was used to compare the differences in means. For the light manipulation study, proportion bleaching was averaged over the ten individuals in each the control and treatment group for each day. Means were and plotted over the four-day period for comparison in rate of increase.

Habitat description

A description of reef topography and composition was accomplished through visual observations while snorkeling. Notes were made with regards to sea floor, habitat distinctions, and rubble size.

RESULTS

Pilot studies

Collection from the various habitats yielded more *P. pertusus* from coral rubble than any other substrate (Table 1). When the four samples from the sieve were examined individually for abundance of *Peneroplis pertusus* they yielded nine, nine, eight, and nine individuals respectively. The two studies evaluating the ten-minute washing period are summarized in Table 2. The collection at nine meters depth yielded three individuals. Two were found to be 100% bleached and the other was 25%.

Distribution and bleaching in the field

Statistically significant values, $P<0.05$ were found when comparing the differences of proportion bleaching by size, proportion of bleaching by site, and abundance of *P. pertusus* found at each site (Table 3).

Habitat	Number of Individuals Found
Halimeda sp.	0
Sand	1
Coral Head	0
Coral Rubble	11

Table 1. Number of *Peneroplis pertusus* collected from different substrates.

Wash	Study 1	Study 2
1	9	14
2	1	0
3	1	0

Table. 2. Number of individuals found after each wash. First study showing approximately 90% of all organism were obtained in the first wash, second study shows 100%.

The Tukey-Kramer results showed that bleaching in individuals was significantly different when comparing site ten to sites three and four. Site 10 had the highest average bleaching of 74% and sites 3 and four showed the lowest with 32% and 38% respectively. Differences in abundance by site showed no significance when using the Tukey-Kramer test. Bleaching by size analysis showed medium and small to be significantly less bleached than large but not differ from xl and xxl. Bleaching differed significantly with size of an individual but not with the depth at which it was collected. The number of individuals found in each size category is shown in Figure 1. Temperature readings taken at one, two and three meters did not vary and were found to be 28.6°C.

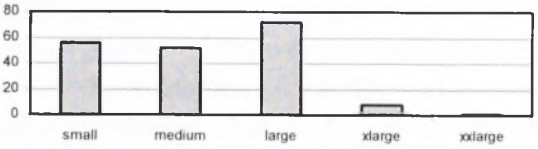


Figure 1. Number of individuals found in each size category showing similar results for small, medium, and large.

Light manipulation

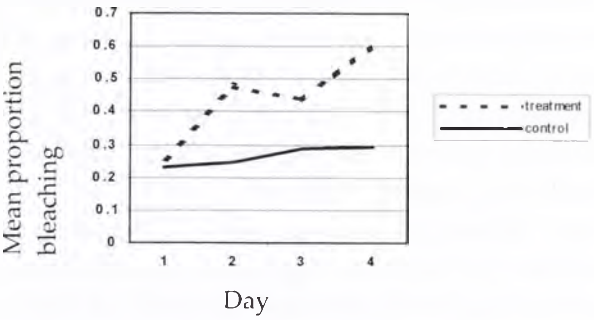


Figure 2. Mean proportion bleaching in treatment and control showing greater increase of bleaching in treatment group.

Light experiments showed a significant difference between the control and treatment group. Mean proportion bleaching increased at a much greater rate in the sample underneath constant photic stress (Figure 3).

One-way Analysis	**P value**
Depth by Size	0.1406
Bleaching by Size	<0.0001
Abundance by Depth	0.6217
Bleaching by Site	0.0021
Abundance by Site	0.0017
Bleaching by Depth	0.9748

Fig 3. Analyses performed and P values.

Habitat Description

Although the northern and southern sections of the fringing reef have different depth gradients, the substrate and samples collected from each had strong similarities. For both sections, the density of coral rubble covering the floor declined with an increase in depth. Filamentous algae cover on individual pieces of coral rubble appeared uniform throughout. The majority of rubble collected from one and two-meter depths had 100% of the upper facing surface covered in filamentous algae. Algae cover declined as water depth increased and samples from the three-meter collection typically had 50-100% cover. The northern reef had more interstitial sand due to a lower density of rubble. Although there was a difference in rubble density between the sides, the size variance between individual pieces appeared uniform throughout. Size of rubble collected from both sides ranged from $10cm^2$ to $216\ cm^2$ in top facing surface area. One major difference between the north and south sections of reef was the presence of the brown alga *Padina pavonea*. This alga has a distinctive fan shaped thallus and was present on approximately 75% of the coral rubble collected from the north reef at one and two-meters depth. *P. pavonea* was found to be completely absent in the south section of the reef. *Halimeda* used in collection was also found only on the shallow sloping north reef. It was found on larger coral rubble with a top surface area between $0.09m^2$ and $0.37m^2$ with a depth between 0.5 and 2 meters. No difference was detected in the size and number of larger coral heads between the sides.

DISCUSSION

Pilot studies

The study examining all samples of sediment within the sieve showed intra-sample variability to not be an issue. The washing study showed ten minutes to be a suitable time frame to obtain a high percentage of the study organism. Coral rubble was found to yield the highest abundance of *P. pertusus*. This is consistent with previous studies that found the organism to mainly inhabit coral rubble (Renema 2003). Hohenneger *et al* (1998) found various species Peneroplids including *P. pertusus* to be better adapted to coral rubble environments when compared to sand. The SCUBA collection at nine meters resulted in only a few specimens. This could be for a few reasons. Filamentous algae are the preferred habitat of Peneroplids in high-energy environments (Hoheneggar 2004). Having weak pseudopods, this habitat provides protection and a mechanism for attachment (Hohenneger 2004). Coral rubble collected from nine meters showed little (< 20%) to no algae cover. The sediment obtained from washing the rubble was much finer and possibly did not provide the protection or habitat composition desired. Light availability could be another factor. Nine meters depth might not provide the light penetration that *P. pertusus* is best suited. Further sampling at this depth would allow for greater comparisons.

Distribution and bleaching in the field

Abundance distribution did not vary with depth. An explanation could be that light penetration does not vary significantly at 1-3 meters. Light is an important limiting factor affecting distribution in larger foraminifera (Hohennegar *et al* 1998). Being host to photosynthetic symbionts restricts *P. pertusus* to the photic zone of the ocean. Logistics prevented light measurements from being taken at the varying depths in this study but Hohenegger *et al.* (1999) found *Peneroplis pertusus* to be highly adapted to extreme (80-100%) and very strong (60-80%) surface irradiance. This could also explain why bleaching was not found to be a factor with depth. The adaptation more intense light could serve as an indication of light availability at depths. Population shifts towards greater depths could signal an increase in the solar radiation that reaches the ocean floor. Evidence for light induced bleaching in corals has been documented. The studies show that greater bleaching occurs on more exposed portions of coral than in areas lying under shadows of fixed objects (Williams *et al* 1990). It is possible that the increase in light also correlates to an increase in temperature.

Temperature variance between one and three meters was not detected in this study. This could be due to instrument sensitivity or the shallow depth gradient being investigated. Temperature typically decreases with depth and is a key factor limiting all symbiont-bearing foraminifera (Hohenegger 2004). Temperature influences the abundance of dissolved organic and inorganic particulate matter available to foraminifera (Hohenegger 2004). Being an ectoderm, temperature strongly affects the metabolic rate of *P. pertusus*. When temperatures are low, more dissolved nutrients are present but metabolism is slowed (Hohenegger 2004). Increased temperatures raise metabolic rates but lower the amount of dissolved nutrients. Temperature also controls the abundance of dissolved CO_2 in the ocean. Concentrations are low in warmer waters and this can hinder photosynthesis (Hohenegger 2004). The lack temperature variance could explain why abundance and bleaching in *P. pertusus* does not vary with depth.

There is a supposed temperature niche optimum of 28°C for foraminifera (Hohenegger 2004), which is close to temperature recorded in Mo'orea. This study supports this finding and suggests that *Peneroplis pertusus* is living under suitable temperatures for normal population dynamics. Little is known about the temperature niche of foraminifer's symbionts or their response to extreme highs and lows (Hohenegger 2004). Investigation into temperature tolerance in foraminifera would allow for better understanding how it effects the population and of its usefulness as a bioindicator in coral reefs.

Test size and abundance were not found to significantly vary by depth in this study except when looking at the very large individuals. The stable distribution of individuals found with small, medium and large test size shows level population distribution. This may be explained by even an even distribution in generations and steady reproductive dynamics (Hohenegger *et al* 1999). The years of highest bleaching incidences in *Amphistigina*, showed the lowest proportion of juveniles (Hallock et al 2006). Other studies found that severe bleaching in *Amphistegina* spp. showed reproductive failure (Williams *et al.* 2004). When partially bleached *Amphistegina* spp. Individuals attempted to reproduce asexually, they either produced hundreds of tiny cells that failed to calcify, or produced a few malformed offspring (Hallock et al 2006). Healthy unbleached specimens can produce hundreds of identical viable offspring. If *Peneroplis* spp. was experiencing these conditions, a skewed size distribution with preference towards larger individuals would be expected. The few individuals found in the largest size category are not suspected to a result of this and a larger sample size would help confirm this. If trends hold true for all symbiont bearing foraminifera, it suggests that *Peneroplis pertusus* is not experiencing stressors that effect natural reproduction. Bleaching found is thought to a natural occurrence of the population.

The strongest correlation in this study was found between bleaching in an individual and its test size. An explanation of this could be natural aging of the individual. Deterioration as the organism ages might lead to break down or loss of the endosymbiont. Another reason could be the demands of increased sized. As the organism grows, its energy needs increase possibly putting a strain on the symbiotic relationship. Studies in another larger symbiont bearing foraminifera *Amphistegina* showed that color loss was rarely seen in specimens smaller than 0.5mm but most specimens whose diameter exceeded 0.8mm showed signs of bleaching (Hallock 2006). This study result suggests the same trends occurring in *Peneroplis pertusus*.

Light Manipulation

A significant difference between bleaching in the control and treatment group was found. This supports previous studies that found solar radiation to be a key factor bleaching in larger foraminifera. Studies on experimental bleaching of *Amphistegina gibbosa* in a laboratory setting showed a very limited optimum irradiance level (Williams *et al.* 2004). Talge *et al.* (2003) demonstrated that light induced bleaching seen in the laboratory showed similar physiological responses as bleaching in specimens collected in the field. Under prolonged photic stress, bleaching occurred even when under optimal temperatures (Talge *et al.* 2003). The connection between bleaching in the laboratory and bleaching in the field is an important link when applying experimental observations to specific habitats.

Habitat Description

The main difference between the two reefs was the depth gradient. Other factors include the presence of *Padina pavonea* and the slightly lower density of coral rubble on the north reef. It is unlikely that these factors alone contributed to the difference in proportion bleaching found between the specific sites: three, four, and ten. With no overall trend in bleaching between the two main reef sites it is difficult to know if habitat was actually a factor. A larger sample size would strengthen the correlation.

CONCLUSION

This study suggests that the abiotic stresses experienced between one and three-meter depths are not severe enough to alter population dynamics in *Peneroplis pertusus* of Mo'orea. Experimental evidence was found to support the correlation between increased solar radiation and bleaching in foraminifera. This can indicate *P. pertusus* usefulness as a bioindicator in the future. Comparison with similar studies in the future will allow for better understanding of changes in Mo'orea's population of *Peneroplis pertusus*.

ACKNOWLEDGEMENTS

I would like to thank all the professors of IB 158 for their guidance, patience, and knowledge: Jaime Bartelome, Vince Resh, Carole Hickman, Brent Mishler, and Jere Lipps. I would also like to thank the graduate student instructors: Liz Perotti, Erica Spotswood, and Alison Purcell, for all of their assistance. My field partners Maya Almaraz and Sara Chinn. The University of California Berkeley Gump Research Station for hosting me during my research.

LITERATURE CITED

Cockey, E., P. Hallock, and B.H. Lidz. 1996. Decadal-scale changes in benthic foraminiferal assemblages off Key Largo, Florida. Coral Reefs **15**:237-248.

Fujita, Kazuhiko. 2004. A field colonization experiment on small-scale distribution of algal symbiont-bearing larger foraminifera on reef rubble. Journal of Foraminiferal Research **34**:169-179.

Hallock, Pamela and D.E. Williams. 2006. Bleaching in foraminifer with algal symbionts: implications for reef monitoring and risk assessment. Anuário do Instituto de Geociêncieas **29**:108-128.

Hallock, Pamela, B.H. Lidz, E.M. Cockey-Burkhard, and K.B. Donnelly. 2003. Foraminifera as bioindicators in coral reef assessment and monitoring: the foram index. Environmental Monitoring and Assessment **81**:221-238.

Hallock, Pamela. 2000. Symbiont-bearing foraminifera: harbingers of global change? Micropaleontology **46**:95-104.

Hawkins, Eva Konrad, and J.J. Lee. 1990. Fine structure of the cell surface of a cultured endosymbiont strain of Porphyridium sp. (Rhodophyta). Transactions of the American Microscopical Society **109**:352-360.

Hoegh-Guldberg, Ove. 1999. Climate change, coral bleaching and the future of the world's coral reefs. The coral reef research institute: executive summary. Accessed December 2006. <http://www.reef.edu.au/OHG/res-pic/HG%20papers/Hoegh-Guldberg%201999.pdf>

Hogenegger, Johann. 1995. Depth estimation by proportion of living larger foraminifera. Marine Micropaleontology **26**:31-47.

Hohenegger, Johann, E. Yordanova, Y. Nakano, and F. Tatzreiter. 1998. Habitats of larger foraminifera on the upper reef slope of Sesoko Island, Okinawa, Japan. Marine Micropaleontology **36**:109-168.

Hohenegger, Johann. 2004. Depth coenoclines and environmental considerations of western pacific larger foraminifera. Journal of Foraminiferal Research **34**:9-31.

Kerr, J.B. and C.T. McElroy. 1993. Evidence for large upward trends of ultraviolet-B radiation linked to ozone depletion. Science **262**:1032-1034.

Renema, W. Larger foraminifera on reefs around Bali (Indonesia). 2003. Zool. Verh. Leiden **345**:337-366.

Renema, Willem. 2006. Habitat variables determining the occurrence of large benthic foraminifera in the Berau area (East Kalimantan, Indonesia). Coral Reefs **25**:351-359.

Talge, Helen K., and P. Hallock. 2003. Ultrastructural responses in field-bleached and experimentally stressed *Amphistegina gibbosa* (Class Foraminifera). The Journal of Eukaryotic Microbiology **50(5)**:324-333.

Tappan, Helen and A.R. Loeblich Jr. 1988. Foraminiferal evolution, diversification, and extinction. Journal of Paleontology **62**:695-714.

Venec-Peyre, M.T. 1990. Distribution of living benthic Foraminifera on the back-reef and outer slopes of a high island (Moorea, French Polynesia). Coral Reefs **9**:193-203.

Williams, Dana E., and P. Hallock. 2004. Bleaching in *Amphistegina gibbosa* d'Orbigny (Class Foraminifera): observations from laboratory experiments using visible and ultraviolet light. International Journal on Life in Oceans and Coastal Waters **4**:1351-13

DISPLAYS OF DEFENSE: BEHAVIORAL DIFFERENCES IN ANTAGONIST AVOIDANCE IN FOUR OPISTHOBRANCH MOLLUSKS

Sameen R. Ghazali

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

Abstract. The defensive behaviors of four opisthobranchs (*Glossodoris cincta, Risbecia imperials, Stylochelius striatus*, and *Dolabrifera dolabrifera*) were observed and categorized. The displays studied were mantle flexation, mucus production, mantle secretion, inking, and rearing. Members of each species were placed in two laboratory situations containing two different antagonists. The antagonists (*Dardanus lagopodes* and *Lutjanus fulvus*) were chosen because they were carnivorous, abundant, and found in the same ecology as the opisthobranchs studied. Additionally, they were chosen because they differed phylogenetically, physiologically, and behaviorally and, therefore, represented two very different predators. In some cases, individuals exhibited different defensive behaviors in the presence of different antagonists. Differential responses could reflect physiological, biological, or phylogenetic differences between the four observed opisthobranch species. In some instances, defensive displays were observed across lineages.

Key words: defense; inking; mantle flexations; rearing; opisthobranch; nudibranch; sea hare; Chromodorididae; Aplysiidae; *Moorea; French Polynesia*

INTRODUCTION

Although nudibranchs lack the protective shell characteristic of numerous gastropods, many possess chemical signals or toxins to deter predators (Mallet & Joron 1999, Penney 2004). Evidence suggests that many opisthobranchs use sequestered chemical compounds from food sources as a means for defense (Faulkner & Ghiselin 1983, Avila 1995). In some cases, the compounds involved in defense are known to be toxic, and in other cases they are not, but still appear to act as effective deterrents for predators (Gimenez-Casalduero et al. 1999, Long & Hay 2006). Additionally, the bright colors of many opisthobranch species may have evolved to warn predators against attacking these soft-bodied marine invertebrates (Rosenberg 1989, Tullrot 1994, Lindstrom 2001). Since opisthobranchs cannot see color, evidence suggests that it is unlikely that bright markings and bold patterns would have evolved from sexual selection or species recognition (Servedio 2000, Behrens 2005). Aside from the present theories surrounding chemical defense and aposematic coloration, the multiple behaviors associated with many defensive displays are still poorly understood.

This study is aimed at examining some of the behavioral responses exhibited by opisthobranchs when in the presence of an antagonist. This paper centers on two major questions. The first and preliminary question being, what are the different predator defense mechanisms exhibited by four opisthobranch species found in Moorea? This study identifies and compares the behaviors exhibited by various opisthobranchs in the presence of an antagonist. Many behaviors such as the release of mucus or ink should be observed only in the presence of an antagonist, since the likely cost of producing the compounds for these compounds is great.

My second major question is how do these opisthobranchs behave when presented with different antagonists? That is, does defensive behavior change in the presence of antagonists that differ phylogenetically, physiologically, and behaviorally? I expect prey subjects to display a variety of defensive behaviors, some of which will only be apparent in the presence of a particular antagonist. If this is the case, I expect to observe differential response to antagonists because of phylogenetic, developmental, or physiological differences between the opisthobranch species. I expect defensive displays to be lineage-specific and to observe the same behaviors in organisms that are more closely related.

METHODS

Study site

All opisthobranch species were collected while snorkeling at Cook's Bay (FIG. 1) in Moorea, French Polynesia. Opisthobranchs were found in close proximity to the Gump Research Station, primarily between the waterfront bungalows and the small grove of trees just north of the station. All subjects were collected in the late morning or early afternoon.

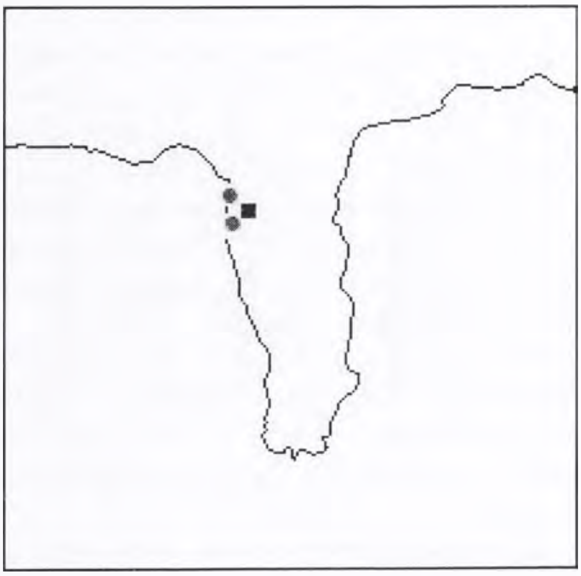


FIG. 1 Site map of Cook's Bay (grove and bungalows noted by ● and field site noted by ■).

Description of species

Two nudibranch species (Chromodorididae) and two species of sea hares (Aplysiidae) were observed in this study (FIG. 2).

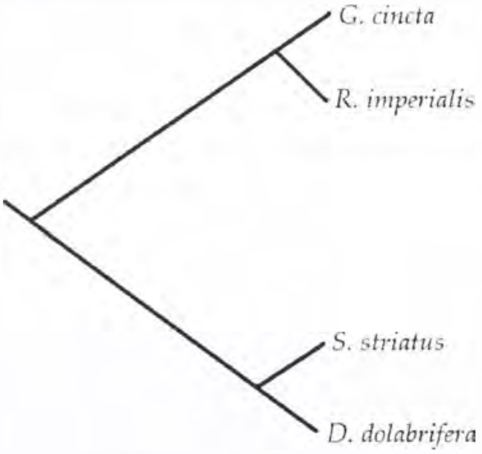


FIG. 2. Phylogeny of opisthobranch species (adapted from Johnson 1994).

Glossodoris cincta (Bergh, 1888) (FIG. 3) is the first of two conspicuously colored opisthobranch species used in this study. This nudibranch is characterized as having three colored bands around the mantle border: an outermost pale blue, middle black, and innermost yellow or khaki band (Rudman 2000). Most of its body is mottled dark brown and light pink in color. Its rhinophores and gills appear to be dark brown speckled with light pink. *Glossodoris* nudibranchs excrete some secondary metabolites from their diets which are thought to deter some though not all potential predators (Rogers 1991). Subjects were found feeding on algae on rock rubble. This species was the most abundant at the collection sites.

FIG. 3. *Glossodoris cincta*

Risbecia imperialis (Pease, 1860) (FIG. 4) is the second brightly colored opisthobranch species used in this study. This species was also found feeding on algae growing on rocks and on the sandy substrate near coral heads. These nudibranchs were often found occurring in pairs, and were observed tailing—a behavior in which one opisthobranch follows the mucus trail of another, using the sensory cells in its oral tentacles (Behrens 2005). This nudibranch is characterized as having a primarily white mantle and foot with yellow dots. Its gills are white edged with dark blue, and its rhinophores are dark blue with white flecks. The zigzagged mantle border of *R. imperialis* is dark blue and encompasses small yellow dots.

FIG. 4. *Risbecia imperialis*

Stylochelius striatus (Quoy and Gaimard, 1832) (FIG. 5) is the first of two inconspicuous opisthobranch species examined in this study. This sea hare can be characterized as having a mottled green and brown body. Also characteristic of *S. striatus*, are dark longitudinal lines and scattered royal blue dots along its mantle. This species was found feeding on blue-green algae that forms film over rocks or muddy surfaces.

FIG. 5. *Stylochelius striatus*

Dolabrifera dolabrifera (Cuvier, 1817) (FIG. 5) is the second cryptic opisthobranch observed in this study. The body of *D. dolabrifera* is flattened and a mottled green in color. The posterior half of the organism is rounded and narrows closer to the head. Its parapodia are fused except for a portion in the posterior midline where two flaps form a respiratory opening (Rudman 2003). This species is known to have glands incapable of producing ink or its associated anti-predator proteins (Prince 2006). The species can be found attached to rock rubble.

FIG. 6. *Dolabrifera dolabrifera*

To address my primary question, does defensive behavior in opisthobranchs change when different antagonists are present, I used two antagonists in this study. I chose hermit crabs (*Dardanus lagopodes*) (Forskål, 1775) because they represent a predator that crushes and chews its prey. It is likely that an opisthobranch would have a lower probability of surviving an encounter with a hermit crab than with a predator that swallows its prey whole (Penney 2004). This is also why I chose the blacktail snapper (*Lutjanus fulvus*) (Scneider, 1801) for my second antagonist. This snapper is representative of fish predators that mouth and swallow their prey. While neither of these species may represent natural opisthobranch predators, they still posed a potential threat and were expected to trigger a defense mechanism in the prey subject. Furthermore, both antagonists are carnivorous, abundant, and share the same habitat as the four opisthobranch species used in this study. There is also very little information available on natural opisthobranch predators.

For the purposes of this study, six hermit crabs were collected while snorkeling in Cook's Bay. Eight blacktail snappers were also caught off of the Gump Station dock using lunchmeat for bait and a bamboo fishing pole. Antagonists were not injured during collection and were maintained in tanks with continuously flowing seawater. They were returned to the site of capture at the conclusion of the experiment.

Experiment design

This experiment was conducted between 10/1/06 and 11/12/06. While in captivity, all opisthobranchs were kept in plastic containers. The sides of each container were punctured using a piece of heated metal to allow for adequate water flow through the container. The container lid was replaced with a rubber-banded mesh sheet to generate additional flow between the water in the container and the water in the tank. Each container contained one opisthobranch and one algae-covered rock to provide the subject with food. The containers were placed in a large outdoor tank. This was necessary because sunlight was needed for the algae to photosynthesize. Rocks were changed out twice weekly and were collected at the sites where opisthobranchs were found.

Each observation tank contained only natural rocky substrate collected where the opisthobranch was found, one antagonist, and one opisthobranch. The tanks used in the crab trials were large plastic containers with the lids removed. The tanks used in the fish trials had fresh salt water flowing though them constantly to ensure that the water contained enough oxygen to sustain the fish. Six subjects of each opisthobranch species were exposed to antagonists in three-hour intervals. I observed the actions of the prey subjects for the first and last half hour of each trial. Chi-square analyses were used to test for significant differences between crab and fish trials.

The opisthobranchs were first exposed to the hermit crabs. The hermit crab was placed in an observation tank and allowed to acclimate for a half hour. The opisthobranch was then placed in the tank in close proximity to the antagonist. Upon completion of the hermit crab trials, the same opisthobranch subjects were then exposed to the second group of antagonists, the blacktail snappers. The fish were also allowed to acclimate for a half hour before the trial. The fish were released after being kept in the lab for one to two days. The control experiment for both sets of trials followed the same time frame and set-up. The opisthobranch was left in the observation tank with a rock while no antagonist was present. The control trial lasted for three hours and opisthobranch behaviors were recorded for the first and last half hour of the trial.

RESULTS

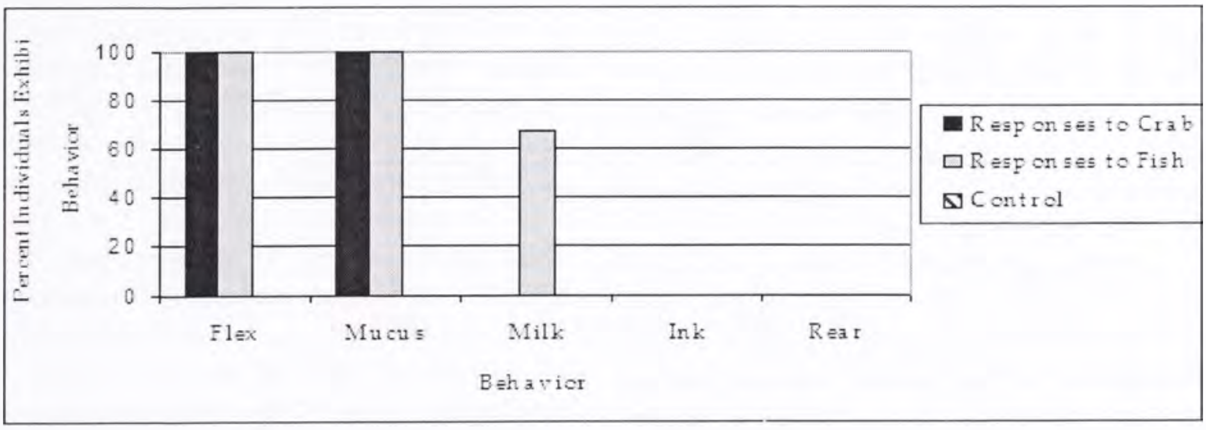


FIG. 7. Behaviors observed in *G. cincta*.

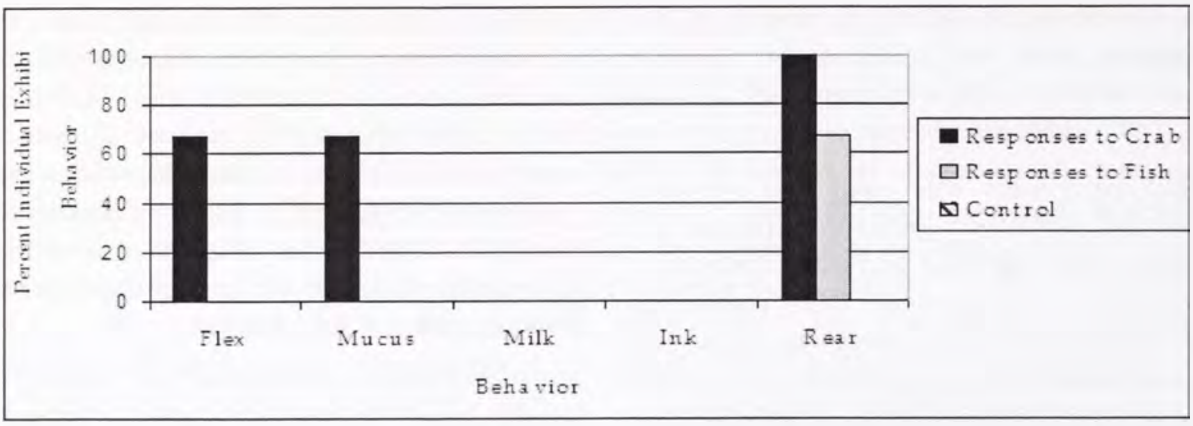


FIG. 8. Behaviors observed in *R. imperialis*.

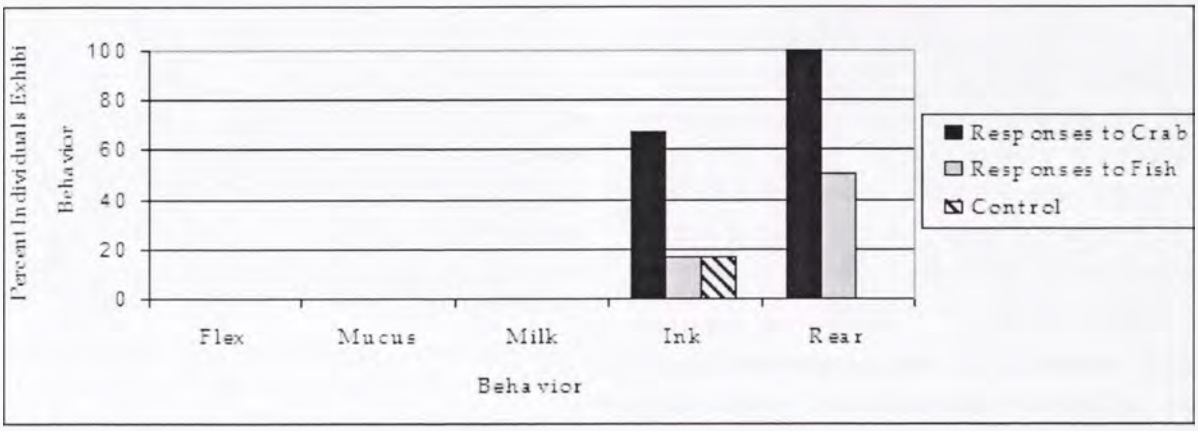


FIG. 9. Behaviors observed in *S. striatus*.

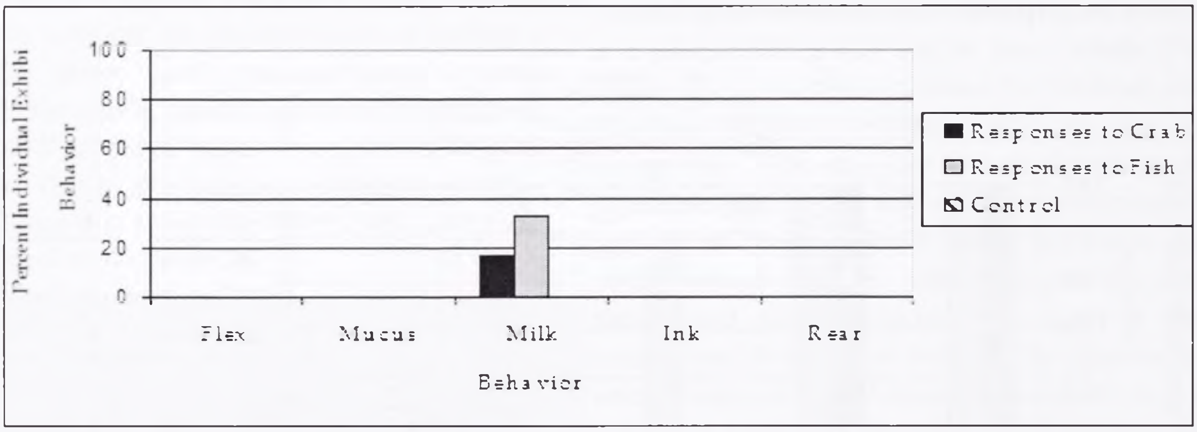


FIG. 10. Behaviors observed in *D. dolabrifera*.

In *G. cincta*, mantle flexations and mucus production occurred in all individuals during both crab and fish trials (FIG. 7). The secretion of a milky substance from the organism's mantle was seen in 67% of the fish trials and was not present in the crab trials (FIG. 7). Inking and rearing were absent in all trials (FIG. 7).

Mantle flexing and the production of mucus was observed in 67% of the *R. imperials* specimens during the crab trials (FIG. 8). Both behaviors were absent in the fish trials (FIG. 8). Rearing was seen in both crab and fish trials, but was more frequent in the crab trials (FIG. 8). Inking and mantle secretions were not present in any *R. imperialis* individuals (FIG. 7).

In *S. striatus*, rearing and inking were present in both crab and fish trials, though both behaviors were more prevalent in the crab trials (FIG. 9). Inking also occurred once in the control setting (FIG. 9). Mantle flexations, mucus production, and mantle secretions were absent from all trials (FIG. 9).

The only behavior recorded in *D. dolabrifera* was the secretion of a white substance from the organism's mantle (FIG. 10). This occurred more frequently in the fish trials, but was also present during the crab trials (FIG. 10).

Chi-square analyses reflected no significant differences between crab and fish trials.

DISCUSSION

Defensive displays

The displays I observed are mantle flexation, mucus production, mantle secretion, inking, and rearing.

Mantle flexations were only observed in the two nudibranch species (*G. cincta* and *R. imperialis*). Although *G. cincta* appeared to react to both antagonists, it is interesting that *R. imperialis* only displayed this behavior in the presence of the crab antagonist. A mantle flexation involved the nudibranch lengthening and twisting its body. It appeared to flex so that its head was directed downward, foot twisted, and the body's posterior portion faced up. During this display, the organism's gills and rhinophores were alert and moving. This may indicate that the organism was taking in sensory information about its environment and, perhaps, its antagonist. Little has been written on mantle flexations, though I speculate that the behavior may be a defensive display that allows individuals to appear longer or bigger in the presence of an antagonistic force. This theory is supported an earlier observation that when a nudibranch is disturbed there is a general contraction, which causes the mantle edge to appear more prominent (Crozier 1916).

Like mantle flexing, mucus production also occurred only within the two nudibranch species. It appeared to co-occur with the flexing display in both *G. cincta* and *R. imperialis*. This is interesting because perhaps the flexing display is a necessary physiological component of mucus production in these nudibranch species. Mucus production is known to be element of locomotion in opisthobranchs, but its role in predator defense has been understudied. The distinct mucus trails left by *G. cincta* and *R. imperialis* appeared to be entirely different from the clear, gel-like substance usually associated with locomotion (Behrens 2005). Additionally, *R. imperialis* produced mucus only during the crab trials, possibly indicating that the organism was more stressed in the situation with the more tactile of the two antagonists.

Both *G. cincta* and *D. dolabrifera* were observed secreting a white, milky substance from their mantles when an antagonist was present. This is interesting because *G. cincta* is a nudibranch and *D. dolabrifera* is a sea hare. It is unclear as to whether the two excretions contained the same compound, though it is thought that such secretions are associated with the sponges that the opisthobranchs feed on (Becerro et al. 2006). Additionally, opisthobranchs are known for secreting chemical compounds that deter predators (Gosliner et al. 1996). I grouped both occurrences as one behavior, since they appear to be the product of the same situational cause—the milky substance was only secreted in the presence of an antagonist. Interestingly, the substance was secreted more often in the presence of the fish antagonist. Perhaps a chemical cue from the fish triggered this reaction. This theory is supported by the observation that mantle secretions occurred almost immediately after the opisthobranch was placed in the tank with the antagonist.

Inking was observed only in *S. striatus* and occurred more frequently in the crab trials than in the fish trials. This could suggest that the organism was more stressed in the situation with the more exploratory and tactile antagonist. Inking is a known defense mechanism in sea hares, and it is thought that the ink distracts a predator long enough for the prey to flee (Rodhouse 1998). This behavior is noted in many other mollusks, perhaps most famously in octopuses (Rodhouse 1998).

Rearing is a behavior associated with repulsion or escape (Behrens 2005). The posture involved the posterior region of the opisthobranch remaining attached to a surface while its mantle and anterior region was lifted up. During this display the organism's oral tentacles were highly active. Because of this, I speculate that this display does not only signal repulsion, but may also be involved in obtaining sensory information about the organism's environment. The behavior was only seen in *R. imperialis* and *S. striatus*. Based on a fundamental understanding of the body plans of all of the observed species, this is not surprising. Both of these species have a defined neck-like region of the mantle, which is absent from the other two species. Interestingly, rearing was seen in all *R. imperialis* and *S. striatus* individuals during the crab trials, but was less prevalent in the fish trials. Perhaps the sensory information about the crab was more accessible, and therefore, specimens were more active during these trials.

Conclusions

Because many behaviors are poorly understood in opisthobranchs, information regarding why these animals act as they do in the presence of antagonistic organisms is relevant to better understanding the physiology and effectiveness of defensive displays.

I expected to observe a variety of defensive behaviors in the four opisthobranch species studied. It is interesting that no species exhibited every behavior recorded in this study. This may be due the differing physiologies of each species.

Though I expected to see the same behaviors exhibited by more closely related species, this was only true some of the time. For example, mantle flexing and the production of distinct mucus trails were only seen in the two nudibranch species (*G. cincta* and *R. imperialis*). Perhaps this behavior is physiologically impossible for the other two species to exhibit, or perhaps they excrete a less visible form of mucus that does not co-occur with a highly visible display. On the other hand, the secretion of a milky discharge was observed in both lineages (in *G. cincta* and *D. dolabrifera*). The secreted compounds could indicate that the nudibranch and sea hare feed on similar sponges and may be parts of similar trophic systems. Or perhaps the display evolved in more than once because it proved to be an effective defense against predation. Rearing was also seen across lineages (in *R. imperialis* and *S. striatus*). Perhaps the behavior evolved in both species because their similar physiologies allowed for sensory information to be collected in a similar way.

I also expected that the defensive behaviors exhibited would be different in the presence of different antagonists. While there was a differential response for many species between the two antagonists, the particular frequency of responses was species-specific. It was interesting to find that in certain cases, there was a differential response of prey to different antagonists. For example, *G. cincta* only exhibited a milky secretion in the presence of the fish antagonist, and *R. imperialis* only flexed and produced mucus in the presence of the crab antagonist. This might be attributed to the organism experiencing more stress in one particular interaction.

For the most part, none of the defensive behaviors occurred in the absence of an antagonist. This suggests that all the observed behaviors are defensive displays, or at the very least, are in response to the presence of another organism in the tank. Only one individual inked in at the start of the control trial, indicating that the individual was stressed during the transfer from the holding tank to the observation tank.

Although chi-square analyses revealed no significant differences between crab and fish trials, differences in behavioral responses are clearly visible in graphs of the original data. Perhaps a larger sample size and further analysis would show a more statistically sound trend.

This paper sought to examine the various defensive behaviors exhibited by four species of opisthobranchs. Further research on the natural predators of these species, if any exist, is necessary in better understanding how they fit into their trophic system and ecology. Additionally, it would be interesting to see if any of the recorded displays are prevalent in behavioral arenas other than defense, such as mating, predation, and intraspecific communication.

ACKNOWLEDGEMENTS

I thank James Bartolome, Roy Caldwell, Terrence Gosliner, Carole Hickman, Jere Lipps, Brent Mishler, Elizabeth Perotti, Alison Purcell, Erica Spotswood, and Vince Resh for their guidance and collaboration throughout this project. I also thank Thomas Bell, Jessica Castillo, Sarah Chinn, Andrew Greene, Margaret Groff, and Julie Himes for their assistance in collections.

LITERATURE CITED

Avila, C. 1995. Natural products of opisthobranch molluscs: a biological review. Oceanography and Marine Biology: an Annual Review 33: 487-559.

Becerro, M.A., J.A. Starmer, and V.J. Paul. 2006. Chemical Defenses of Cryptic and Aposematic Gastropterid Molluscs Feeding on their Host Sponge *Dysidea granulosa*. Journal of Chemical Ecology 32: 1491-1500.

Behrens, D.W. 2005. Nudibranch Behavior. New World Publications, Jacksonville, FL.

Crozier, W.J. 1916. On the Immunity Coloration of Some Nudibranchs. Proceedings of the National Academy of Sciences 2: 672-675.

Faulkner, D.J. and M.T. Ghiselin. 1983. Chemical defense and evolutionary ecology of dorid nudibranchs and some other opisthobranch gastropods. Marine Ecology, Progress Series: 13 (2-3): 295-301.

Gimenez-Casalduero F., R.W. Thaker, and V.J. Paul. 1999. Association of color and feeding deterrence by tropical reef fishes. Chemoecology 9: 33-39.

Gosliner, T.M., D.W. Behrens, and G.C. Williams. 1996. Coral Reef Animals of the Indo-Pacific. Sea Challengers, Monterey, CA.

Johnson, Rebecca. 1994. A Phylogeny of Moorean Nudibranchs with a test of nudibranch palatability. Biology and Geomorphology of Tropical Islands Class Papers 1994: 98-109.

Lindstrom, L. 2001. Experimental approaches to studying the initial evolution of conspicuous aposematic signaling. Evolutionary Ecology 13: 605-618.

Long, J.D., and M.E. Hay. 2006. Fishes learn aversions to a nudibranch's chemical defense. Marine Ecology Progress Series 307: 199-208.

Mallet, J. and M. Joron. 1999. Evolution of Diversity in Warning Color and Mimicry: Polymorphisms, Shifting Balance, and Speciation. Annual Review of Ecology and Systematics 30: 201-233.

Penney, B.K. 2004. Individual selection and the evolution of chemical defense in nudibranchs: experiments with whole *Cadlina luteomarginata*. Journal of Molluscan Studies 704: 399-402.

Prince J.S., and P.M. Johnson. 2006. Ultrastructural comparison of *Aplysia* and *Dolabrifera* ink glands suggests cellular sites of anti-predator protein production and algal pigment processing. Journal of Molluscan Studies 72: 349-357.

Rodhouse, P.G. 1998. Physiological progenesis in cephalopod molluscs. Biological Bulletin 195 (1): 17-20.

Rogers, S.D., and V.J. Paul. 1991. Chemical Defenses of 3 *Glossodoris*-Nudibranchs and Their Dietary Hyrtios Sponges. Marine Ecology Progress Series 77 (2-3): 221-232.

Rosenberg, G. 1989. Aposematism Evolves by Individual Selection: Evidence from Marine Gastropods with Pelagic Larvae. Evolution 483: 1811-1813.

Rudman, W. B. 2000. *Glossodoris cincta* (Bergh, 1888). Sea Slug Forum. Australian Museum, Sydney. www.seaslugforum.net/factsheet.cfm?base=gloscinc

Rudman, W. B. 2003. *Dolabrifera dolabrifera* (Cuvier, 1817). Sea Slug Forum. Australian Museum, Sydney. www.seaslugforum.net/factsheet.cfm?base=doladola

Servedio, M.R. 2000. The Effects of Predator Learning, Forgetting, and Recognition Errors on the Evolution of Warning Coloration. Evolution, 543: 751-763.

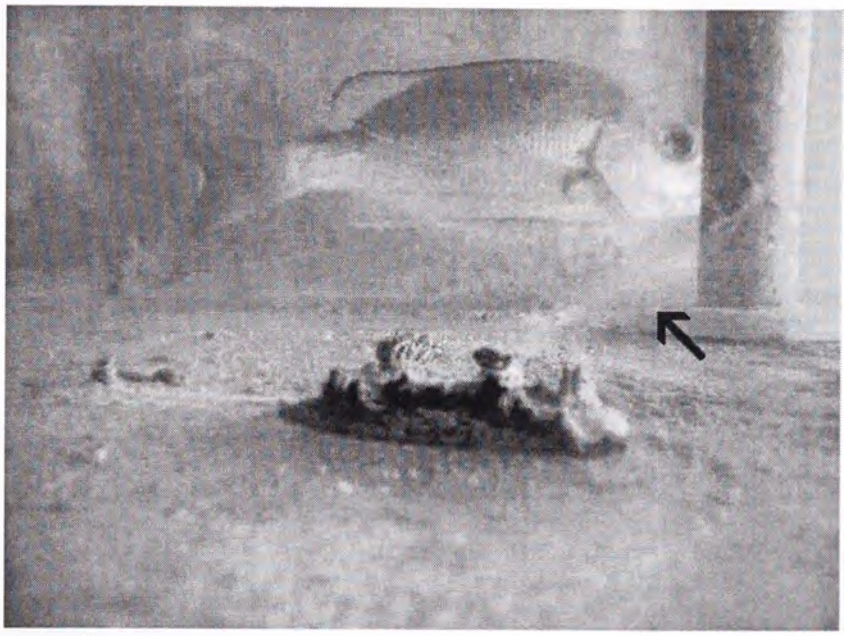
Tullrot, A. 1994. The Evolution of Unpalatability and Warning Coloration in Soft-Bodied Marine Invertebrates. Evolution 483: 925-928.

Appendix A

The following list provides photograph and brief description of some of the defensive displays observed in this study.

Mantle flexing by *G. cincta* in the presence of *D. lagopodes*.

One *D. lagopodes* inspects a distinct mucus trail left by *G. cincta*.

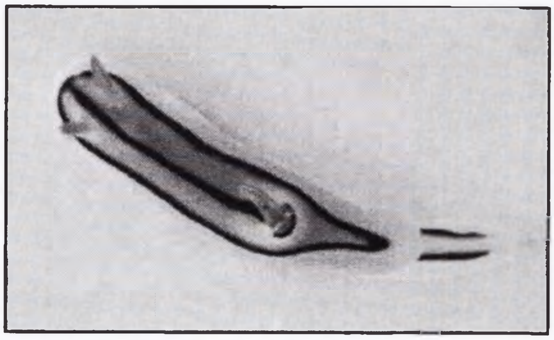

A *G. cincta* specimen secretes a milky discharge in the presence of *L. fulvus*.


Rearing display by *S. striatus* in the presence of *D. lagopodes*.

Appendix B

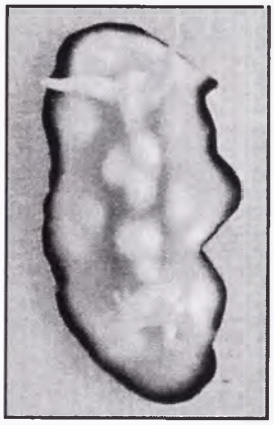
The following list provides a photograph (left) and brief description (right) of every opisthobranch species I encountered during my time on Moorea, and is intended as a reference for future students. I do not presume that these are the only species present around the island, or that these species cannot be found at different depths or locations than I have specified.

***Cyerce elegans* (Bergh, 1870)**
Found: 5-10 feet, Cook's Bay
Length: 15mm
Primary color: Pale yellow/white
Distinguishing characteristics: bifurcate enrolled rhinophores; opaque cerata

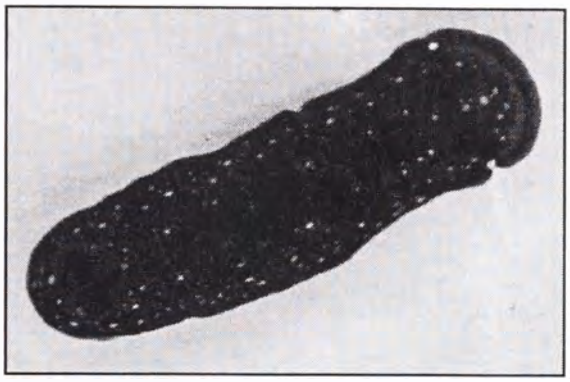


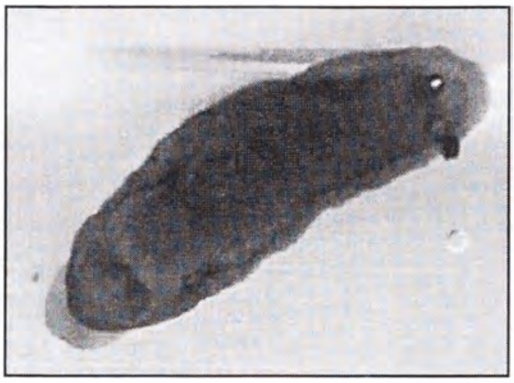
***Chromodoris lochi* (Rudman, 1982)**
Found: 25-30 feet, Cook's Bay
Length: 35mm
Primary color: Light blue/white
Distinguishing characteristics: Black longitudinal lines; orange gills and rhinophores

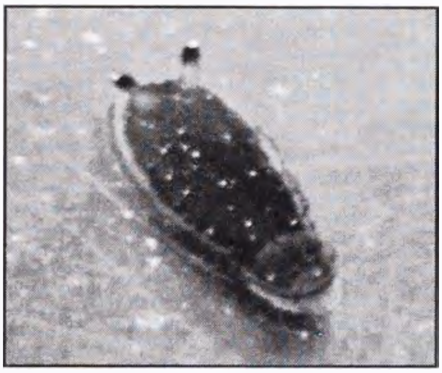
Chromodoris sp.1 (possibly closely related to *C. virbrata*)
Found: 5-10 feet, Cook's Bay
Length: 30mm
Primary color: White/yellow
Distinguishing characteristics: Dark purple mantle border, gills, and rhinophores



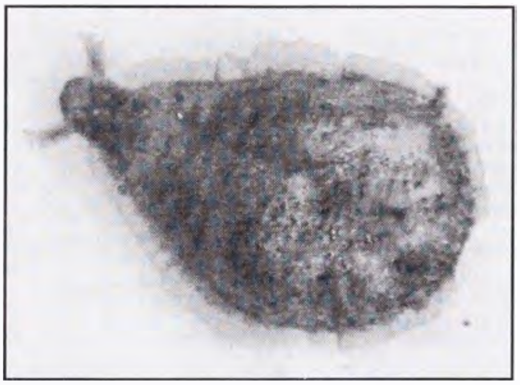
Chromodoris sp.2
Found: 5-10 feet, Cook's Bay
Length: 30mm
Primary color: Yellow
Distinguishing characteristics: White marks, gills, and rhinophores; dark purple mantle border



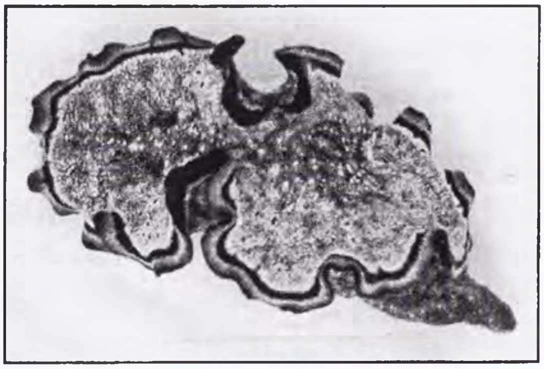




Dendrodoris nigra **(Stimpson, 1855)**
Found: 5-10 feet, Cook's Bay
Length: 25mm (adult)
Primary color: Black (above); Dark brown (juvenile, left)
Distinguishing characteristics: White tips of rhinophores; white spots along mantle; red mantle border (juvenile)



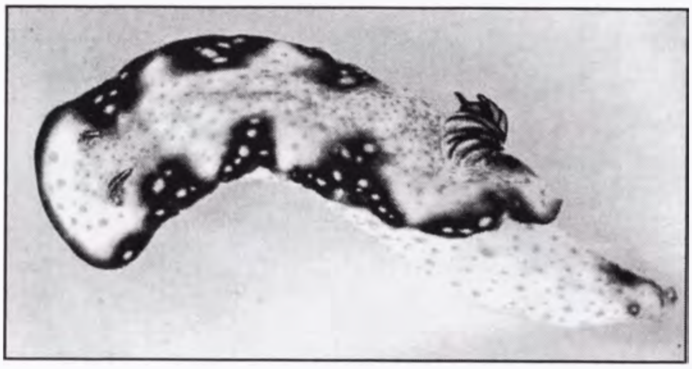
Dolabrifera dolabrifera **(Cuvier, 1817)**
Found: 5-10 feet, Cook's Bay
Length: 20mm
Primary color: Mottled green
Distinguishing characteristics: Flat; rounded; body narrows closer to head



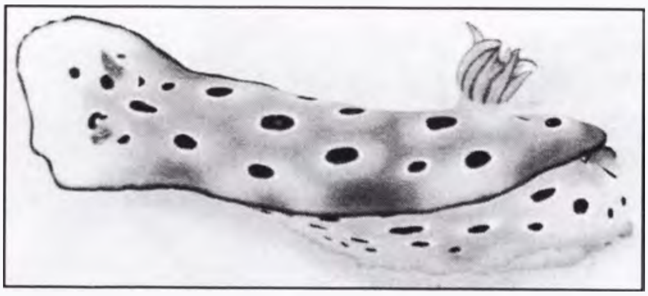
Glossodoris cincta **(Bergh, 1888)**
Found: 5-10 feet, Cook's Bay
Length: 50mm
Primary color: Mottled pink/brown
Distinguishing characteristics: Dark black/blue mantle borders; rhinophores and gills are dark brown speckled with pink

Hexabranchus sanguineus **(Ruppell & Leuckart, 1828)**
Found: 5-10 feet, Cook's Bay
Length: 35cm
Primary color: Red
Distinguishing characteristics: Swimmer; grows to large sizes; white mantle border; red with white gills
Photo courtesy of Sarah Chinn

Hypselodoris zephyra **(Gosliner & Johnson, 1999)**
Found: 4-6 feet, Opunohu Bay
Length: 25mm
Primary color: Beige/lavender
Distinguishing characteristics: Black longitudinal lines; orange rhinophores and gills

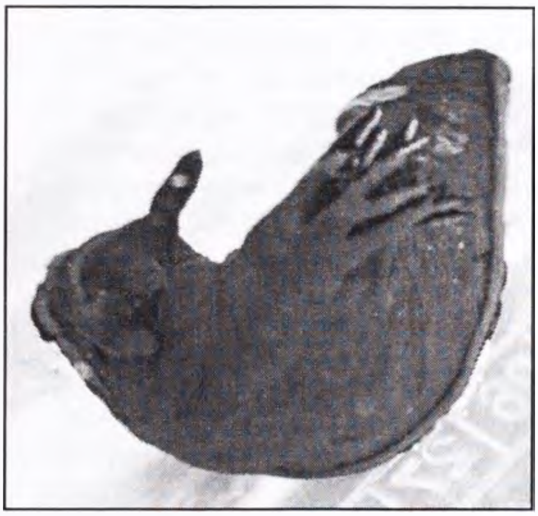


Risbecia imperialis **(Pease, 1860)**
Found: 5-10 feet, Cook's Bay
Length: 45mm
Primary color: White
Distinguishing characteristics: Yellow dots along mantle and in zigzagged blue mantle border; gills are white edged with dark blue; rhinophores are dark blue speckled with white



Risbecia tryoni **(Garrett, 1873)**
Found: 30 feet, Cook's Bay
Length: 60mm
Primary color: Crème/white
Distinguishing characteristics: Purple mantle border; dark spots on mantle; gills are crème edged in brown.

***Stylochelius striatus* (Quoy and Gaimard, 1832)**
Found: 5-10 feet, Cook's Bay
Length: 55mm
Primary color: Mottled green/brown
Distinguishing characteristics: Small royal blue dots along mantle; dark longitudinal lines



***Tambja morosa* (Bergh, 1877)**
Found: 30 feet, Cook's Bay
Length: 70mm
Primary color: Black
Distinguishing characteristics: Royal blue markings near mouth and along mantle border; green/blue gills

RESOURCE PARTITIONING BY WINTERING SHOREBIRDS: A BEHAVIORAL COMPARISON OF TWO SPECIES IN A TROPICAL ESTUARY

ANDREW D. GREENE

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

Abstract. Shorebirds often feed in multispecies groups that display interesting niche dynamics. On Moorea, French Polynesia, the Wandering Tattler (*Tringa incana*) and Pacific Golden Plover (*Pluvialis fulva*) are the dominant shorebird species during the northern winter. These species' feeding behavior was observed at the Temae estuary on the northeast side of the island. Relative abundance of the two species was determined using transect counts at the estuary and an adjacent beach. *T. incana* displayed more striking and sprinting behavior, while *P. fulva* displayed more picking and walking behavior. *T. incana* also consumed more crabs than *P. fulva*. The two species existed in relatively equal abundance in the estuary; *T. incana* was more common on the beach. Though these data suggest some differences in feeding niche, a great deal of overlap was observed. The degree of niche partitioning appears to be greater in this study than in similar studies conducted on these species' breeding grounds.

Key words: feeding behavior; Pluvialis fulva; Tringa incana; *Moorea, French Polynesia; resource partitioning; estuary*

INTRODUCTION

The niche concept, first suggested in the early 1900s, evolved greatly in the first half of the 20th century (Vandermeer 1972). Hutchinson (1957) defined the niche as the place where all environmental variables are within the survival limits of a given species. Gause's axiom states that no two species can inhabit the same niche (Vandermeer 1972). Schoener (1974) found that the most important niche parameters that segregate species are habitat, food type, and timing of habitat use.

Shorebirds often form dense, multispecies aggregations, making them a good model system for the study of niche dynamics. Resource partitioning, particularly regarding feeding behavior, has been studied extensively in shorebirds. For example, Thomas & Dartnall (1970) showed that when multiple species of sandpipers feed in one area, they segregate by species into different microhabitats. These microhabitats reflect the spatial distribution of each bird species' preferred prey (Ribeiro et al. 2004).

The migratory nature of many shorebirds' life histories presents an extra factor in determining the extent of niche partitioning. Holmes and Pitelka (1968) found that the diets of four sympatric sandpipers overlapped considerably during breeding season on the Arctic tundra. During migration, shorebirds are often opportunistic feeders with a great deal of dietary flexibility (Davis & Smith 2001, Isacch et al. 2005). Many species come together during this period, forming multispecies aggregations in which competition for food intensifies (Recher 1966). Baker & Baker (1973) found that shorebirds are usually pushed into a narrower, more specialized feeding niche during winter due to food scarcity. Because of this, tropical wintering grounds are an ideal place to study niche partitioning in migratory shorebirds.

The island of Moorea, in French Polynesia, hosts wintering Wandering Tattlers (*Tringa incana*, (Gmelin 1789)) and Pacific Golden Plovers (*Pluvialis fulva*, Gmelin 1789), both of which nest on Arctic tundra during the northern summer. The two species are not closely related, belonging to different families in the order Charadriiformes. *P. fulva* is a member of Charadriidae, the plover and lapwing group; *T. incana* is a member of Scolopacidae, the sandpiper group. The two species are also significantly different morphologically: *T. incana* has a long, narrow bill, while *P. fulva* has a shorter, thicker bill.

Despite these differences, the two species seem to inhabit superficially similar niches at the Temae beach and estuary on Moorea. Moreover, these species show little difference in resource utilization during the breeding season, both feeding almost exclusively on insects (Johnson and Conners 1996, Gill et al. 2002). The purpose of this study was to identify significant differences in resource utilization of these two species on their wintering grounds by studying their feeding behavior and distribution within a small study site.

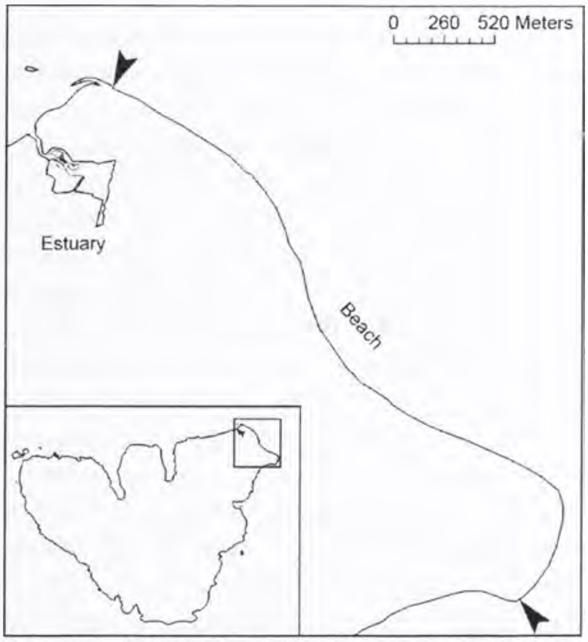


FIG. 1. Map of study site. Inset shows position of site relative to the entire island of Moorea. Arrows indicate boundaries of the beach transect.

METHODS

Study site

All behavioral observations were made at the Temae estuary, in the northeast corner of Moorea (Fig. 1). This site was selected because both species were present in sufficient numbers to conduct a behavioral study there. The estuary is 350 m long and 200 m wide. The water depth in the estuary averages 20-30 cm. The substrate is primarily calcium carbonate sand (L. LaRue, pers. comm.). The eastern half of the estuary is heavily impacted by runoff by a nearby golf course (LaRue 2006), so behavioral observations and transect counts were limited to the western half.

Transect counts were also carried out along a 4 km stretch of beach adjacent to the Temae estuary.

The substrate type along the shore is a mixture of coral sand, loose coral rubble, and aggregated coral rubble. The reef crest is approximately 40 m from the shore along the entire length of the study area.

Study organisms

The Wandering Tattler (*Tringa incana*) and Pacific Golden Plover (*Pluvialis fulva*) are the dominant migratory shorebird species found on Moorea during the northern winter. They are also the only shorebirds commonly found at the Temae estuary. *P. fulva* breeds in northeastern Siberia and western Alaska, whereas *T. incana* breeds only in Alaska (Johnson and Conners 1996, Gill et al. 2002). Both have large winter ranges that extend from the California coast to the Pacific islands. Both also are found in a wide array of open habitats within this range (Johnson and Conners 1996, Gill et al. 2002). *T. incana*, however, more strongly favors rocky intertidal habitats for foraging (Gill et al. 2002).

Behavioral sampling

Feeding behavior of individual birds was observed in 10-minute observation sessions.

Observations were made through 8-power binoculars. The estuary was arbitrarily divided into 3 zones: the south shore, the north shore, and the islands in between. In a typical morning or evening of observations, one bird of each species was observed in each zone. When a zone was sampled, an actively-foraging bird was randomly selected within it and observed for 10 minutes. The nearest individual of the other species was then observed as well. This procedure was designed to avoid biased sampling of any one section of the estuary.

A systematic procedure was followed for the behavioral observations. When an individual bird was selected for observation, the date and time were noted, as well as the bird's location. The following pieces of data were recorded each minute for 10 minutes thereafter:

- Distance of bird from shore: measured in meters. Positive numbers indicated the bird was on land, negative numbers indicated the bird was in the water.
- Distance moved: The distance the bird moved, in meters, from the previous minute's observation.
- Water depth: The bird's depth in the water, measured in centimeters and based on the proportion of the bird's legs that were submerged. A value of 0 indicated the bird was on land.
- Behavior type: Behavior type was assigned based on the dominant behavior during the last ten seconds of each minute. A behavior category was chosen from the following list of stereotyped behaviors:
    - Scanning: Bird is visually searching for prey, but bill use is negligible.
    - Picking: Bird is using bill to pick items off the substrate.
    - Striking: Bird is aggressively using bill to strike a prey item.
    - Eating: Bird is manipulating and/or consuming a recently-caught prey item.
    - Preening: Bird is using bill to groom feathers, or is bathing.
    - Resting: Bird has ceased activity, and bill is tucked between wings.
- Movement class: Bird was noted to be standing still, walking, running, or sprinting. As with the behavior types above, the movement class was assigned based on the dominant movement class during the last ten seconds of each minute.

In addition to these specific behavioral data, incidental observations were also recorded. These included any interactions with other birds, the distance of any flights made, and, when possible, type of prey caught. After the 10 minutes had elapsed, a GPS waypoint was recorded at the bird's location.

A total of 46 10-minute observation sessions were carried out for *P. fulva* and 49 for *T. incana*. Equal numbers of observations were made in the morning (5:00-9:00 am) and evening (4:00-6:00 pm), when the birds were most active. Five observations of each species were also made during the middle of the day, but midday observations were terminated because the birds were less active during this time.

In order to statistically compare different observation sessions, the mean distance moved per minute, mean distance to shore, and mean water depth were all calculated for each session. The frequencies of each behavior and movement type were also determined for each session. A series of Student's t-tests were used to determine the significance of any differences in these means and frequencies between the two species. These tests were performed using JMP software (SAS Inc. 2004).

Transect counts

In order to determine the relative abundance of the two species studied, transect counts were performed both in the estuary and along the shoreline. In the estuary, two parallel 225 meter transects were performed, one on the north shore, the other on the south shore. Each visible individual of the two species was marked on a map of the estuary as it was seen. The two transects were sufficient to cover the east side of the estuary, therefore every individual within that area was counted. This procedure was carried out twice, once in the morning, once in the evening.

A 4 km linear transect count was performed along the beach north of the estuary (see Fig. 1). Every bird seen was noted in a notebook and a GPS unit was used to mark its location. GIS software was used to project these points onto a map of the shoreline. This procedure was also performed twice.

The large difference in the length of the transects (225 m vs. 4 km) made direct comparison of the results impossible. To make comparison possible, two random 225 m pieces were selected from each of the beach transects, and these were compared directly to the two 225 m transects from each of the estuary counts.

RESULTS

Behavioral Sampling

T-tests determined the significance of the differences between the two species' positions and movement rates while foraging. *T. incana* had a significantly higher mean water depth while foraging (p=0.0004) (Fig. 2). *P. fulva* on average was farther inland, though not significantly. *T. incana* had a slightly higher movement rate, but this difference was also not significant.

There were significant differences in the frequencies of the various behavior types between the two species. The frequency of scanning behavior was significantly higher in *T. incana* (p=0.0041) (Fig. 3). Picking behavior was significantly more frequent in *P. fulva* (p=0.0003). The rarely-seen striking behavior was observed only in *T. incana*, and this was consequently significant (p=0.0007). There were no significant differences in the frequency of eating, preening, or resting behaviors between the two species.

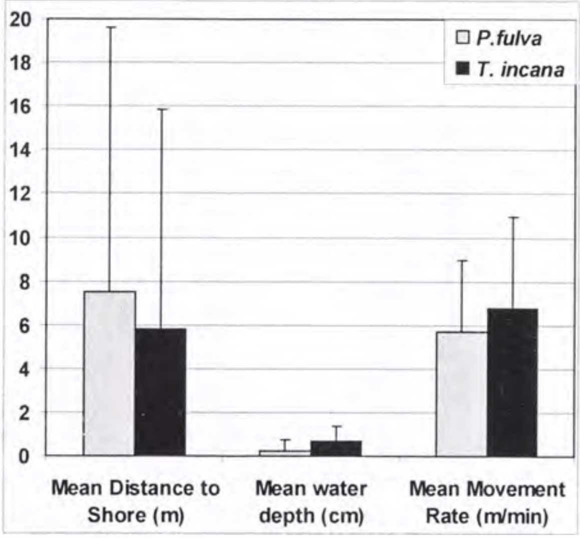


FIG. 2. Mean distance to shore, water depth, and movement rate during feeding observations, by species.

Among movement classes (Fig. 4), there were no significant differences in the amount of time spent still or running between the two species. Walking was slightly, yet significantly, more common in *P. fulva* (p=0.0493). Sprinting was much more frequent in *T. incana* (p=0.0002).

Transect Counts

Table 1 shows the number of individuals of each species counted during the estuary transects and in an equivalent length of beach transects. Density for both species was much higher in the estuary. The proportional abundance of *P. fulva* was slightly higher in the estuary, while *T. incana* was more abundant along the shore. Figures 5 and 6 show the results of one estuary transect and one beach transect, respectively.

Incidental Observations

During the course of the behavioral observations, additional items were noted outside of the prescribed data collection scheme. The identity of prey items was the most important of these. Small crabs were taken by both species. *T. incana* took

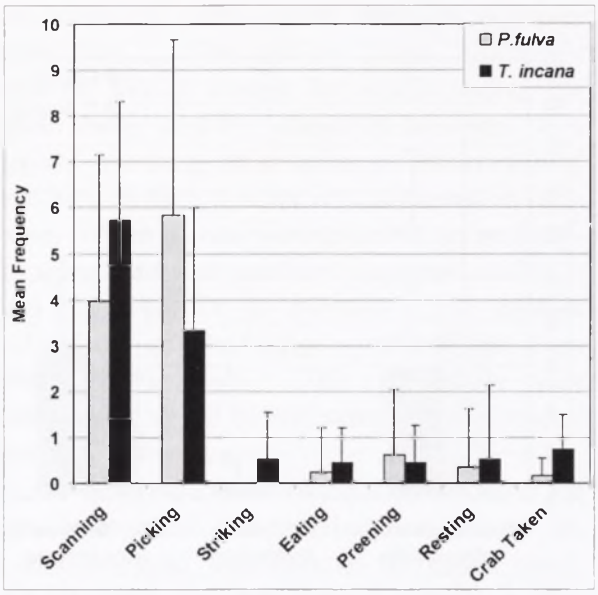


FIG. 3. Mean frequencies of behavior types, by species.

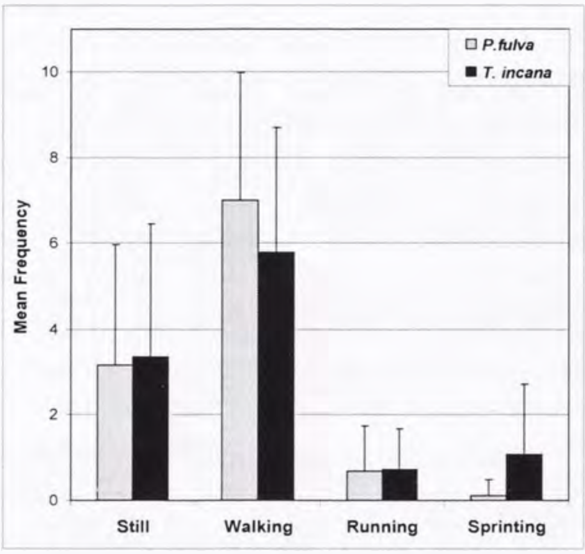


FIG. 4. Mean frequencies of movement types, by species.

Estuary Transects

	Transect 1	Transect 2
P. fulva	11	15
T. incana	10	11

Beach Transects

	Transect 1	Transect 2
P. fulva	0	1
T. incana	3	2

TABLE 1. Results of beach and estuary transects. Each value represents the number of birds seen along a 450 m transect length.

significantly more crabs than *P. fulva* ($p<0.0001$), averaging nearly one per 10-minute observation session (see Fig. 3). *P. fulva* typically took smaller, unidentifiable prey, though this could not be quantified. *P. fulva* was also seen pulling a worm out of a burrow in the substrate on two occasions. On four occasions, *P. fulva* was seen chasing *T. incana* after the latter had caught a crab.

DISCUSSION

These data clearly show a difference in feeding behavior between *P. fulva* and *T. incana*. The underlying food preferences of these species can be inferred from these behavioral differences. The dominant behavior regime displayed by *P. fulva*, deliberate walking and picking, suggests a preference for non-mobile prey. Byrkjedal & Thompson (1998) report that, when foraging on estuarine wintering grounds, *P. fulva* primarily take snails, bivalves, insects, and amphipods. Since the food items taken by *P. fulva* were generally too small to see, they were likely amphipods, insects, and perhaps very small snails. Although Kato et al. (2000) characterized polychaete worms as the most important food item for *P. fulva*, consumption of such worms was observed only twice in the course of the present study. *T. incana* showed a higher frequency of scanning, striking and sprinting. These behaviors all suggest a preference for larger, more mobile prey. The small burrowing crabs that are common in the estuary are one such prey item. Indeed, the behaviors observed are reflected in this species' preference for crabs.

The distributional data show a higher density of *T. incana* along the shoreline, which is consistent with its preference for intertidal habitat (Gill et al. 2002). Though feeding behavior was not observed on the beach, it is reasonable to infer that prey availability has some affect on the two species' distributions. Crabs were observed on the beach, although

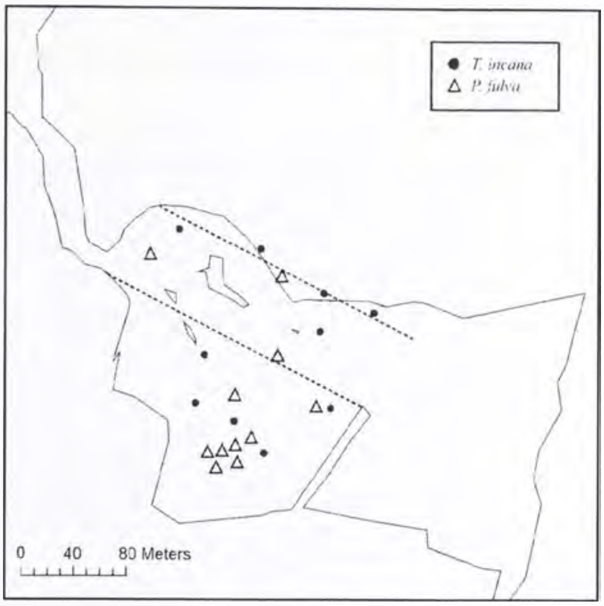


FIG. 5. Map showing birds counted during one estuary transect (n = 21). Dashed lines represent the location of the transects. Map from LaRue 2006.

FIG 6. Map showing results of one beach transect. Cross symbols represent the beginning and end of transect.

not in the same density as in the estuary. The beach's rockier substrate likely did not support the same suite of small prey organisms favored by *P. fulva* in the estuary.

Many studies (Thomas & Dartnall 1970, Davis & Smith 2001, Skagen and Oman 1996, Isacch et al. 2005) have emphasized that shorebirds, as a group, display a great deal of opportunism and flexibility in their feeding habits. The apparent attempted kleptoparasitism observed by *P. fulva* on *T. incana* is one example of such opportunism. These species also showed a degree of dietary flexibility: crabs were occasionally taken by *P. fulva*, and *T. incana* spent an appreciable amount of its foraging time picking small items off the substrate. Therefore, the two species do not occupy entirely separate feeding niches.

Recher (1966) suggested that competitive exclusion does not occur readily in migrant shorebirds due to both relative abundance of food in their habitats and the temporary nature of their sympatry. In this case, *T. incana* and *P. fulva* may not occupy exclusive feeding niches because they share a relatively rich feeding habitat and do so only seasonally.

In conclusion, the degree of overlap in resource utilization by these species clearly varies throughout the year. The degree of niche partitioning between these two species, while not absolute, is higher on wintering grounds than on breeding grounds, which is consistent with the pattern established by Baker & Baker (1973). Further study is needed to determine whether this pattern can be applied to other shorebirds, and perhaps to other migratory bird groups as well.

ACKNOWLEDGMENTS

I thank all of the instructors of the 2006 Moorea field course for guidance on the data collection and analysis techniques used in this paper. Thanks also to Lori LaRue for assistance in the field and for sharing maps and data about the estuary. Thanks as well to Maggie Groff for assistance in gathering behavioral data. A special thanks to Sheri Lubin and Morgan Tingley for invaluable help in developing protocols for this project.

LITERATURE CITED

Baker, M. C. and A. M. Baker. 1973. Niche relationships among six species of shorebirds on their wintering and breeding ranges. Ecological Monographs **43**:193-212

Byrkjedal, I. and D. B. A. Thompson. 1998. Tundra Plovers. T&AD Poyser Ltd, London.

Davis, C. A. and L. M. Smith. 2001. Foraging strategies and niche dynamics of coexisting sandpipers at stopover sites in the southern Great Plains. The Auk **118(2)**:484-495.

Gill, R. E., B. J. McCaffery, and P. S. Tomkovich. 2002. Wandering Tattler (*Heteroscelus incanus*). *in* A. Poole and F. Gill, editors. The birds of North America, No. 642. The Birds of North America, Inc., Philadelphia, PA.

Holmes, R. T. and F. A. Pitelka. 1968. Food overlap among coexisting sandpipers on northern Alaskan Tundra. Systematic Zoology **17(3)**:305-318.

Hutchinson, G. E. 1957. Concluding Remarks. Cold Spring Harbor Symposia on Quantitative Biology **22**:415-427.

Isacch, J. P., C. A. Darrieu, and M. M. Martinez. 2005. Food abundance and dietary relationships among migratory shorebirds using grasslands during the non-breeding season. Waterbirds **28(2)**:238-245.

Johnson, O. W. and P. G. Connors. 1996. American Golden-Plover (*Pluvialis dominica*), Pacific Golden-Plover (*Pluvialis fulva*). *in* A. Poole and F. Gill, editors. The birds of North America. The Academy of Natural Sciences, Philadelphia, and The American Ornithologists Union, Washington, D.C.

Kato, K., K. Omori, and M. Yoneda. 2000. Feeding ecology of the Pacific Golden Plover (*Pluvialis fulva*) in the Sg. Labu River on the west coast of the Malay Peninsula. Hydrobiologia **437**:221-233.

LaRue, L. 2006. Sedimentation in Moorea's Temae estuary. Biology and Geomorphology of Tropical Islands, research paper. UC Berkeley.

Recher, H. F. 1996. Some aspects of the ecology of migrant shorebirds. Ecology **47(3)**: 393-407.

Ribeiro, P. D., O. O. Iribarne, D. Navarro and L. Jaureguy. 2004. Environmental heterogeneity, spatial segregation of prey, and the utilization of southwest Atlantic mudflats by migratory shorebirds. Ibis **146**:672-682

Schoener, T. W. 1974. Resource partitioning in ecological communities. Science **185**:27-39.

Skagen, S. K. and H. D. Oman. 1996. Dietary flexibility of shorebirds in the western hemisphere. Canadian Field-Naturalist **110(3)**:419-444.

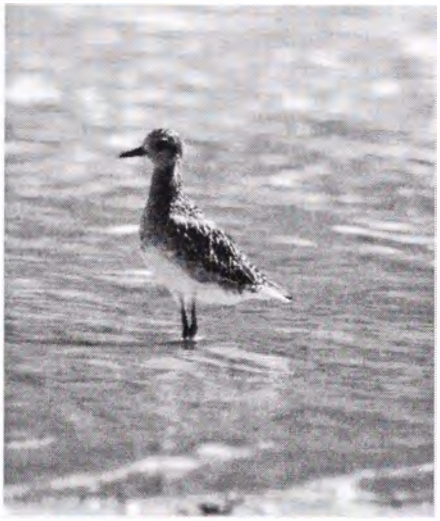
SAS Institute Inc. (2004) JMP: The Statistical Discovery Software, version 5.1.2.

Thomas, D. G. and A. J. Dartnall. 1970. Ecological aspects of the feeding behavior of two calidritine sandpipers wintering in south-eastern Tasmania. Emu **71**:20-26

Vandermeer, J. H. 1972. Niche theory. Annual review of Ecology and Systematics **3**:101-127.

APPENDIX A

Tringa incana



Pluvialis fulva

DOES THE RIVER CONTINUUM CONCEPT WORK IN SMALL ISLAND STREAMS? FUNCTIONAL FEEDING GROUP VARIATION ALONG A LONGITUDINAL GRADIENT

MARGARET H. GROFF

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

Abstract. The River Continuum Concept (RCC) predicts that as the form of particulate organic matter available in streams and rivers varies longitudinally, so will the functional feeding groups (FFGs) of benthic macroinvertebrates. The RCC was developed based on data from continental streams; therefore, its applicability to the unique ecology of island streams is virtually untested. The purpose of this study was to discover if the RCC works in the small streams of Moorea, French Polynesia. Three sites along an elevational gradient were sampled for benthic macroinvertebrates in five streams of similar catchment size. Each sample was sorted and all taxa were assigned to a FFG. Species richness and FFG variation along a longitudinal gradient were compared to RCC predictions. Patterns in the longitudinal variation of crustacean/mollusc species richness and shredder, grazer, and predator percent composition were found to match RCC predictions. However, total species richness, insect species richness, and the percent composition of collecting organisms did not. Therefore, an alternative theoretical framework is needed to accurately describe FFG variation in tropical streams.

Key words: River Continuum Concept, benthic macroinvertebrates, functional feeding groups, species richness, island streams, Moorea

INTRODUCTION

The River Continuum Concept (RCC) was proposed to explain the longitudinal variation of benthic macroinvertebrate communities in freshwater catchments (Vannote et. al. 1980). The RCC postulates that as stream size increases and the composition of available organic matter changes, the functional feeding groups (FFGs) will also change to reflect the increase in habitat diversity and the shift in food availability (Vannote et. al. 1980). The RCC assumes an abundance of macroinvertebrate taxa, especially insects, and a substantial catchment size.

Because the RCC was developed using data collected from large continental streams in North America, its usefulness to the unique biology of small island streams is largely untested. Island streams have many particular features that may affect the application of the RCC. First, their watersheds are generally small, so streams meet the ocean no larger than mid-sized, fifth-order streams. Second, the banks of island streams are often heavily vegetated, providing a continuous supply of organic matter, but frequent flooding washes away the accumulated plant material (Resh 1995). This is different from continental streams, where riparian vegetation is often reduced as stream order increases. Third, the majority of organisms found within island streams are not insects but diadromous crustaceans and molluscs (Greathouse 2006).

As with most other islands, it is unknown whether the RCC applies in the streams on Moorea, French Polynesia, an island located 18 kilometers west of Tahiti (Resh 1990). Most streams on Moorea are small, reaching only third order before reaching the Pacific. The Opunohu River to the north has the largest catchment on the island, reaching only fifth order as it enters Opunohu Bay.

The objectives of this study were to: (1) describe the macroinvertebrate fauna of the small streams of Moorea; (2) determine how the functional feeding groups of macroinvertebrates change along a longitudinal gradient in these streams; and (3) determine if the River Continuum Concept applies to the streams on Moorea. I postulate that the RCC will not apply on Moorea because of its low aquatic insect diversity and the diadromous nature of the majority of its in-stream fauna.

METHODS

Study sites

Five streams with similar catchment sizes were selected from different regions of the island (Fig. 1). Each stream was divided into three different sites: (1) a low-gradient, 50-meter reach starting at the first riffle from the mouth; (2) a high-gradient, 25-meter mid-reach; and (3) a high-gradient, 12.5-meter reach at the headwaters, the highest reach possible to sample with a D-frame net. Each site was partitioned into three subsites based on stream morphology (riffle, run, pool, and step). The physical parameters describing each site are found in Table 1.

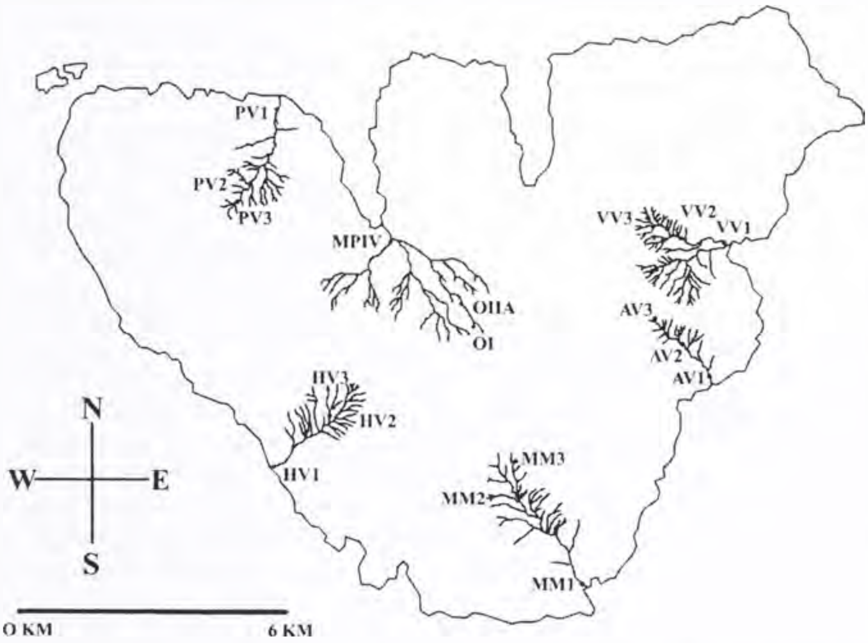


Fig. 1: Map of Moorea and 15 study sites

Maatea-Mahaerua (MM)

The town of Maatea is located on the east side of Moorea, just above the southernmost point of the island (Fig. 1). The Mahaerua River follows the Toto Valley, starting from the south-facing slope of Mount Tohiea, and meets the Pacific as a third-order stream at the southwest end of Maatea. Site MM1 is located approximately 109 meters upstream from the circum-island road. The furthest downstream reach is bisected by an artificial riffle and both banks are leveed. The morphology is primarily composed of runs (78%) and a large shallow pool (20%) created by the riffle (Table 1). Flow is highly influenced by the tide, causing the depth to fluctuate approximately 20 cm every few minutes, but the salinity is unaffected (Table 1).

Sites MM2 and MM3 were both accessed by the road approximately 150 meters from the left bank of the Mahaerua; a tall brick wall is to the south, a convenience store with a large Hinano sign is to the north. Site MM2 is located 1.5 kilometers from the mouth. It is reached by following the road past a farm to a large dip in the road before a steep hill. A small tributary stream bisects the road at this point. The sampled reach extends 25 meters upstream from the confluence. Site MM3 is 2.2 kilometers from the mouth of the Mahaerua. It is accessed by hiking upstream from the end of the road for approximately 15 minutes. The right branch is followed at each confluence. A small island splits the channel in the upper eight meters of the 12.5-meter reach.

Papetoai-Vaihana (PV)

The town of Papetoai is located on the north side of Moorea, immediately west of the entrance to Opunohu Bay (Fig. 1). The Vaihana River flows from the northern face of Mount Tautuapae down the northwest facing Terahimaua Valley. The mouth of the Vaihana River outlets into the Pacific Ocean as a fourth-order stream just east of "Chez Kiri" in Papetoai. Site PV1 is approximately 30 meters upstream from the circum-island road. A road approximately 50 meters west of the mouth of the Vaihana leads to site PV2; it intersects the main road between a residence surrounded by tall hedges and a low grey-brick wall. It is one of two paved roads in Papetoai. Site PV2 is located 10m upstream from the end of this road. Site PV3 is reached by hiking approximately 2.5 hours upstream from site PV2. Site PV3 is unique from the other headwater sites sampled because the channel was restricted within a narrow bedrock canyon.

Vaiare-Vaipohe (VV)

The town of Vaiare is located on the east side of Moorea at the base of Vaiare Bay, near the Gare Maritime. The Vaipohe River flows from the ridge between Mount Tearai to the north and Mount Mouaputa to the south. Approximately twenty meters from the mouth

Table 1: Abiotic characteristics of study sites. Measurements were made from mid-October to mid-November 2006. Ru=run, Ri=riffle, P=pool, S=step. Opunohu data was collected by Resh et al. (1990)

Site	MM1	MM2	MM3	PV1	PV2	PV3
Reach length	50	25	12.5	50	25	12.5
pH	6	6	6	6	6	6
Conductivity (uS/cm)	122.9-132.6	89.7	95.6	123.5	95.5	93.2
Salinity (ppt)	0.1	0	0	0.1	0	0
Turbidity (NTU)	5.7	2.25	8.64	1.6	3.62	1.62
Width (m)	7.1	2.2	2.5	5	7.6	2
Depth (m)	.27-.62	.12-.45	.02-.18	.05-.15	.07-.76	0.25
Temperature in air/water (°C)	33/26.2	27/23.2	26/21.8	22/22	23/22	22/22.3
Elevation (m)	5	73	141	13	80	268
Distance from mouth (km)	0.109	1.5	2.19	0.03	0.94	2.29
Stream order	3	3	1	4	3	2
Morphology (%)	78Ru, 2Ri, 20P	30Ri, 25P, 45S	60Ri, 40S	35Ru, 65Ri	30Ri, 30P, 40S	45Ru, 25Ri, 30P

Site	VV1	VV2	VV3	AV1	AV2	AV3
Reach length	50	25	12.5	50	25	12.5
pH	6	6	6	5-6	6	7
Conductivity (uS/cm)	148	134.9	114.6	94.5	78.7	66.1
Salinity (ppt)	0.1	0.1	0.1	0	0	0
Turbidity (NTU)	3.94	2.43	2.4	2.8	7.8	3
Width (m)	2.5	3.5	2	3.6	3	2.7
Depth (m)	0.01	0.25	0.07	.07-.17	.08-.73	.1-.43
Temperature in air/water (°C)	33/25.5	29/24.1	27/23.5	26/24.4	25/23.7	25-22.9
Elevation (m)	11	75	190	1	50	311
Distance from mouth (km)	0.2	0.9	1.6	0.075	0.73	1.26
Stream order	4	4	2	2	2	1
Morphology (%)	45Ru, 55Ri	40Ru, 20Ri, 20P	80Ri, 20P	40Ru, 60Ri	20Ru, 60Ri, 20P	30P, 70S

Site	HV1	HV2	HV3	MPIV	OIIA	OI
Reach length	50	25	12.5	--	--	--
pH	6	6	6	7.6	7.6	9.2
Conductivity (uS/cm)	128.1	119.4	115.1	120	120	113
Salinity (ppt)	0.1	0.1	0.1	--	--	--
Turbidity (NTU)	3.6	1.5	4.5	--	--	--
Width (m)	6.5	2.4	1.2	12	2	5
Depth (m)	0.01	.09-.25	.05-.17	0.5-1.0	0.2-0.3	0.001-0.3
Temperature in air/water (°C)	28/25.1	25/24.2	24/23	25/23	27/23	26/26
Elevation (m)	16	118	225	2	122	335
Distance from mouth (km)	0.02	1	2	0.4	3.1	4.0
Stream order	3	2	1	4	2	1
Morphology (%)	40Ru, 60Ri	50Ru, 20Ri, 30P	40Ru, 20Ri, 40 P	--	--	--

it intersects the Nuuati, entering the Pacific Ocean as a fifth-order stream. Site VV2 is approximately 200 meters from the mouth. The reach extends 50 meters from the upstream side of the bridge on the road immediately to the right of the mouth. Site VV2 is reached by following the road to the head of the Vaiare-Paopao cross-island trail. It is to the right of the end of the road through the stand of coconut palms. Site VV3 is reached by following the main channel upstream about 45 minutes from site VV2. The reach extends from the base of a four-meter waterfall 12.5 meters downstream.

Afareiaitu-Vaioro (AV)

The town of Afareiatu is on the eastern side of Moorea. The Vaioro River flows from the southeast face of Mount Mouaputa and meets the Pacific Ocean as a second-order stream at the north end of Afareiatu. Site AV1 is 75 meters upstream from the mouth. There was significantly more garbage within the reach than observed at other low-gradient sites. Site AV2 is approximately 750 meters from the mouth of the Vaioro River and extends 25 meters upstream from the base of the trail to the top of Mouaputa. The head of the Mouaputa trail branches off to the left across the stream from the trail to the Vaioro waterfall. Site AV3 is reached by hiking for approximately two hours up the Mouaputa trail. The trail follows the stream for most of its length. The sampling site is located just downstream of the confluence where the trail leaves the stream and extends up a steep hill with a coconut palm at the top. The trail crosses just upstream of the site, but causes minimum impact.

Haapiti-Vairemu (HV)

The town of Haapiti is located on the western side of Moorea. The Vairemu River runs from the southwest face of Mount Mouapu and enters the Pacific Ocean as a third order stream. The mouth is located in the middle of town to the north of a soccer field. Site PV1 is 20 meters upstream from the mouth and there is a road along the unvegetated right bank. Sites PV2 and PV3 are reached by following the road on the right bank. Approximately one kilometer up, there is a fork in the road. Site PV2 is reached by taking the fork to the right until the main channel is reached. The reach extends 25 meters upstream from the road. Site PV3 is reached by taking the left fork for another kilometer until it can no longer be traveled by car. The site is past the length of road covered by herbaceous vegetation. A dry streambed crosses the road at the top of the hill. The dry bed is followed until running water is reached. The right bank is mostly sediment and highly susceptible to erosion.

The OpunohuRiver catchment

The Opunohu river catchment is the largest on Moorea. The Opunohu is located on the north side of the island. It flows from the northern slope of Mt. Tohiea and meets the Opunohu Bay as a fifth-order stream. Resh et al. (1990) surveyed this stream, and it is used as a comparison to the five streams that I sampled. Comparable sites are: site MPIV, which is located 0.4 km upstream from the mouth of the Opunohu on a fourth-order tributary that flows down the Mouapu valley; Site OIIA, which is a second-order site near Marae de Titiroa; and site OI, which is a first-order stream reached by following a south-bound trail from the Belvedere (Resh et al. 1990).

Functional feeding groups

Benthic macroinvertebrates are assigned to FFGs based on feeding mode and food preference (Vannote et al. 1980). Shredders feed on living and dead plant material, breaking down the coarse particulate organic matter (CPOM) into fine particulate organic matter (FPOM). Collectors gather or filter the FPOM. Grazers scrape off the algae attached to available surfaces within the stream. Predators feed on living organisms (Merritt and Cummins 1996).

Benthic macroinvertebrate sampling

Benthic macroinvertebrates were collected at each site using a D-frame net and a small aquarium net. Each of 15 sites was divided into three subsites. Each subsite was sampled with three one-minute kicks of the D-net across the width of the stream. If the stream was too narrow, the three kicks were done longitudinally starting downstream. In addition, a five-minute search was performed in each sub-site. Instream rocks were inspected for gastropods and an aquarium net was used to catch shrimp. Organisms were sorted and identified in the laboratory using a 10x microscope. Each specimen was identified to the species level if possible. Others were identified to family.

Data analysis

The total number of individuals and biomass estimates were calculated for each sample. The latter was determined by assigning each species identified a biomass equivalence (BME) number, with one Chironomid (Diptera) as the basic unit (Appendices A–E). The BME was a rough estimate; gastropods and shrimp were divided into size classes in five-millimeter increments. Crabs were assigned a BME based on the size of their carapace.

Each taxon was assigned to a FFG. All sub-samples from each site were combined, and the percentage of each FFG in each site was calculated based on the number of individual organisms and the BME estimate.

Statistical analyses of species richness and FFG percent composition were performed using JMP 5.1 (SAS Institute 2003). Normally distributed data were analyzed using ANOVA and the student's t-test. All other data were analyzed using the Wilcoxin and Tukey tests.

RESULTS

Species richness

A total of 52 taxa were found in the 15 sites sampled (Appendices A–E). Species richness ranged from 14 to 23 in the headwaters, 14-19 in the mid-reaches, and 15 to 29 near the mouth (Appendices A–E). Highest species richness was found at the mid-elevation sites in the Vairemu and Vaioro Rivers (HV2 and AV2) and the site with the least lowest species richness was the mid-elevation site on the Vaihana River (PV2, Fig. 2). The number of taxa was relatively constant in the Vaipohe River (VV).

The highest insect taxa richness was found at downstream site PV1, the lowest at downstream site AV1 (Fig. 3). The highest species richness of crustaceans and molluscs occurred at downstream sites MM1, PV1, and AV1, while the lowest occurred at upstream sites MM1 and AV3 (Fig. 4). Fifteen taxa occurred exclusively at one site. Overall, the number of individual species did not change much from headwaters to mouth, but the species richness of insects was highest in high-elevation sites and the species richness of crustaceans and molluscs was highest in the lowest-elevation sites (Fig. 2-4). Statistical analysis found a significant difference only in mollusc and crustacean richness between low, mid, and high elevations.

Functional feeding group longitudinal variation

Macroinvertebrate FFG composition showed variation from headwaters to mouth. The percentage of grazers increased in three of the five streams sampled (i.e. PV, VV, and AV) based on number of individual organisms (Fig. 5) and the BME estimate (Fig. 6). The percentage of shredding organisms decreased from upstream to downstream along the continuum both by number and BME. The percentage of predators remained constant in three of five streams (MM, AV, HV), ranging from approximately five to ten percent of each sample. The BME estimate of predators showed no similar trends between the different catchments sampled. The percentage of collectors decreased in three of the five streams sampled (PV, VV, and AV) by individual numbers. Like the predators, the BME composition of collectors showed no distinctive trend. Statistical analysis found no significant difference between the elevational variations observed in FFGs.

The Opunohu River catchment

In the Opunohu, total species richness increased from 19 in the high-gradient site to 28 in the low-gradient site (Fig. 7). Crustacean and mollusc taxa increased from 3 in the headwaters (OI) to 13 near the mouth (MPIV). The number of insect taxa varied; the highest species richness (12) was found in the high-gradient site, while the lowest (6) was found in the mid-reach site (OIIA). The percentage of shredding organisms stayed constant across the continuum (Fig. 7). The percentage of grazers increased from headwaters to mouth. Both predators and collectors showed drastic variability between the three sites. The percentage of predators decreased from 86% in site OI to 5% in site OIIA. The collectors increased from 13% in site OI to 85% in site OIIA. Collector and grazer proportions did not vary much between sites OIIA and MPIV (Fig. 8).

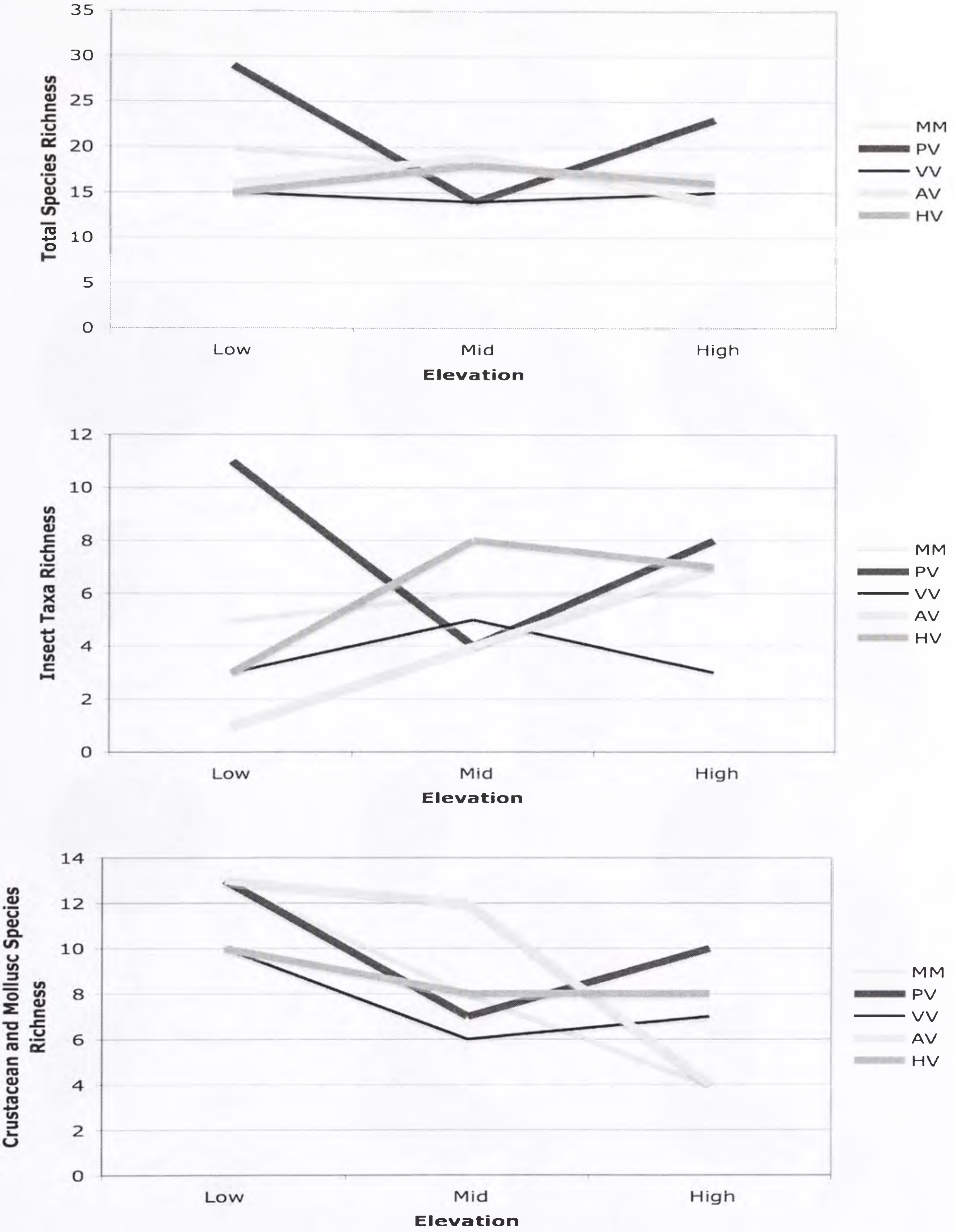


Fig. 2: Species richness at fifteen study sites. Richness was calculated using the total number of taxa found, the number of insect taxa and the number of mollusc and crustacean taxa.

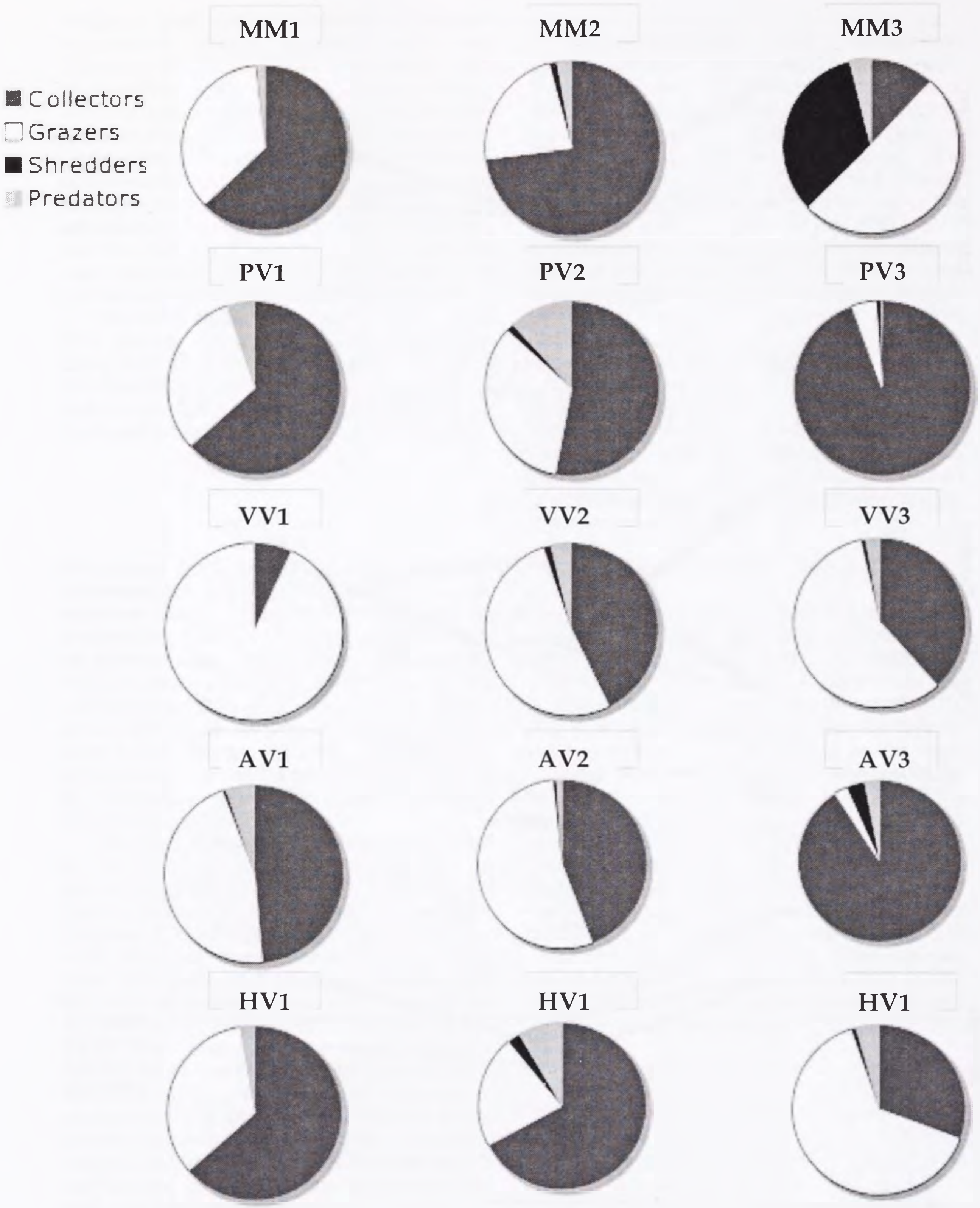


Fig. 3: Functional feeding group composition by abundance of individuals.

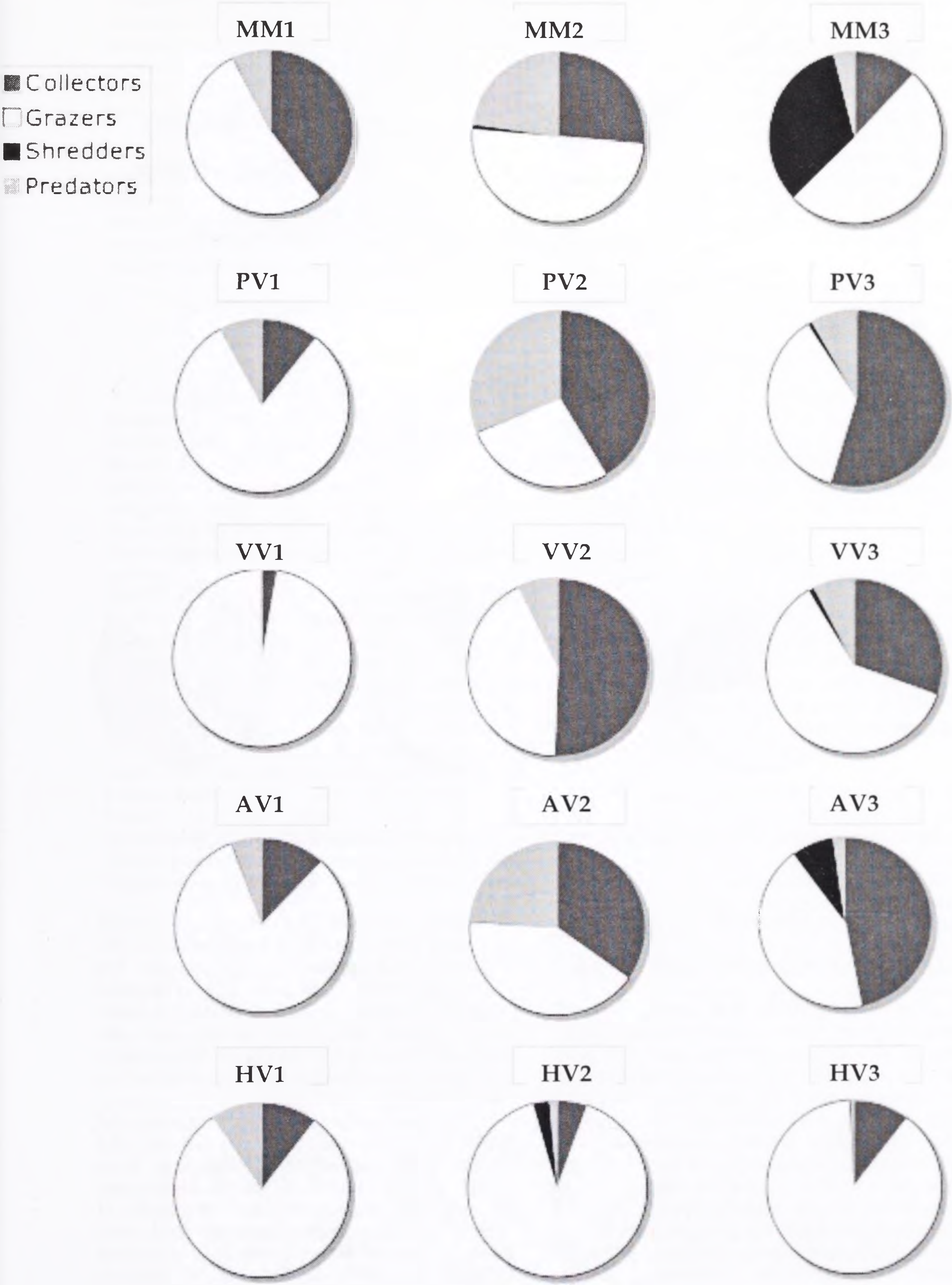


Fig. 4: Functional feeding group composition by biomass equivalence.

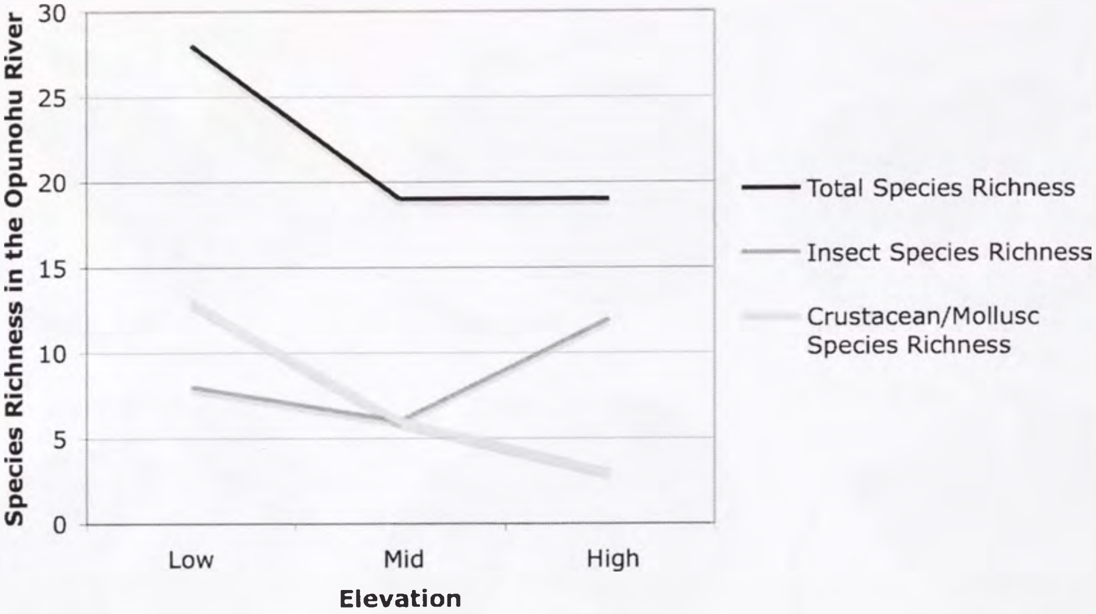


Fig. 5: Species richness in the Opunohu River catchment

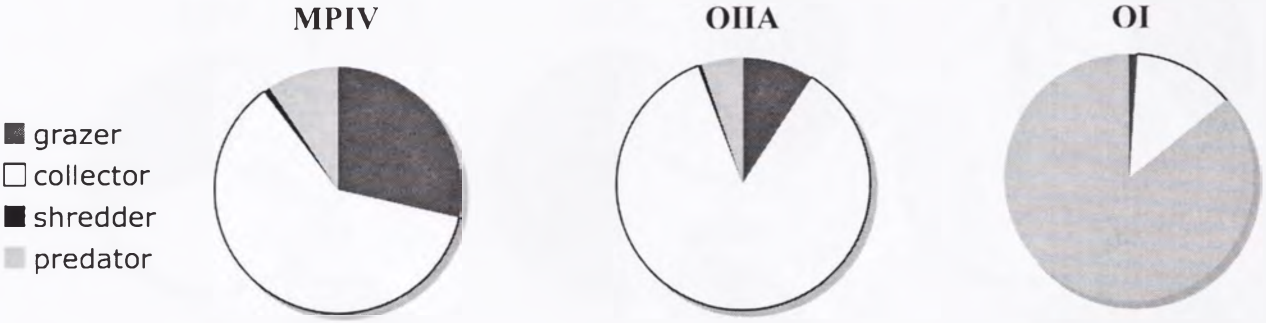


Fig. 6: Functional feeding group composition by abundance of individuals in the Opunohu River catchment.

DISCUSSION

Does species richness match RCC predictions?

The RCC predicts that species richness increases from upstream to downstream as the size of the stream increases. In the small streams sampled on Moorea, the total species richness did not vary significantly between high-gradient, mid-reach, and low-gradient sites. The highest variation was seen in the PV catchment, where species richness ranged from 14 to 29 (Fig. 2), but the lowest richness was found in the mid-elevation site. The pattern of insect species richness also did not match RCC predictions. Although ANOVA found no significant difference between elevations, the number of insect species increased from mouth to headwaters in HV and AV and remained constant in the Mahaerua River (MM) and VV. This trend could be attributed to the small size of many insect species in these rivers and the susceptibility of insect larvae to the frequent disturbances caused by flooding observed in island streams.

The pattern of species richness for gastropod molluscs and crustaceans did match RCC predictions, increasing from headwaters to mouth down the continuum. The aquatic gastropods and decapods of Moorea are all marine in origin and their larvae must still return to the sea to mature (Greathouse 2006). This is a possible explanation for why the highest concentration of species was found closer to the mouth. The streams were also large at lower elevations, providing more habitat and allowing resource partitioning between more species of grazing gastropods.

In the Opunohu River catchment, patterns of total species richness did follow RCC predictions, increasing from 19 in the headwaters to 28 at the mouth (Fig. 7). The number of mollusc and crustacean species increased down the continuum while the number of insect species decreased.

Do FFGs match RCC predictions?

Shredders

The pattern of shredders found along an elevational gradient in Moorean streams matched RCC predictions. The percentage of shredding individuals decreased from upstream to downstream in all streams sampled, with the exception of HV. According to the RCC, shredding organisms should compose one-third of the functional community in high elevation sites, but shredder biomass was depauperate in all headwaters sites sampled except MM3 (Fig. 6). Shredding organisms were 18% of individuals found and 34% of the biomass (Figs 5-6).

Shredding insects were mostly absent on Moorea. Only one family of shredders was present, Pyralidae (Lepidoptera). Only two individuals of Pyralidae were found, one in a headwaters site, one in a site near the mouth (Appendices A-B). The major shredders found in this study were Taltrids (Amphipoda) and two species of millipede, *Oxidus gracilis* Koch (Paradoxosomatidae) and *Glyphiulus grnulatus* Gervais (Cambalopsidae).

Grazers

The pattern of grazers along the streams sampled also matched RCC predictions, increasing down the continuum in three of five streams. In MM and HV the number of individuals decreased but the rough estimate of BME remained relatively constant from headwaters to mouth. It's not an issue of canopy cover because all headwaters sites had similar coverage. Grazers were the only FFG that matched the predictions made by the RCC in the Opunohu.

Predators and collectors

The RCC predicts that the proportions of predators and collectors should remain constant for streams smaller than fifth order. The prediction for predators applied in all but one stream, PV. Predators should compose about ten percent of all samples, but they only appeared in that abundance in two samples, HV2 and PV2. The low percentage of predatory species may result from competition with fish for food (Greathouse and Pringle 2006), an aspect not considered in this study.

The functional composition of collectors should either stay the same or increase down the continuum. In all streams sampled, the proportion of collectors varied greatly and did not show a trend in abundance or BME. Atyid shrimp were classified as collectors 2/3 of the time and *Macrobrachium* shrimp and crabs were classified as spending 1/2 of their time collecting (Greathouse and Pringle 2006).

A potential problem with the results of this study is that the exact role of many of the macroinvertebrates found in the functional communities of Moorea is not known. Crustaceans, such as shrimp and crabs, are generally omnivores and it is not known how much of their time is divided between each feeding mode or how life history affects feeding preference. The role of crustaceans in the continuum can only be estimated. The large crustaceans composed a high proportion of the biomass in the system, resulting in a higher percentage of collectors (Fig. 5).

Some aspects of the RCC applied to the small streams of Moorea, but others did not. Longitudinal patterns of crustacean/mollusc species richness and shredder, grazer, and predator percent composition matched RCC predictions. Total species richness, insect species richness, and the percent compostion of collecting organisms did not. Although statistical analysis found no significance between the longitudinal variations observed in FFGs, the RCC was developed only to show trends, some of which were observed in the streams on Moorea. A more comprehensive study by Greathouse and Pringle (2006) in Puerto Rico found similar results even though the island of Puerto Rico is much larger than Moorea, much closer to a major land mass, and contains higher-order streams. The Puerto Rico study also found a greater diversity of insects, including beetles (Coleoptera), mayflies (Ephemeroptera), and caddisflies (Trichoptera) (Greathouse and

Pringle 2006); these insect orders were not found in Moorean streams.

In conclusion, the overall findings of tropical island stream studies demonstrate that the RCC generally applies to tropical island streams, but that it functions through top-down control, a process not included in the current RCC (Greathouse 2006). Therefore an alternative theoretical framework is needed to accurately describe FFG variation in tropical streams.

ACKNOWLEDGMENTS

I thank Tom Bell, Sameen Ghazali, Julie Himes, Lori LaRue Joel Nitta, Alison Purcell and Mike O'Dowd for sampling assistance and Matthew Cover, Kaua Fraiola, Kevin Lunde, and Alison Purcell for help with identification. Special thanks to Dr. Vincent Resh and all the instructors of the Moorea Class of 2006 for advice and support throughout this process. This project was funded by Maureen Groff.

REFERENCES

Greathouse, E. A., and C. M. Pringle. 2006. Does the river continuum concept apply on a tropical island? Longitudinal variation in a Puerto Rican stream. Canadian Journal of Fisheries and Aquatic Sciences 63: 134-152.

Merritt, R. W., and K. W. Cummins, editors. 1995. Aquatic Insects of North America, Third Edition. Kendall/Hunt Publishing. Dubuque.

Resh, V. H., and F. de Szalay. 1995. Streams and Rivers of Oceania. Ecosystems of the World. Elsevier. New York.

Resh, V. H., J. R. Barnes, and D. A. Craig. 1990. Distribution and ecology of benthic macroinvertebrates in the Opunohu river catchment, Moorea, French Polynesia. Annals of Limnology 26 (2-3): 195-214.

Vannote, R. L., G. W. Minshall, K. W. Cummins, J. R. Sedell, and C. E. Cushing. 1980. The river continuum concept. Canadian Journal of Fisheries and Aquatic Sciences 37: 130-137.

APPENDIX A: Benthic macroinvertebrate species found in the Mahaerua River. Samples were collected 17 October and 18 October 2006. BME= Biomass Equivalence.

SPECIES	**FUNCTIONAL FEEDING GROUP**	**SITE**		
		MM1	**MM2**	**MM3**
MOLLUSCA				
Family Neritidae				
Neritina auriculata Lamarck	grazer	N (58) BME (4800)		
Septaria porcellana Linnaeus	grazer	N (1) BME (15)		
Clithon spinosa Budgin	grazer	N (7) BME (2370)		
Neritina canalis Lamarck	grazer	N (12) BME (780)	N (2) BME (2880)	N (1) BME (15)
Neritina turrita Gmelin	grazer	N (2) BME (6)	N (3) BME (750)	
Neritilia rubida Pease	grazer	N (10) BME (150)	N (47) BME (715)	N (68) BME (1020)
Family Thiaridae				
Thiara granifera Lamarck	grazer	N (36) BME (660)	N (1) BME (15)	N (1) BME (15)
Melanoides tuberculata Müller	grazer	N (2) BME (50)		
Family Planorbidae	grazer			
Family Limnaeidae				
Species A	grazer			
Species B	grazer			
CRUSTACEA				
Family Atyidae				
Atyoida pilipes Newport	1/3 grazer, 2/3 collector	N (484) BME (11000)	N (1) BME (150)	N (1) BME (50)
Caridina weberi De Man	1/3 grazer, 2/3 collector		N (1) BME (60)	
Family Palaemonidae				
Macrobrachium lar Fabricius	1/2 collector, 1/2 predator	N (3) BME (320)	N (1) BME (4000)	
Macrobrachium australe Guérin-Méneville	1/2 collector, 1/2 predator	N (5) BME (650)		
Macrobrachium latimanus von Martens	1/2 collector, 1/2 predator			
Famiy Grapsidae				
Geosesarma angustifrons larva Milne-Edwards	collector	N (4) BME (12)		
Geosesarma angustifrons Milne-Edwards	1/2 collector, 1/2 predator	N (20) BME (2500)		
Labuanium trapesoideum Milne-Edwards	1/2 collector, 1/2 predator			
Order Isopoda	shredder	N (1) BME (10)		
Order Copepoda	collector			
Order Ostracoda	grazer			
Family Talitridae	shredder		N (1) BME (15)	N (29) BME (435)
INSECTA				
Order Odonata				
Family Libellulidae	predator		N (1) BME (4)	N (2) BME (8)
Family Coenegrionidae	predator		N (4) BME (24)	N (11) BME (66)
Order Hemiptera				

SPECIES	FUNCTIONAL FEEDING GROUP	SITE		
		MM1	MM2	MM3
Family Saldidae				
Saldula tahitiensis Cobben	predator			
Order Diptera				
Family Chironomidae				
Chironomini sp. A	collector	N (27) BME (27)	N (151) BME (151)	N (27) BME (27)
Chironomini sp. B	collector	N (32) BME (32)	N (5) BME (5)	N (14) BME (14)
Orthocladinae	collector	N (1) BME (1)		
Family Tabanidae	predator			
Family Ephydridae				
Apuvillus cheesemanae Edwards	grazer		N (2) BME (4)	N (1) BME (2)
Family Psychodidae	collector			
Family Stratiomyiidae	collector			
Family Dolichopodidae	predator			
Family Ceratapogonidae	collector			
Family Simuliidae				
Simulium dussertorum Craig	collector			
Simulium sp.	collector			
Simulium exasperans Craig	collector			
Simulium malardei Craig	collector			
Simulium lotii Craig	collector		N (6) BME (18)	N (48) BME (144)
Order Lepidoptera				
Family Pyralidae				
Species A	shredder	N (1) BME (2)		
Species B	shredder			
Order Collembola	collector	N(1) BME (2)		
NEMATODA	collector			
OLIGOCHAETA				
Species A	collector	N (118) BME (300)	N (14) BME (35)	N (12) BME (30)
Species B	collector			
Worm with many spines	collector		N (1) BME (4)	
ACARINA				
Species A	predator		N (1) BME (0.5)	N (3) BME (1.5)
Species B	predator			N (3) BME (1.5)
Species C	predator			
Species D	predator			N (1) BME (0.5)
DIPLOPODA				
Family Cambalopsidae				
Glyphiulus grnulatus Gervais	shredder		N (2) BME (40)	N (12) BME (240)
Family Paradoxosomatidae				
Oxidus gracilis Koch	shredder			N (1) BME (40)
TOTAL NUMBER OF TAXA		20	18	17

APPENDIX B: Benthic macroinvertebrate species found in the Vaihana River. Samples were collected 27 October 2006. BME= Biomass Equivalence.

SPECIES	**FUNCTIONAL FEEDING GROUP**	**SITES**		
		PV1	**PV2**	**PV3**
MOLLUSCA				
Family Neritidae				
Neritina auriculata Lamarck	grazer			
Septaria porcellana Linnaeus	grazer	N (28) BME (14885)		
Clithon spinosa Budgin	grazer	N (17) BME (12280)		
Neritina canalis Lamarck	grazer	N (15) BME (5820)	N (1) BME (2000)	N (2) BME (2880)
Neritina turrita Gmelin	grazer			
Neritilia rubida Pease	grazer	N (51) BME (765)	N (15) BME (295)	N (17) BME (255)
Family Thiaridae				
Thiara granifera Lamarck	grazer	N (246) BME (5200)	N (19) BME (430)	N (11) BME (375)
Melanoides tuberculata Müller	grazer	N (1) BME (300)	N (12) BME (1130)	N (4) BME (460)
Family Planorbidae	grazer	N (1) BME (2)		
Family Limnaeidae				
Species A	grazer	N(1) BME (2)		
Species B	grazer			N (1) BME (3)
CRUSTACEA				
Family Atyidae				
Atyoida pilipes Newport	1/3 grazer, 2/3 collector	N (59) BME (1270)	N (11) BME (2720)	N (23) BME (6090)
Caridina weberi De Man	1/3 grazer, 2/3 collector			N (5) BME (620)
Family Palaemonidae				
Macrobrachium lar Fabricius	1/2 collector, 1/2 predator	N (1) BME (60)	N (3) BME (4300)	N (3) BME (2850)
Macrobrachium australe Guérin-Méneville	1/2 collector, 1/2 predator	N (13) BME (7250)	N (7) BME (6400)	
Macrobrachium latimanus von Martens	1/2 collector, 1/2 predator			
Famiy Grapsidae				
Geosesarma angustifrons larva Milne-Edwards	collector			
Geosesarma angustifrons Milne-Edwards	1/2 collector, 1/2 predator			
Labuanium trapesoideum Milne-Edwards	1/2 collector, 1/2 predator	N (1) BME (50)		
Order Isopoda	shredder			N (1) BME (10)
Order Copepoda	collector			
Order Ostracoda	grazer	N (1) BME (1)		
Family Talitridae	shredder			N (3) BME (45)
INSECTA				
Order Odonata				
Family Libellulidae	predator	N (1) BME (4)		
Family Coenegrionidae	predator	N (36) BME (216)	N (12) BME (72)	N (1) BME (6)
Order Hemiptera				

SPECIES	FUNCTIONAL FEEDING GROUP	SITE		
		PV1	PV2	PV3
Family Saldidae				
Saldula tahitiensis Cobben	predator	N (2) BME (8)		
Order Diptera				
Family Chironomidae				
Chironomini sp. A	collector	N (236) BME (236)	N (58) BME (58)	N (13) BME (13)
Chironomini sp. B	collector	N (465) BME (465)	N (3) BME (3)	N (1) BME (1)
Orthocladinae	collector			
Family Tabanidae	predator	N (1) BME (1)		
Family Ephydridae				
Apuvillus cheesemanae Edwards	grazer	N (2) BME (4)		
Family Psychodidae	collector	N (2) BME (3)		
Family Stratiomyiidae	collector	N (1) BME (2)		
Family Dolichopodidae	predator	N (1) BME (1)		
Family Ceratapogonidae	collector			
Family Simuliidae				
Simulium dussertorum Craig	collector			N (1) BME (3)
Simulium sp.	collector			N (6) BME (18)
Simulium exasperans Craig	collector			N (827) BME (3305)
Simulium malardei Craig	collector			N (1) BME (3)
Simulium lotii Craig	collector		N (5) BME (15)	N (26) BME (78)
Order Lepidoptera				
Family Pyralidae				
Species A	shredder			
Species B	shredder			
Order Collembola	collector	N (3) BME (6)		
NEMATODA	collector			
OLIGOCHAETA				
Species A	collector	N (19) BME (47.5)	N (2) BME (5)	N (14) BME (35)
Species B	collector			N (1) BME (40)
Worm with many spines	collector			N (1) BME (4)
ACARINA				
Species A	predator	N (4) BME (2)	N (1) BME (0.5)	
Species B	predator	N (2) BME (1)		
Species C	predator	N (5) BME (2.5)		
Species D	predator	N (5) BME (2.5)		
DIPLOPODA				
Family Cambalopsidae				
Glyphiulus grnulatus Gervais	shredder		N (2) BME (40)	N (3) BME (60)
Family Paradoxosomatidae				
Oxidus gracilis Koch	shredder			N (1) BME (40)
TOTAL NUMBER OF TAXA		29	14	23

APPENDIX C: Benthic macroinvertebrate species found in the Vaipohe River. Samples were collected 2 November 2006. BME= Biomass Equivalence.

SPECIES	FUNCTIONAL FEEDING GROUP	SITES		
		VV1	VV2	VV3
MOLLUSCA				
Family Neritidae				
Neritina auriculata Lamarck	grazer			
Septaria porcellana Linnaeus	grazer			
Clithon spinosa Budgin	grazer	N (4) BME (4480)		
Neritina canalis Lamarck	grazer	N (53) BME (37150)	N (8) BME (6020)	N (2) BME (1080)
Neritina turrita Gmelin	grazer	N (8) BME (22200)		
Neritilia rubida Pease	grazer	N (1003) BME (15045)	N (55) BME (825)	N (110) BME (1650)
Family Thiaridae				
Thiara granifera Lamarck	grazer	N (372) BME (7575)	N (13) BME (275)	N (4) BME (185)
Melanoides tuberculata Müller	grazer	N (1) BME (100)	N (3) BME (130)	N (6) BME (715)
Family Planorbidae	grazer			
Family Limnaeidae				
Species A	grazer			
Species B	grazer			
CRUSTACEA				
Family Atyidae				
Atyoida pilipes Newport	1/3 grazer, 2/3 collector	N (110) BME (3300)		
Caridina weberi De Man	1/3 grazer, 2/3 collector		N (2) BME (180)	N (30) BME (2400)
Family Palaemonidae				
Macrobrachium lar Fabricius	1/2 collector, 1/2 predator	N (3) BME (300)		N (1) BME (600)
Macrobrachium australe Guérin-Méneville	1/2 collector, 1/2 predator	N (4) BME (360)	N (7) BME (2430)	N (3) BME (460)
Macrobrachium latimanus von Martens	1/2 collector, 1/2 predator			
Famiy Grapsidae				
Geosesarma angustifrons larva Milne-Edwards	collector			
Geosesarma angustifrons Milne-Edwards	1/2 collector, 1/2 predator			
Labuanium trapesoideum Milne-Edwards	1/2 collector, 1/2 predator			
Order Isopoda	shredder			
Order Copepoda	collector			
Order Ostracoda	grazer			
Family Talitridae	shredder	N (1) BME (15)		
INSECTA				
Order Odonata				
Family Libellulidae	predator		N (1) BME (4)	
Family Coenegrionidae	predator		N (1) BME (6)	N (2) BME (12)
Order Hemiptera				

SPECIES	FUNCTIONAL FEEDING GROUP	SITE		
		VV1	VV2	VV3
Family Saldidae				
Saldula tahitiensis Cobben	predator			
Order Diptera				
Family Chironomidae				
Chironomini sp. A	collector	N (7) BME (7)	N (35) BME (35)	N (51) BME (51)
Chironomini sp. B	collector	N (6) BME (6)	N (10) BME (10)	N (2) BME (2)
Orthocladinae	collector			
Family Tabanidae	predator			
Family Ephydridae				
Apuvillus cheesemanae Edwards	grazer			
Family Psychodidae	collector			
Family Stratiomyiidae	collector			
Family Dolichopodidae	predator			
Family Ceratapogonidae	collector			
Family Simuliidae				
Simulium dussertorum Craig	collector			
Simulium sp.	collector			
Simulium exasperans Craig	collector			
Simulium malardei Craig	collector			
Simulium lotii Craig	collector		N (1) BME (3)	
Order Lepidoptera				
Family Pyralidae				
Species A	shredder			
Species B	shredder			
Order Collembola	collector	N (3) BME (6)		
NEMATODA	collector		N (1) BME (1)	N (1) BME (1)
OLIGOCHAETA				
Species A	collector	N (18) BME (45)	N (14) BME (35)	N (11) BME (27.5)
Species B	collector			
Worm with many spines	collector	N (1) BME (4)		
ACARINA				
Species A	predator			N (1) BME (0.5)
Species B	predator			
Species C	predator			N (1) BME (0.5)
Species D	predator			
DIPLOPODA				
Family Cambalopsidae				
Glyphiulus grnulatus Gervais	shredder		N (2) BME (40)	
Family Paradoxosomatidae				
Oxidus gracilis Koch	shredder			N (2) BME (80)
TOTAL NUMBER OF TAXA		15	14	15

APPENDIX D: Benthic macroinvertebrate species found in the Vaioro River. Samples were collected 7 November and 11 November 2006. BME= Biomass Equivalence.

SPECIES	**FUNCTIONAL FEEDING GROUP**	**SITES**		
		AV1	**AV2**	**AV3**
MOLLUSCA				
Family Neritidae				
Neritina auriculata Lamarck	grazer	N (38) BME (3915)		
Septaria porcellana Linnaeus	grazer	N (1) BME (260)	N (1) BME (260)	
Clithon spinosa Budgin	grazer	N (22) BME (1665)		
Neritina canalis Lamarck	grazer	N (28) BME (10600)	N (3) BME (1280)	N (1) BME (880)
Neritina turrita Gmelin	grazer	N (11) BME (610)	N (7) BME (2760)	
Neritilia rubida Pease	grazer	N (7) BME (105)	N (80) BME (1200)	
Family Thiaridae				
Thiara granifera Lamarck	grazer	N (11) BME (165)	N (1) BME (35)	
Melanoides tuberculata Müller	grazer		N (2) BME (135)	
Family Planorbidae	grazer			
Family Limnaeidae				
Species A	grazer			
Species B	grazer		N (1) BME (3)	
CRUSTACEA				
Family Atyidae				
Atyoida pilipes Newport	1/3 grazer, 2/3 collector	N (63) BME (1860)	N (9) BME (1280)	N (5) BME (1040
Caridina weberi De Man	1/3 grazer, 2/3 collector	N (7) BME (180)	N (12) BME (1250)	N (6) BME (680)
Family Palaemonidae				
Macrobrachium lar Fabricius	1/2 collector, 1/2 predator		N (1) BME (1500)	
Macrobrachium australe Guérin-Méneville	1/2 collector, 1/2 predator	N (1) BME (120)		
Macrobrachium latimanus von Martens	1/2 collector, 1/2 predator		N (1) BME (6000)	
Famiy Grapsidae				
Geosesarma angustifrons larva Milne-Edwards	collector	N (1) BME (3)		
Geosesarma angustifrons Milne-Edwards	1/2 collector, 1/2 predator	N (30) BME (2270)		
Labuanium trapesoideum Milne-Edwards	1/2 collector, 1/2 predator	N (1) BME (50)		
Order Isopoda	shredder			
Order Copepoda	collector		N (1) BME (1)	
Order Ostracoda	grazer			
Family Talitridae	shredder	N (2) BME (30)		N (6) BME (90)
INSECTA				
Order Odonata				
Family Libellulidae	predator			
Family Coenegrionidae	predator			N (13) BME (78)
Order Hemiptera				

SPECIES	FUNCTIONAL FEEDING GROUP		SITE	
		AV1	AV2	AV3
Family Saldidae				
Saldula tahitiensis Cobben	predator			
Order Diptera				
Family Chironomidae				
Chironomini sp. A	collector	N (71) BME (71)	N (25) BME (25)	N (353) BME (35
Chironomini sp. B	collector		N (1) BME (1)	N (14) BME (14)
Orthocladinae	collector			
Family Tabanidae	predator			
Family Ephydridae				
Apuvillus cheesemanae Edwards	grazer		N (1) BME (2)	N (6) BME (12)
Family Psychodidae	collector			
Family Stratiomyiidae	collector			
Family Dolichopodidae	predator			
Family Ceratapogonidae	collector			N (1) BME (1)
Family Simuliidae				
Simulium dussertorum Craig	collector			
Simulium sp.	collector			
Simulium exasperans Craig	collector			
Simulium malardei Craig	collector			
Simulium lotii Craig	collector		N (1) BME (3)	N (30) BME (90)
Order Lepidoptera				
Family Pyralidae				
Species A	shredder			
Species B	shredder			N (1) BME (4)
Order Collembola	collector			
NEMATODA	collector			
OLIGOCHAETA				
Species A	collector	N (16) BME (40)	N (41) BME (102.5)	N (2) BME (5)
Species B	collector			
Worm with many spines	collector			
ACARINA				
Species A	predator		N (1) BME (0.5)	
Species B	predator			
Species C	predator			N (1) BME (0.5)
Species D	predator			
DIPLOPODA				
Family Cambalopsidae				
Glyphiulus grnulatus Gervais	shredder		N (1) BME (20)	N (9) BME (180)
Family Paradoxosomatidae				
Oxidus gracilis Koch	shredder			
TOTAL NUMBER OF TAXA		16	19	14

APPENDIX E: Benthic macroinvertebrate species found in the Vairemu River. Samples were collected 10 November and 14 November 2006. BME= Biomass Equivalence.

SPECIES	FUNCTIONAL FEEDING GROUP	SITES		
		HV1	HV2	HV3
MOLLUSCA				
Family Neritidae				
Neritina auriculata Lamarck	grazer	N (2) BME (860)		
Septaria porcellana Linnaeus	grazer		N (1) BME (120)	
Clithon spinosa Budgin	grazer	N (17) BME (2180)		
Neritina canalis Lamarck	grazer	N (38) BME (17560)	N (11) BME (5420)	N (3) BME (1620)
Neritina turrita Gmelin	grazer	N (10) BME (6225)	N (12) BME (2010)	N (2) BME (2700)
Neritilia rubida Pease	grazer	N (3) BME (45)	N (28) BME (420)	N (55) BME (825)
Family Thiaridae				
Thiara granifera Lamarck	grazer	N (22) BME (290)	N (1) BME (15)	N (2) BME (30)
Melanoides tuberculata Müller	grazer	N (1) BME (100)	N (2) BME (200)	N (5) BME (370)
Family Planorbidae	grazer			
Family Limnaeidae				
Species A	grazer			
Species B	grazer			
CRUSTACEA				
Family Atyidae				
Atyoida pilipes Newport	1/3 grazer, 2/3 collector	N (7) BME (210)	N (3) BME (290)	
Caridina weberi De Man	1/3 grazer, 2/3 collector		N (2) BME (170)	N (10) BME (980)
Family Palaemonidae				
Macrobrachium lar Fabricius	1/2 collector, 1/2 predator	N (1) BME (5000)		
Macrobrachium australe Guérin-Méneville	1/2 collector, 1/2 predator	N (5) BME (1400)		
Macrobrachium latimanus von Martens	1/2 collector, 1/2 predator			
Famiy Grapsidae				
Geosesarma angustifrons larva Milne-Edwards	collector			
Geosesarma angustifrons Milne-Edwards	1/2 collector, 1/2 predator			
Labuanium trapesoideum Milne-Edwards	1/2 collector, 1/2 predator			
Order Isopoda	shredder			N (1) BME (10)
Order Copepoda	collector			N (1) BME (1)
Order Ostracoda	grazer			
Family Talitridae	shredder			
INSECTA				
Order Odonata				
Family Libellulidae	predator			
Family Coenegrionidae	predator	N (5) BME (30)	N (23) BME (138)	N (5) BME (30)
Order Hemiptera				

SPECIES	FUNCTIONAL FEEDING GROUP	SITE		
		HV1	HV2	HV3
Family Saldidae				
Saldula tahitiensis Cobben	predator			
Order Diptera				
Family Chironomidae				
Chironomini sp. A	collector	N (62) BME (62)	N (140) BME (140)	N (6) BME (6)
Chironomini sp. B	collector	N (38) BME (38)	N (18) BME (18)	N (6) BME (6)
Orthocladinae	collector			
Family Tabanidae	predator			
Family Ephydridae				
Apuvillus cheesemanae Edwards	grazer		N (1) BME (2)	N (1) BME (2)
Family Psychodidae	collector			
Family Stratiomyiidae	collector			
Family Dolichopodidae	predator			
Family Ceratapogonidae	collector			
Family Simuliidae				
Simulium dussertorum Craig	collector			
Simulium sp.	collector			
Simulium exasperans Craig	collector			
Simulium malardei Craig	collector			
Simulium lotii Craig	collector	N (4) BME (12)	N (10) BME (30)	N (2) BME (6)
Order Lepidoptera				
Family Pyralidae				
Species A	shredder			
Species B	shredder			
Order Collembola	collector		N (1) BME (2)	
NEMATODA	collector			
OLIGOCHAETA				
Species A	collector	N (69) BME (172.5)	N (7) BME (17.5)	N (7) BME (17.5
Species B	collector			
Worm with many spines	collector		N (1) BME (4)	N (5) BME (20)
ACARINA				
Species A	predator			
Species B	predator			
Species C	predator			
Species D	predator			
DIPLOPODA				
Family Cambalopsidae				
Glyphiulus grnulatus Gervais	shredder		N (1) BME (20)	N (1) BME (20)
Family Paradoxosomatidae				
Oxidus gracilis Koch	shredder		N (6) BME (240)	
TOTAL NUMBER OF TAXA		15	18	16

FEEDING PREFERENCE OF THE CUSHION STAR, *CULCITA NOVAEGUINEAE* IN MO'OREA

SIGRID V. HAWKINS

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

Abstract. Previous studies of the feeding biology of *Culcita novaeguineae* Muller & Troschel in Hawai'i have shown that the cushion star prefers to prey on coral species of the genus *Pocillopora* over the genus *Porites*. Distribution and feeding biology studies of *C. novaeguineae* in Cook's Bay on the island of Mo'orea, French Polynesia have shown that it habitats near *Porites spp.* coral in areas where *Porites spp.* coral is sparse, and prefers to prey on *Acropora spp.* coral. The purpose of this study was to further examine coral prey preference of *C. novaeguineae* in Mo'orea, using specimens from four areas on the island for better representation of its feeding ecology on Mo'orea, French Polynesia. My studies showed that *C. novaeguineae* in Mo'orea prefers *Acropora spp.* and *Pocillopora spp.*, over *Porites spp.* ($p=0.0046$), and showed no preference for *Acropora spp.*, over *Pocillopora spp.*. The study further determined that *C. novaeguineae* primarily feeds nocturnally, and moves persistently in search for food. As a coral predator, *C. novaeguineae* can affect abundance of certain coral species, and change the overall reef ecology in Mo'orea.

Key words: asteroid, Culcita novaeguineae; *feeding preference; locomotion; Moorea, French Polynesia; predation*

INTRODUCTION

Changes in abundance of certain species of echinoderms can have large-scale effects on reef community structure. To understand the variations in structure and function of coral-reef communities, it is necessary to understand the ecology of coral reef echinoderms (Birkeland 1988). For example: Lessios *et al.* (1984) reported a several-fold increase in benthic algae following the widespread mortality of an echinoid species on Caribbean reefs. Likewise, increases in abundance of an asteroid species can bring about major changes in community structure of corals (Birkeland 1988). A good example of this occurred in Mo'orea, French Polynesia in the 1980s, when the predatory sea star *Acanthaster planci* killed entire coral communities (Moran et al 1988).

C. novaeguineae is an echinoid that is also known to prey on coral. Studies by Goreau et al (1972), Glynn & Krupp (1986) and Birkeland (1988) determined that *C. novaeguineae's* coral feeding is relatively specialized, because it has a large biomass to support in relation to its stomach surface. The studies showed that *C. novaeguineae* prefers to prey on small or encrusting scleractinians, especially pocilloporids and acroporids (Goreau et al 1972, Glynn & Krupp 1986 as quoted in Birkeland 1988).

A study in Mo'orea, French Polynesia determined that the cushion star preferred habitats where *Porites spp.* was present (Roberge 2000). In the study Roberge observed that *C. novaeguineae* was found within 1m of the *Porites spp.* thirty five percent of the time, even though *Porites* represented only 3.75% of the surveyed area. His focus was the distribution and locomotion of the cushion star, and he did not examine food preference as a factor in habitat selection.

The first feeding biology study on Mo'orean cushion stars was done in 2003 (Bertics 2003). In the study Bertics conducted laboratory feeding trials in which the cushions stars were offered three species of coral, as well as algae, sediment, rocks, and fungi. Her study showed that C. *novaeguineae* preferred *Acropora spp.* over all other food choices.

While both studies contributed to the better understanding of the ecology of cushions stars on Mo'orea, French Polynesia, their scope was limited to C. *novaeguineae* found in Cook's bay, and was not representative of the island of Mo'orea. The reef ecology of Cooks Bay differs from other areas on the island, because of the extensive development along the bay, and the associated pollution. For example, in their study sites, Roberge and Bertics found very little *Pocillopora spp.* and *Porites spp.*, whereas I found both coral in abundance in other areas on the island. I also frequently observed cushion stars in the vicinity of *Pocillopora spp.* and *Acropora spp.*

The purpose of my study was to first examine if cushion stars feed diurnally or nocturnally, and then to determine C. *novaeguineae's* coral preference using specimens from different areas on the island for a more representative sample of Mo'orea's cushion star population. In conjunction with coral preference I also observed C. *novaeguineae* movements to determine if cushion stars searching for food move in a persistent pattern, or randomly. My hypotheses were: (1) C. *novaeguineae* feeds nocturnally. During my initial surveys which were all conducted during the day, I did not observe any cushion stars feeding, indicating they prefer feeding at night. (2) C. *novaeguineae* in Mo'orea prefer to prey on *Acropora spp.*, over *Pocillopora spp.*, or *Porites spp.* During field surveys in search of cushion stars, I found more cushion stars near *Acropora spp.*, specifically *Acropora cervicornis* than other coral species indicating that *Acropora spp.* might be a choice prey coral. (3) C. *novaeguineae* moves in a persistent pattern toward food. As a relatively slow mover, cushion stars need to be able to detect their food and move toward it consistently to survive as a predator.

METHODS

Study sites

C. novaeguineae specimen collection, coral gathering, and field observations were conducted at four sites on Mo'orea (Fig. 1) during the period from Oct 11-30, 2006. The first site was located at Cook's Bay near the Richard B. Gump Research Station, were I collected four specimens at distances between 7-50 meters from shore. Another study site, Faimano Beach, was located approximately two miles east of the Gump Research Station; I collected three specimens there, at distances between 20 and 50 meters from shore. The third site was Tamae beach on the northeastern side of Mo'orea, near the airport, ca 15 km from the research station. Here, I captured three cushion stars in an area 250-300 meter off shore. The last study site was near the Vaihapu reef crest approximately two kilometers north of the Gump Research Station; I collected three cushion stars there.

FIG. 1. Collection and field observation sites in Mo'orea, French Polynesia

All *C. novaeguineae* were found in water between 2.5 and seven meters deep.

Coral gathering

Coral for feeding experiments were collected by hand using a mesh bag. Only live coral fragments that were broken off by natural processes were collected, in accordance with laws protecting these organisms. Specimens were transported in plastic tubs with seawater, and then stored in large outdoor rectangular tanks with continuous flowing seawater, 51 cm deep. Specimens from three genera were used: *Acropora, Pocillopora,* and *Porites*. *Porites* was primarily represented by *Porites lobata*, which was abundant at all locations, *Pocillopora spp.* which was scarce near the Gump Research Station, but relatively abundant at the other locations, and *Acropora spp.*, specifically *Acropora cervicornis,* which I found only at Tamae at approximately 300 meters off shore.

Preference for feeding diurnally or nocturnally

My first experiment determined if *C. novaeguineae* prefers to feed during the day or at night. *C. novaguineae* was observed continuously for a period of three hours in the morning between 4:00 am and 8:00 am, and again between 6:00 pm and 10:00 pm.. Thirteen feeding trials were held using the same specimens for both day and night feeding experiments. The experiments were conducted in a 3.5 ft. diameter round tank, and an 8.0 X 4.0 X 2.0 ft. rectangular outdoor tank with continuously flowing seawater. The rectangular tank was divided into three compartments using mesh screen and bricks; one of the compartments was used as a holding tank for newly captured cushion stars, another as storage for food coral, and one for feeding trials. Newly captured cushion stars were isolated from food for 12 hours before starting feeding experiments to purge their digestive system. During the experiments I placed one coral specimen from each species of comparable size at the opposite end of the compartment as the cushion star. In the round tank, I placed the cushion stars around the center pole of the tank, and three coral specimens (one per species) for each cushion star around the outer perimeter of the tank. At the end of the observation period, I removed the corals from the compartments.

Coral preference

My second experiment was to examine coral preference when *C. novaeguineae* was offered three choices of coral: *Acropora spp. Pocillopora spp.*, and *Porites spp.* I held 39 trials in which I placed one coral specimen from each species of comparable size at the opposite end of the compartment as the cushion star in the rectangular tank. In the round tank, I placed the cushion stars around the center pole of the tank, and three coral specimens (one from each species) for each cushion star around the outer perimeter of the tank. I continuously observed the cushion stars for three hours between 5:30 pm and 10:00 pm recording which coral was visited and how often. At the end of the observation period, I removed the corals from the compartments. The cushion stars remained without food for 12 hours.

After I determined that *C. novaeguineae* prefers both *Acropora spp.* and *Pocillopora spp.* over *Porites spp.*, I conducted another experiment. I held 48 trials using the same experimental design, but with only two coral species, to determine if *C. novaguineae* prefers either *Acropora spp.* or *Pocillopora spp.*

I statistically analyzed the results using Wilcoxon tests for 3-coral experiments, and the Rank-sums test for 2-coral experiments.

Locomotion

To determine if *C. novaeguineae* moves toward its food in a pattern or haphazardly, I observed and recorded their movements for

18 days during feeding trials. I recorded the distance each specimen moved during each observation period, whether it moved toward coral or away from it, if it climbed the side of the tank, and if it moved clockwise or counterclockwise. Most locomotion experiments were held in the round tank, therefore it was more practical to use clock, and counterclockwise directions rather than right or left.

Field observations

To observe *C. novaeguineae* in their natural environment, four field observations were made between 29 Oct and 6 Nov, 2006. In Cook's Bay three transects covering approximately 5 X 200 meters were surveyed for *C. novaguineae* at night, using underwater flashlights. Transects started 25 meters from shore at the station and concluded 200 meters north in a path parallel to the shore. Four transects covering approximately 10 X 50 meters were surveyed at Tamae beach. Transects started at the left-hand-side of the beach 300 meters from shore, and paralleled the shoreline. At Faimano beach three transects approximately 7 X 150 meters were surveyed, starting 50 meters from shore near the boat channel marker on the left side of the beach, and progressed parallel to the shore.

For each specimen observed during transects I recorded the substrate composition it was found on, its distance from coral heads, whether of not it was feeding, and what it was eating. To determine if it was eating, I dove down, picked up and turned over *C. novaeguineae* to see if its stomach was extruded.

Turning

To determine a relationship between *C. novaeguineae* size, and the time it takes turn when it is flipped on its back, I conducted 40 experiments with 12 specimens. Each trial I turned the cushion stars over onto their backs and monitored their behavior and how much time they needed to "get back on their feet". To analyze the relationship between *C. novaguineae* size and the time it takes to turn over, I performed a linear regression analysis.

RESULTS

Day vs. night feeding preference

Cushion stars prefer to feed at night. I calculated the frequencies of individuals feeding during the day, and compared them with the frequencies of individuals feeding at night. The results showed that 81.6 percent of the time *C. novaeguineae* was eating was at night, and 23.1 percent during early morning hours (Fig. 2).

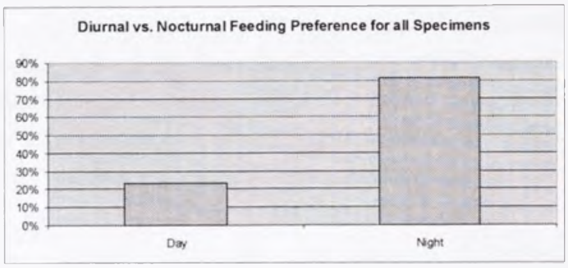


FIG. 3. Diurnal/nocturnal preference

Coral preference

When *C. novaeguineae* was offered three species of coral, it showed a strong preference for *Acropora* and *Pocillopora spp.* over *Porites spp.*. A Wilcoxon test determined a significant difference between groups (p= 0.0046). Of 39 trials overall, *C. novaeguineae* chose *Acropora spp.* 46.2 percent of the time, *Pocillopora spp.* 30.8 percent, and *Porites spp* 10.3 percent. It did not eat 12.8 percent of the trials, and fed on other things such as detritus materiel in the tank, and even pieces of plaster in the tank from a recent repair 11.8 percent. Individual feeding frequencies are depicted in Table 1 in the appendix.

In the experiments when *C. novaeguineae* was offered two species of coral, it showed a slight preference for *Pocillopora spp.* over *Acropora spp.* A Rank-sums test showed insignificant preferences between the two groups (p = 0.4482). Of 48 trials the cushion stars frequented *Pocillopora spp.* 47.9 percent of the time and *Acropora spp.* 39.6 percent. Individual frequencies are depicted in Table 2 in the appendix.

Locomotion

In 219 observation hours combined, *C. novaeguineae* moved frequently and in a persistent pattern (Fig 3).

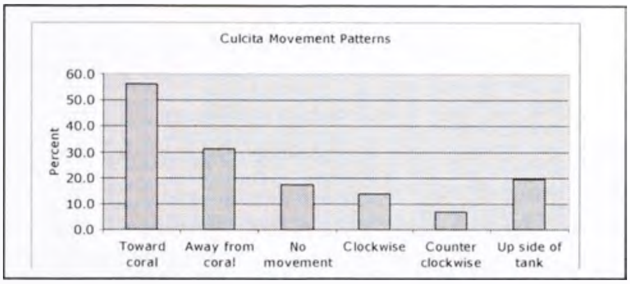


FIG. 3. Movement patterns of *C. novaeguineae.*

Overall the cushion stars moved on average 34.5 cm per hour. The furthest an individual moved in one hour was 4.9 meters. In 56.3 percent of the trials, *Culcita novaeguineae* moved toward coral, in 31 percent of the trials it moved away from coral. 17.2 percent of the trials it did not move, and it moved clockwise 13.8 percent of the time and counter clockwise 6.9 percent. During 19.5 percent of the experiments it climbed up the side of the tanks.

Turning

There was no clear correlation between *C. novaeguineae* size and the time it takes to turn over. Using linear regression analysis P= 0.2317, and Rsquare was 0.03843 (Fig. 4).

DISCUSSION

The hypothesis that *C. novaeguineae* prefers to feed at night rather than during the day was confirmed in both my laboratory experiments and during field observations. *C. novaeguineae* fed approximately four times more often in the evening than in the morning. In fact, of the 23.1 percent when *C. novaeguineae* was eating during the early morning, it did so before sunrise. Once daybreak advanced, the cushion stars stopped eating, and moved away from coral in search for shelter. During daytime field observations, I rarely observed *C. novaeguineae* on open sand, but generally found them next to coral heads or wedged underneath. During evening feeding and locomotion experiments I observed that cushion stars would stop moving when a flashlight was pointed toward them, and individuals that were climbing the side of the tank would release their hold, and tumble down. *C. novaeguineae* is photosensitive, and it is possible it experiences disorientation in bright light.

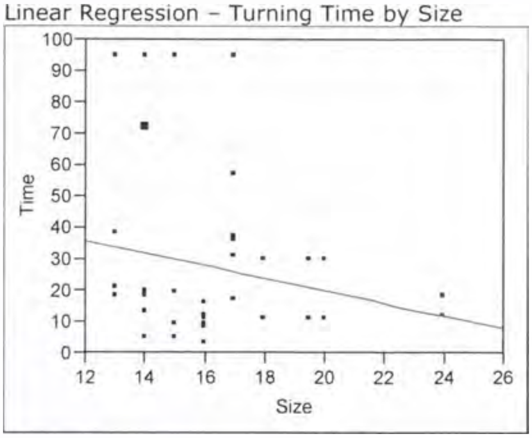


FIG. 4. Correlation experiment between size and time turning over.

In my study *C. novaeguineae* showed a clear preference for *Acropora spp.* and *Pocillopora spp.* over *Porites spp.*. When it had a choice, *C. novaeguineae* would prefer not to eat *Porites spp.* coral even though it was relatively abundant in three of my study sites. It was not abundant in Cook's Bay. In fact, the reef in front of the Gump Research Station in Cook's Bay does not have much live coral. It is mostly composed of sediment, calcified rock, and coral rubble (Bertics 2003). Roberge's study in 2000, suggested *Porites spp.* might be a potential choice prey of *C. novaeguineae,* because of its close proximity to the coral, however this does not seem to be the case. *Porites* coral is considered a large species, and it is possible that *C. novaeguineae* has difficulties climbing it, and therefore does not eat it (Glynn and Krupp 1986). It might be using *Porites* coral heads as shelter in Cook's Bay.

Bertics' (2003) feeding experiments using only specimens from Cooks Bay, showed a strong feeding preference for *Acropora spp.* In my experiments, *C. novaeguineae* showed a slight, but statistically insignificant (p=0.4482) preference for *Pocillopora spp* over *Acropora spp.*, .suggesting that cushion stars will eat both coral if they are readily available.

C. novaeguineae move in a persistent pattern in search for food. My experiments showed that cushion stars moved toward food more often than not. Eighty percent of the time when *C. novaeguineae* moved away from food it was a new specimen. New specimens generally moved toward a corner of the rectangular tank where they wedged themselves into the corner and remained motionless. In the round tank, the cushion stars climbed up the side of the tank to the waters edge, and then crept along the edge. Once cushion stars sensed that there was no escape, they would change patterns, and either crawl back to the center pole and remain there, or crawl next to another individual and "huddle" together. Somewhat "habituated" cushion stars generally moved and mounted coral within the first hour of an observation period. This suggests that *C. novaeguineae* detects food, possibly by smell, and then purposefully moves toward it.

There was no clear correlation between *C. novaeguineae's* size and turn-over time, but figure 4 shows a slight pattern: larger individuals turned over faster than smaller ones. Turning from their aboral side back onto their oral side likely requires significant energy which larger individuals are more likely capable of.

CONCLUSION

C. novaeguineae in Mo'orea is a coral predator that has exhibited coral preference. Its selective predation on *Pocillopora spp.* and *Acropora spp.* can potentially limit the abundance of these coral species on the island of Mo'orea, especially the coral *Acropora cervicornis,* which in northern Mo'orea, only occurs in the Tamae region. Changes in the abundance of the two preferred coral can affect the overall reef ecology and fish habitat.

ACKNOWLEDGMENTS

I thank the professors in the course for their guidance and leadership throughout the semester, April Dobbs and Lori LaRue for their help gathering corals, and the GSI's Erica Spotswood, ESPM, Liz Perotti, IB, and Alison Purcell, ESPM for statistical and editing help. I also want to thank Valentine and Toni at the Gump Research station. They were great!

LITERATURE CITED

Bertics, Victoria. (2003). Feeding Biology of the Cushion Sea Star, *Culcita novaeguineae,* in Moorea, French Polynesia. U.C. Berkeley, Student Research Papers, Fall 2000.

Birkeland, C (1988). The influence of echinoderms on coral-reef communities. Mar Lab

Glynn, PW & Krupp, DA. (1986). Feeding biology of a Hawaiian sea star corallivore, Culcita Novaeguineae. *Experimental Marine Biological Ecology*. vol 96: 75-96.

Lessios, HA, JD Cubit, DR Robertson, MJ Shulman, MR Parker, SD Garrity & SC Levings (1984a) Mass mortality of *Diadema antillarum* on the Caribbean coast of Panama. Coral Reefs. 3:173-182

Roberge, Paul. (2000). Locomotion and Distribution of the Cushion Star Culcita novaeguineae in Cook's Bay, Moorea, French Polynesia. U.C. Berkeley, Student Research Papers, Fall 2000.

APPENDIX A

TABLE 1. Percent frequency by individuals

Individual Feeding Frequency -Three Coral Species

Specimen	Location	% Acropora	% Pocillopora	% Porites	% Other	% not eating
1	Cooks Bay	33.3	0.0	0.0	33.3	33.3
2	Cooks Bay	33.3	0.0	33.3	0.0	33.3
4	Cooks Bay	100.0	0.0	0.0	0.0	0.0
7	Cooks Bay	28.6	71.4	0.0	14.3	14.3
5	Maharepa	22.2	33.3	0.0	11.1	33.3
6	Maharepa	44.4	22.2	22.2	11.1	22.2
3	Tamae	30.8	23.1	7.7	7.7	38.5
8	Tamae	20.0	20.0	20.0	0.0	20.0

TABLE 2. Percent frequency with Two Corals

Feeding Frequency with Two Coral Species

Specimen	Location	% Acropora	%Pocillopora
7	Cooks Bay	33.3	66.7
9	Faimano	50.0	33.3
10	Faimano	16.7	33.3
12	Faimano	0.0	50.0
5	Maharepa	33.3	50.0
6	Maharepa	33.3	50.0
11	Maharepa	25.0	25.0
3	Tamae	66.7	50.0
8	Tamae	66.7	33.3

DISTRIBUTION AND DISPERSAL OF THE SOUTH PACIFC TREE, *FAGRAEA BERTERIANA* (LOGANIACEAE)

ZACHARY R. HANNA

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

Abstract. Tahitian legend states *Fagraea berteriana* is a gift of repentance from the god, Tane. The scientific community knows little more about this tree than its inter-island distribution in the South Pacific. I surveyed the island of Moorea to map an intra-island distribution of *F. berteriana* and quantify environmental characteristics surrounding the tree's growth. I tested dispersal hypotheses by collecting bird observations and conducting seed germination experiments that included a number of seed scarification treatments. The tree occurred in densities ranging from 44 to 244 trees/hectare and at elevations spanning from approximately 300 to 900 m. Density differed with significance between two sites, Tohiea and the Cross Island Trail. Tree density increased with greater elevations and more southerly aspects. Ninety-five percent of the trees sampled grew on slopes greater than 80%. There was no correlation between tree density and slope or between density and tree height. Tree density did not significantly differ between three substrate types: rock, rocky soil, and soil. I observed Silvereyes and Red-vented bulbuls consuming *F. berteriana* fruits and germination from seeds, although at a less than 1% rate. The germination success and bird observations served as a preliminary investigation of the dispersal of *F. berteriana* and fostered ideas concerning worthwhile future directions of study.

Key words: Fagraea berteriana; tree; distribution; Moorea, French Polynesia; density; elevational gradient; germination; bird dispersal;

INTRODUCTION

Many factors can influence the distribution of plants in the tropics (Clark et al. 2005). It can be difficult to parse out these factors. Islands can offer a solution to this dilemma.

Fagraea berteriana (A. Gray ex Benth), spelling as per Welsh 1998, is a little-known tree of the South Pacific. Currently, only broad distributional data exists for this organism. In order to obtain an understanding of how *F. berteriana* is distributed on the islands to which it is native, this study was conducted on Moorea, French Polynesia.

The belief of a number of Mooreans in rural agriculture occupations is that *F. berteriana* does not germinate from seed. Instead, the plant spreads asexually, growing

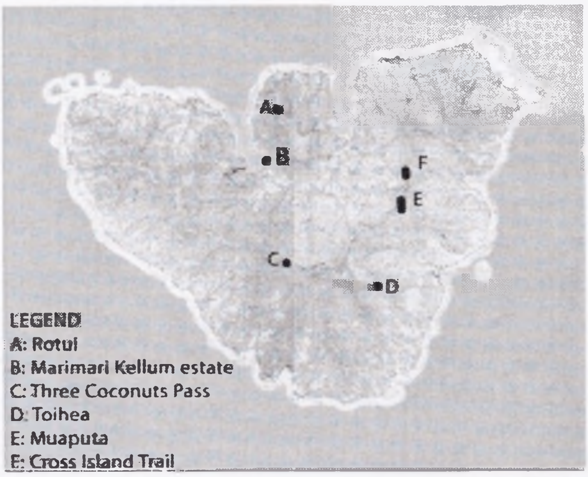


FIG. 1. Five sites sampled for *Fagraea berteriana* density and other associated environmental .variables on Moorea, French Polynesia.

from clippings of the plant's vegetative structure (Tapu, pers. comm.). One elder that was later interviewed believed that *F. berteriana* can grow from seed, but it takes much longer than growth from a clipping of the plant's vegetative structure. Mooreans thus prefer to use clippings when planting the tree in their gardens as part of their cultural tradition (Murphy, pers. comm.). A source in the agroforestry literature states that *F. berteriana* can be cultivated from seed (Elevitch and Wilkinson 2000). Steidl, in 2005, attempted to germinate 2840 seeds, scarifying 80 mechanically and 80 with an acid treatment and planting the seeds on various substrates. Steidl observed no germination (Steidl 2005).

Whether this tree germinates from seed is an integral piece of the study of this plant's distribution. Accordingly, this study sought to map the distribution, quantify associate environmental variables, germinate seeds, and study possible dispersal pathways of *F. berteriana*.

METHODS

Study organism

F. berteriana, in the family Loganiaceae, is a tree indigenous to the south Pacific. Its native range spans from French Polynesia west to Samoa and north to New Guinea (Welsh 1998, Elevitch and Wilkinson 2000). The plant's flowers are white and fade to yellow after five to six days (Hargreaves and Hargreaves 1970). It produces red, roughly-spherical, indehiscent fruits. Voucher specimens were deposited in the Jepson Herbarium, Univerisity of California, Berkeley.

Study sites

I conducted this study on Moorea, French Polynesia from 3 October – 15 November 2006. Moorea is a high, volcanic island in the Society archipelago in the South Pacific. Its tallest peak, Tohiea extends above 1000 m. The majority of native plants on islands in this region reside in the high elevation wet zone cloud forests and the subalpine zones at the high elevation ridges and peaks (Meyer and Florence 1996).

Previous study of this tree on Moorea documented its presence in the garden on the estate of Marimari Kellum located in southeast Opunohu Bay (Figure 1). I obtained all fruits supplying seeds for the germination experiment from Marimari Kellum's. The *F. berteriana* population on her property exceeds two-hundred individuals and these are hypothesized to be of the lineage of one tree planted in Kellum's garden thirty years ago (Steidl 2005, Kellum, pers. comm., Mishler pers. comm.).

Distribution

I searched for *F. berteriana* within 15 m of either side of the trails and roadways on Moorea (Figure 2). While searching, I used a GPS and compass to record the coordinates of all sites visited. This spatial data was then plotted on a map (produced by Ministere de L'Urbanisme des Transports Terrestres et de L'Administration Generale, 1989) from which I obtained elevational information.

Environmental Data

In addition to the distributional sampling of *F. berteriana*, I quantified a number of ecological variables associated with the plant's growth. At sites supporting populations of <10 individuals, I recorded the slope, aspect, height from ground to extent of canopy, growth substrate for the trees present, number of trees within a 5 m radius.

Where populations of >10 individuals were found, I also took density data by measuring the distance from a random point to the nearest *F. berteriana* individual (Cottam and Curtis 1956). This nearest *F. berteriana* individual was then my sample tree at which I logged the same environmental data previously mentioned as recorded at the sites with <10 individuals. I took ten samples in each of five study sites, except for the Cross Island Trail, where the terrain conditions allowed me to only take seven samples.

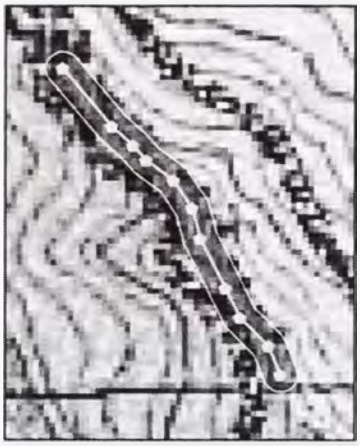


FIG. 2. Map of Rotui sample site on Moorea, French Polynesia. Points along line are the random points from which the distance to the nearest *F. berteriana* individual was measured. The buffer is the 15 m on either side of the trail that was searched.

Disperser observations

Fruit doves are present on Moorea and in order to see if they could be possible dispersers of *F. berteriana* seed, I observed birds on fruiting *F. berteriana* individuals at high and low elevation sites. On three days in November, beginning between 5:40 and 6:15 AM, I observed fruiting *F. berteriana* individuals for four hours. Observations were conduced at two high elevation sites, Three Coconuts Pass and Cross Island Trail, and at the low elevation site, Marimari Kellum's garden. On the tree being observed in Marimari's garden, I counted the total number of *F. berteriana* fruits present and the subset of these fruits that displayed evidence of consumption by birds.

Germination Experiment

I attempted to germinate individual seeds between moist filter paper in petri dishes by placing twenty-five seeds in each of six petri dishes, for a total of 150 seeds. These germinated from 10 October – 17 November 2006. Seed quantity was maximized in order to address the question of whether *F. berteriana* can propagate from seed. I approximated the number of seeds in *F. berteriana* fruits by first measuring the diameter of a fruit. I then weighed the flesh of the fruit, excluding the rind. A small section of this flesh was then weighed and the seeds were counted. I calculated that section's proportion of the total weight and used this to estimate the total number of seeds in the fruit. This procedure was repeated for 10 fruits and used to construct an approximate number of seeds per unit diameter of fruit.

I planted approximately 27,900 untreated seeds in soil and 28,600 spread out on coconut bark and these germinated from 27 October – 17 November. Approximately 16,400 seeds were spread out on a rock surface and these germinated from 6 November – 17 November.

To simulate passage through a bird's digestive tract, I treated seeds with 6 M HCl for 15, 30, and 45 second time intervals and followed the HCl treatment with rinsing. I treated approximately 10,200 seeds for 15 seconds with HCl, 11,900 seeds for 30 seconds, and 10,800 seeds for 45 seconds. I planted these seeds in soil on 8 November and they germinated until 17 November.

The final seed treatment was coffee. Seeds were submerged in a coffee solution for 19.5 hours and then rinsed. I spread approximately 17,500 seeds on rock, 17,100 seeds on coconut bark, and I planted 16,600 seeds in soil. These germinated from 7 November – 17 November.

I planted the seeds in 35x15 cm trays, which I placed in an area with partial shade and watered twice a day. I elevated the trays on brick with water moats surrounding them after an initial ant invasion resulting in the loss of a few seeds. Sterilized potting soil was utilized for the soil treatments and also in the trays under the rocks and coconut bark in treatments involving those materials. As a control, I left one tray with just sterilized soil and no seeds.

In total, the germination experiments involved approximately 160,000 seeds.

Ant dispersal

Fire ants colonized the untreated seed experiments on 28 October, the second day of the experiment. For approximately 12 hours, they removed seeds from the experiment and carried them away along their ant trail. I counted the number of seeds that the ants carried past an arbitrary point on their ant trail. Beginning at 4:00 PM on 28 October, I counted seeds for five minutes and I replicated this interval 5 times, for a total of 25 minutes of observation.

Statistical analyses

I chose to use the continuous, plotless density measure of the distance between a random point and the nearest *F. berteriana* individual instead of the defined area measure of the number of trees within a 5 m radius of the sample tree because the plotless measure is able to pick up changes in density on a larger and finer scale than the fixed radius method. To test the validity of this, I ran a linear regression between the log of the distances and the number of trees in the plots. In order to obtain a normal distribution, I first applied a log transformation of the distances to the nearest *F. berteriana* individual. Henceforth, the distances to the nearest *F. berteriana* individuals will be referred to as density.

I ran linear regression analyses of density and elevation, density and aspect, density and height, and density versus slope. I ran ANOVA's for density versus substrate and density versus sample site and employed Student's t-test with a Bonferroni correction to reveal which categories differed with significance. All statistical analyses were computed with JMP IN version 5.1.2.

RESULTS

Distribution

Five sites supported populations of > 20 *F. berteriana* individuals. These five sites were the Cross Island Trail, Muaputa, Tohiea, Three Coconuts Pass, and Rotui (Figure 1). The *F. berteriana* individuals found at these sites was almost exclusively confined the ridge tops and just down from the ridgelines. The low end of the elevational range for natural populations of *F. berteriana* began at about 300 m and extended to 700 m. Scattered individuals occurred up to approximately 900 m elevation, such as just below the summit of Rotui. *F. berteriana* grew along the coast of Moorea, but the instances in which this was observed were all associated with cultivation of the plant as an ornamental (Appendix 1).

TABLE 1. *Fagraea. berteriana* densities at five sites on Moorea, French Polynesia.

Site	Tohiea	Three Coconuts Pass	Cross Island Trail	Rotui	Muaputa
Density (trees / ha)	244	44	50	67	118

Environmental data

F. berteriana density, as calculated using the nearest individual method, varied between the five sites supporting >10 individuals (Table 1). I found *F. berteriana* on three different substrates: rock, rocky soil (soil with numerous, large rocks), and soil. Out of the 47 trees at the five sample sites for which slope was measured, only 2 trees were growing on <80% slopes. Seventeen of the 47 trees were found on >150% slopes. Heights of

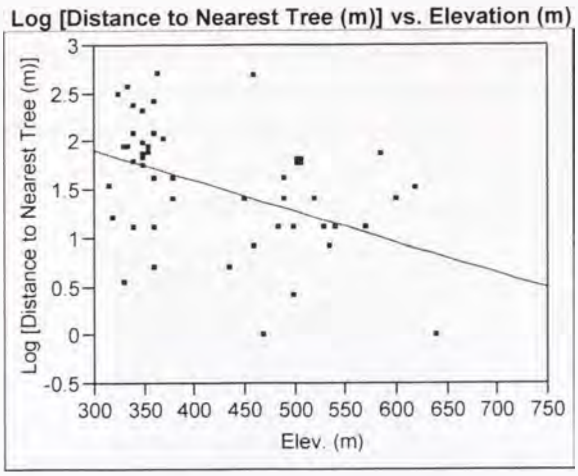


FIG. 3. The log of the distance from a random point to the nearest *F. berteriana* individual versus the elevation at which the measurement took place on Moorea, French Polynesia.

sampled *F. berteriana* ranged from 1 m to 12 m, but the average height was 4.3 m. Aspect measurements did not reveal that *F. berteriana* was strictly confined in its orientation to the sun.

Disperser observations

No birds were observed consuming *F. berteriana* fruits at either the Three Coconuts Pass or the Cross Island Trail site. While I did not sight any birds on or near the observation tree on Three Coconuts Pass, I did identify 5 Red-vented bulbuls, *Pycnonotus cafer*, and 9 Silvereyes, *Zosterops lateralis*, within a 5-meter radius of the tree observed at the Cross Island Trail site. At Marimari Kellum's garden, 6 Red-vented bulbuls and 5 Silvereyes consumed *F. berteriana* fruits. Eleven additional Red-vented bulbuls and 41 other Silvereyes were visitors to the observation tree. Out of 80 *F. berteriana* fruits on the observed tree, 40 displayed evidence of bird consumption.

Germination experiment

The average *F. berteriana* fruit size was 23.5 mm and this contained approximately 1200 seeds. *F. berteriana* can germinate from seed on Moorea. The untreated seeds were the only seeds that germinated. One untreated seed in soil sprouted on 7 November and a second on 12 November. One untreated seed on coconut bark germinated on 7 November and four germinated on 11 November. No other seeds germinated. The three HCl treatments exhibited the growth of a light green mold in the soil around and on the seeds.

Ant dispersal

The ants removed an average of 7 seeds per minute from the untreated seed experiments. Some ants were trapped on the trays when I isolated the experiment within a moat. These ants continued to move seeds around. By 17 November, they had removed virtually all of the seeds from the coconut bark and cashed them in the soil beneath the bark. Four of the five germinations that occurred with the coconut bark treatment took place under the bark after ants moved the seeds there. One occurred on the bark.

Statistical analyses

F. berteriana density was significantly correlated with elevation (P=0.0013, R^2=0.2082) (Figure 3) and aspect (P=0.0458, R^2=0.0876) (Figure 4). There was no significant correlation of density and tree height (P=0.9129, R^2=0.0003) or density and slope (P=0.2758, R^2=0.0263).

Mean *F. berteriana* density did not differ significantly by substrate type (P=0.8924, R^2=0.0061). Between some sites, density did vary significantly (P=0.0113, R^2=0.2610). Toihea differed significantly from Three Coconuts Pass (P=0.0070) and the Cross Island Trail (P=0.0036).

DISCUSSION

Moorea hosts natural populations of *F. berteriana* of considerable densities (Table 1).

The tree density at Tohiea, the densest site, was similar to the density of Douglas-fir (200 stems/ha) in an old-growth, mixed-conifer stand in the Sierra Nevada of California, USA (Ansley and Battles 1998). These natural *F. berteriana* populations were generally confined to at or just below ridge tops. Not once did I find natural populations on lowland slopes.

F. berteriana density increased with higher elevations and with a more southerly exposure to the sun (Figures 3 and 4). A rapid increase in rainfall corresponds with moving higher in altitude on high islands, such as Moorea (Meyer and Florence 1996). On Moorea, in the southern hemisphere, slopes facing the northwest receive the most intense solar radiation. This was the aspect with the lowest *F. berteriana* density. Higher *F. berteriana* densities were correlated with increased rainfall and shade conditions.

Density only differed significantly between two pairs of sites: Tohiea and Three Coconuts Pass and Tohiea and the Cross Island Trail. Tohiea's *F. berteriana* population reached 500 m, approximately 180 m higher than the populations at Three Coconuts Pass and the Cross Island Trail. This difference in elevation may correspond with a drastic difference in rainfall (Meyer and Florence 1996).

The observation that Red-vented bulbuls and Silvereyes will consume *F. berteriana* fruits is a first step in a study of its dispersal. The fact that these birds are also found where there are natural populations of *F. berteriana* allows for the possibility that the birds may be consuming the fruits there also. It is likely that the seeds of *F. berteriana* on Moorea will be viable after passing through a bird's digestive tract since the plant is documented as dispersed by pigeons in the Marquesas (Petard 1986, Whistler 2001). Red-vented bulbuls and Silvereyes are both introduced, non-native birds on Moorea (Meyer and Florence 1996). The bird that once may have dispersed *F. berteriana* on Moorea may now be extinct.

There are nine native ant species present on Moorea (Ledoux et al. 2006). Ant dispersal of *F. berteriana* does not explain the growth of the tree on rock substrate. I observed the ants removing seeds from a non-soil substrate (coconut bark) and storing them in the soil. Germination on rock would be unlikely in the case of ant dispersal.

Seven successful germinations out of approximately 160,000 seeds is quite a low germination rate. With 1200 seeds in the average fruit, it may be that the germination rate is really this low. Alternatively, it may be that the partially shaded conditions in which the experiment was conducted were not optimal for germination.

The distribution of *F. berteriana* on Moorea is a patchy one, which may be indicative of dispersal by frugivores (McConkey et al. 2004). The tree appears to thrive at high elevations with shade and ample rainfall. This study suggests that the germination rate of *F. berteriana* is quite low. If the tree is dispersed by birds, then very few of the dispersed seeds develop.

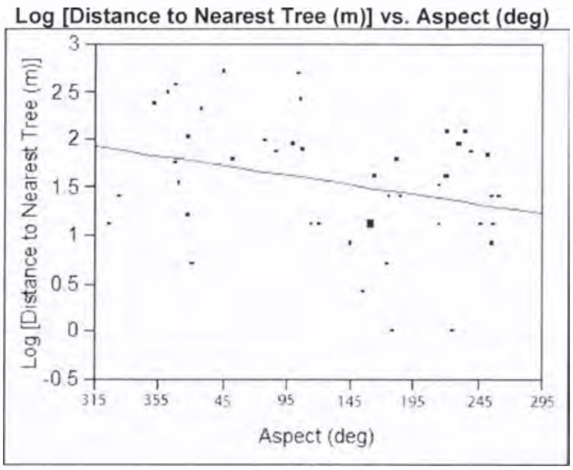


FIG. 4. The log of the distance from a random point to the nearest *F. berteriana* individual versus the aspect of the tree on Moorea, French Polynesia.

Why natural populations of *F. berteriana* are almost exclusively confined to the very tops of ridges still remains to be explained. Study of the viability of seeds after passage through Silvereyes and Red-vented bulbuls seems the next logical step that must be made before gaining an idea of the dispersal of *F. berteriana*. This question of dispersal may play a key role in addressing the ridge top distribution phenomenon.

ACKNOWLEDGMENTS

I thank Tom Bell, Joel Nitta, Mike O'Dowd, and Jessica Castillo for field assistance. I thank the Gump Station for hosting me during my time in Moorea. Also deserving much gratitude is Marimari Kellum for allowing me to collect fruits on her property. Finally, I thank all of the GSI's and professors involved with the Moorea course for their encouragement and essential advice and inputs into this project.

LITERATURE CITED

Ansley, Jolie-Anne S. and John J. Battles. 1998. Forest Composition, Structure, and Change in an Old-Growth Mixed Conifer Forest in the Northern Sierra Nevada. *Journal of the Torrey Botanical Society* **125**: 297-308.

Clark, C. J., J. R. Poulsen, B. M. Bolker, E. Connor and V. T. Parker. 2005. Comparative Seed Shadoes of Bird-, Monkey-, and Wind-Dispersed Trees. *Ecology* **86**: 2684-2694.

Cottam, G., and J.T. Curtis. 1956. The use of distance measures in phytosociological sampling. *Ecology* **37**: 451-460.

Elevitch, Craig R. and Kim M. Wilkinson, Eds. 2000. *Agroforestry Guides for Pacific Islands*. Permanent Agriculture Resources: Holualoa, Hawaii.

Hargreaves, Dorothy and Bob Hargreaves. 1970. *Tropical Trees of the Pacific*. Hargreaves, Kailua, Hawaii.

Ledoux, Stephanie, Yohann Allouche, Jean-Yves Meyer, Rudolph Putoa and Herve Jourdan. 2006. An update of the ant fauna of Tahiti and Moorea (French Polynesia). http://iussi.confex.com/iussi/2006/techprogram/P2498.HTM

Mayfield M. M., D. Ackerly, and G. C. Daily. 2006. The diversity and conservation of plant reproductive and dispersal functional traits in human-dominated tropical landscapes. Journal of Ecology 94:522-536 ER.

McConkey, Kim R., Hayley J. Meehan and Donald R. Drake. 2004. Seed dispersal by Pacific Pigeons (*Ducula pacifica*) in Tonga, Western Polynesia. *Emu* **104** : 369-376.

Meyer, Jean-Yves and Jacques Florence. 1996. Tahiti's native flora endangered by the invasion of *Miconia calvescens* DC. (Melastomataceae). *Journal of Biogeography*. **23**: 775-781.

Petard, Paul. 1986. *Quelques Plantes Utiles de Polynesie Francaise et Raau Tahiti*. Editions haere po no Tahiti. Papeete.

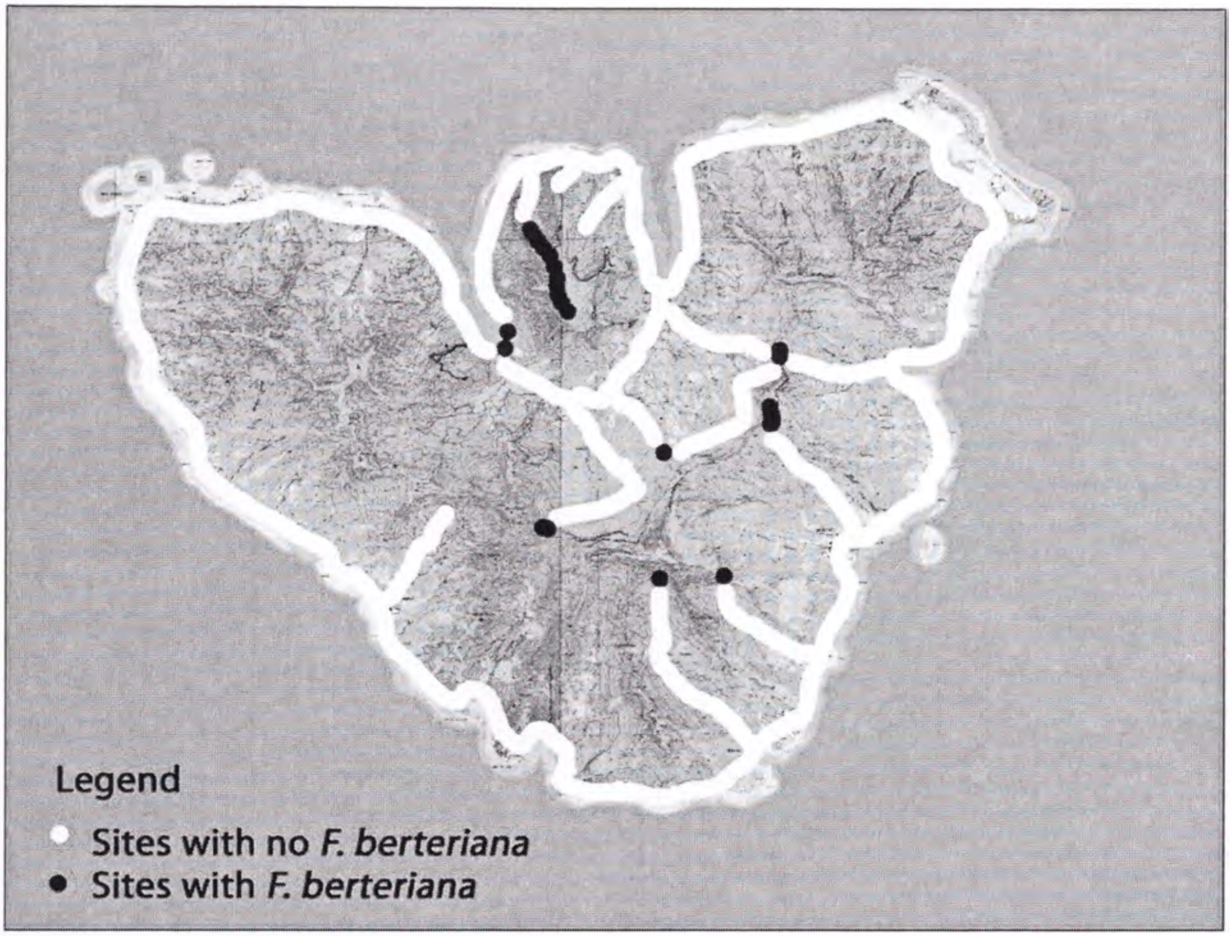
Steidl, Sara A. 2005. Biology of *Fagraea berteroana*: habitat specialization and conditions for biological success of an anomalous population. Moorea Biology and Geomorphology of Tropical Islands, Student Research Papers. University of California, Berkeley.

Welsh, Stanley L. 1998. Flora Societensis: a summary revision of the flowering plants of the Society Islands. E.P.S. Inc., Orem, Utah.

Whistler, W. Arthur. 2001. *Plants in Samoan Culture: The Ethnobotany of Samoa*. Isle Botanica, Honolulu, Hawaii.

APPENDIX A

Map of the sites searched on Moorea, French Polynesia. The white areas are where no *F. berteriana* was found and the black areas are where the tree was found.



FUNCTION, DESIGN, SCALING, AND SEXUAL DIFFERENCES OF DIMORPHIC CHELAE IN THE LAND CRAB, *CARDISOMA CARNIFEX*

TOM HATA

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

Abstract. Crab chelae are a model system for studying the relationship between the biomechanics of an organism's structure and its ecological role. This study investigated how chelae dimorphism may correlate with specialization in function in the land crab *Cardisoma carnifex* (Herbst 1791). This was achieved by comparing field observations of preferential claw usage during diurnal activities to a mechanical model derived from anatomical claw measurements, claw closing effort of captured specimens, and calculations of expected closing force. Behavior, mechanical scaling, and effort were also compared between males and females. Foraging, eating, and lead claw entering burrow showed significant differences in claw use frequency. It was also found that the major and minor claws scaled differently with respect to carapace length, with the major claw displaying positively allometric scaling and the minor claw displaying near-isometric scaling. Measurement of claw closing effort with respect to claw length showed high correlation in male minor and female major claws. In males, expected force measurements showed a greater rate of growth in the minor claw than in the major claw with respect to claw length, but in females, expected force in the major claw exhibited a greater rate of growth. A possible explanation for the differences in design between sexes may be that there are functional differences between male and female chelae, such as the primary use of male minor claw and female major claw in gripping objects when stressed.

Key words: biomechanics; Brachyura; functional morphology; force generation; sexual dimorphism

INTRODUCTION

Ecological functions of anatomical structures are often closely tied to the mechanical design of these structures. The relationships between form and function have been studied for centuries in many vertebrates, invertebrates, and plants. Most recently, arthropods–particularly crabs—have been the focus of thorough investigation. Crab chelae, or claws, serve many ecological functions, such as foraging (Schenk and Wainwright 2001), mate acquisition (Schenk and Wainwright 2001), defense (Barnes 1974), offense (Meglitsch 1972), and manipulating objects (Meglitsch 1972). Despite the multipurpose nature of the chelae, they are mechanically simple, and so they are a model system for exploring the relationship between morphology and ecology (Schenk and Wainwright 2001).

Many Brachyuran crabs exhibit claw dimorphism, which can often be attributed to some specialization in function between the claws. The larger claw is commonly designated as the "crusher" claw (major claw) and the smaller claw is designated as the "cutter" claw (minor claw). This dimorphic design is assumed to be a tradeoff between closing speed and force generation, with the minor claw adapted for speed and the major claw adapted for power (Schenk and Wainwright 2001). The most extreme example of claw dimorphism in crabs is observed in the fiddler crab (family Ocypodidae, genus *Uca)*. In this case, the claw dimorphism is also sexually dimorphic, with only males possessing a large major claw. In this case, form of the major claw is a consequence of sexual selection (Levinton and Judge 1993, Levinton et al. 1995, Levinton and Allen 2005). Diet is also a factor associated with claw design (Yamada and Boulding, 1997). Studies have shown that crabs that feed on fast moving prey have fast, relatively weak claws, while crabs that feed on hard-shelled prey have slow, powerful claws that operate at higher mechanical advantage (Seed 1995, Yamada and Boulding, 1997).

Cardisoma carnifex (Herbst 1791), a member of family Gecarcinidae, is a burrowing land crab that is common on the island of Moorea, French Polynesia (Bickel 1997) as well as throughout the Pacific (Denhoy and Battersby 1992). Adult crabs are eight to thirteen centimeters in carapace width (Denhoy and Battersby 1992), and they are opportunistic scavengers, feeding on leaves, plant parts, detritus, and mud (Bickel 1997). *C. carnifex* individuals of both sexes exhibit dimorphic chelae. Although there have been several studies on its ecology (Denhoy and Battersby 1992; Bickel 1997; Cheng 2000; Elitzur 2001), no claw mechanical studies have been done, and it remains a poorly studied species.

The main objective of this study was to determine relationships between function, closing force generation, and anatomical scaling of the dimorphic claws in *C. carnifex*. The first objective was to observe *C. carnifex* in the field in order to establish the ecological roles of the major and minor claws. I expected to see preferential usage of one claw over the other depending on the activity. Another objective was to determine whether there were differences between the major and minor claws in scaling of claw size relative to crab size and in scaling of claw shape relative to claw size. Based on a study of fiddler crabs by Levinton and Allen (2005), I expected the major claw to scale allometrically, while the minor claw would scale isometrically. Preliminary observations of crabs led me to expect that the major claw would exhibit changes in claw shape relative to claw size, while the minor claw's shape would be independent of size. The third objective of this study was to determine differences in closing force generation between the major and minor claws by measuring claw closing effort and calculating expected closing force at the claw tip by using a mechanical model from anatomical measurements. Again, based on Levinton and Allen's 2005 study of fiddler crabs, I expected the major claw to allometrically scale in force generation as a function of claw length, becoming weaker relative to an isometric scaling of force as claw size increased. In contrast, I expected the minor claw to scale isometrically in force generation. The final objective of this study was to determine whether there were mechanical claw differences between sexes. Since both sexes exhibited claw dimorphism, I expected anatomical scaling and closing force generation between sexes to be similar for the major and minor claws.

FIG. 1. Map showing the location of the study site at Gump Biological Research Station on Moorea, French Polynesia (17°30' W Lat., 149°50'S Long.)

External Claw Measurements

External claw measurements and carapace width were measured in order to determine whether claw scaling was isometric or allometric. In order to prepare crabs for dissection, they were put into containers of seawater and placed in the freezer for approximately two hours, or until they stopped moving. Crabs were sexed by abdomen shape and their carapace widths were recorded. For this study, carapace width was used as a proxy for crab size. Claws were identified as either major or minor and separated from the rest of the body using dissecting scissors. External claw measurements were chosen from Levinton and Allen (2005). Claw length, dactyl length, dactyl height, pollex length, manus height, manus width, and manus length (fig. 2a) were measured to the nearest .05mm using vernier calipers.

Muscle Pennation Angle

Crab chelae can be described as a simple lever system. Pennate muscles attached to an apodeme contract to rotate the dactyl (moveable finger) around a pivot point to either open the claw or close it against the pollex (fixed finger) (Levinton and Allen 2005) (Fig. 2b). An internal morphological property that affects closing force is the pennation angle (α) of the claw closer muscle, which, in arthropods, is the angle at which muscle fibers connect to the apodeme (Schenk and Wainwright 2001). The calculation of force in relationship to pennation angle can be written as (Schenk and Wainwright 2001):

$$F_m = A \sigma \sin 2\alpha \qquad (2)$$

F_m is the force exerted by the muscle, A is the muscle cross-sectional area (MCA), and σ is the stress generated per unit MCA. As the equation shows, force generation is maximized as muscle pennation approaches 45° (Schenk and Wainwright 2001).

To stiffen muscles fibers to measure closing muscle pennation angle, the claws were then preserved in the closed position in 10% formol for at least 24 hours. The manus, but not the dactyl, was then cut along line X-X (Fig. 2a) using dissecting scissors for smaller claws or a handsaw for larger claws. Dactyls were removed carefully from the claw, since the closing muscle apodeme usually remained attached to the dactyl. In order to determine pennation angle, the bottom portion of each claw was placed over a vertical strip of tape, with the area where the closing apodeme used to lie placed along one edge of the tape. A string tied to two dissecting pins was pinned down across the claw, parallel to the visible muscle fibers. The claw was then removed and another piece of tape was placed down, with one edge of the tape lying along the path of the string. The string was removed and the intersecting pieces of tape were digitally photographed. The pennation angle was measured by using the program ImageJ to determine the angle of intersection.

Muscle Cross-sectional Area

Force generation in equation 2 is proportional to a second internal morphological property: muscle cross-

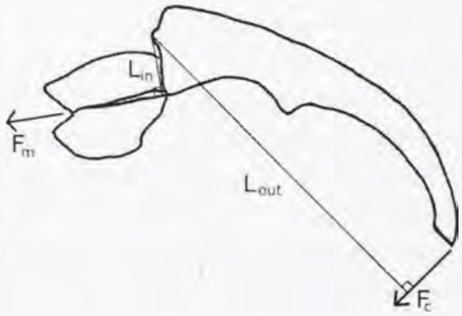


FIG. 3. Diagram of a major claw dactyl and its closer apodeme. Forces and lever arms are labeled for the closing lever system. L_{in} is the in-lever, the distance from the pivot to the apodeme joint perpendicular to muscle contractile force F_m. L_{out} is the out-lever, the distance from the pivot to the dactyl tip perpendicular to force exerted at claw tip, F_c.

sectional area, which is equal to apodeme area (Levinton and Allen 2005). In order to measure apodeme surface area, the closing muscle apodeme was separated from the dactyl after the dactyl was extracted from the rest of the claw. The apodeme was then scraped clean of muscle fibers and digitally photographed along with a known scale. Using these photographs, ImageJ (Rasband, 2006, National Institutes of Health), was used to calculate the apodeme surface area. Surface areas of excessively damaged apodemes were not included.

Mechanical Advantage

The force generated by the muscle is transmitted through the lever system, where the mechanical advantage (MA) of the lever system becomes yet another morphological factor that influences closing force. In a lever system, lever-arm length is the distance from the fulcrum to the force vector, perpendicular to the force. MA is determined by the relative proportion of lever-arm length (in-lever/out-lever) (Levinton and Allen 2005). Values of MA greater than one represent a proportional increase in force exerted on the load and a corresponding decrease in velocity in moving the load, while MA values less than one represent a decrease in force exerted and increase in velocity (Vogel 2003).

In order to measure mechanical advantage of the closing lever system, the in-lever and out-lever of the lever were measured, as shown in figure 3. In-lever distance was measured from the pivot point to the middle of the thick, central line of the apodeme, perpendicular to this central line. Out-lever distance was measured from the pivot point to an imaginary line perpendicular to the dactyl tip, parallel to the dactyl tip. Measurements were taken using vernier calipers to the nearest .05mm. Mechanical advantage was calculated using equation x.

Force Calculation

Expected maximum closing force at the tip of the claw (F_c) was determined by first calculating the expected maximum force exerted by the claw closing muscle (F_m) using equation 2. For the maximum stress value (σ), 200 kilopascals was used. Testing of isometric stress has shown that this value is relatively constant among a variety of animals (Vogel 2003). As F_m is the force input and F_c is the force output in the closing lever described in figure 3, F_m can be determined by multiplying F_c by mechanical advantage (MA).

$$F_c = MA\ F_m \qquad (3)$$

Statistical Analysis

To obtain claw use frequencies, claw usage data were grouped by time of day recorded and summed together within each group. This avoided an overabundance of frequencies of 1 due to small sample sizes. For each size class, claw usage was sorted by activity. For each activity, claw usage for major claw, minor claw, and both claw were divided by the total claw usage for that activity to obtain a frequency of claw usage.

In order to determine whether different activities had different frequencies of claw usage, the claw usage frequency data were analyzed using Kruskal-Wallis tests for group differences and Tukey-Kramer HSD for multiple paired tests. The Tukey-Kramer tests were corrected for multiple tests at the .95 significance level. Kruskal-Wallis tests were used, because data was not normally distributed, even with transformations. For this analysis, the groups used were the six observed activities, the response was claw frequency, and the tests were done by claw. The activity "lead claw entering burrow" was removed as a group for the analysis of both claws used, because it is physically impossible for these crabs to enter their burrows with both claws leading.

To determine whether claw frequencies differed within activities, the usage frequency data was once again analyzed using Kruskal-Wallis test and Tukey-Kramer HSD. The groups used were the three categories of claw use, the response was claw frequency, and the tests were done by activity. For the analysis of the activity "lead claw entering burrow", the group "both claws" was removed for the same reason stated in the previous paragraph. Kruskal-Wallis and Tukey Kramer HSD were also used to compare relative frequencies of claw usage for ambidextrous activities.

Linear regressions of log-log plots were used to analyze scaling of the anatomical and mechanical data. All regressions were performed by sex in order to determine if there were differences in scaling between sexes. Excluding the field data, all other data were log transformed. First, claw size was analyzed as a function of carapace width. Next, all other measurements (external claw measurements, indentation volume of polystyrene, apodeme surface area, pennation angle, measurements of mechanical advantage, effort exerted, and calculated closing force) were analyzed as a function of claw length. For measurements showing no linear correlation for either claw of either sex, measurements were analyzed using Kruskal-Wallis and Tukey HSD tests, grouped by claw and sex. Specifically, this was done for measurements of mechanical advantage.

RESULTS

Field Observations

Kruskal-Wallis tests showed that for each category of claw use, claw usage frequencies were significantly different between activities (p<0.0001 for both claws used, p=0.0198 for major claw, and p=0.0008 for minor claw) (Appendix A). For activities performed using both claws, the usage frequency of removing dirt was significantly higher than those of threat display, and fighting. For activities performed using only the major claw, usage frequency for fighting was significantly higher than frequencies for lead claw entering burrow, foraging, eating, and removing dirt. Also, usage frequency during threat display was significantly higher than the frequency during eating. Finally, minor claw frequency of usage in "lead claw entering burrow" was significantly higher than the other five activities.

For Kruskal-Wallis tests of claw usage by activity, eating (p=0.0244), foraging (p=0.0025), and lead claw entering burrow (p<0.0001) showed significant differences in claw usage (Appendix B). For the activity of eating, the frequency of use of both claws was significantly higher frequency than the frequency of use of just the major claw. As for foraging, both claws were used at a significantly higher frequency than both the major claw and the minor claw used alone. Finally, for lead claw entering burrow, minor claw frequency of usage was significantly higher than major claw frequency.

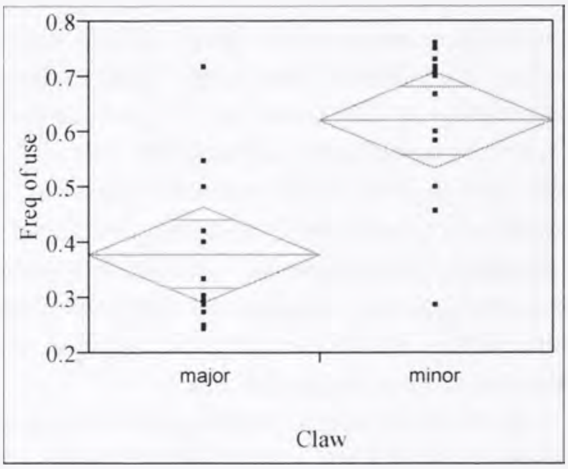


FIG. 4. Claw use frequency of ambidextrous feeding, grouped by claw. Means and quantiles are shown for each group. Analysis by Kruskal-Wallis test showed significant difference between treatments (p=0.0027).

Eating was the only ambidextrous activity observed where relative usage rates between claws could be measured. Kruskal-Wallis test showed the usage rate between major and minor claw to be significantly different ($p=0.0024$), with minor claw usage frequency (0.621) significantly higher than major claw usage frequency (0.379) (Fig. 4).

Closing Effort

Linear regressions of log-log plots of volume of polystyrene indentation as a function of claw length showed that in males, there was no correlation with the major claw ($p=0.7275$) but there was a strong correlation with the minor claw ($p<0.0001$) (Fig. 5). In contrast, females exhibited no correlation with the minor claw ($p=0.1308$) but did exhibit strong correlation with the major claw ($p=0.0069$).

External Claw Measurements

Linear regressions of log-log plots of claw length (CL) as a function of carapace width (CW) and external claw measurements as functions of claw length displayed strong correlations ($p<0.05$) (Fig. 6) for both sexes. For males, the major claw scaled to $CW^{1.395}$ ($R^2=.991$) and the minor claw scaled to $CW^{1.12}$ ($R^2=.988$). For females, the major claw scaled to $CW^{1.210}$ ($R^2=1.21$) and the minor claw scaled to the $CW^{1.04}$ ($R^2=1.04$). Excluding female major claw manus width, scaling of external claw measurements ranged from $CL^{0.877}$ to $CL^{1.148}$ (Appendix C). Scaling for female major claw manus width is claw length to the $CL^{0.773}$ ($R^2=0.974$) (Appendix C).

Muscle Pennation Angle

Linear regressions of log-log plots of muscle pennation angle as a function of claw length displayed no correlation in males (major: $p=0.438$; minor: $p=0.735$), but displayed strong correlation in females (major: $p=0.0264$; minor: $p=0.0345$) (Fig. 7). Female major claw closing muscle pennation angle scaled to $CL^{-0.204}$ ($R^2=0.4382$) and minor claw pennation angle scaled to $CL^{-0.234}$ ($R^2=0.7352$) (Appendix C).

Muscle Cross-sectional Area

Linear regressions of log-log plots of apodeme surface area as a function of claw length showed strong correlations in both major and minor claws of males and females ($p<0.05$) (Appendix C). In males, major claw apodeme area scaled to $CL^{1.798}$ ($R^2=0.629$) and minor claw apodeme area scaled to $CL^{2.084}$ ($R^2=0.922$). In females, major claw apodeme

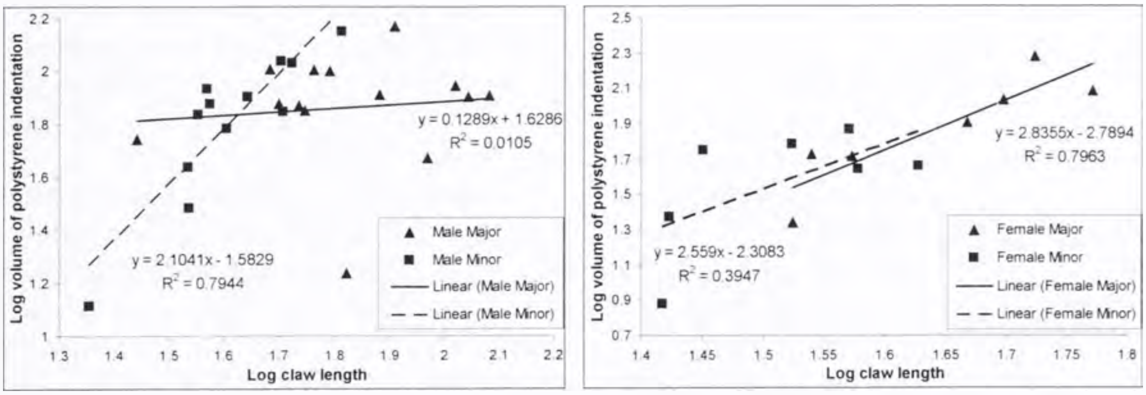


FIG 5. Log-log plot of volume of polystyrene indentation as a function of claw length. Indentation volume was used as a measure claw closing effort. In males, the major claw displayed poor correlation ($n=14$, $p=0.7275$), and the minor claw displayed high correlation ($n=12$, $p<0.0001$). In females, the major claw displayed high correlation ($n=7$, $p=0.0069$), and the minor claw displayed poor correlation ($n=7$, $p=0.1308$).

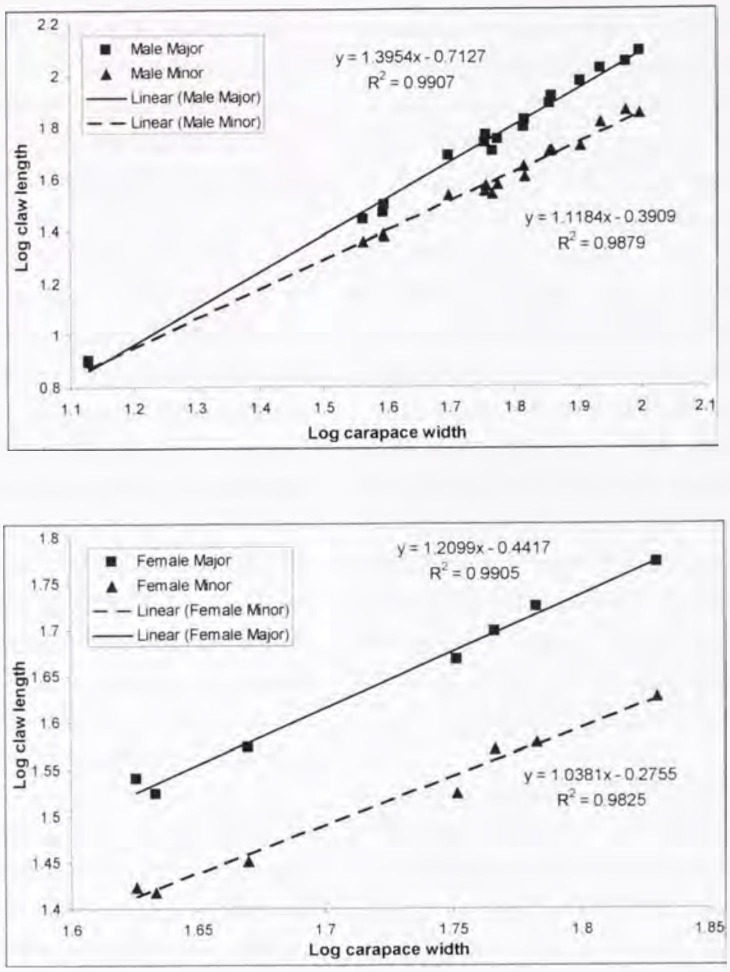


FIG. 6. Log-log plot of the claw length as a function of carapace width. In males, both the major claw and the minor claw displayed high correlations ($n=17$, $p<0.0001$ for both graphs). In females, the major claw and the minor claw exhibited high correlations as well ($n=7$, $p<0.0001$ for both graphs).

area scaled to $CL^{1.609}$ ($R^2=0.896$) and minor claw apodeme area scaled to $CL^{1.420}$ ($R^2=0.785$).

Mechanical Advantage

Linear regressions of log-log plots of the in-lever, out-lever, and mechanical advantage as a function of claw length showed strong correlations for in-levers and out-levers in both major and minor claws of males and females ($p<0.05$), but showed almost no correlation for mechanical advantage for any claw of either sex (Appendix C). In males, major claw in-lever scaled to $CL^{0.962}$ ($R^2=0.983$) and minor claw in-lever scaled to $CL^{1.12}$ ($R^2=0.976$). Major claw out-lever scaled to $CL^{0.936}$ ($R^2=0.985$) and minor claw out-lever scaled to $CL^{1.081}$ ($R^2=0.976$). In females, major claw in-lever scaled to claw length to $CL^{0.986}$ ($R^2=0.974$) and minor claw in-lever scaled to $CL^{1.248}$ ($R^2=0.935$). Major claw out-lever scaled to $CL^{0.989}$ ($R^2=0.939$) and minor claw out-lever scaled to $CL^{1.026}$ ($R^2=0.994$).

Kruskal-Wallis test and Kramer HSD analysis of mechanical advantage showed that MA in male and female major claws was significantly higher than MA in male and female minor claws (Fig. 8). MA in female major claws was also significantly higher than MA in male major claws.

Force Calculation

Linear regressions of log-log plots of

expected maximum closing force as a function of claw length showed strong correlations in both major and minor claws of males and females ($p<0.05$) (fig. 9). In males, major claw force scaled to $CL^{1.666}$ ($R^2=0.893$) and minor claw force scaled to $CL^{2.194}$ ($R^2=0.845$). In females, major claw force scaled to $CL^{1.684}$ ($R^2=.943$) and minor claw force scaled to $CL^{1.577}$ ($R^2=0.719$).

Discussion

My results show that there are strong anatomical and mechanical differences between the major claw and minor claw. The major claw length scaled allometrically as a function of carapace width, and several external claw measurements scaled allometrically as functions of claw length as well. The minor claw, however, appeared to scale nearly isometrically with respect to both claw length against carapace width and external measurements against claw length.

Positively allometric scaling of the major claw not only demonstrates that the claw becomes proportionally larger relative to the carapace as size increases, but it also shows that there is some degree of shape change as claw size increases. For both sexes, there are relative increases in dactyl and pollex length and a relative decrease in manus length with respect to increasing claw length. Empirically, I also observed that the curvature of the dactyl seemed to increase, as well as the relative slenderness of the dactyl and pollex when compared to the claw size. On the other hand, the nearly isometric scaling of the minor claw, especially so in males, shows that minor claw shape is relatively independent of claw size.

Measurements of internal anatomy also exhibited differences in scaling. An example of this is apodeme surface area, which is also defined in this study as the muscle cross-sectional area. As equation 1 showed, force generation at the muscle is directly proportional to muscle cross-sectional area. Analysis of regression involved correlating an area as a function of a distance, so for isometric scaling the expected value would be CL^2. Scaling of apodeme surface area in the major claw was negatively allometric, which suggests that unless there were other compensating factors, larger claws were weaker per square of unit length. However, the minor claw apodeme in male crabs scaled very close to the 2nd power in males, so it

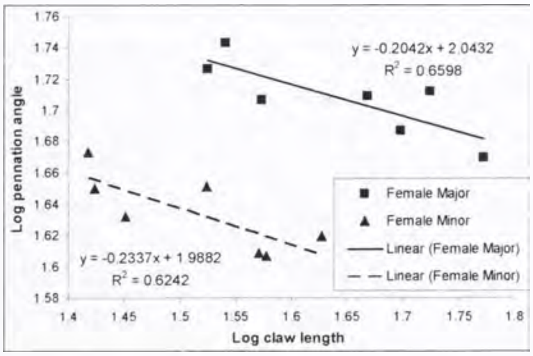


FIG. 7. Log-log plot of pennation angle as a function of claw length in females. . Both the major claw (n=7, p=0.0264) and the minor claw (n=7, p=0.0345) exhibited strong correlations. The graph for pennation angle in males is not shown, because both claws exhibited poor correlations.

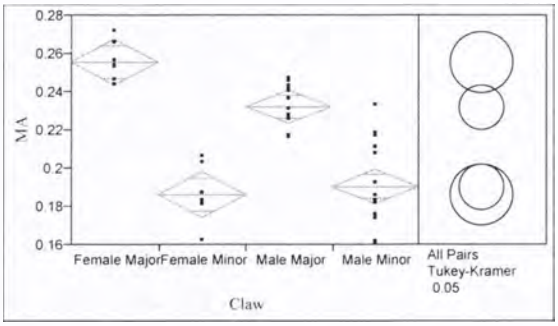


FIG. 8. Mechanical advantage (MA) of claws grouped by claw and sex, showing Tukey Kramer HSD analysis. Means and quantiles are shown for each group. Kruskal-Wallis test showed significant difference between treatments ($p<0.0001$). The major claw treatments in both sexes were significantly greater than minor claws treatments in both sexes. Female major claw treatment was also significantly greater than male major claw treatment ($\alpha=0.05$)

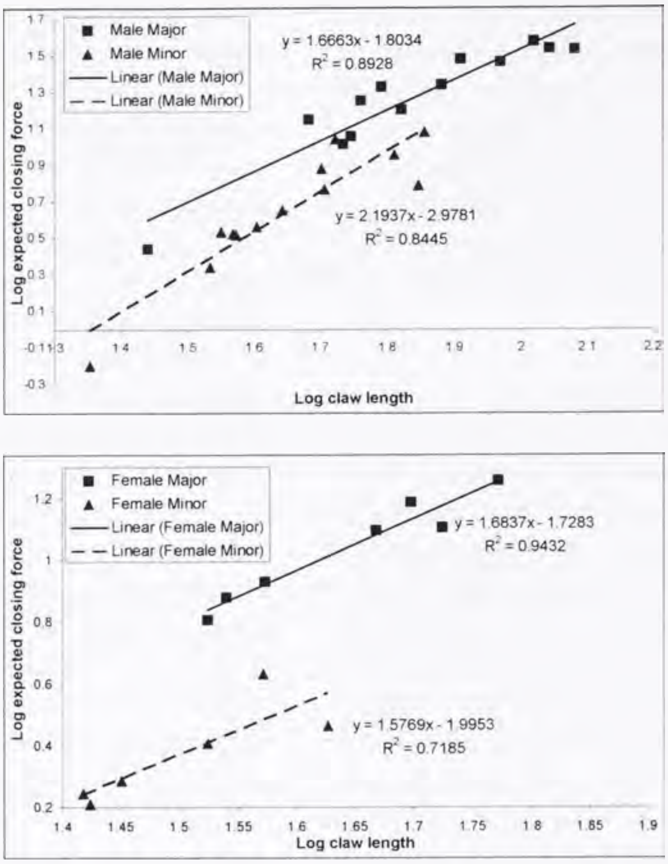


FIG. 9. Log-log plot of expected claw closing force at claw tip as a function of claw length. In males, both the major claw (n=13, $p<0.0001$) and the minor claw (n=13, $p<0.0001$) displayed high correlations . In females, the major claw (n=7, $p=0.0003$) and the minor claw (n=6, $p=0.0330$) exhibited high correlations as well.

could be considered isometric. Female minor claw closing apodemes scaled more negatively isometrically than did female major claw closing apodemes, which raises an issue of differences between sexes which will be discussed later in the paper. The male minor claw's isometric scaling means that, with regard to muscle cross-sectional area, force generation per square of unit claw length is independent of crab size.

As mentioned earlier, there are potentially several factors that could provide force generation compensation for negatively allometric scaling of muscle cross-sectional area. One such factor is the muscle pennation angle, which maximizes force production as it approaches 45°. In males, no correlation was found between pennation angle and claw length. However, the mean pennation angle for minor claw was closer to the ideal 45° (45.141°) than the mean pennation angle for the major claw (53.407°). Thus, in this respect the minor claw closing muscle is more efficient in generating force than the minor claw muscle in males.

Female measurements of pennation angle provided an unexpected result. In females, there are strong correlations between pennation angle and claw length. It is important to note that for this study, there was a low female sample size, with only seven females measured. The smallest female measured had pennation angles of 53.255° major and 47.095° minor and the largest female had pennation angles of 46.701° major and 41.643°. This shows that as female crabs increase in size, force generation efficiency in terms of pennation angle moves from favoring the minor claw to favoring the major claw. The decreasing pennation angle can be partially explained by observing the anatomical scaling of the manus width ($CL^{0.774}$) in relation to the scaling of the manus length ($CL^{0.886}$). Division of manus length scaling by manus width scaling results in a ratio of $CL^{0.112}$, which may be a significant difference in scaling. Thus, with the manus length growing much more rapidly than the width, pennation angle may have to decrease in order for the muscle to fit inside of the manus. However, this explanation would not apply to the female minor claw, as the ratio of scaling for manus length to manus width is $CL^{-0.057}$. An expected result of this ratio would be that pennation angle would increase with an increase in claw length. However, this ratio may not be large enough to significantly affect pennation angle, and lack of correlation in male pennation angle shows that the ratio of scaling for manus length to manus width may not necessarily have a large effect on pennation angle. More accurate measurements of pennation angle and larger sample sizes may show that like males, pennation angle does not scale in females relative to claw size.

Mechanical advantage is not a factor that determines force generation, but rather it affects force transmission and amplification from muscle to claw tip. It may be important to note that my method of measuring mechanical advantage differs from the methods of previous studies. When I measured mechanical advantage, the two lever arms I measured were perpendicular to the contractile force and the load. This runs contrary to previous studies of mechanical advantage in crab claws, which have measured from the pivot point to the tip of the claw for the out-lever, which is not necessarily perpendicular to the load(see Yamada and Boulding 1997, Schenk and Wainwright 2001, Levinton and Allen 2005), which may lead to inaccurate values of mechanical advantage. This may also have no significant effect when comparing relative values, but it would affect calculations of predicted force. No correlation was found between mechanical advantage and claw length. This could be attributed to variability in claw shape between crabs. However, one-way analysis of mechanical advantage showed significant differences between major and minor claws by sex. As observed by Levinton and Allen (2005) and Schenk and Wainwright (2001), mechanical advantage was significantly higher in the major claw than in the minor claw. This supports the "crusher and cutter" model, where the major claw is designed to be more powerful by being physically larger of the two claws and by operating at higher mechanical advantage.

Recent studies, however, have not explored sex differences in mechanical advantage within species that exhibited dimorphism in both sexes. No significant difference was found between the minor claw MA values between sexes, which suggests similarity in design in this respect. However major claw MA was significantly different between sexes, and female major claws operated at a significantly higher mechanical advantage. This may correlate with differences in major claw function between sexes. Although it was almost impossible to positively identify the sex of crabs observed in the field, I mostly observed territoriality in male crabs, judging sex by claw and carapace size, as well as claw shape. These crabs exclusively fought using their major claws. Assuming that females do not exhibit the same degree of territoriality, perhaps male major claw design may be additionally influenced by pressure exerted by sexual selection. In fiddler crabs, it was found that major claw MA decreased and claw closing speed increased as claw size increased (Levinton and Allen 2005). Levinton and Allen concluded that this decreased MA was related to the increased closing speed and that faster claws may be advantageous during fights between males. While *C. carnifex* does not exhibit scaling of MA in its claw closing system, Levinton and Allen's conclusions may be a possible explanation for lower MA values in the male major claw. Even if the male claw design did not assist in male-to-male fighting, its exaggerated length may be important as a form of display. In larger males, it appeared as if the dactyl and pollex of the major claw became disproportionately long and slender. A disproportionate increase in length with respect to height would cause a likely decrease in mechanical advantage.

Differences in claw performance based on sex are confirmed by measurement of effort. Recent studies used direct measurements of claw closing force to compare claw differences (Levinton and Judge 1993, Levinton et al. 1995, Yamada and Boulding 1998, Levinton and Allen 2005), but this option was unavailable during this experiment. Instead, effort, an indirect measurement of force, was used. It is important to note that during these tests, it is very likely that the crabs acted under varying degrees of motivation. This means that this test was by no means a measurement of maximum effort, but rather a measure of effort that the crabs chose to exert. In males, minor claw effort displayed high correlation as a function of claw length, while major claw effort displayed very poor correlation. A predicted result would be that both the major and minor claws would exhibit correlations of effort with respect to claw length, and that the correlations would be distinct from each other. A possible explanation for the major claw's poor correlation may be that relative to the minor claw, the major claw requires more energy to operate. Depending on how threatened it felt, the crab may not be inclined to clamp down using full force, especially if it had to do it over a time interval. Another possible reason may be that the structural design of the larger major claws may not be able to support a full exertion of force at the tip. As noted earlier, the dactyl and pollex become relatively long and slender as claw size increases, to the point where they appear relatively fragile. If this were the case, then crabs may be more inclined to exert some closing force that is less than their maximum. This may also show a functional difference between claws, where the minor claw could be predominantly used to clamp on to various objects when the crab is stressed. This was witnessed on several occasions when male crabs would grab tightly onto nearby objects primarily using their minor claws while being handled, holding onto these objects for minutes at a time.

In females, the opposite phenomenon was observed, with major claw effort displaying high correlation and minor claw showing poor correlation. In this case, the major claw may be better adapted to prolonged clamping than the minor claw due to a variety of factors. Muscle cross-sectional area grows more rapidly in the major claw than in the major claw and pennation angle approaches the ideal 45° in the major claw as claw size increases in females. Also, it appears that female major claws do not exhibit the same exaggeration of major claw shape present in males. This may be due to the fact that extremely large females were not found, but it may also be true that extremely large females do not exist. These differences in effort between sexes may suggest that there might be functional claw differences between sexes.

Scaling of expected claw closing force once again displays similarities between the major claw of one sex and the minor claw of the other sex. In males, expected force in the minor claw scaled with claw length to a greater exponent than the major claw. In females, the expected closing force of the major claw grew more rapidly with respect to claw length than the minor claw. If we accept the previously mentioned notion that the male minor claw and female major claw are primarily responsible for strongly gripping onto objects when the crab is stressed, then larger increases in force generation for those claws would be more beneficial to the organism's performance than if closing force scaled in a different manner. The data also showed that out of the four claw and sex combinations, the male minor claw exhibited the greatest rate of growth. A possible explanation for this is that the minor claw's high rate of growth compensates for the relatively low value of force that the minor claw could potentially generate when compared to the major claw.

Regardless of sex, the major claw did appear to be important in the role of attack and defense. In observed crabs, threat displays were primarily made using primarily what seemed like body size to intimidate other crabs. If the degree of threat display escalated, then the major claw was thrust forward, often culminating in a fight between crabs, which exclusively involved their major claws. The defensive value of the major claw can perhaps be observed by the orientation of the crabs as they enter their burrows. Crabs entered their burrows with their major claw facing the entrance significantly more often than with their minor claw facing the entrance. Although there are many possible reasons behind this, one reason may be that having the major claw at the entrance may provide a greater threat deterrent than the minor claw would. Other possibilities include that the crabs dig with their minor claw, that they eat stored food with a certain claw, or that the crabs are simply programmed to enter their burrows in this manner. An unexpected observation was made several times in the field, when crabs would exit their burrows in one orientation and enter their burrows in the opposite orientation. Again, there could be various explanations for this phenomenon, including the importance of orientation for various activities while inside of their burrows or perhaps the lack of importance of orientation.

While the major claw may be important for attack and defense, the use of claws in other activities seems to be more dependent on both claws being used in conjunction. When crabs foraged, they most often used both claws to secure their grip on their food. This may be attributed to the fact that there was high competition for food at the two study sites, so foraging crabs needed to secure their food and run back to their burrows as quickly as possible to prevent their food from being stolen by other crabs. The act of removing dirt from the burrow during digging is also an activity where both claws were observed to be used at the same time. This is most likely due to the fact that carrying out dirt with both claws at once would be the most efficient way to do this activity.

Unlike the previously discussed activities, eating appears to favor the use of the minor claw. Although the activity was done most frequently using both claws, with the exclusive use of the minor claw second in frequency, the minor claw was used more significantly more frequently than the major claw during ambidextrous feeding. A possible explanation for this is that the minor claw is more efficiently designed for this activity, such as in its ability to shred food. It may also be more energetically favorable to use the minor claw; since it is smaller, it may require less energy to operate. On several occasions while observing feeding in the field, I saw crabs picking at grass and eating with both claws. Although the minor claw was used more frequently, it seemed as if the major claw was primarily used to pull out tougher grass. In other words, the crabs used the additional force generated by the major claw during feeding when it was needed, instead of using it as often as it used the minor claw. The simultaneous use of the major and minor claws also shows how the two claws can overlap in function. Other than the activity of fighting, of which there was not a statistically significant sample size, it appeared that there were no activities that were claw exclusive.

Although the field observations provide a general idea of how *C. carnifex* uses its major and minor claws in nature, the design of the study has provided limited insight as to the direct relationship between claw usage as a function of crab size (due to limited sample size) or as a function of sex (due to the difficulty of identifying sex in the field).

Future studies of crab behavior may involve more vigorous observations of activity in order to determine functional differences by size or sex. One set of activities I could not observe was the act of digging as well as other activities performed while crabs were in their burrows. Correlating this information with biomechanical data could provide further insight in the relationship between claw design and function. Future biomechanical studies could involve direct measurements of closing force using devices such as force transducers and measuring claw closing speed. Also, similar studies could be done on the other species of crabs present on Moorea.

In summary, the dimorphic chelae in *C. carnifex* displayed differences in function, anatomical scaling and force generation. In addition, although sexual dimorphism may have seemed subtle at first, many differences in claw design were found between sexes, and possible differences in function could be inferred. Through this study, we saw how mechanical design related to ecological function through correlations between dimorphic structures and their functional specialization.

ACKNOWLEDGEMENTS

I would like to thank the University of California, Berkeley and the Gump Station for the opportunity to perform this study. Thanks to the professors and graduate student instructors, especially Liz Perotti for her major contributions to my project. Thanks to Melissa Riley, Sarah Chinn, and Daniel Song for assisting me in my field and lab work. Thanks to Professor Mimi Koehl for providing the inspiration for this project. Finally, thanks to Robert Dudley and Jeff Levinton for help with my methods.

LITERATURE CITED

Barnes, R. D. 1974. Invertebrate Zoology, Third Edition. W.B. Sanders Company, Pennsylvania.

Bickel, S. A. 1997. Burrowing ecology and behavior of the land crab, *Cardisoma carnifex* in Moorea, French Polynesia. Biology and Geomorphology of Tropical Islands. **6**: 116-122.

Cheng, L. 2000. Changes in territoriality with food supplementation in a land crab, *Cardisoma carnifex*, on Moorea, French Polynesia. Biology and Geomorphology of Tropical Islands.**9**: 38-47.

Denhoy, R. and G. Battersby. 1992. Factors influencing density and distribution of *Cardisoma carnifex* in established areas, including an assessment of burrow fidelity, and a temporal survey of the road kill of *Cardisoma carnifex* on the island of Moorea, French Polynesia. Biology and Geomorphology of Tropical Islands **1**:pages unnumbered.

Elitzur, B. 2001. Habitat and its effect on burrow distribution of the land crab, *Cardisoma carnifex* (Herbst 1791). Biology and Geomorphology of Tropical Islands **10**:101-08.

Levinton J. S., M. L. Judge, Josepha P. Kurdziel. 1995. Functional differences between the major and minor claws of fiddler crabs (*Uca*, family Ocypodidae, Order Decapoda, Subphylum Crustacea): A result of selection or developmental constraint? Journal of Experimental Marine Biology and Ecology **193**: 147-160.

Levinton, J. S. and B. J. Allen. 2005. The paradox of the weakening combatant: the trade-off between closing force and gripping speed in a sexually selected combat structure. Functional Ecology **19**: 159-165.

Levinton, J. S. and M. L. Judge. 1993. The relationship of closing force to body size for the major claw of *Uca Pugnax* (Decapoda: Ocypodidae). Functional Ecology **7** (3): 339-345.

Meglitsch, P. A. 1972. Invertebrate Zoology Second Edition. Oxford University Press, New York.

Schenk, S. C. and P. C. Wainwright. 2001. Dimorphism and the functional basis of claw strength in six brachyuran crabs. Journal of Zoology, London **255**: 105-119.

Seed, R. and R.N. Hughes. 1995. Criteria for prey size-selection in molluscivorous crabs with contrasting claw morphologies. Journal of Experimental Marine Biology and Ecology **193**:177-195.

Vogel, S. 2003. Comparative Biomechanics: Life's Physical World. Princeton University Press, Princeton.

Yamada, S. B. and E. G. Boulding. 1998. Claw morphology, prey size selection, and foraging efficiency in generalist and specialist shell-breaking crabs. Journal of Experimental Marine Biology and Ecology **220**:191-211.

APPENDIX A

Kruskal-Wallis test and Tukey-Kramer test analysis of claw use frequency against observed activity. * signifies a Kruskal-Wallis P-value between .001 and .05, ** signifies a P-value over .05.

Claw(s) Used	Activity	Group	Mean Freq
Both Claws	Removing Dirt	A	1.000
	Foraging	A B	0.590
	Eating	A B	0.500
	Threat Display	B	0.214
	Fighting	B	0.000
* **Major Claw**	Fighting	A	1.000
	Threat Display	A B	0.595
	Lead Claw Entering Burrow	B C	0.228
	Foraging	B C	0.218
	Eating	C	0.132
	Removing Dirt	B C	0.000
Minor Claw	Lead Claw Entering Burrow	A	0.772
	Eating	B	0.368
	Foraging	B	0.192
	Threat Display	B	0.190
	Fighting	B	0.000
	Removing Dirt	B	0.000

APPENDIX B

Kruskal-Wallis test and Tukey-Kramer test analysis of claw use frequency against claw(s) used. by activity. * signifies a Kruskal-Wallis P-value between .001 and .05, ** signifies a P-value over .05.

Treatment	Tukey-Kramer Test	Group	Mean Frequency
* Eating	Both Claws	A	0.500
	Minor Claw	A B	0.368
	Major Claw	B	0.132
Threat Display	Major Claw	n/a	0.595
	Both Claws	n/a	0.214
	Minor Claw	n/a	0.190
Foraging	Both Claws	A	0.590
	Major Claw	B	0.218
	Minor Claw	B	0.192
Lead Claw Entering Burrow	Minor Claw	A	0.772
	Major Claw	B	0.228
	Both Claws	╳	n/a
** Removing Dirt	Both Claws	n/a	1.000
	Major Claw	n/a	0.000
	Minor Claw	n/a	0.000
** Fighting	Major Claw	n/a	1.000
	Both Claws	n/a	0.000
	Minor Claw	n/a	0.000

APPENDIX C

Scaling of claw measurements as a function of claw length. Scaling is reported as exponent (e) of base carapace length (CL) to which the measured parameter (X) scales in the equation:

$$X=bCL^e$$

b is a constant. * signifies a P-value between .001 and .05, ** signifies a P-value over .05.

Claw	Measurement	Sex	e	R^2
Major	dactyl length	Male	1.102	0.996
	*	Female	1.148	0.848
	dactyl height	Male	0.966	0.985
		Female	1.068	0.965
	Pollex length	Male	1.091	0.978
		Female	1.139	0.960
	manus height	Male	0.966	0.981
		Female	0.939	0.989
	Manus length	Male	0.901	0.969
		Female	0.886	0.956
	Manus width	Male	0.928	0.971
		Female	0.773	0.974
	in-lever	Male	0.962	0.983
		Female	0.986	0.974
	out-lever	Male	0.936	0.985
		Female	0.989	0.939
	** MA	Male	0.027	0.075
	**	Female	-0.003	0.000
	* apodeme area	Male	1.798	0.629
	*	Female	1.609	0.896
	** muscle pennation angle	Male	-0.048	0.051
	*	Female	-0.204	0.660
Minor	dactyl length	Male	1.094	0.988
		Female	1.073	0.999
	dactyl height	Male	0.974	0.985
		Female	1.046	0.986
	Pollex length	Male	1.109	0.937
		Female	1.108	0.992
	manus height	Male	0.959	0.963
		Female	0.960	0.625
	Manus length	Male	1.050	0.866
		Female	0.877	0.961
	Manus width	Male	0.904	0.992
		Female	0.934	0.939
	in-lever	Male	1.124	0.971
		Female	1.248	0.935
	out-lever	Male	1.082	0.976
		Female	1.026	0.994
	** MA	Male	0.042	0.017
	**	Female	0.222	0.282
	apodeme area	Male	2.084	0.922
	*	Female	1.420	0.785
	** muscle pennation angle	Male	0.039	0.010
	*	Female	-0.234	0.624

ONTOGENY OF DEFENSE:
DOES LIFE HISTORY AFFECT PREDATOR RESPONSE BEHAVIOR IN THE PYGMY OCTOPUS, *OCTOPUS BOCKI*?

JULIE E. HIMES

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

Abstract. Organisms experience physiological and ecological changes during ontogenesis, and studies have shown that such changes have an impact on behavior over the life cycle. However, little is known about how octopus behavior changes during ontogeny. The pygmy octopus, *Octopus bocki* (Adam 1941), expresses differences in chromatophore development and mantle length between developmental stages. These changes may be important in predator defense, therefore I hypothesized that predator response behavior also changes over the life cycle. Timed interactions between an octopus and a fish predator were used to compare the behaviors and color displays exhibited by three different size categories of *O. bocki*; juvenile, sub-adult, and adult. Color display diversity was analyzed using the Shannon-Weiner diversity index. The analysis revealed a negative correlation between color display diversity and size during predator interactions, supporting the hypothesis that behavior changes over the life cycle. Counter-intuitively color display diversity decreases while chromatophore development increases. To broaden the context in which behavior was examined, interactions between adult *O. bocki* were used to investigate how adults use color displays. The number of color/texture combinations was graphed to compare displays expressed during predator interactions with those expressed during intraspecific interactions. A Wilcoxon test revealed that adult *O. bocki* used significantly more color and texture displays during intraspecific interactions than during predator interactions. I concluded that adult octopuses use color displays for communication rather than predator defense and that these displays change during ontogenesis.

Key words: ontogeny; behavior; communication; Octopus bocki; *pygmy octopus; predator response; Moorea, French Polynesia*

INTRODUCTION

Predator avoidance is critical during any stage in an organism's life. Juvenile animals that cannot depend on parental protection are under strong selection pressure to exhibit fully developed predator avoidance behavior at an early age (Impekoven 1976, Miller and Blaich 1986, Göth 2001). Because some organisms change drastically as they age, altering size, appearance, and even habitat, that predator response behavior may also change during the life cycle is not surprising. The ontogenetic aspect of behavior has been studied in social organisms such as mammals, birds, and insects (Pongracz and Altbacker 2000, Mondor and Roitberg 2002), however little is known about how behavior changes over the ontogenetic stages of solitary marine invertebrates.

A cephalopod's life cycle involves changes in body size and chromatophore number and density (Packard 1985). These physiological changes may influence predator response behavior; for example, Hanlon and Messenger (1988) suggest that the use of chromatophores and iridophores differs between juvenile and adult cuttlefish, *Sepia officinalis* (Linnaeus 1758). Juvenile cuttlefish were more likely to exhibit inappropriate body patterning when placed on a uniform background than adults. Like cuttlefish, octopuses also have the ability to change color, pattern, and texture, yet little is known about the ontogenetic changes in octopus behavior.

In order to study the ontogeny of octopus predator response behavior, *Octopus bocki* (Adam, 1941) was used as the model species. This species is a pygmy octopus commonly found in shallow coral rubble on reef crests around Moorea, French Polynesia. Because *O. bocki* reproduces year-round (Cheng 1996), it was possible to collect juvenile, sub-adult, and adult individuals simultaneously, facilitating life history studies.

During preliminary observations, adult *O. bocki* exhibited a large variety of possible color patterns while sub-adults and juveniles were more limited in the range of displays they could produce. This physiological difference reflects Packard's (1985) findings that chromatophore number and density increases over the life cycle of cephalopods. These observations led to the hypothesis that adult *O. bocki* will show a wider range of color patterns in response to a predator than immature individuals. My goals were to determine whether ontogeny affects the way in which an octopus responds to a fish predator, and also to investigate how color patterns and texture are used in *O. bocki*.

METHODS

Octopus collection

The specimens for this study were collected from three sites: the reef by the village of Maharepa and two locations on the Vaipahu reef crest (Fig. 1). These sites were chosen based on the abundance of *O. bocki* found in previous studies (Pearson 1994, Cheng 1996).

Site 1 is close to the Gump Research Station and very shallow (0.5 m) with many exposed slabs of coral rubble. Sites 2 and 3 are deeper (3 m) and had to be accessed by boat. At Site 1, four large garbage cans were placed on flat exposed coral rubble on the reef crest at wading distance from each other. A non-porous cinderblock was placed at the bottom of each can. Pieces of coral rubble were then selected from the surrounding crest and stacked in the cans. The rubble drained for at least 30 minutes and no more than 45 minutes, allowing the octopuses and other organisms to crawl into the shallow pool of water that collected at the bottom of the can. After 30 minutes the rubble was removed and replaced on the reef. Octopuses and potential food items such as crustaceans were collected in sealed containers and transported to the laboratory.

Octopus care

A dissecting microscope was used to determine sex (by the presence of the hectocotylized third right arm on males) and mantle length (from the center of the eyes to tip of mantle). The octopuses were separated into three categories based on mantle length: juvenile (five mm or less), sub-adult (six to nine mm), and adult (ten mm or larger). Juveniles and sub-adults were kept in plastic cups and adults were kept in Tupperware containers. Each cup or container was covered with a lid to prevent escape. Seawater in the cups and containers was taken directly from the ocean and changed twice daily. Adults and sub-adults were fed crustaceans collected from the rubble, and juveniles were fed plankton or minute crabs each night. Factors such as temperature, light, salinity, and pH remained uniform for all animals.

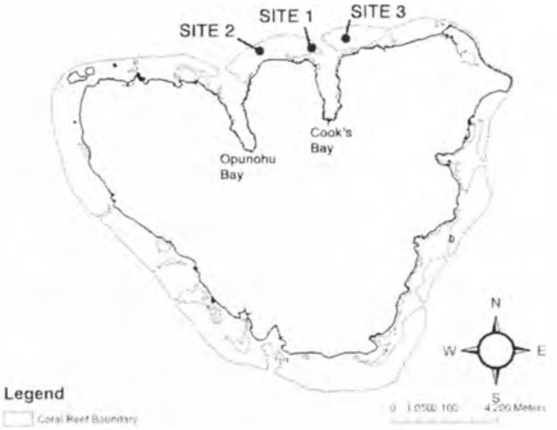


FIG. 1. Three collection sites were located on the Vaipahu reef crest (Sites 1 and 2) and the Maharepa reef (Site 3), Moorea, French Polynesia.

Response to predators

A transparent 19-liter observation tank was used for experimentation. The sample size was 21 - seven octopuses per age group. An octopus was placed in a small empty transparent jar with many holes to allow water flow, and set in the middle of the tank. Opaque barriers surrounded the observation tank so that no outside stimuli could disturb the octopus. A small window (eight x five cm) was cut to allow observation with minimal disturbance. The octopus acclimated for at least 30 mins, at which time a fish was added to the tank for a ten minute timed trial. I assumed that any species of fish is perceived as a predator to the octopus, whether it naturally eats octopuses or not. The species of fish used in these experiments was the blacktail snapper, *Lutjanus fulvus* (Scneider 1801). During the ten minute trial, observations on the movements, color changes, texture, and other behaviors such as inking were recorded using voice recording software. These behaviors along with descriptions of each are listed in Table 1. The fish were kept up to four days before release. The order of experimentation was randomized using Excel's random number generator, and the test was repeated three times per octopus. Experiments took place during the day, and to avoid stress no single octopus was tested more than once per day.

TABLE 1. Names and descriptions of behaviors observed during interactions between *O. bocki* and a fish predator.

Behavior	Description
Colors	
Red	Red chromatophores contracted on mantle and/or arms
White	No chromatophores contracted
Red With White Spots	Chromatophores contracted on mantle except at certain locations
Flickering Red	Chromatophores rapidly contract and relax on mantle and/or arms
Flickering Blue	Iridophores rapidly contract and relax near eyes or on mantle
Flash Red	Chromatophores contract once simultaneously on mantle and arms
Textures	
Smooth	No papillae on mantle, eyes, or arms
Mantle Papillae	Small papillae on mantle
Eye Papillae	One papilla above each eye
Movements	
Still	Mantle location does not change
Crawling	Mantle location changes
Positions	
Normal	Mantle is relaxed, arms are resting
Ballooning	Mantle rises above the substrate and web forms a "balloon" or "parachute" position
Other	
Inking	Octopus releases a pseudomorph or diffuse ink

Response to intraspecifics

To observe chromatophore usage during intraspecific interactions in adult *O. bocki*, an adult was added to the home container of another adult. Hiding places such as coral pieces or shells were removed from the home container before the second adult was added. The different colors and textures displayed by the resident adult were observed and recorded for five minutes. Testing was performed during the day, and each adult was observed interacting with every other adult.

Data analysis

The average percent time each age group spent exhibiting different color patterns during predator interactions was graphed for comparison. The predator response data were then analyzed using the Shannon-Weiner diversity index. This test allowed for comparison of the color pattern diversity across a size gradient. In order to find correlations between color display diversity and mantle length, a bivariate fit analysis of the average H' values was run using JMP 5.1 (SAS Institute Inc. 2003).

A count of the number of color and texture combinations was totaled to compare the displays exhibited by adults when encountering a predator to the displays exhibited by adults when encountering another adult. A Wilcoxon test was then run using JMP 5.1 to find significant differences between the number of colors and textures displayed during intraspecific interactions and predator interactions.

RESULTS

Response to predators

During interactions between the octopus and fish a total of 14 behaviors were observed, and six of these behaviors were color patterns. The average percent time each age group spent displaying each color pattern is graphed in Fig 1.

The Shannon-Weiner diversity index analysis compared color pattern diversity across a range of octopus mantle lengths. A bivariate fit of the average H' values showed a significant correlation between color display diversity and mantle length (R^2 = 0.65, p = 0.003). These results are shown along with a linear fit line in Fig. 2.

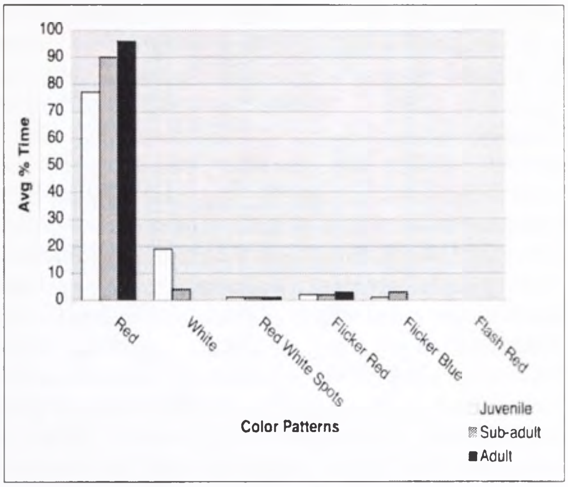


FIG. 1. The average % time each age group spent displaying each color pattern during predator response trials.

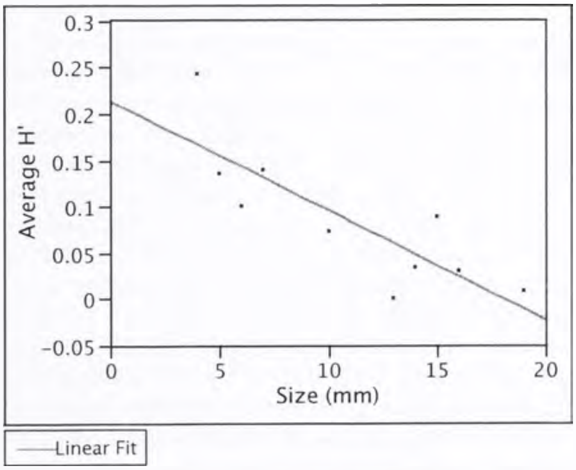


FIG. 2. Bivariate fit of average H' values is graphed by size (mm) (R^2=0.65, p=0.003), illustrating a negative correlation between octopus size and color display diversity in response to predators.

Response to intraspecifics

During adult intraspecific interactions, nine color patterns, four textures, and three body positions were observed. I present a comparison of behaviors that were seen in predator interactions with those seen in intraspecific interactions in Table 2. Three colors, one texture, and two body positions were observed which were not seen in predator interactions. Iridophores, producing blue iridescent spots, were displayed on the mantle and at the base of the arms. A single papilla was also sometimes observed on the tip of the mantle. The two new postures observed in predator interactions were "erect mantle" and "clubbed mantle." The mantle was described as erect when it was elongated and held upright. A "clubbed mantle" described an erect mantle with a large, rounded end. In Fig. 3, I compare the average number of color/texture combinations of each individual adult recorded during predator interactions to those recorded during intraspecific interactions. A color/texture combination was defined as each unique pair of behaviors, one color and one texture, displayed simultaneously. For example, a common color/texture combination was red/smooth. The results of the Wilcoxon test found a significant difference in the number of different color/texture combinations displayed during predator interactions and intraspecific interactions ($p < 0.0001$).

TABLE 2. A list of the colors, textures, and positions expressed by adult *O. bocki* during predator interactions and intraspecific interactions. An "X" indicates observed behaviors and "-" indicates that the behavior was not observed.

	Predator	Intraspecific
Color		
Red	X	X
Flickering Red	X	X
Flash Red	X	X
Red With White Spots	X	X
Red With Blue Mantle Spots	-	X
Red With Blue Arm Base Spots	-	X
White	X	X
White With Blue Mantle Spots	-	X
Flickering Blue	X	X
Texture		
Smooth	X	X
Mantle Papillae	X	X
Eye Papillae	X	X
Mantle Tip Papilla	-	X
Position		
Normal	X	X
Ballooning	X	-
Erect Mantle	-	X
Clubbed Mantle	-	X
Total	**11**	**16**

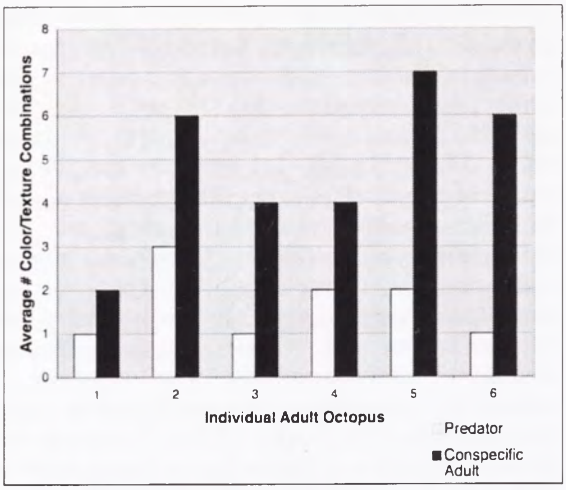


FIG. 3. Average # of color/texture combinations expressed during predator interactions (light bars) and intraspecific interactions (dark bars). The number of displays given during intraspecific interactions is significantly different than during predator interactions ($p < 0.0001$).

DISCUSSION

The resulting H' values of the Shannon-Weiner diversity index are strongly correlated with size. There was a negative correlation between mantle length and color pattern diversity (Fig. 3). Thus, as the octopus grows, the diversity of color patterns that it displays behaviorally when encountering a predator decreases linearly. These results are opposite of the proposed hypothesis that more mature *O. bocki* will display more color variations, yet support the general hypothesis that ontogeny affects predator response behavior.

The trend found in the Shannon-Weiner index prompted further exploration into how color patterns are used in adult *O. bocki*. From preliminary observations, this species has the ability to display more patterns as an adult than as a sub-adult or juvenile because chromatophores are less developed in immature octopuses (Packard 1985). However, the increase in color pattern ability as this species grows does not result in an increase in the color diversity displayed when the octopuses interacted with a predator.

The results of intraspecific interaction experiments between adult *O. bocki* suggest that color patterns are used for communication rather than predator deterrence (see Table 2 and Fig. 4). The numbers of colors, textures, and positions observed in trials where two adults were allowed to interact are greater than those observed in predator interactions (Table 2). I graphed the number of color/texture combinations for each individual (Fig. 5). In every adult, the number of color/texture combinations displayed is greater by at least double in the intraspecific interaction than in the predator interaction. I included texture in this graph because, although textures were rarely observed during predator interactions, textures such as mantle papillae were common during intraspecific interactions. The results of a Wilcoxon test showed a highly significant difference between displays observed during intraspecific interactions and predator interactions.

Moynihan (1985) defines systems of communication as the transmission and recognition of signals, which encode specific information. Octopuses and other coleoid cephalopods have an amazing ability to communicate by sending visual signals through the use of chromatophores (Moynihan 1985). Communication, rather than noncommunication (or crypsis) or aposematic display, provides an explanation supported by the results of this study. The adults use their developed chromatophores for intraspecific communication, such as sexual or hostile displays, rather than for predator deterrence.

This conclusion helps to explain why adults display less color diversity than juveniles and sub-adults when faced with a predator. One possible explanation for the trend found in Fig. 2 is that juveniles and sub-adults do not need to use their chromatophores for communication. They are not sexually mature, therefore intraspecific interactions are not an important part of their lives. The color patterns used for communication may still be developing during these early life stages, but have not been differentiated toward the correct behavioral context.

Hanlon and Messenger (1988) proposed that the cuttlefish, *Sepia officinali,s* demonstrated a shift from crypsis to communication during the life cycle. All age groups of *O. bocki* in my study exhibited red coloration for the majority of predator tests (Fig. 1), however juveniles and sub-adults exhibited red for a smaller percentage of time than adults. *O. bocki* is a nocturnal species, and red is a difficult color to detect at night. Therefore, red may represent the cryptic or normal coloration of this species. Thus, Hanlon and Messenger's (1988) study is not supported by my data, as juveniles and subadults showed less cryptic coloration than adults (Fig. 1). While there is not a shift from crypsis to communication in *O. bocki*, there does seem to be a shift toward communication over the life history of this species. Furthermore, there seems to be a shift from less specific to more specific differentiated behavioral responses to certain situations as an octopus matures.

The results of these predator and intraspecific interaction experiments demonstrate the effects of ontogeny on the behavioral displays of pygmy octopuses. Display behaviors change as the octopus grows, and these changes may be attributed to sexual maturity and the development of chromatophore usage in intraspecific communication.

ACKNOWLEDGEMENTS

I thank the professors, Jere Lipps, Brent Mishler, Vincent Resh, and James Bartolome, who made this course possible. I also thank the graduate student instructors, Elizabeth Perotti, Alison Purcell O'Dowd, and Erica Spotswood for their help and support. I especially thank Roy Caldwell, Becky Williams, and Christine Huffard for their involvement in developing my project and identifying octopus species. Finally I would like to thank the students; Sameen Ghazali, Andrew Greene, Margaret Groff, and Natalie Valencia, who supported me and assisted me in collecting my specimens.

LITERATURE CITED

Cheng, M. A. W. 1996. The reproductive biology of two species of pygmy octopuses *Hapalochlaena lunulata* and *Octopus bocki*. Ph.D. dissertation, Dept. Integrative Biology, University of California, Berkeley.

Göth, A. 2001. Innate predator-recognition in Australian brush-turkey (*Alectura lathami*, Megapodiidae) hatchlings. *Behaviour* 139: 117-136.

Hanlon, R. T. and J. B. Messenger. 1996. *Cephalopod Behavior*. Cambridge University Press.

Hanlon, R. T. and J. B. Messenger. 1988. Adaptive coloration in young cuttlefish (*Sepia officinalis* L.): the morphology and development of body patterns and their relation to behavior. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* 320(1200): 437-487.

Impekoven, M. 1976. Responses of laughing gull chicks (*Larus atricilla*) to parental attraction calls and alarm calls, and effects of prenatal auditory experience on responsiveness to such calls. *Behaviour* 56: 250-278.

Miller, D. B. and C. F. Blaich. 1986. Alarm call responsivity of mallard ducklings III: Acoustic features affecting behavioral inhibition. *Developmental Psychobiology* 19: 291-301.

Mondor, E. B. and B. D. Roitberg. 2002. Pea aphid, *Acyrthosiphon pisum*, cornicle ontogeny as an adaptation to differential predation risk. *Canadian Journal of Zoology* 80 (12): 2131-2136.

Moynihan, M. 1985. *Communication and Noncommunication by Cephalopods*. Indiana University Press.

Norman, M. D. and Sweeney, M. J. In press. The shallow-water octopuses (Cephalopoda: Octopodinae) of the Philippines. *Invertebrate Zoology*.

Packard, A. 1985. Sizes and distribution of chromatophores during post-embryonic development in cephalopods. *Vie et Milieu* 35: 285-298.

Pearson, J. A. 1994. Food preferences of juvenile *Octopus bocki*. University of California, Berkeley, IB c158: *Biology and Geomorphology of Tropical Islands*.

Pongracz, P. and V. Altbacker. 2000. Ontogeny of the responses of European rabbits (*Oryctolagus cuniculus*) to aerial and ground predators. *Canadian Journal of Zoology* 78 (4): 655-665.

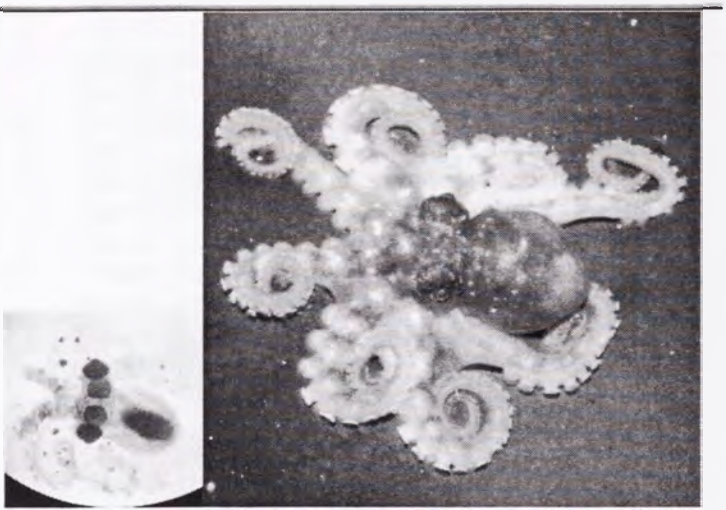
Roper, C. F. E. and Hochberg, F. G. 1988. Behavior and systematics of cephalopods from Lizard Island, Australia, based on color and body patterns. *Malacology* 29(1): 153-193.

OCTOPUSES OF MO'OREA, FRENCH POLYNESIA

Julie Himes

Octopus bocki

Common Name: Bock's pygmy octopus
Mantle Length: Up to 2.5 cm
Characteristics: This species normally appears dark red, but can exhibit white spots on the mantle and/or arms, or blue iridescent spots on the mantle and/or arms. *O. bocki* is characterized by a pair of dark spots inside the mantle cavity.
Habitat: *O. bocki* is very common around Mo'orea. This species inhabits coral rubble on shallow barrier reef crests with high flow.
Depth: Intertidal – 5 m
Distribution: The Philippines to French Polynesia



Julie Himes

Above Left: Transparent hatchling *O. bocki* are common in coral rubble on barrier reef crests around Mo'orea. Hatchlings are usually 4-5 mm mantle length when they settle out of their pelagic paralarval habitat.

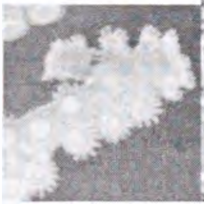
Octopus cyanea

Common Name: Day octopus, big blue octopus, Cyane's octopus
Mantle Length: 16 cm or larger
Characteristics: This ocellate species often displays false eye-spots. It can exhibit a variety of colors including gray, white, red, and mottled brown.
Habitat: This species inhabits dens in coral rubble crevices. It forages during the day and during the full moon. On Mo'orea, most adults were found on fringing reefs at depths of 1-10 m.
Depth: Intertidal – 18 m
Distribution: *O. cyanea* is an Indo-Pacific species found from Hawai'i to French Polynesia and west to the east coast of Africa.

Christine Huffard

Octopus wolfi

COMMON NAME: Star-sucker pygmy octopus
MANTLE LENGTH: Up to 1.5 cm
CHARACTERISTICS: This species shows red or brown chromatophores and can display white markings. The function of stellate suckers on males of this species is unknown.
HABITAT: *O. wolfi* inhabits coral heads and coral rubble. Specimens were found on barrier reefs of Mo'orea.
DEPTH: Intertidal to at least 30 m
DISTRIBUTION: Red Sea to French Polynesia, and Philippines to the Great Barrier Reef.



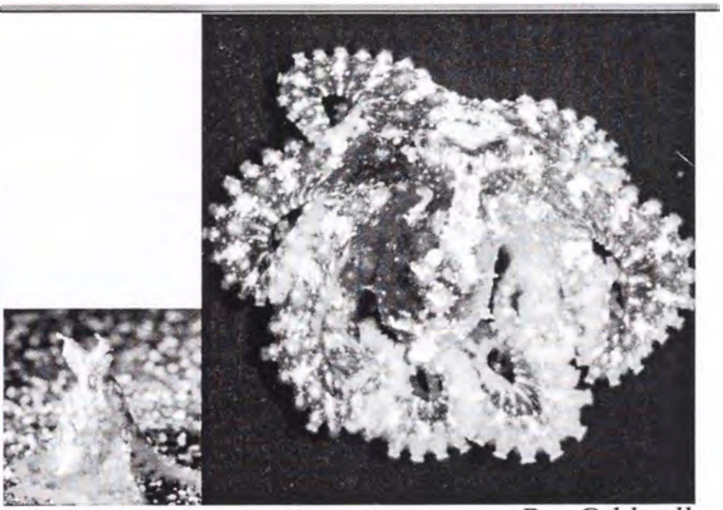
Roy Caldwell

Julie Himes

ABOVE LEFT: Stellate suckers at the tips of the arms are a distinctive feature of *O. wolfi* males.

Octopus cf *abaculus*

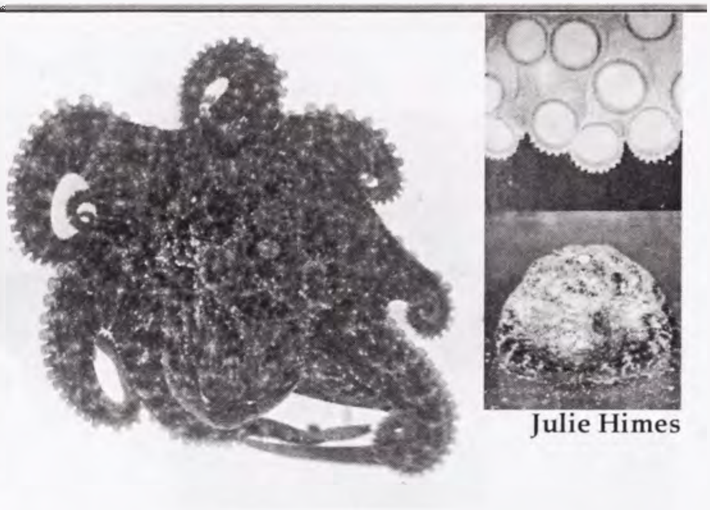
COMMON NAME: Undescribed species
MANTLE LENGTH: Up to 2.5 cm
CHARACTERISTICS: This species is red with well-defined white spots on the mantle as well as along the arms. It can also display mottled yellow and white. This species has long arms and can exhibit papillae on the mantle and above the eyes.
HABITAT: Coral rubble
DEPTH: 25 m
DISTRIBUTION: Only known from French Polynesia



Roy Caldwell

Octopus cf *vitiensis*

COMMON NAME: No common name
MANTLE LENGTH: At least 1.5 cm
CHARACTERISTICS: This species is usually mottled gray/brown and can turn entirely black. A light spot can be seen between the eyes as well as two light spots at the end of the mantle.
HABITAT: Only four female specimens are known. These specimens were all found in coral rubble on Mo'orea's shallow barrier reef crests where flow is very high.
DEPTH: Intertidal
DISTRIBUTION: Only known from Mo'orea, French Polynesia


Julie Himes

TOP RIGHT: All suckers of this species possess small fringes (known only from female specimens). The function of these fringes is unknown.

BOTTOM RIGHT: *O.* cf *vitiensis* disguising itself as a rock. The white spot between the eyes is visible in this photograph.

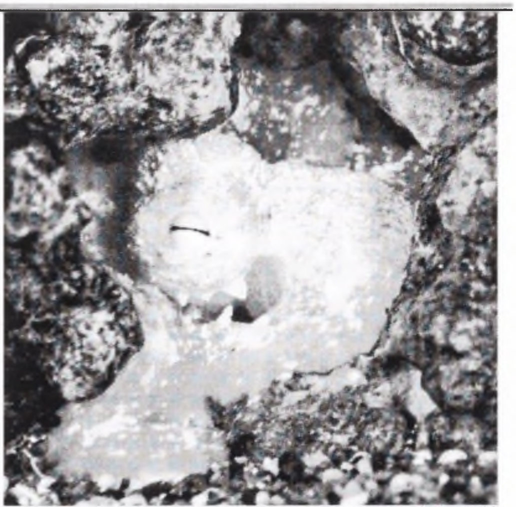
Octopus sp.

COMMON NAME: No common name
MANTLE LENGTH: Unknown
CHARACTERISTICS: This species, part of the macropus group, has very long arms and can display red stripes along the arms. Red squid-like paralarvae can be found on coral reefs around Mo'orea in October 4-6 days after the full moon. Nothing more is known about this species.
HABITAT: This species has not been observed as an adult in its natural environment.
DEPTH: Unknown
DISTRIBUTION: Only known from French Polynesia


Roy Caldwell

Octopus sp.

COMMON NAME: White octopus
MANTLE LENGTH: At least 4 cm
CHARACTERISTICS: The mantle and arms are normally white and this species displays papillae below the eyes.
HABITAT: This species inhabits coral rubble on white sand. It forages at night and has been observed swimming over the sand resembling a flatfish.
DEPTH: 3 m
DISTRIBUTION: Only known from French Polynesia



Roy Caldwell

IS THE TAMANU LOSING TURF? DISTRIBUTION AND PROPOGATION OF THE ECONOMICALLY IMPORTANT *CALOPHYLLUM INOPHYLLUM OF MOOREA*

VALERIE B. HOWELL

Environmental Science, Policy, and Management, University of California, Berkeley, California 94720 USA. valeriehowell@berkeley.edu

Abstract. French Polynesia's indigenous tamanu Tree (*Calophyllum inophyllum)* is an important natural resource harvested for lumber, resin, and oil. Being a marine-seed dispersed species it self-propagates and can be found growing along the coastline of Moorea. Development and harvest patterns on Moorea may be slowing the natural reproductive rate of the species. Fifty years ago it was recommended as a species to include in management programs as it had been noted to be in decline due to its slow growth and high use rates. Interviews with elders, carvers and healers indicated that the range has indeed diminished. A total island survey was performed and the resulting map of C. *Inophyllum's* distribution indicates that the range is healthy- but it will continue to compete with human development for the diminishing resource of coastal terrain.

Key words: Tamanu; Calophyllum inophyllum; *Moorea, French Polynesia; ethnobotony*

INTRODUCTION

Calophyllum inophyllum, known to Tahitians as "tamanu", or, "Beware" when the strong wood is discussed, or "Poro Ati" when referring to its beautiful "perfectly round ball" shaped fruit, is an evergreen member of the family Clusiacea. Highly valued by the native culture for its lumber and medicinal values, and also for its pleasing beach canopy, it is distributed along rocky coastline throughout French Polynesia. Economically promising for its' anti-microbial, anti-viral, anti-cancerous and anti HIV-1 (Ishikawa 2000) action, researchers in French Polynesia are keen to make use of new chemical analysis which will allow for developing standardized therapeutic uses (Laure 2005). Construction of tikis, canoes, bowls, furniture, musical instruments and houses (Whistler 1992) as well as production of traditional medicines for curing eye infections, skin infections and even broken bones, however, attracts many harvesters who gather up the reproductive nuts, remove nearly girdling squares of bark or outright chop the tree down. And then there's the loss of its preferred coastal habitat: Self-propagated by marine seed dispersal, like the coconut, this is one of the first trees to colonize newly formed islands in the pacific region (Gunn 1976), yet it has been suggested for more than fifty years that due to impact of wild harvesting and competition for turf, it is necessary to undertake appropriate management measures to insure the tamanu will remain an important potential source of revenue for French Polynesia (Petard 1985). Seeing that the tamanu was recently red-listed by the International Union for the Conservation of Natural Resources as a threatened species (Stephens 1998) I was compelled to investigate the robustness of the population on Moorea.

METHODS

Study site

The island of Moorea is located 17 kilometers northwest of Tahiti and is a typical "high island" with mountains punching upwards in the center and sloping downward on all sides with watershed valleys giving out onto a ring of white coralline sandy beaches. It is on these beaches that the tamanu and other marine - seed dispersed seedlings mark their arrival, and to a large extent, where they stay- due to heavy nature of floating fruits which wouldn't easily be carried inland by scavengers or birds (Gunn, 1976). Moorea has 62 kilometers of this littoral as natural habitat for *C. inophyllum* and I chose, due to its manageable size, to walk the entire periphery making GIS waypoints of every specimen on the island- a complete census-taking data on the location, dimensions, substrate, fruit presence, and evidence of impact by harvesters along the way. With this data I plan to test the hypothesis that tamanu is evenly distributed around the island.

Interviews

Knowing that the tamanu was once sacred and guarded by taboo, planted around ceremonial sites and reserved for the use only of religious and political leaders, I wished also to learn of any interior sites which might host tamanu trees growing in areas of former or current human plantation. To this end I conducted a series of interviews with elders, woodworkers, agro-foresters and healers for clues as to what the former range of the species might have been. Mr. Pierre Nardi arrived just over fifty years ago to the island of Moorea where he found a sparsely populated island with plenty of opportunity for farming. In addition to introducing modern pineapple culturing methods, he bought land and set to work planting hardwoods teak and mahogany. "Tamanu was everywhere back then. Nowadays there are none left of the old plantations in the hills and there are none of the giants left anywhere on the island." Mr. Nardi indicated to me that the last ocean-going vessel to be made of tamanu on Moorea, "The Maire", was sourced from Vaiare from land belonging to Monique Donasson- Princess Pomare.

Henri Jones, longtime resident of Temae beach which was hit by an enormous hurricane in 1985, indicated that the exposed beach was once dense with tamanu where there are now young coconuts and even younger noni trees.

An elder of the community, Papa Mita, communicated to me through a translator that there are fewer trees than before but a stand of about ten could possibly remain surrounding an ancient, undiscovered ceremonial terrace known as a marae, in an inaccessible valley behind Mt. Rotui.

I also spoke with an expert on traditional resources, culture and medicine, Hinano Murphy, and learned that the tamanu trees which once provided her afternoon labor (gathering the seeds to place in the fire to keep mosquitoes at bay) have been reduced in numbers in the region of Afareaitu by roughly 80%. At the Agricultural vocational school on the island, which is very much concerned with conservation of useful plant resources, I was shown the stumps of two very large *C. inophyllum* which had fallen prey to negligence, were burned near the base by a bar-b-que and cut down due to their dangerous proximity to school buildings.

I learned from Gerard Brigant,, a tamanu product manufacturer in Papeete, who buys his seeds from Bora Bora, and chemist Jean-Pierre Bianchini of the University of French Polynesia that while tamanu harvesting might benefit the economy, Tahiti and Moorea do not have adequate supply of the resource to fulfill manufacturing needs.

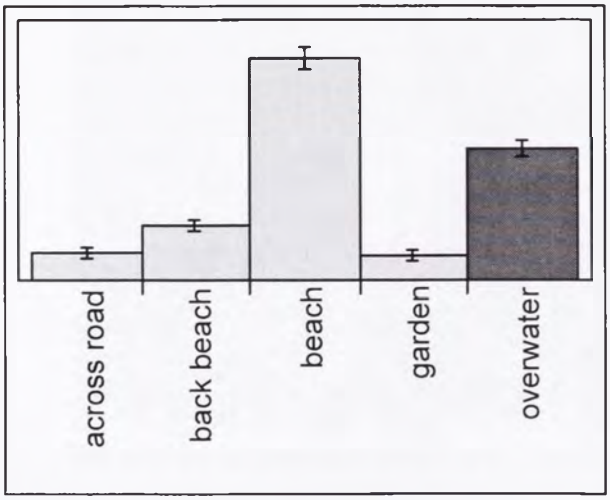


Fig. 4 Distribution of tamanu trees by location

Woodcarvers however, remarked to me that the Society for Rural Development had undertaken the propagation of tamanu at a site near the hospital in Afareaitu and that there was a plantation of trees behind the soccer field in Pao Pao.

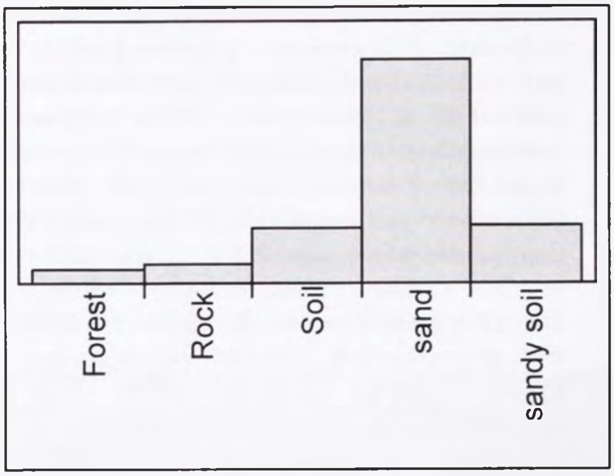


Fig. 2 Distribution by habitat

Germination

C. inophyllum propagates by conveyance of buoyant salt-water tolerant seeds on ocean currents. Seeds prefer to germinate in coralline salt-saturated soils (Elevitch) so beaches are sites of first colonization of the species. I also undertook a small germination study to compare known germination rates available in the scientific literature with local seeds and conditions. One study found average germination times of 22 days for seeds fully shelled, 38 days for seeds in cracked shells and 57 days for seeds still in their shells (Parras). To mimic this experiment I planted the nuts in three states: Whole fruit, seed with endocarp, and naked seed in beach matrix/ soil mixture. Six seeds each were sown.

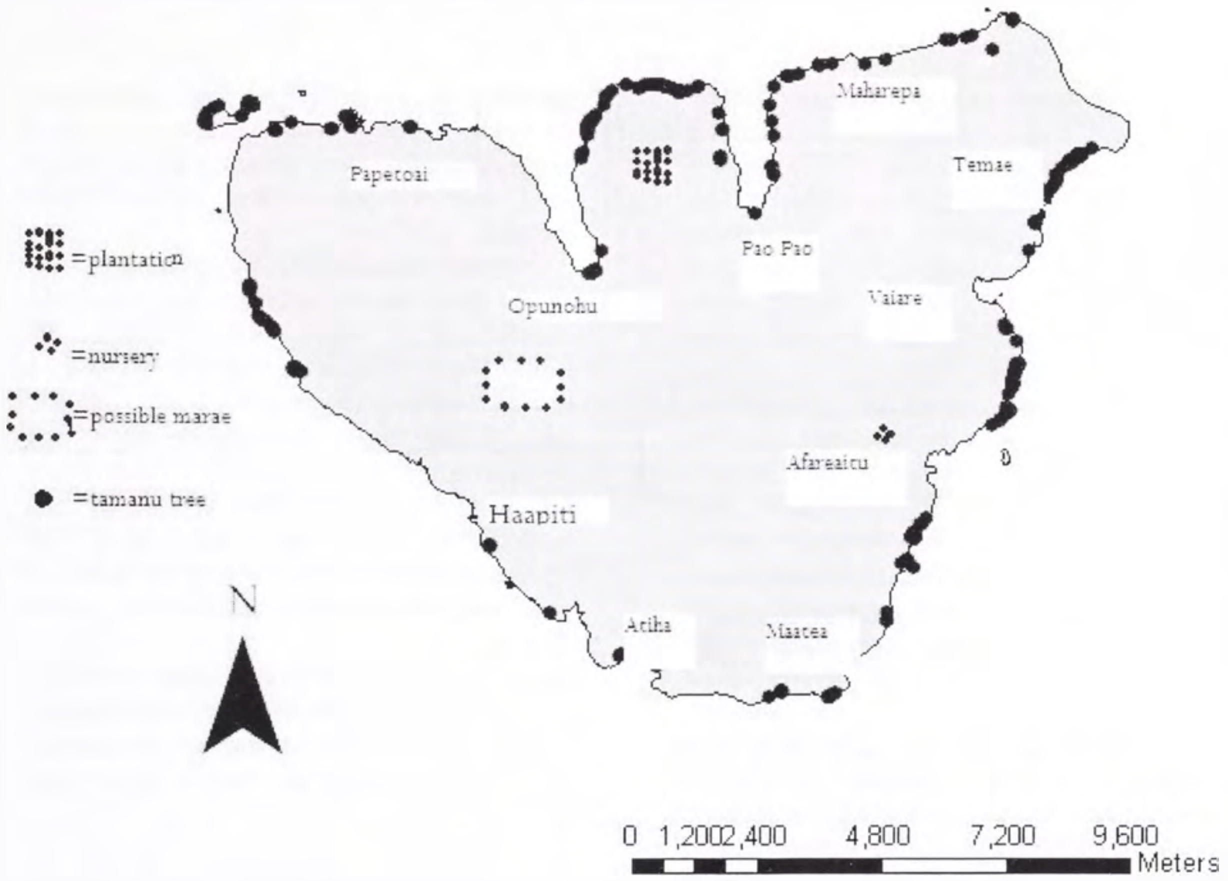


Fig. 1 Distribution of *Calophyllum inophyllum* around the island of Moorea, French Polynesia

RESULTS

Distributional survey

As can plainly be seen in figure one, I discovered more than 400 tamanu trees distributed fairly evenly around the entire island and all of the locations exposed in the interview process as being former strongholds of tamanu; Afareaitu, Vaiare, PaoPao. The oldest and grandest grove of the former princess is on protected land and has plenty of sprouts coming up in its' shade. Motu (Tahitian for island) Fareone and Tiahura which appear of the coast of Papetoai also had presence of tamanu, which is consistent with the known former range (Burford), however, on Fareone the were all lined up in a border fence strung with barbed wire creating cutting across the center of the island. On Motu Tiahura they were found growing in more natural positions ringing the island where there were no rock walls.

A histogram showing that more trees grow where retaining walls are absent (Fig. 2) demonstrates that that is indeed the case consistently around the island. This iimportant because, as you can see in Fig. 3 that the tamanu trees preferred habitat is by far the beach. The second preferred habitat is also the beach, closer to the water, with branches hanging over the high water line. From these two positions the fruit of the tree could drop into the water and float away for possible germination elsewhere. Corroborating habitat preferences is another histogram spread sorting the trees by which substrate on which they were growing. You can see in Fig. 4 that of 379 trees found (excluding the plantation and nursery sites which are unnatural locations perturbing the data) 227 were found growing in sand.

Island wide there were not many *C. inophyllum* seedlings found. The majority of

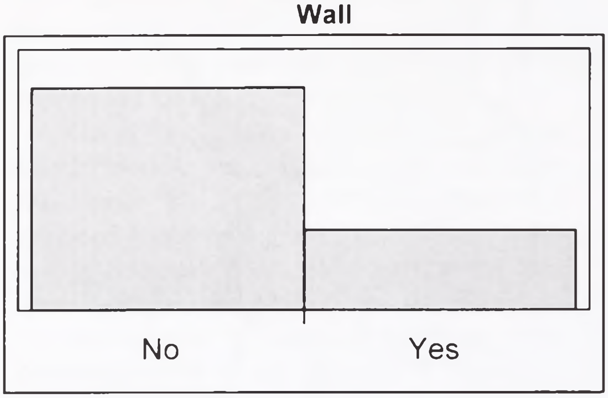


Fig. 2 The majority of Tamanu trees on Moorea grow where there is no retaining seawall- suggesting it is easier to germinate where there is no wall capturing the coast.

trees had no sprouts around them (Fig. 5) In Fig. 6 and 7 it is clear that most sprouts were found growing in the forest, on rock, or even on sandy soil. With a significant P value of .0199 you can see that sprouts are not found growing in sand or, less significant (P.4188), on the beach.

It is on the back beach, in a garden, or even across the coastal road that one would finsprouts. The likely explanation for this apparent contradiction has to do with the observation that Mooreans regularly clean their beachfronts of debris and even rake the tamanu almonds into piles and remove them from the environment for personal use. It is on rocky or grassy substrate that the locals don't rake away the seeds- potential sprouts.

An analysis of where one can find seeds confirms this. Most trees, in fact, were found with no nuts in trees and no nuts on ground (Fig. 8 and 9). In Fig. 10, a one way analysis of where one does find most nuts, indicates a with a highly significant P value of .0005, that, once again as was with the sprouts, it is on rocks in the forest or even sandy soil, but not sand (Fig. 11). Again, I believe this to be due to human activity- the nuts being removed diligently and daily from beachOne can clearly see in Fig. 12 (significant P value of .0109) that the larger, therefore older, trees as determined by diameter at breast height, can be found growing on sand. In Fig. 13, less significant (P value of .8683) but showing a trend, there are more larger trees growing on the beach and over the water than in any other location.

As for the health of the population. Fig. 14, a distribution of trees by diameter, shows that the mean diameter was 62 cm (with a standard deviation of 49.3) with a few individuals topping the scale near 400 cm. These larger individuals often had several large trunks diverging from each other at ground level and sprouting nuts wedged into crevices in the aging bark. The trees appeared to overall healthy with no pests or indications of disease of any kind. The only stress observed was that caused, again by human activity, with 36 individuals (nearly ten per cent of trees) having had portions of their bark harvested for medicinal use (see photo next page).

Germination

Fig. X Tanamu germination experiment

The seeds in my germination study sprouted at a rate consistent with past findings. However, there was not enough time to see if seeds in cracked shells and seeds still in their shells would sprout, also, at consistent rates. Incidentally, seeds sprouted for study were planted at Atatia

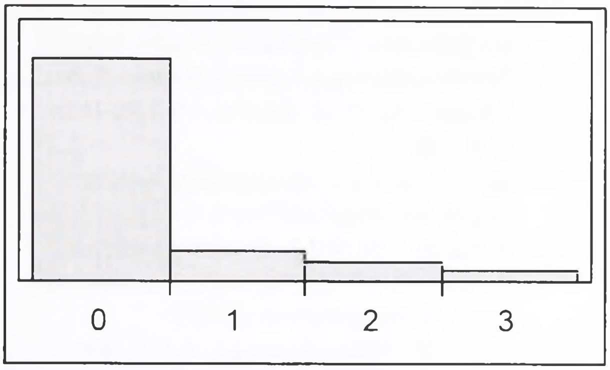


Fig. X. Distribution of numbers of sprouts around Tamanu trees. 1 corresponds to less than 5 sprouts. 2 corresponds to between 5 and 10 and 3 corresponds to greater than ten srpouts.

cultural center by students from the Agricultural vocation high school.

DISCUSSION

It is evident at a glance of the distributional map of Moorea that the tamanu's preferred habitat on Moorea is its coastline. Undeveloped coastline on Moorea is becoming ever more scarce. An examination by researchers from University of California at Berkeley's Environmental Planning Department found that historic growth and development patterns on the island confirmed that most of the land along Moorea's coastline was built upon, with development creeping inland up river valleys. And furthermore, at the observed rate of development, since 1986, Moorea may reach build-out in 50 years (Timothy Duane 2006). It is clear that since development follows the coastline in a high island setting, all native coastal species will be feeling the pressure from human activity. Chiefly valued today for its wood and medicinal products, the tamanu is also a valuable native coastal stabilizer. The tamanu tree is an excellent urban forestry tree for large spaces in coastal environments.

The tree's large spreading crown and horizontal branches make it a good shade tree and focal point for parks and other open areas (Friday, 2006). Since the tree is adapted to shallow, often flooded, salty and windy environments, it is highly recommended to be planted as a coastal species windbreak. Where *Calophyllum inophyllum* was not represented in great numbers on the coastline there were in its place planted single- row windbreaks of ironwood (Casuarnia spp.). New development on the island, namely at the golf course in Temae, has removed all coastal vegetation whatsoever and replaced it with four non-native species- including two which are on Tahiti's own list of invasive plants forbidden to be planted and sited for removal. This includes the octopus or chenille tree and the tulip tree. But Moorea's communes own land use management "best management practices" recommends using native species and specifically calls for the leaving of rough wild vegetation in all new coastal development and to establish buffer strips along wetland perimeter. It is also specifically called for to discourage the constructions of rock walls on beaches. Analysis of Moorea's terrestrial zoning plan (PGA) policies reveal that current planning regulations for tourism zones are inconsistent with the goals of the Marine Management Plan (PGEM) as they encourage large scale tourism development in the vicinity of important marine protected areas (MPAs) and fail to control inputs from reaching the marine environment that potentially could cause signifcant harm to marine ecosystems (Duane 2006).

In this study I found that there is a healthy population of tamanu trees on Moorea, but their preferred habitat is the coastal areas which are diminishing. It germinates well when humans don't disrupt their natural propagation and does not seem to grow as often where there are rock walls. Because it is a species highly recommended to be included in Moorea's coastal plans for protection from cyclones and capture of coastal runoff, it is hoped that consideration will be made in future developments to leave existing tamanu trees were they grow and encourage the dissemination of young trees being grown for the public at the native plant nursery run by the Society of Rural Development.

ACKNOWLEDGEMENTS

I would like to thank all of the professors, graduate student instructors and my fellow students, as well as staff of the Gump station for their guidance and encouragement. I would also like to thank Debbie Giles of University of California at Davis for her help formatting this paper.

LITERATURE CITED

Bhat,P.R., Murali, K.S., Hegde, G.T., Shastri, C.M., Bhat, D.M., Murthy, Indu K., Ravindranath, N. H., Annual variation in non-timber forest product yield in Western Ghats, Karnataka, India. Current Science, Vol. 85, No. 9, 10 November 2003.

Bianchini, Jean-Pierre, Professor Emeritus, Chemistry, University of French Polynesia, Papete. Personal communication

Brigant, Gerard, producer of tamanu products, Papeete. Personal communication. Burford, Martha, Vascular plants of the mo'orean motu species list and location

Duane, Timothy 2005. Land use planning to promote marine conservation of coral reef ecosystems in Moorea French Polynesia. Pacific Rim Research Program.

Elevitch, Craig R. and Wilkinson, Kim, Agroforestry Guides for Pacific Islands, Sustainable Agroforestry Reaserch in Education, pp 41, 46,, 51, 86, 128, 161, 164, 218, 228

Friday, J.B., and D. Okano, *Calophyllum inophyllum* (kamani), ver. 2.1 In: Elevitch C.R. (ed.). Species profiles for Pacific island agroforestry. Permanent agriculture resources (PAR) www.traditionaltree.org, April, 2006.

Gunn, C.R. and Dennis, J.V., World guide to tropical drift seeds and fruits, 1976.

Ishikawa, T. 2000. Anti-HIV-1 active *Calophyllum* coumarins: distribution, chemistry and activity. Heterocycles 53/2: 453-474.

Laure, Frederic, Etude de la composition chimique et de la biodiversite du *C. inophyllum* de Polynesie Francaise. Nice, March 3, 2005.

Murphy, Hinano, Directrice, Atatia Cultural Center. Personal communication.

Nardi, Pierre, farmer. Personal communication.

Parras, V., Comparison of different methods of germinating bitaog. The Philipine Journal of Forestry, undated.

Petard, Paul, 1986. Plantes Utiles de Polynesie Francaise et Raau Tahiti, pp 27, 44, 66, 225-235. Pi'ito, woodcarver. Personal communication.

Societe' de Development Rurale. Personal communication.

Whistler, Dr., W., Flowers of the pacific island seashore. Isle Botanica, 1992.

World Health Organization (WHO) Regional office for Western Pacific, Medicinal Plants in the South Pacific, 29.

APPENDIX

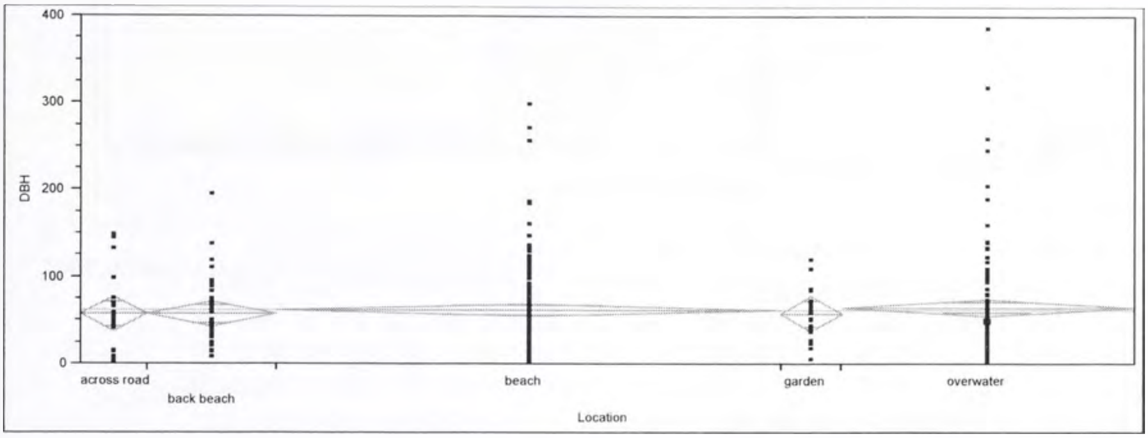


Fig. 1 Analysis of tree size (diameter at breast height) By Location

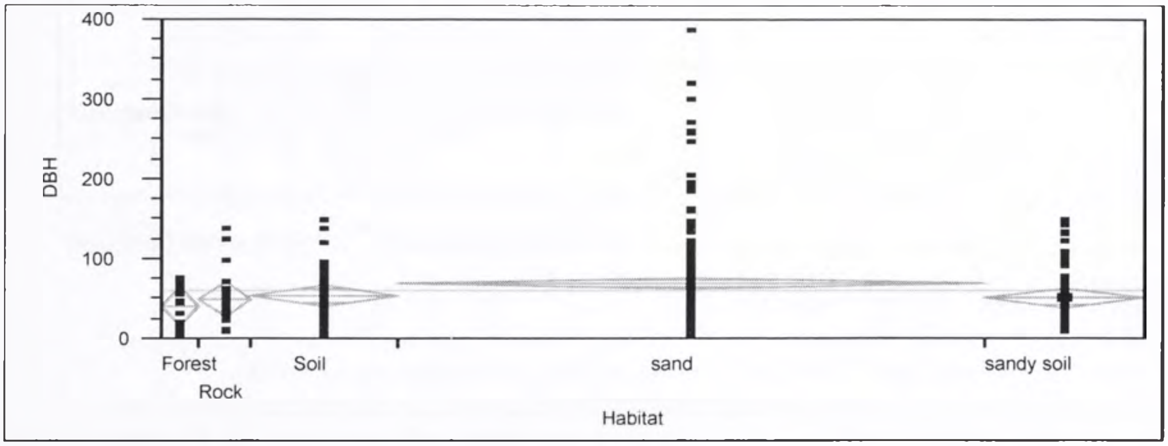


Fig. 2 Analysis of tree size (diameter at breast height) By Habitat

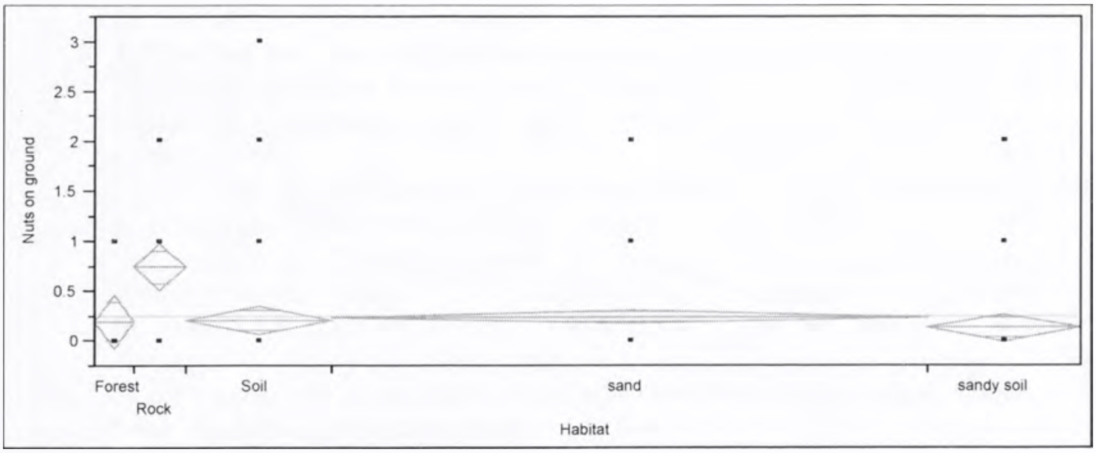


Fig. 3 Analysis of Nuts on ground By Habitat

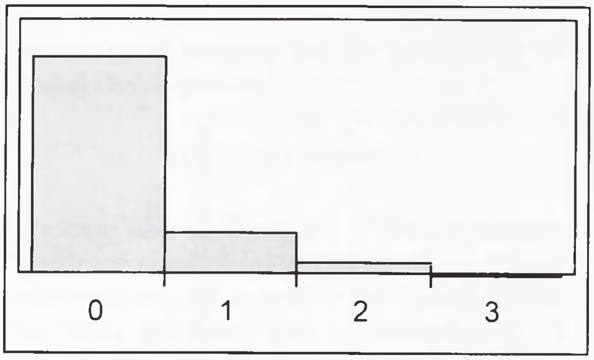


Fig. 4. Distribution of trees with fallen nuts on the ground.

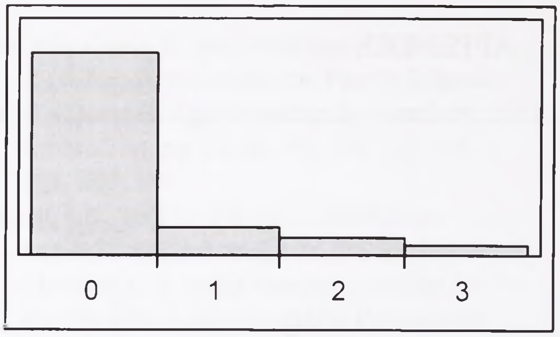


Fig. 6 Island trees without sprouts outnumber those with many.

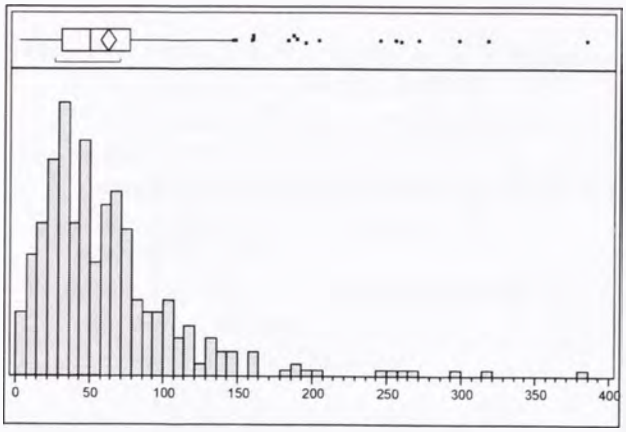


Fig. 5. Distribution of tree size by DBH in centimeters.

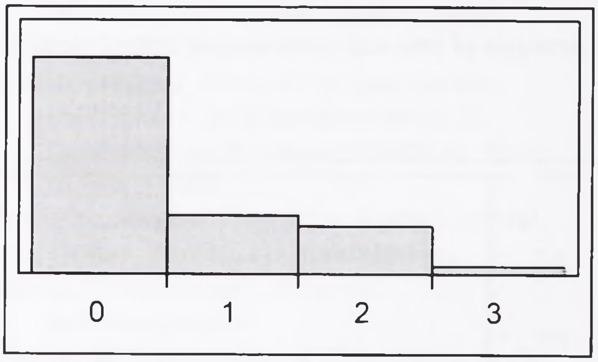


Fig. 7 Distribution of fruiting trees (nuts on branches).

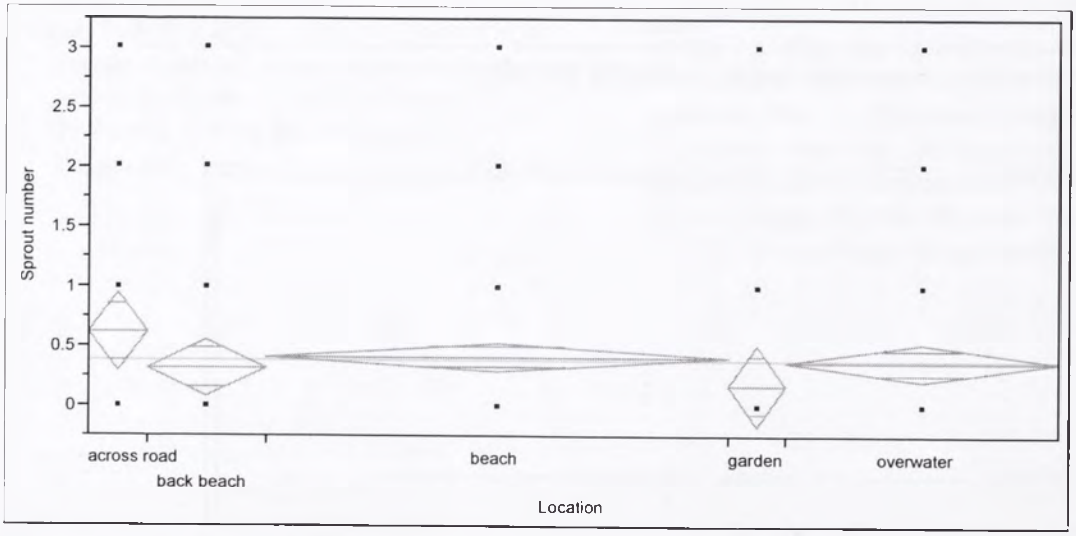


Fig. 8. Analysis of seedling quantity by Location (not significant)

A SEDIMENTATION STUDY OF MOOREA'S TEMAE ESTUARY

LORI LARUE

Environmental Science Policy and Management, University of California, Berkeley, California 94720 USA
lorilarue@berkeley.edu

Abstract. Large inland estuaries are a rare occurrence in the South Pacific's Society Islands. Moorea is one of the few islands that has a large inland estuary. Moorea's Temae estuary is undergoing change as new development projects in the area are on the rise. Usually, as development increases, sediment patterns change. This study looked for changes in the sediment distribution patterns of the Temae estuary. GPS waypoints and transects were used to create GIS maps of the estuary's location, boundaries, depth, and sediment distribution. Sieving and settling tubes were used to determine the estuary's sediment grain sizes; loss on ignition tests examined the sediment's organic matter; and core samples were taken to inspect for influxes of new sediment. The estuary's observed sediment distribution matches typical estuarine sediment distribution patterns. It could not be determined if the new developments have caused any deviations in sediment patterns. The core samples did not show any obvious new layers of sediment inside the estuary. The new developments have drastically altered the estuary's natural boundaries; it is possible the sedimentation patterns within the estuary will change over time as a result of the disturbance.

Key words: Estuary, Sediment distribution; Sediment cores; Moorea, French Polynesia

INTRODUCTION

Sediments are substances that settle to the bottom of a body of water. Particles that run off of land into the water are a major contributor of sediment. As vegetation is stripped from land to make way for anthropogenic development, eroded soils can be carried into local waterways by rain. The factors that influence the way that sediment is distributed along the bottom once it enters an estuary include tides, winds, and currents (Yaacob, 2005).

This study examined the patterns of sediment distribution in the Temae estuary on the island of Moorea, French Polynesia. Estuaries have a source of fresh water flowing in, usually a river or stream, which flows through the estuary's brackish water out to sea. In the case of the Temae estuary, the source of fresh water is not from a natural source. We therefore examined the sedimentation patterns to see if they followed the classic estuarine sediment distribution patterns (Sokal and Rohlf, 1969).

The Temae estuary project was conducted because of the perceived importance of documenting an area that is threatened by new development. A major goal was to describe this unique area before it is drastically changed or obliterated.

The objectives of this study were 1) to create a GIS map of the current Temae estuary including location, boundaries, and depth; 2) to describe sediment distribution throughout the estuary; and 3) to examine core samples taken within the estuary for signs of influxes of new sediment, possibly caused by the new developments in the area. This study has captured a snapshot of the Temae estuary during a time of change.

METHODS

Study area

This study was conducted in the Temae estuary on the northeast corner of the island of Moorea, French Polynesia. Moorea lies in the tropics of the southern hemisphere (17° 52S, 149° 56W). The study took place over two months (October and November, 2006) during the end of the dry season and the beginning of the wet season. Moorea experiences on average between 3000-4000 mm of rain per year (Pasturel, 1993). The mean annual temperature (near the ocean) is 26°C (Meyer, 1996).

The Temae estuary is the only estuary of its kind on the island. Large inland estuaries are rare in French Polynesia. There are only two large inland estuaries in the Society Islands; the Temae estuary on Moorea and a similar estuary on Tahiti.

Until recently, the estuary was connected to nearby Lake Temae. In January of 2005, construction began on an 18-hole golf course (Moorea Golf Resort, 2006). The golf course was built on the land where Lake Temae met the estuary, severing the link between the estuary and the lake. At the time of this study, there was no longer a natural connection between the lake and the estuary. The fresh water that flows into the estuary comes mainly from manmade sources, like golf course sprinklers. In June 2006, construction began on a 154 room hotel. By the conclusion of this study, in November 2006, construction had begun on a development of 115 residential units (Moorea Golf Resort, 2006).

Approximately fifty percent of the perimeter of the estuary was altered by either rock retaining walls or other human impacts (earthen dams, dredging). The estuary at low tide had an area of 40,503 m/sq; high tide was 59,131 m/sq.

The estuary is home to a wide diversity of wildlife; including shore birds, fiddler crabs, and eels. According to Edison Pearson, a fisherman and resident, there is a species of green crab that is only found in the Temae estuary. There is also an active benthic community inside the estuary.

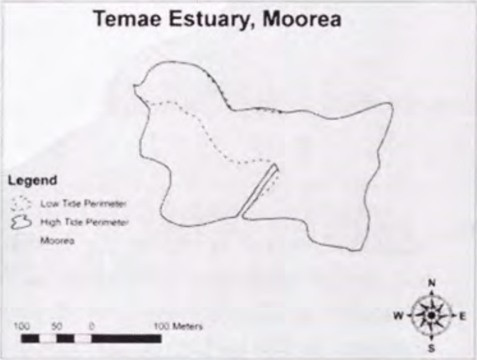


FIG. 1. GIS map of Temae Estuary at high and low tide.

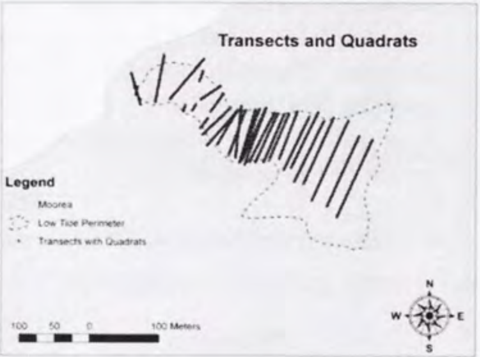


FIG. 2. GIS map of transects and quadrats used to collect data (low tide).

Wentworth Grain Size Scale	
Size (mm)	*Wentworth Size Class*
256	Boulders (B)
64	Cobbles (C)
4	Pebbles (P)
2	Very Coarse Sand (SVC)
1	Coarse Sand (SC)
½	Medium Sand (MS)
¼	Fine Sand (SF)
1/8	Very Fine Sand (SVF)
1/16	Silt (S)
1/256	Clay (CL)

FIG. 3. Wentworth grain size scale

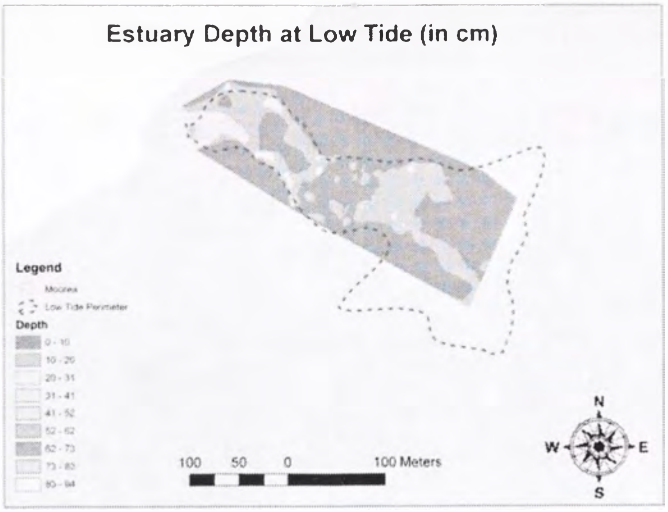


FIG. 4. GIS map of Temae estuary depth at low tide.

Sampling

The project began by making maps of the perimeter of the estuary at both high and low tide. A GPS unit was used to mark waypoints on a hand drawn map. The data collected were used to make a GIS map of the estuary (Fig. 1).

Using the GIS map, transects of the estuary were made to map surface sediment and depth. The transects ran parallel from north to south every ten meters. Every two meters along each transect a surface sample was scooped, then observed and recorded (Fig. 2). Prior to transecting the estuary, a preliminary sediment sampling was done. Using the Wentworth scale (Krumbein and Sloss, 1951), the sediment in the estuary was categorized into ten sediment sizes (Fig. 3). Sediment surface grain size was determined using sieves in the laboratory. Once a basis for visual observation and differentiation was established, the transects began. Thirty transects, over 1,100 quadrats, were examined.

In order to discover evidence of new deposition of sediment, thirty three core samples were taken throughout the estuary. Sites chosen for samples included areas where each of the Wentworth grain size categories of sediment was found in high abundance as well as from each of the estuary's islands and around the shore. The cores were taken with a 35cm long piece of 53mm wide PVC pipe and placed into plastic bags.

Analysis

Surface sediment samples were collected in triplicate. Every sediment type was collected except boulders, cobbles, and pebbles. The first set of samples was analyzed using the dry sieving method (Krumbein and Sloss, 1951). Seven sieve sizes were used, ranging from 2mm for coarser sediments to 1/256mm for finer sediments (Fig. 6). The second set of samples was analyzed using settling tubes (Krumbein and Pettijohn, 1938). Each sample was treated with hydrogen peroxide to remove any excess organic material before adding the samples to the settling tubes. After placing sediment in the settling tubes, a hydrometer was used to take measurements (g/L) at 30 seconds, 2 hours, and 24 hours (Gee and Bauder, 1986). The sediments that settled to the bottom of the cylinder were collected, dried, and weighed (Fig. 6). Both the dry sieving and settling tube techniques examined sediment grain size. The third surface sediment sample was used to test for organic material using the loss on ignition technique. This technique required heating approximately six grams of each sample to 450°C for four hours. The samples were weighed before and after ignition to determine the percent of material that was lost.

Each of the collected core samples was measured, photographed, and dissected. The cores were sliced in half vertically and sediment samples from the top, middle, and bottom were collected.

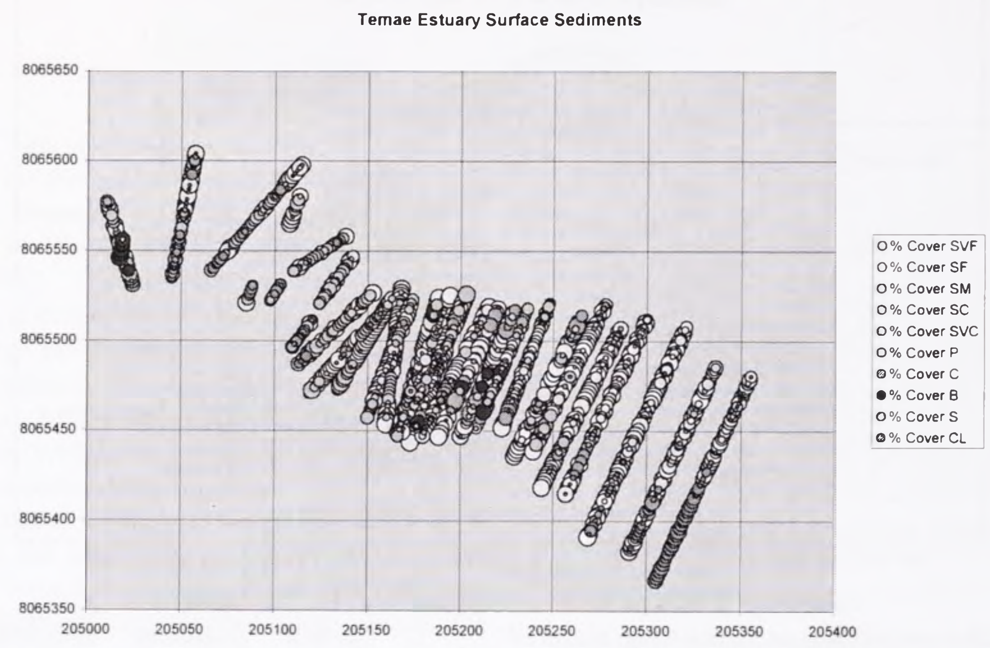


FIG. 5. Surface sediments by percent coverage, graphed on UTM grid.

See Fig. 3 for legend abbreviation key

RESULTS

GIS Mapping

A GIS map of the estuary at the time of the study was created. In addition to mapping the Temae estuary's location on Moorea and perimeter at high and low tide (Fig. 1), maps of the depth (Fig. 4) and sediment distribution (Fig. 5) were also produced.

Sediment distribution

The sediments found near the mouth, or the west side, of the estuary tend to be larger than the sediments found inland (Fig. 5), or the east side, of the estuary (near the golf course). This is consistent with the classic estuarine sediment distribution patterns.

The GIS maps showed several channels of deeper water. The sediment grains in these channels are larger than the sediment grains adjacent to the channels in the shallower water.

The surface sediments that were sieved show a trend of increasing grain size. The smaller grains (1/2048mm, 1/256mm, and 1/16mm) are predominately found in the clay, silt, and fine sands (Fig. 7).

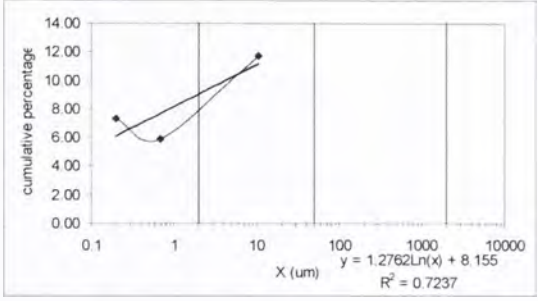
Settling tubes provided settling velocity information for the samples (Fig. 6). Of the surface samples that underwent loss on ignition tests, results show a trend of higher percentage of material lost in the smaller grain sediments (Fig. 8).

Microscopic examination showed that the sediments were primarily made up of calcium carbonate. The samples included small pieces of shells and coral.

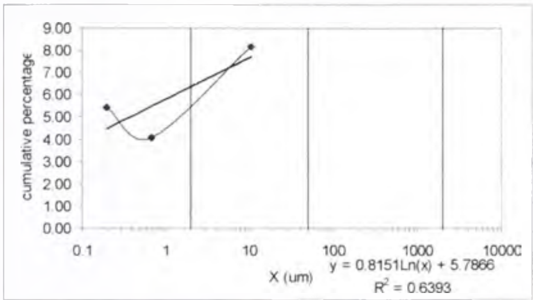
Sediment cores

One of the major objectives of this study was to examine sediment cores to look for evidence of new sediment layers. However, visual and microscopic examination of the cores through dissection did not provide any conclusive results. The sediment occasionally showed signs of stratification, but more often remained the same throughout. For example, a core sample would appear to have the same grain size throughout the depth of the core, but the top 2mm would be a slightly different color from the rest of the core. The sediment cores showed evidence of worm and clam burrowing.

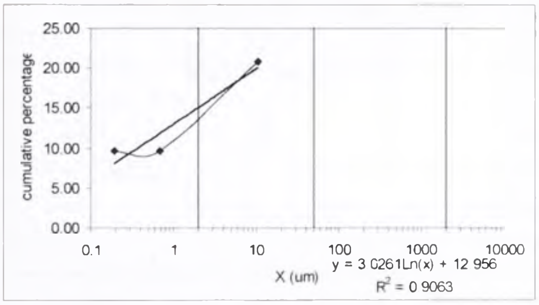
The salinity of the estuary was 31.3ppt near the mouth and 20.8ppt inland (near the golf course). The runoff from the golf course into the estuary had a salinity reading of 0.5ppt.



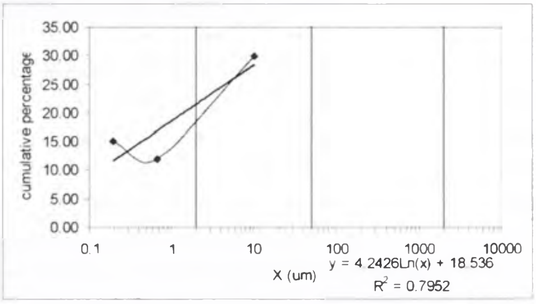
Settling tube results for very coarse sand (SVC).



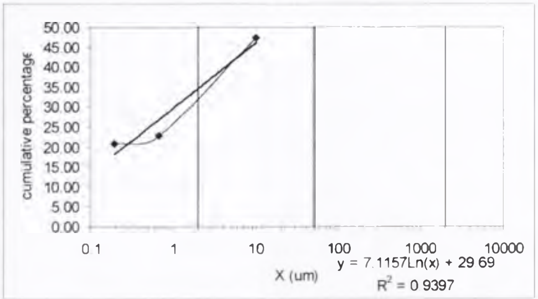
Settling tube results for coarse sand (SC).



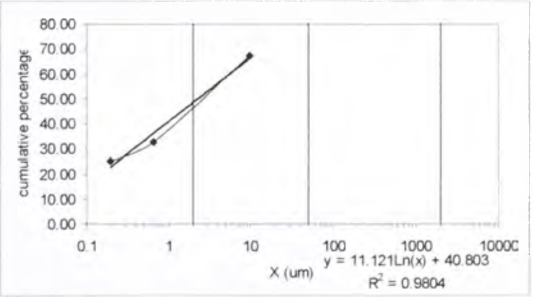
Settling tube results for medium sand (SM).



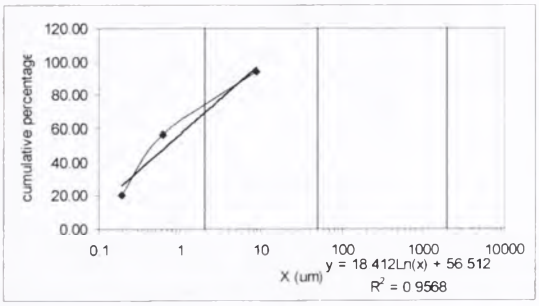
Settling tube results for fine sand (SF).



Settling tube results for very fine sand (SVF).



Settling tube results for silt (S).



Settling tube results for clay (CL).

Each of the sediment types was examined (except boulders, cobbles, and pebbles).

The y-axis is the hydrometer reading (g/L) divided by the sample weight (x 100 to get %).

The x-axis is the mean particle size diameter.

FIG 6. Settling Tube Results

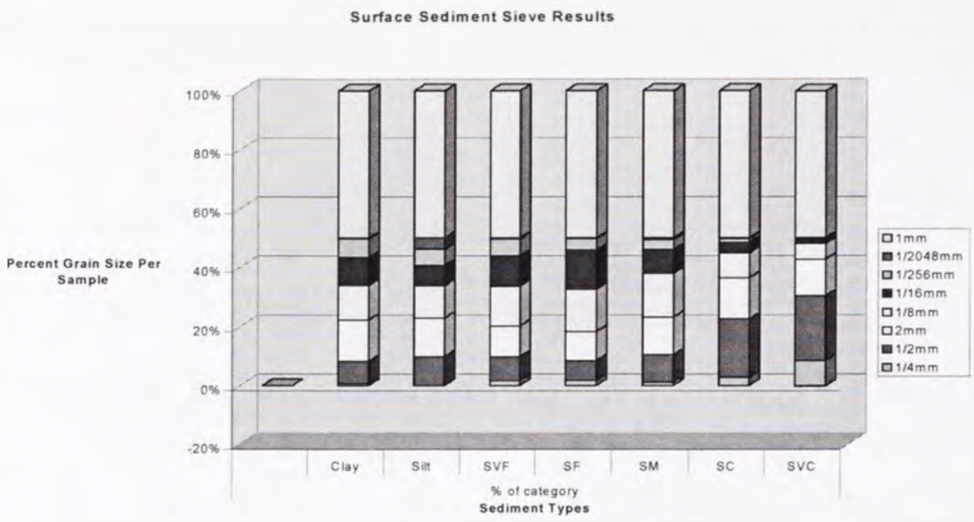


FIG. 7. Surface sediment sieve results. Sediments go from smaller grain on left to larger grain on right of X axis.

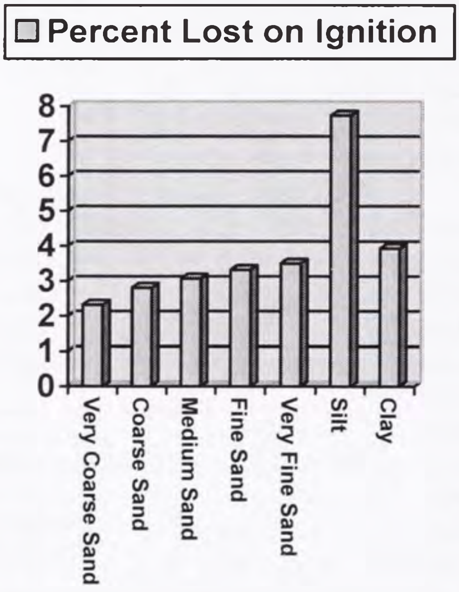


FIG. 8. Percent material lost on ignition by sediment type. X-axis shows grain size categories, y-axis shows percent of material lost on ignition.

DISCUSSION

GIS mapping

GPS and GIS technology played a major role in this project. By using GIS maps, patterns were exposed. Once patterns were established, the data collected were more readily interpreted. For example, a pattern emerged that showed larger grain sizes tended to be found in areas of deeper water. This supports to the idea that larger, heavier grains sink while finer grains continue to remain in solution (Selley, 1982).

The GIS depth maps showed channels of deeper water inland. These deeper channels may have been formed earlier when the estuary was connected to the lake.

Sediment distribution

The sediment patterns exhibited in the estuary are similar to what would be expected based on standard estuarine characteristics (Sokal and Rohlf, 1969). In general, the larger grain sediments, such as coarse sands, were located near the mouth of the estuary whereas the finer grain sediments, such as clay, are found inland. The sediment found near the shores often had coarser grain sizes than the sediment found in the center of the estuary.

In contrast, the deeper channels were characterized by larger grain sediments. This could be because the larger grains tend to fall out of suspension in faster moving currents. It could also be a result of the finer grain sediments falling out of suspension only to be swept back into the water column as the tide changes.

Unfortunately for the study, the area of the estuary that approaches the new developments was dredged from the shore to approximately 20 meters into the water. The area was dredged three meters deep before any surface sediment data were collected. If there were any drastic sediment changes caused by the new developments, it would have been most evident in that area.

Sediment cores

The sediment cores proved to be inconclusive. The expected layers of new sediment were not observable. No clear link could be drawn between the new developments near the estuary (golf course, hotel, or housing) and the sediment layers of the estuary.

CONCLUSION

The Temae estuary is undergoing dramatic changes. It was not possible to determine if these changes have affected the estuary's sedimentation because before this study, no previous research had been done on the Temae estuary's sediment distribution. Therefore, there were no historical references for comparison. In lieu of site specific records, general estuarine characteristics were referenced. The sediment patterns in the Temae estuary were consistent with classic estuarine sediment patterns. Sediment cores were taken but did not provide any conclusive results.

The estuary's sediments are primarily made up of calcium carbonate. Microscopic investigation showed that the sediment was filled with broken pieces of shells, coral and urchin spines. This information leads to the conclusion that the primary source of sediment has been coming from the ocean. The new developments may also be introducing sediment into the estuary, but this could not be proven that with any certainty.

The development of the golf course has cut off the major source of fresh water, Lake Temae, from the estuary. Without a constant outward flow of freshwater, the estuary could potentially fill up with incoming marine sediment. In addition to filling with sediment, the salinity and chemistry of the estuary could drastically change. If so, the estuary's benthic communities and other wildlife may be reduced in numbers. As this is the only estuary of its kind on Moorea, some of the species that inhabit this unique niche may soon be threatened.

ACKNOWLEDGMENTS

I thank the GUMP station and staff for hosting me while I did my research. Also, thank you to Jere Lipps, Brent Mishler, Jaime Bartolome, Carole Hickman, Vince Resh, Liz Perotti, Allison Purcell, Erica Spotswood, Jonathan Sanderman, and Karen Tuxin. Special thanks to my partners for all their help, support, and companionship: Josh Walker, Andrew Greene, April Dobbs, Maggie Groff, Erin Prado, Sigrid Hawkins, Valerie Howell, and Carol Chang.

LITERATURE CITED

Gee, G.W. and J.W. Bauder, 1986. Particle-Size Analysis in Methods in Soil Science: Physical and Mineralogical Methods. SSSA, Madison, WI.

Krumbein, W. C. and F.J. Pettijohn. 1938. Manual of Sedimentary Petrography. Appleton-Century-Crofts, Inc., New York.

Krumbein, W.C. and L.L. Sloss. 1951. Statigraphy and Sedimentation. W.H. Freeman and Company, San Francisco.

Meyer, J. 1996. Tahiti's native flora endangered by the invasion of Miconia *calvescens* DC. (Malastomataceae). Journal of Biogeography, pg. 775.

Moorea Golf Resort. 2006. http://www.mooreagolf-resort.com/public/en/index.php

Pasturel, J. 1993. La climatologie des isles. Editions de l'ORSTOM, Paris

Selley, R.C. 1982. An Introduction to Sedimentology. Academic Press, London.

Sokal, R.R. and F.J. Rohlf. 1969. Biometry. W. H. Freeman and Company, San Francisco.

Yaacob, R. 2005. The relationship of sediment texture with coastal environments along the Kuala Terengganu Coast, Malaysia. Enviromental Geology 48: 639-645.

DISTRIBUTION, ECOLOGY, AND SYSTEMATICS OF THE FILMY FERNS (HYMENOPHYLLACEAE) OF MOOREA, FRENCH POLYNESIA

JOEL H. NITTA

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

Abstract. Ferns present an interesting case in island biogeography because of their unusually high dispersal ability. A general survey of the filmy ferns (Hymenophyllaceae) of Moorea, French Polynesia was undertaken by observing distribution in the field, conducting tests of desiccation tolerance, and inferring a phylogeny based on morphological characters. A total of 12 filmy fern species (including three new records for Moorea) were found to occur in moist, well-covered habitats throughout the island, with distinct sets of high elevation and low elevation species. Five species were included in an experiment to determine relative degree of desiccation tolerance; results indicate that terrestrial species have lower desiccation tolerance than epiphytic or epipetric species. In order to place the Moorea filmy ferns in the larger archipelago context, eight additional Society Island species were included in the phylogeny for a total of 20 taxa based on 21 morphological characters. The resulting tree was used to interpret distributional and experimental data and to infer biogeographic patterns. Filmy fern species are widely dispersed throughout the Society Islands, and there is no indication of an adaptive radiation on Moorea.

Key words: Filmy Ferns; Hymenophyllaceae; Moorea, French Polynesia; Society Islands, French Polynesia, desiccation tolerance; systematics; island biogeography

INTRODUCTION

Ferns are interesting plants to consider from a biogeographical perspective because of their extremely high dispersal capability. The spores of most fern species are small, lightweight, and can remain viable for months in an air-dry condition; these characteristics have enabled ferns to become widely dispersed, even to oceanic islands located thousands of kilometers from the mainland (Tryon 1970). Ferns on remote islands are of particular significance because they represent a disharmonic subset of the source flora (Whittaker 1999). Although many potentially colonizing fern species may disperse their spores over a wide area, only those that can tolerate the conditions of the new habitat will be able to survive and successfully establish a population (Wolf et al. 2001).

One such group of ferns that seems to be restricted in its potential habitat is the filmy ferns (Hymenophyllaceae). These ferns derive their name from their extremely thin lamina, which is only one cell layer thick. In addition, filmy ferns also lack any differentiated epidermis or stomata. Thus, they are highly dependent on environmental moisture and occur only in moist (but not completely wet) areas. The filmy ferns have diversified extensively within this niche, displaying an amazing degree of variation in both morphology (erect to long-creeping rhizome, robust roots or rootless, simple to divided fronds, overall size minute to large) and growth habit (terrestrial, epipetric, epiphytic, and liana) (Dubuisson et al. 2003). These characteristics make filmy ferns an ideal model system for studying processes of evolutionary adaptation and dispersal.

Although the fern flora of many Pacific islands such as the Hawaiian Islands has been extensively studied (Robinson 1914, Palmer 2002), those of the Society Islands have received relatively little attention. The most comprehensive review of the ferns of the

Society Islands is Copeland's (1932) Pteridophytes of the Society Islands, however, extensive taxonomic revisions since that time have rendered it nearly unusable. More recently, Murdock and Smith (2003) compiled a checklist for ferns on Moorea based on field work, a literature review, and survey of herbarium specimens. Two additional filmy fern species were described on the island just last year (Ranker et al. 2005)

This study was conducted on the island of Moorea, French Polynesia at the University of California, Berkeley Gump Research Station. My goal was to describe the diversity of Hymenophyllaceae on Moorea by 1) observing distribution throughout the island, 2) conducting tests of desiccation tolerance, and 3) inferring a phylogenetic tree based on morphological characters that can serve as a framework to interpret distributional and experimental data. In order to place the Moorean filmy ferns in a larger Society Island context, herbarium samples from various locations outside Moorea are also included in the tree.

Family Characteristics

Filmy ferns are an ancient group, with putative fossils dating back to the Triassic (Axsmith et al. 2001). They occupy a basal position in the leptosporangiate clade, sister to the most basal family Osmundaceae (Pryer et al. 1995). The Hymenophyllaceae family is highly diverse (>600 species).

Members of the Hymenophyllaceae family are characterized by their simplified lamina, which is only a single cell thick and lacks any differentiated epidermis or stomata. Filmy ferns also have unique sori. Unlike many ferns which bear sori on the underside of the leaf, filmy fern sori occur at the edge of the leaf, on the tips of veins. The sori are contained within involucres (synonymous with indusia for this family). The receptacles that bear the sporangia may either be exserted or included. Although the family contains a range of morphological diversity, many filmy ferns are reduced in size, with fronds reaching lengths of less than 2 cm (reviewed in Dubuisson et al. 2003).

FIG. 1. Mat-like covering of *Crepidomanes minutus* on a tree branch halfway up the trail to Mt. Mouaputa summit. Photo by Joel Nitta.

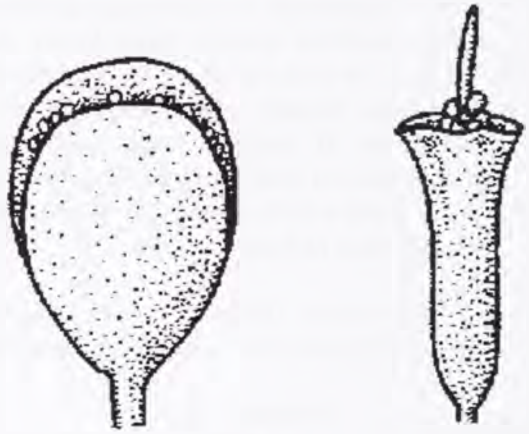


FIG. 2. Bivalved (left) vs. tubular (right) involucres. Involucres of most species are no more than a few mm long. Adapted from Palmer 2002.

The ecology of filmy ferns reflects their morphology. The thin lamina of filmy ferns dries out quickly, so they are restricted to perennially moist habitats. Most filmy ferns are either epiphytic or epipetric, with a few terrestrial and liana species. If the habitat is favorable, ferns may occur in a dense mat of interweaving rhizomes and cover the substrate (Fig. 1).

Taxonomy of the Hymenophyllaceae

Although the monophyly of Hymenophyllaceae as a family is undisputed, there has been a great deal of debate concerning intrafamilial relationships. Two main genera have been traditionally recognized based on involucre morphology, *Trichomanes* having tubular involucres and *Hymenophyllum* having bivalve involucres (Fig. 2). Over the years, several modifications have been proposed to this bigeneric scheme. Morton (1968) added four monotypic genera, and defined multiple subgenera, sections, and subsections within *Trichomanes* and *Hymenophyllum*. Iwatsuki (1984) contended that involucre morphology within the family actually varies over a range from tubular to bivalve, and proposed eight genera to match this finer gradation in morphology. Other classification schemes have involved upwards of 30 to 40 different genera (Copeland 1938, Pichi-Sermolli 1977).

Recent molecular phylogenetic studies have shed new light on systematic relationships within the family. A molecular phylogeny based on the chloroplast rbcL gene contains two large monophyletic lineages, which correspond to the traditional *Trichomanes / Hymenophyllum* split (Pryer et al. 2001). Other studies have elucidated relationships within genera (Dubuisson et al. 2003, Ebihara et al. 2004), and Ebihara et al. have proposed yet another classification system based on monophyletic groupings found using rbcL data (Ebihara et al. 2006).

As the taxonomy of this group is still in flux, I have chosen to use Ebihara's recently published revised taxonomy of the family, which is supported by molecular data. This taxonomy largely retains the *Hymenophyllum* genus sensu lato, while splitting the Trichomanoid group into several genera.

Prior to this study, a total of nine species of filmy ferns were known to occur on Moorea (Copeland 1938, Murdock and Smith 2003,

- Family Hymenophyllaceae
  - Genus *Abrodictyum*
    - Subgenus *Abrodictyum*
      - *Abrodictyum caudatum* (Brack.) Ebihara & K. Iwats.
    - Subgenus *Pachychaetum*
      - *Abrodictyum dentatum* (Bosch) Ebihara & K. Iwats.
  - Genus *Callistopteris*
    - *Callistopteris apiifolium* (C. Presl) Copel.*
  - Genus *Crepidomanes*
    - Subgenus *Crepidium*
      - *Crepidomanes humile* (G. Forst.) Bosch
    - Subgenus *Crepidomanes*
      - Section *Crepidomanes*
        - *Crepidomanes bipunctatum* (Poir.) Copel.
        - Section *Gonocormus*
          - *Crepidomanes minutum* (Blume) K. Iwats.
    - *Crepidomanes sp. 1*
    - *Crepidomanes sp. 2*
  - Genus *Didymoglossum*
    - Subgenus *Didymoglossum*
      - *Didymoglossum tahitense* (Nadeaud) Ebihara & K. Itwats.
  - Genus *Hymenophyllum*
    - Subgenus *Mecodium*
      - *Hymenophyllum polyanthos* (Sw.) Sw.
    - Subgenus *Pleuromanes*
      - *Hymenophyllum pallidum* (Blume) Ebihara & K. Iwats.
    - Subgenus *Sphaerocionium*
      - *Hymenophyllum digitatum* (Sw.) Fosberg**

FIG. 3. Taxonomic relationships of the filmy ferns of Moorea sensu Ebihara et al. (2006). * *Trichomanes apiifolium* is listed as *Trichomanes societense* in Murdock and Smith (2003), but they report that the two are very similar and most likely the same species. ** *H. digitatum* is also listed as *H. taeniatum*, but this is most likely a species complex.

Ranker et al. 2005). I discovered three filmy ferns previously unreported in the literature for Moorea, bringing the total to 12. The Moorean filmy ferns are a diverse group, representing five of Ebihara's nine genera. Classification of the Moorean filmy ferns following Ebihara's system is outlined in Figure 3.

Desiccation Tolerance

Most animals and flowering plants cannot survive being dried to equilibrium with the air; however, many organisms under 5 mm in size posses the ability to survive complete desiccation (defined as 10% or less of original water content) (Alpert 2006). Desiccation tolerance is truly a remarkable feature, as it requires complete suspension of metabolism and the preservation of organelles that are normally bathed in fluid (Hoekstra et al. 2001). Desiccation tolerance is known in a few animal species, approximately 350 species of adult flowering plants and ferns, and the majority of lichens and mosses (Alpert 2006).

Filmy ferns, although normally restricted to moist, shady areas, are also known to survive long intermittent periods of drying due to sunlight (Shreve 1911). Filmy ferns occur in similar habitat and have even adapted similar growth forms to many mosses, so it is unsurprising that they have also evolved desiccation tolerance as well. Proctor (2003) conducted one of the only physiological studies of filmy ferns, and found two species with different overlapping ranges in south-west England to have different degrees of desiccation tolerance as measured by chlorophyll fluorescence.

The ability to tolerate drying is likely to be linked to geographic range. In this study, I have conducted desiccation tolerance experiments in the laboratory on each species to determine if they have different desiccation tolerances and how these relate to their distribution on the island.

METHODS

Distribution Analysis

Distribution analysis was primarily conducted by observing ferns near hiking trails. Filmy ferns only occur in shaded, moist areas, and are thus restricted to forests on the island's interior. There are many hiking trails traversing different parts of the interior of Moorea, ranging in elevation from sea level to 1207 m, at the top of the highest peak, Toihea. Trails I hiked included Three Coconuts Pass, Mt. Rotui, Mt. Mouaputa, and the Vaiare to Paopao cross island trail (Fig 4). I also sampled ferns from Marimari Kellum's property and the Mahaeru stream valley on the south face of Mt. Tohiea. These areas lacked trails, but I was able to sample by hiking cross-country. Sampling involved gathering ecological data such as relative percent cover, aspect, habitat, height, and population density, as well as taking GPS coordinates for each specimen.

Desiccation Tolerance Experiment

I conducted a laboratory experiment in

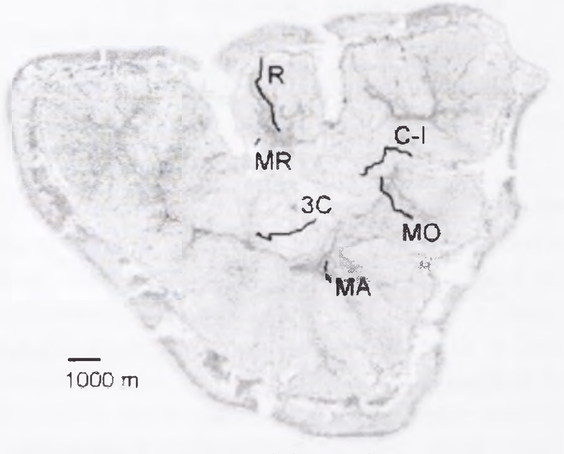


FIG. 4. Location of filmy ferns study sites on Moorea. All sites indicated by heavy black lines. Key: 3C = Three Coconuts Trail, C-I = Cross Island Trail, MA = Mahaeru Creek, MO = Mt. Mouaputa Trail, MR = Marimari Kellum's property, R = Mt. Rotui Trail. Adapted from Ministere de L'Urbanisme des Transports Terrestres et de L'Administration Generale.

order to determine if the various species of filmy ferns on Moorea have different abilities to tolerate desiccation that might influence their distribution. The experiment relied on ion leakage as a measure of membrane damage due to desiccation, a technique commonly used in mosses and lichens (Shirazi et al. 1996). In these plants, intracellular cations have been shown to leak from cells following desiccation stress; plants that are desiccation tolerant show significantly less leakage than those that are sensitive (Brown and Buck 1979). In addition, given enough time, desiccation tolerant plant species will be able to repair the damage to their membranes and ion leakage will decrease or cease completely (Leopold et al. 1981).

Due to time restrictions, it was not possible to include all 12 Moorean filmy fern species in the experiment. Instead, 5 species representing a variety of habitats and morphology were selected. Fern specimens were immediately placed in airtight containers in the field upon collection, and used within 12 - 24 h to prevent any desiccation damage before the experiment began. All samples were soaked in tap water in the laboratory overnight to insure maximum hydration prior to the experiment. Each sample consisted of a minimum of 0.015 g (wet weight) of frond material, with a maximum sample wet weight of 0.080 g. 8 samples (n = 8) were used per replicate. In order achieve a homogenous sample containing only lamina and veins, fronds were cut so that no part of the stipe or rachis was included in the sample. Samples were washed with deionized water, blotted dry, and weighed. These were placed in airtight containers, kept at 24°C in the dark at either 100% or 20% relative humidity. Humidity was maintained by placing deionized water or silica gel respectively inside the containers. Degree of desiccation damage and subsequent repair was measured twice, after periods of 7 and 14 days. At each of these times, fronds were removed from the containers, weighed, placed in 12.5 mL deionized water, allowed to sit for 2 hours, and removed. A Corning Checkmate II conductivity meter (Corning Inc., Corning, USA) was used to measure conductivity of the resulting solution. After this measure of membrane damage due to desiccation, samples were soaked in tap water for 24 hours in a controlled setting (63% RH, 24°C, darkness) to allow frond membranes to recover. Conductivity of the samples was then measured again following the protocol described above. Finally, samples were dried overnight in an oven at 65°C and weighed to determine dry weight.

For statistical analysis, ion leakage was expressed by dividing conductivity (μS/cm3) by dry weight (g) for each sample to correct for dependence of leakage on membrane mass. Data were found to deviate from a normal distribution by the Shapiro-Wilk test ($p < 0.0001$). A log transformation of the data still did not approximate a normal distribution ($p < 0.0001$). The Wilcoxon test was used to determine if there was any significant difference between groups in the data. Significance of difference between specific groups was determined using the Tukey test.

Morphological Phylogeny

Outgroup selection was limited because of extremely low degree of homology between Hymenophyllaceae and its sister family Osmundaceae. Instead of choosing a specific outgroup, tree rooting was based on current molecular phylogenetic studies at the sub-generic and species level (Ebihara et al. 2006).

Terminal taxa of the phylogeny include all Moorean filmy fern species observed in this study as well as several species known to occur on other islands in the Society Island archipelago.

Morphological characters were chosen based on the criteria that they be 1) heritable, 2) unique, and 3) significantly different between taxa (Brent Mishler, pers. comm.) Several characters were chosen based on those used in the morphological phylogeny of *Trichomanes* taxa by Dubuisson (1997). Character states for each Moorean filmy fern species were scored by examining specimens collected in the field. Character states for non-Moorean filmy ferns were scored by examining specimens collected on Tahiti and by examining herbarium specimens deposited at Tahiti (TA) and Berkeley (UC). After all characters were scored, a data matrix was compiled using

MacClade 4.0 (Sinauer Associates Inc., Sunderland, USA).

The data matrix was loaded PAUP 4.0 (Sinauer Associates Inc., Sunderland, USA) and hypothetical phylogenetic trees were generated by using maximum parsimony analysis; these were then compiled into one single consensus tree.

RESULTS

Distribution Analysis

Below I describe the filmy fern occurrence at and condition of each study site. Filmy fern occurrences are summarized in Table 1.

Three Coconuts Pass - This trail leads from the Belvedre (247 m), a lookout with a vista of Mt. Rotui, to a ridge known as Three Coconuts Pass (357 m). It is mostly flat, meandering through the forest, crossing a few streams, and finally climbing near the end when the pass is reached. The habitat is typical of low to mid-elevation Polynesian forest, dominated by *Inocarpus fagiferus* (Park.) Fosberg. and *Angiopteris evecta* (G. Forst.) Hoffm. *Cr. humile* and *Cr. bipunctatum* were common on tree trunks and rocks (the latter occurring more often on rocks). *D. tahitensis* was slightly more difficult to find, and occurred on the trunks of *Inocarpus fagiferus*.

Cross-Island Trail - This trail connects the port of Vaiare, on the west side of the island, to the town of Paopao, located at the mouth of Cook's Bay to the north. It climbs gradually following a stream valley, then crosses the ridge (331 m) between Mt. Mouaputa and Mt. Tearai, then finally makes a long descent into Paopao. Habitat and filmy ferns observed were similar to the Three Coconuts Pass trail.

Marimari Kellum's Property - This piece of land is situated between Opunohu Bay and the eastern face of Mt. Rotui. There was no trail available, but I hiked toward Mt. Rotui until the slope became too steep to continue. Habitat and filmy ferns occurring here were again similar to the other two low- to mid-elevation sites. One difference is that this site is where I first observed *Cr. sp. 1*, a new record for Moorea. *Cr. sp. 1* occurs at Marimari Kellum's property on rocks within a nearly dry stream bed and on larger boulders up the hillside.

Mt. Rotui Trail - This trail starts on the coast between Cook's Bay and Opunohu Bay and climbs a north-facing ridge to the top of Mt. Rotui (899 m). Unlike the preceding three sites, it lacks a low- to mid-elevation *Inocarpus*

TABLE 1. Distribution of filmy ferns along trails on Moorea. Data are relative abundance and elevations (in meters) for each species by site. Relative abundance was determined by ease with which specimens could be found in the field; white = common, grey = uncommon, black = rare. Elevation is based on GPS readings from collected specimens and field notes.

Site	*A. caudat.*	*A. dentat.*	*Ca. apiifolia*	*Cr. bipunct.*	*Cr. humile*	*Cr. minutus*
marimari	-	-	-	40 - 150	40 - 150	-
x island	-	-	-	40 - 330	40 - 330	-
3 cocos	-	-	-	250 - 300	250 - 360	-
mahaeru	400 - 500	400 - 500	490	130 - 300	130 - 300	400 - 500
rotui	-	-	-	-	-	600 - 800
mouaputa	600 - 830	460 - 800	400 - 830	100 - 450	100 - 450	500 - 830
	Cr. sp. 1	*D. tahitense*	*H. digitat.*	*H. polyan.*	*H. pallid.*	*Cr. sp. 2*
marimari	40 - 150	40 - 150	-	-	-	-
x island	-	40 - 330	-	-	-	-
3 cocos	-	250 - 360	-	-	-	-
mahaeru	130	130 - 300	400 - 500	400 - 500	-	-
rotui	-	-	650 - 800	600 - 800	-	-
mouaputa	515	100 - 300	500 - 830	500 - 800	820	800

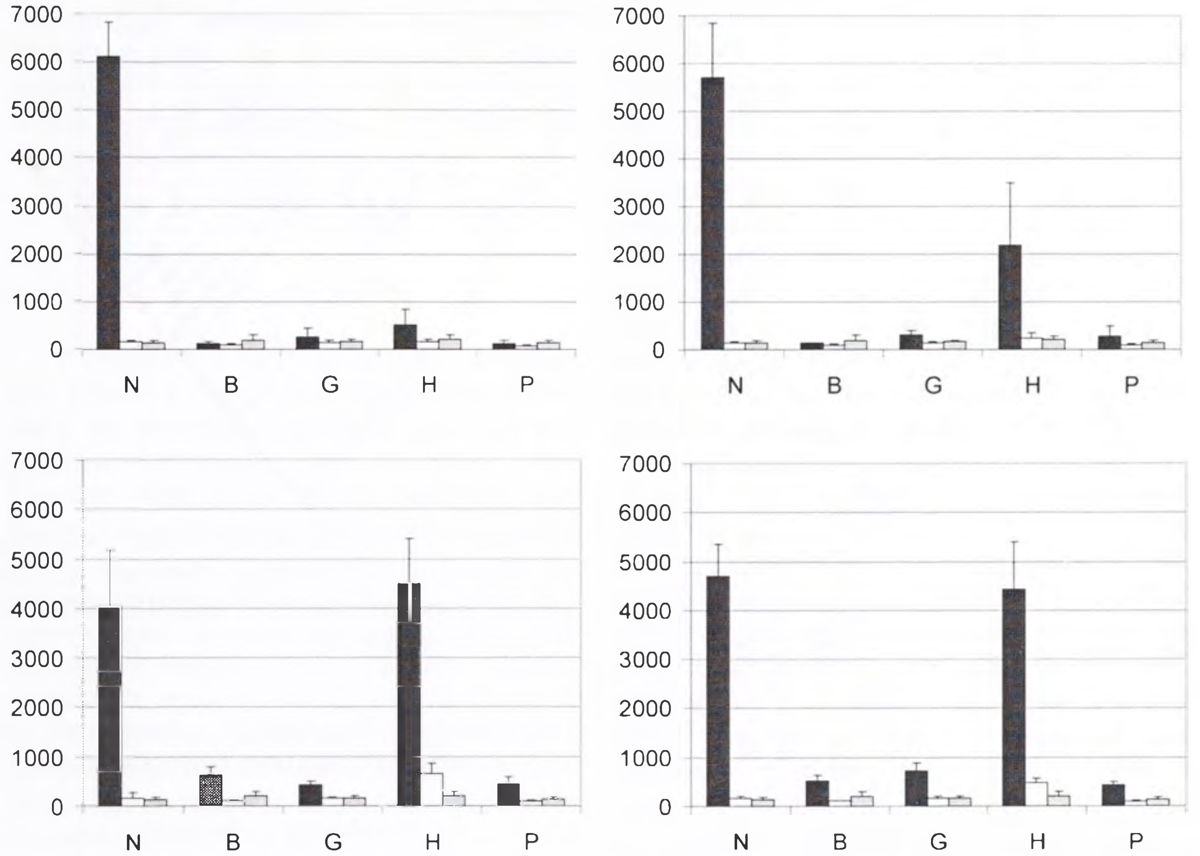


Figure 5. Results of desiccation tolerance test Data are electrical conductivities (μS g^{-1} dry weight) and 95% confidence intervals. Species key: B = *Cr. bipunctatum*, N = *A. dentatum*, G = *H. digitaum*, H = *Cr. humile*, P = *H. polyanthos*. For each species, the black bar is electrical conductivity immediately following desiccation, the white bar is electrical conductivity following a 24 hr recovery period, and the grey bar is control (no desiccation). * indicates results found to be significantly different from others by the Tukey test.

fagiferus forest. Rather, the lower elevation hillside is mostly dry and dominated by *Psidium guajava* Linn. and *Hibiscus tiliaceus* L. Thus, none of the typical low-elevation filmy ferns are to be found on this trail. Filmy ferns did not appear until higher elevations, when the habitat changes to cloud forest. The cloud forest is dominated by smaller trees and shrubs such as *Metrosideros collina* (JR & G. Forst.) A. Gray, *Weinmannia parviflora* Forster, and *Fagraea berteroana* A. Gray. *Dicranopteris linearis* (Burm. f.) Underw. dominates along the trail itself, often growing so thickly that it appears to limit the growth of other plants. *H. polyanthos* was the most common fern, often occurring in mats covering tree trunks and branches. *Cr. minutus* occurred less frequently, and *H. digitatum* (previously unknown in the literature to occur on Moorea) was occasionally spotted underneath overhanging branches or roots.

Mahaeru Creek - Mahaeru creek has its headwaters at the southern base of Mt. Tohiea. This area lacked a trail, but I followed a ridge next to the creek toward Mt. Tohiea until the slope became too steep to allow further progress. This area contained very high fern diversity. *Cr. humile* and *Cr. bipunctatum* were plentiful at low elevations near the creek, and *D. tahitense* was common on *Inocarpus fagiferus* trunks. Once I climbed above 350 m, I began to see *A. dentatum* on steep hillsides, and occasionally *A. caudatum* on tree ferns. Other high-elevation species such as those on Mt. Rotui were also present, but not as numerous.

I only saw one occurrence of *Ca. apiifolia*, mixed in with a population of *A. dentatum*.

Mt. Mouaputa Trail - This trail starts out above the town of Afareaitu (100 m) and climbs to the summit of Mt. Mouaputa (831 m). It follows a creek through a mid-elevation *Inocarpus fagiferus* forest for about 3 km, then climbs a ridge to the top of the mountain. The ridge is initially dry and dominated by *Dicranopteris linearis*, but eventually gives way to a humid cloud forest. Thus, this trail contains a variety of habitats and a high diversity of filmy ferns. Typical low elevation species were found along the creek, with the rare addition of *Cal. apiifolia* and *Cr. sp. 1*. Upon reaching the base of the ridge (460 m), the trail enters a *Miconia calvescens* DC dominated thicket, and *A. dentatum* is common on the exposed soil. Higher up in the cloud forest, some rare species such as *H. pallidum* and *Cr. sp. 2* (previously unknown in the literature to occur on Moorea) were discovered by carefully searching in the area next to the trail.

Desiccation Tolerance Experiment

Average water content following desiccation was 52.8% +/- 2.2% for samples dried at 100% RH and 13.2% +/- 2.2% for samples dried at 20% RH.

At 100% RH, electrical conductivity was significantly higher for *A. dentatum* over all other species after 7 days of drying; by 14 days of drying, *A. dentatum* still showed the greatest amount of ion leakage, but the electrical conductivity of *Cr. humile* also became significantly greater than the other three species (fig. 5). These and other differences were found to be significant by the Tukey test at the 0.05 level, corrected for multiple tests. At 20% RH, both *A. dentatum* and *Cr. humile* had similarly high conductivity values which set them apart from the rest of the species. The electrical conductivity values for the other three species (*Cr. bipunctatum, H. digitatum, H. polyanthos*) never varied from each other significantly following desiccation after any time at either humidity.

All fern species tested were able to completely recover following desiccation (post-recovery ion leakage values were not significantly different from control values).

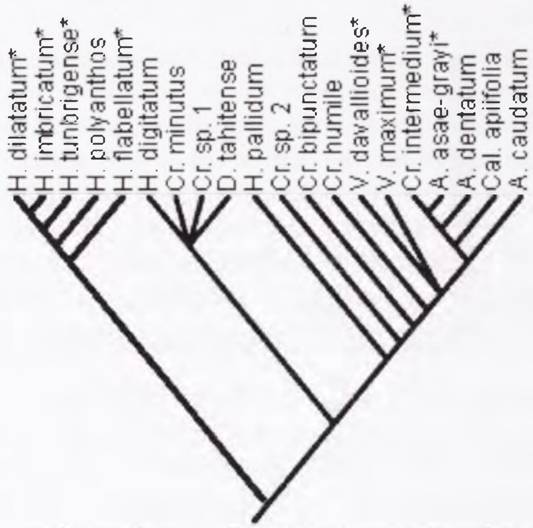


FIG. 6. Consensus tree showing evolutionary relationships between Society Island filmy fern species. * indicates ferns not known from Moorea.

Morphological Phylogeny

A total of 25 morphological characters were defined and scored (see Appendix A). Some characters had to be excluded because they did not meet all three requirements mentioned above in the methods section. Characters were restricted to macroscopic features that could be seen either with the naked eye or under a dissecting microscope (up to 40x magnification). Characters included all major parts of the sporophyte: rhizome and roots, stipe, frond, and sporangia. Spore and gametophyte characters were not used.

21 characters were used to produce a phylogeny with 20 terminal taxa (figure 6). This phylogenetic tree is the consensus tree (consistency index = 0.0500) of 12 equally parsimonious trees, each with a length of 65 steps. Terminal taxa included all 12 Moorean species, plus 8 additional species that do not occur on Moorea but are described from other locations in the Society Island archipelago: *H. dilatatum* (G. Forst.) Sw., *H. imbricatum* Blume, *H. tunbrigense* (L.) Sm., *H. flabellatum* Labill., *Vandenboschia. davallioides* (Gaudich.) Copel., *V.*

maximum Blume, *Cr. intermedium* (Bosch) Ebihara & K. Iwats., and *A. asae-grayi* (Bosch) Ebihara & K. Iwats. Tree structure reflects traditional Hymenophyllaceae taxonomy, splitting *Hymenophyllum* s.l. and *Trichomanes* s.l. species into two sister clades.

Discussion

Elevational Gradient

As I observed the filmy ferns at each study site, I noticed that species composition varied along an elevational gradient, and that this gradient was similar between sites. The Moorean filmy ferns can be split into two major groups: low elevation species occurring below 450 m and moderate to high elevation species occurring above 450 m. These two groups were largely exclusive, with little overlap in range. Low elevation species include *Cr. bipunctatum*, *Cr. humile*, *Cr. sp. 1*, and *D. tahitense*. Moderate to high elevation species include *A. dentatum*, *A. caudatum*, *Cal. apiifolia*, *Cr. minutus*, *H. polyanthos*, *H. digitatum*, *H. pallidum*, and *Cr. sp. 2*.

The physical factors responsible for determining fern distribution along the elevational gradient such as humidity and precipitation are important in determining the species composition of all plants, not just filmy ferns. There is a marked difference in plant communities between the low- to mid-elevation valley forest dominated by *Inocarpus fagiferus*, *Hibiscus tiliaceus*, and *Angiopteris evecta* and the high elevation cloud forest dominated by *Metrosideros collina*, *Weinmannia parviflora*, and a huge variety of epiphytes. This transition in plant communities is reported to occur at 400 – 500 m for Polynesian high volcanic islands (Meyer 2004), a figure which matches the cutoff point between my two filmy fern groups. Thus, the difference in filmy fern species composition between high and low altitude reflects an overall difference in plant communities between high and low altitude.

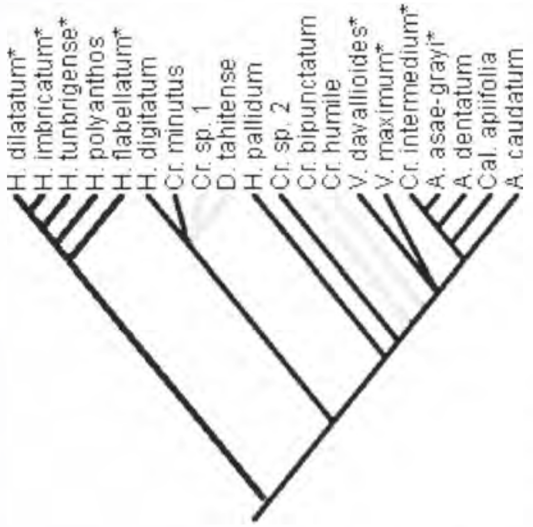


FIG. 7. Distributional data overlaid onto consensus tree. Grey indicates low elevation species; black indicates mid to high elevation species except for non- Moo'orea species noted with an asterisk, for which distribution data are lacking.

It is interesting that the clear elevational gradient in species composition that I observed in the field did not match the mixed results of the desiccation tolerance test, however. High elevation species included in test showed both relatively high (*H. digitatum*, *H. polyanthos*) and low (*A. dentatum*) degrees of desiccation tolerance as indicated by ion leakage values. Similarly, the low elevation species also yielded mixed results; *Cr. humile* displayed a low degree of desiccation tolerance, whereas *Cr. bipunctatum* was much more tolerant. This suggests that relative degree of desiccation tolerance may only be a minimal factor in determining filmy fern distribution. In my test, all five species included were able to completely recover following desiccation, regardless of the degree of initial damage. Perhaps filmy ferns only need to survive a certain minimum amount of desiccation in the field, and relative degree of desiccation tolerance beyond this minimum is unimportant.

The results of the morphological phylogeny can also be used to interpret this elevational pattern. When elevational data from the distribution survey is overlaid on the consensus tree, it is apparent that the low elevation and high elevation species do not form monophyletic groups (figure 7). However, the low elevation species do form two grades each containing a pair of closely related species. This shows that low elevation species have evolved similar morphology that reflects their habitat niche. Although the filmy fern species occupying a single habitat type may not necessarily be more closely related to each other than those from different habitats, they do share at least some of the same morphological adaptations.

Growth Habit

Another pattern I noticed in the course of this study is the correlation of both distribution and desiccation tolerance with growth habit. Of the twelve Moorean filmy fern species, only two (*A. dentatum* and *Cal. apiifolia*) are terrestrial; the rest are epiphytic or occasionally epipetric. [The line between epiphytism and epipetrism is blurred in the field; I observed many species typically described as epiphytic growing on both trees and rocks. The important difference between these and terrestrial species is the habit of growing into the soil (terrestrial) versus growing spread out over the surface of some substrate (epiphytic or epipetric), not the nature of the substrate itself. Therefore, I will refer to these as "epiphytic" species with the intention of including occasional epipetrism.]

Dubuisson (2003) noted that growth habit correlates with habitat within *Trichomanes* s.l., describing terrestrial species as more widespread and epiphytic species as restricted to wet areas or cloud forests. I observed the opposite trend on Moorea, finding the two terrestrial species only at high elevation, and epiphytic species at both high and low elevations. This could be an issue of sample size; Dubuisson's study includes 193 of the 325 members of *Trichomanes* s.l., whereas my study includes only 12 species from both *Trichomanes* s.l and *Hymenophyllum* s.l. Another possibility is that the two terrestrial species I found are relatively weak competitors in the Moorean forest environment. *Cal. apiifolia* seems to be an infrequent reproducer: of the eight specimens of *Cal. apiifolia* that I collected in the field, only two had fronds bearing sporangia. I also noticed that *A. dentatum* showed more evidence of herbivory damage than other species. These observations suggests that perhaps these species are poor competitors and became restricted to high elevation as low elevation areas became crowded with other (non-filmy) terrestrial fern species.

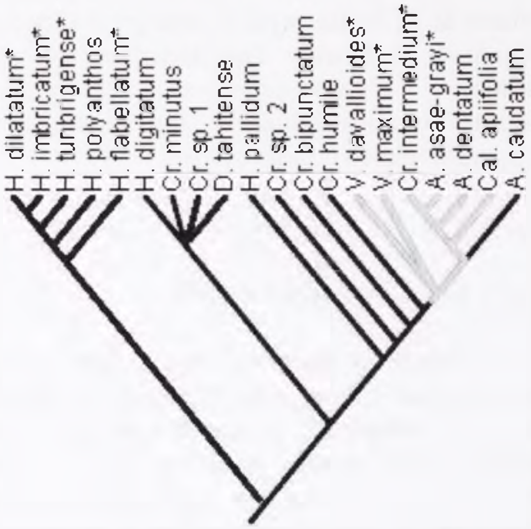


FIG. 8. Growth habit overlaid onto consensus tree. Grey indicates terrestrial species; black indicates epiphytic species.

There is a strong correlation between growth habit and desiccation tolerance. Electrical conductivity values following initial desiccation were generally higher for the terrestrial *A. dentatum* than the other epiphytic species, indicating that terrestrial ferns have a lower desiccation tolerance than epiphytic ferns. Most epiphytic ferns do not possess true roots, and all water gain and loss occurs through frond lamina and rhizomes located near the substrate surface. Therefore, it makes sense that they would evolve countermeasures such as desiccation tolerance to survive prolonged periods of low humidity. Terrestrial species such as *A. dentatum*, however, can still rely on an extensive root system to absorb water from the ground even when atmospheric humidity is low. Thus, terrestrial species have less selection pressure to gain extensive desiccation tolerance. There was one exception to the pattern – *Cr. humile*, an epiphyte, had electrical conductivity values nearly as high or higher than *A. dentatum* after drying at 20% RH. More tests including more representatives of both terrestrial and epiphytic growth habits are needed to clarify this trend.

The importance of growth habit as an evolutionary adaptation can be seen by plotting growth habit states for each species onto the phylogeny. Terrestrial species and epiphytic species each form nearly exclusive groups. This reflects both the unique morphological characters that each group has developed as well as their distinctness as evolutionary lineages. Characters related to growth habit should be useful for continued taxonomic studies within the family.

Island Distribution

By including other Society Island species in the phylogeny and examining distribution data for each species, it is possible to place the Moorean filmy ferns in the larger context of an island chain. The 12 filmy ferns occurring on Moorea are also known to occur on most of the other Society Islands (Copeland 1932, Moore 1933, Copeland 1938, Robertson 1952, Fosberg 1997, Ranker et al. 2005), and Moorea species do not form a monophyletic group within Society Island filmy ferns, as can be seen on the consensus tree. This, plus the fact that Moorea filmy ferns include a wide variety of genera, suggests that filmy ferns on Moorea do not represent the result of an adaptive radiation; rather, they have probably evolved independently in other locations and subsequently become widespread throughout the Society Island archipelago. High dispersal ability conferred via lightweight, durable spores seems to be a characteristic that unites all ferns, reflected in their disproportionately high frequency in island flora and low rate of endemism (Smith 1972). This high dispersal ability does have its limits, however: although the filmy fern flora from nearby island chains such as the Marquesas or Cook Islands are reported to be similar to that of the Society Islands (Robertson 1952, Game et al. 1997), a different set of species occurs on the more distant Hawaiian islands (Palmer 2002). By sampling from a broad area representing several island chains, it should be possible to clarify biogeographical patterns within the Pacific Hymenophyllaceae in future studies.

Conclusion

The family Hymenophyllaceae contains a rich diversity of ecology and morphology, and this diversity is well represented by the filmy ferns of Moorea. Moorean filmy ferns occur from low (30 m) to high (900 m) elevations in epiphytic, epipetric, and terrestrial growth forms. They display a variety of morphological characters that have evolved to match the niche of each species including frond size, loss of roots, and rhizome habit. Within this diversity, certain patterns are evident such as a low degree of desiccation tolerance in terrestrial species and strong elevational preference. By constructing a phylogenetic tree that includes filmy ferns known from other sites within the Society Island archipelago, larger biogeographical patterns become evident. The Moorea filmy ferns do not appear to be the result of an adaptive radiation, but rather a group of species that each evolved elsewhere and immigrated to the island independently. These patterns seen in my study highlight the suitability of filmy ferns as a model system for studying processes in evolution and biogeography. It is certain that more fascinating contributions can be made to these fields by continued investigation of the family Hymenophyllaceae.

ACKNOWLEDGMENTS

I thank members of the Moss Lab: Brent Mishler, Andy Murdock, and Alan Smith for lending their expert knowledge and teaching me the ways of ferns. I am indebted to all the professors and graduate student instructors of the course for providing countless hours of support and advice.

LITERATURE CITED

Alpert, P. 2006. Constraints of tolerance: why are desiccation-tolerant organisms so small or rare? The Journal of Experimental Biology 209:1575 - 1584.

Axsmith, B. J., M. Krings, and T. N. Taylor. 2001. A filmy fern from the Upper Triassic of North Carolina (USA). American Journal of Botany 88:1558 - 1567.

Brown, D. H., and G. W. Buck. 1979. Desiccation Effects and Cation Distribution in Bryophytes. New Phytologist 82:115-125.

Copeland, E. B. 1932. Pteridophytes of the Society Islands. Bernice P. Bishop Museum Bulletin 93:1 - 86.

Copeland, E. B. 1938. Ferns of Southeastern Polynesia. Occasional Papers of Bernice P. Bishop Museum 14:45 - 101.

Copeland, E. B. 1938. Genera Hymenophyllacearum. Philippine Journal of Science 51:2 - 110.

Dubuisson, J. Y. 1997. Systematic relationships within the genus Trichomanes sensu lato (Hymenophyllaceae, Filicopsida): cladistic analysis based on anatomical and morphological data. Botanical Journal of the Linnean Society 123:265-296.

Dubuisson, J. Y., S. Hennequin, E. J. P. Douzery, R. B. Cranfill, A. R. Smith, and K. M. Pryer. 2003. rbcL phylogeny of the fern genus Trichomanes (Hymenophyllaceae), with special reference to neotropical taxa. International Journal of Plant Sciences 164:753-761.

Dubuisson, J. Y., S. Hennequin, F. Rakotondrainibe, and H. Schneider. 2003. Ecological diversity and adaptive tendencies in the tropical fern Trichomanes L. (Hymenophyllaceae) with special reference to climbing and epiphytic habits. Botanical Journal of the Linnean Society 142:41-63.

Ebihara, A., J. Y. Dubuisson, K. Iwatsuki, S. Hennequin, and I. Motomi. 2006. A taxonomic revision of Hymenophyllaceae. Blumea 51:1 - 60.

Ebihara, A., S. Hennequin, K. Iwatsuki, P. D. Bostock, S. Matsumoto, R. Jaman, J. Y. Dubuisson, and M. Ito. 2004. Polyphyletic origin of microtrichomanes (Prantl) Copel. (Hymenophyllaceae), with a revision of the species. Taxon 53:935 - 948.

Fosberg, R. F. 1997. Preliminary checklist of the flowering plants and ferns of the Society Islands. in. University of California, Berkeley.

Game, J. C., W. R. Sykes, and A. R. Smith. 1997. A status report on Cook Island pteridophytes. in. University of California, Berkeley.

Hoekstra, F. A., E. A. Golovina, and J. Buitink. 2001. Mechanisms of plant desiccation tolerance. Trends in Plant Science 6:431-438.

Iwatsuki, K. 1984. Studies in the systematics of filmy-ferns. VII. A scheme of classification based chiefly on the asiatic species. Acta Phytotaxomica et Geobotanica 35:165-179.

Leopold, A. C., M. E. Musgrave, and K. M. Williams. 1981. Solute Leakage Resulting from Leaf Desiccation. Plant Physiology 68:1222-1225.

Meyer, J.-Y. 2004. Threat of invasive alien plants to native flora and forest vegetation of eastern Polynesia. Pacific Science 58:357 - 375.

Moore, J. W. 1933. New and critical plants from Raiatea. Bernice P. Bishop Museum Bulletin 102:1 - 53.

Morton, C. V. 1968. The Genera, Subgenera, and Sections of the Hymenophyllaceae. Contributions from the United States National Herbarium 38:153 - 214.

Murdock, A. G., and A. R. Smith. 2003. Pteridophytes of Moorea, French Polynesia, with a New Species, Tmesipteris gracilis (Psilotaceae). Pacific Science 57:253-265.

Palmer, D. D. 2002. Hawaii's Ferns and Fern Allies. University of Hawaii Press.

Pichi-Sermolli, R. E. G. 1977. Tentamen Pteridophytorum Genera in Taxonomicum Ordinem Redigendi. Webbia 31:313 - 512.

Proctor, M. C. F. 2003. Comparative Ecophysiological Measurements on the Light Responses, Water Relations and Desiccation Tolerance of the Filmy Ferns Hymenophyllum wilsonii Hook. and H. tunbrigense (L.) Smith. Annals of Botany 91:717-727.

Pryer, K. M., A. R. Smith, J. S. Hunt, and J. Y. Dubuisson. 2001. rbcL data reveal two monophyletic groups of filmy ferns (Filicopsida: Hymenophyllaceae). American Journal of Botany 88:1118 - 1130.

Pryer, K. M., A. R. Smith, and J. E. Skog. 1995. Phylogenetic relationships of extant ferns based on evidence from morphology and rbcL sequences. American Fern Journal 85:205–282.

Ranker, T. A., P. G. Trapp, A. R. Smith, R. C. Moran, and B. S. Parris. 2005. New Records of Lycophytes and Ferns from Moorea, French Polynesia. American Fern Journal 95:126.

Robertson, R. 1952. Catalogue des plantes vasculaires de la Polynesie Francaise. Bulletin de la societe de etudes oceaniennes 98:371 - 379.

Robinson, W. J. 1914. A Taxonomic Study of the Pteridophyta of the Hawaiian Islands-IV. Bulletin of the Torrey Botanical Club 41:51-59.

Schneider, H. 2000. Morphology and anatomy of roots in the filmy fern tribe Trichomaneae H. Schneider (Hymenophyllaceae, Filicatae) and the evolution of rootless taxa. Botanical Journal of the Linnean Society 132:29 - 46.

Shirazi, A. M., P. S. Muir, and B. McCune. 1996. Environmental Factors Influencing the Distribution of the Lichens Lobaria oregana and L. pulmonaria. The Bryologist 99:12-18.

Shreve, F. 1911. Studies on Jamaican Hymenophyllaceae. Botanical Gazette 51:184-209.

Smith, A. R. 1972. Comparison of fern and flowering plant distributions with some evolutionary interpretations for ferns. Biotropica 4:4 - 9.

Tryon, R. 1970. Development and Evolution of Fern Floras of Oceanic Islands. Biotropica 2:76-84.

Whittaker, R. 1999. Island Biogeography: Ecology, Evolution and Conservation. Oxford University Press.

Wolf, P. G., H. Schneider, and T. A. Ranker. 2001. Geographic distributions of homosporous ferns: does dispersal obscure evidence of vicariance? Journal of Biogeography 28:263 - 270.

APPENDIX A: CHARACTERS

RHIZOME

1. *Habit: 0 = erect; 1 = creeping.* An erect rhizome is no longer than 5 cm in length for the entire fern, and all fronds are clustered together with very short internode length. An erect rhizome is often associated with the terrestrial growth habit. Creeping rhizomes have no limit on growth length, and fronds are spaced out along the rhizome. Creeping rhizomes are usually associated with the epiphytic or epipetric growth habit. See Figure 9.

2. *Presence of roots: 0 = true roots present; 1 = true roots absent, root-like shoots present.* Root-like shoots are a unique character of the Hymenophyllaceae family. Root-like shoots more closely resemble an extension of the rhizome rather than a true root. Differences cited by Schnieder include thickness compared to the rhizome (true roots are half the diameter of the rhizome or less; root-like shoots range from equal to half in size of the rhizome), presence of a root cap (true roots present; root-like shoots absent), and direction of growth (true roots positive geotropic or negative heliotropic; root-like shoots plagiotropic) (Schneider 2000). This character is also closely associated with growth habit. Most terrestrial species have retained full-sized roots, whereas epiphytic species possess root-like shoots.

3. *Hairs on rhizome: 0 = hairs absent, 1 = some hairs; 2 = thick hairs.* Hairs are defined as one or more elongated single cells in a row. Some species such as *Cr. humile* and *Cr. bipunctatum* have dense hairs such that the rhizome itself cannot be seen because it is completely covered in hairs. Others still possess hairs, but not completely covering the rhizome, whereas others are hairless or nearly so. Ebihara et. al. consider this one character that sets apart Hymenophyllum from the other genera (those previously considered to be in Trichomanes) (Ebihara et al. 2006). This is an ordered character. See Figure 10.

4. *Hairs on roots: 0 = hairs absent, 1 = some hairs; 2 = thick hairs.* This character only applies to those species having true roots; those lacking roots were scored as "?". Hairs are defined as above. This is an ordered character. See Figure 10.

5. *Hairs on root-like shoots: 0 = hairs absent;, 1 = some hairs, 2 = thick hairs.* This character only applies to those species having root-like shoots; those with true roots were scored as "?". Hairs are defined as above. This is an ordered character. See Figure 10.

6. *Rhizome diameter: 0 = filiform (<1 mm thick); 1 = medium (1 – 2.5 mm thick); 2 = robust (>2.5 mm thick).* Reduced root size is usually associated with epiphytism, but not in all cases (such as *A. caudatum*, other *Abrodictyum* species). This is discrete-state, ordered character as shown in Figure 11.

STIPE

7. *Lamina: 0 = not winged; 1 = narrow wing (average 2 cells wide;) 2 = winged (average 5 cells wide).* An extension of the lamina at least halfway down the stipe is called the "wing." This is an ordered character. See Figure 12.

8. *Hairs on stipe: 0 = hairs absent; 1 = hairs present.* There is no case of thick hairs covering the stipe as on the rhizome; only presence or absence is coded for.

9. *Hairs on wing: 0 = hairs absent; 1 = hairs present.* In one case, hairs were found to be growing from the margin of the stipe wing. **EXCLUDED**: only occurs in a single taxon (*Cr. bipunctatum*).

FROND

10. *Type of venation: 0 = dichotomous; 1 = anadromous, 2 = catadromous.* Filmy ferns are known for being the one of the few fern families containing both anadromous and catadromous vein patterns (Dubuisson 1997). In several species, especially those which lack highly dissected fronds, the vein pattern is dichotomous. See Figure 13.

11. *Cell walls: 0 = thin, straight cell walls; 1 = thick, wavy or pitted cell walls.* In most filmy ferns, the lamina appears delicate and translucent; however, in some, the lamina is darker in color and thicker due to thick cell walls, even though it is only one cell layer. This character could only be scored on fresh specimens; lamina of all dry specimens became wavy and toughened due to desiccation.

12. *Dissection of blades: 0 = simple to slightly lobed; 1 = once to twice pinnatifid; 2 = three times or greater pinnatifid.* No filmy ferns included in this phylogeny are completely pinnate. Species with larger fronds are often more dissected. This is treated as an ordered character.

13. *Sub-marginal false vein: 0 = absent;, 1 = present.* Most species lack any sort of specialized cells in the lamina, but false veins do appear in some. False veins appear distinct from other lamina cells and may serve a purpose as supporting tissue (Boer 1962). A sub-marginal vein appears 3 cells below the margin. Sub-marginal false veins may either be continuous (as in Cr. *sp. 1*) or discontinuous (as in Cr. *bipunctatum*). See Figure 14.

14. *False vein in between true veins: 0 = absent; 1 = present.* This type of false vein appears between true veins rather than in the sub-marginal position. **EXCLUDED**: only occurs in a single taxon (*D. tahitense*).

15. *Specialized marginal cells: 0 = absent; 1 = present, but not elongated as compared to non-specialized lamina cells; 2 = present, two to three times longer than non-specialized lamina cells.* This is treated as an ordered character. See Figure 15.

16. *Frond size: 0 = large (>20 cm), 1 = medium (10 - 20 cm), 2 = small (2.5 - 10 cm), 3 = tiny (< 2.5 cm).* Filmy ferns display a range of frond sizes. Most epiphytic and epipetric species are smaller, whereas most terrestrial species are larger. This is a discrete-state, ordered character as shown in Figure 16.

17. *Habit of fronds: 0 = erect; 1 = lax; 2 = adpressed.* This could only be determined based on observations in the field. Treated as an unordered character.

18. *Hairs on veins: 0 = absent; 1 = present.* Small hairs, usually only one to two cells long appear on the veins of some species.

19. *Hairs on margin: 0 = absent; 1 = present.* **EXCLUDED**: only occurs in a single taxon (*H. digitatum*).

20. *Cell shape: 0 = round; 1 = elongated.* Cell shape has been used in other morphological studies of Hymenophyllaceae (Dubuisson 1997), therefore I felt it appropriate to use in this phylogeny.

Sorus

21. *Position of sori: 0 = sori at end of ultimate segment; 1 = sori occurs before end of ultimate segment.* Although there could have been overlap between these two states, I observed that they were mutually exclusive. Sori either occurred at the end of the segment or somewhere near the middle.

22. *Immersion of sori in lamina: 0 = immersed; 1 = not immersed.* Sori may either be immersed in the lamina, or not immersed and on a pedicel.

23. *Position of receptacle: 0 = included to slightly exserted, but not more than 2 X the length of the involucre; 1 = significantly exserted.* Sporangia are clustered around the receptacle. Bivalve involucres usually have included receptacles, and tubular involucres usually have exserted receptacles. See Figure 17.

24. *Shape of involucre: 0 = mostly bivalve; 1 = mostly tubular.* This is a debated character. Iwatsuki proposes 8 different classes representing a range from tubular to bivalve, whereas traditional classification recognizes only two states. **EXCLUDED**: correlates with position of receptacle. Included to slightly exserted receptacles are always associated with bivalve involucres, and significantly exserted receptacles are always associated with tubular involucres.

Various

25. *Rhizoids: 0 = absent; 1 = present.* **EXCLUDED**: only occurs in a single taxon (*D. tahitense*). This is known as an autapomorphy of the *Didymoglossum* genus (Ebihara et al. 2006).

Character Figures

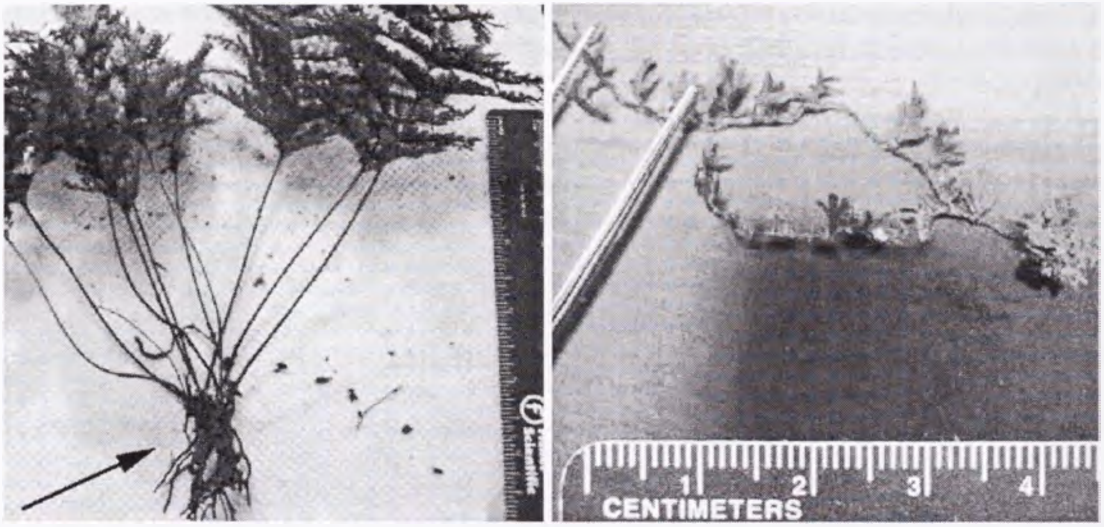


FIG. 9. Examples of erect and creeping rhizomes. *A. dentatum* (left) has an erect rhizome, indicated by the arrow. *Cr. sp. 1* (right) has a creeping rhizome.

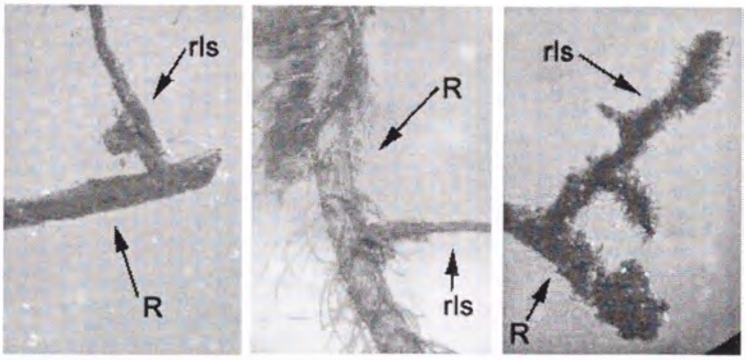


FIG. 10. Examples of hairs on rhizomes and root-like shoots. *H. polyanthos* (left) has a glabrous root and a hairy root-like shoot. *H. pallidum* (middle) has a hairy rhizome and glabrous root-like shoots. *Cr. bipunctatum* (right) has both rhizome and root-like shoots thickly covered in fuzzy, brownish black hairs. All magnification 20x. "R" with an arrow indicates the rhizome; "rls" with an arrow indicates a root-like shoot.

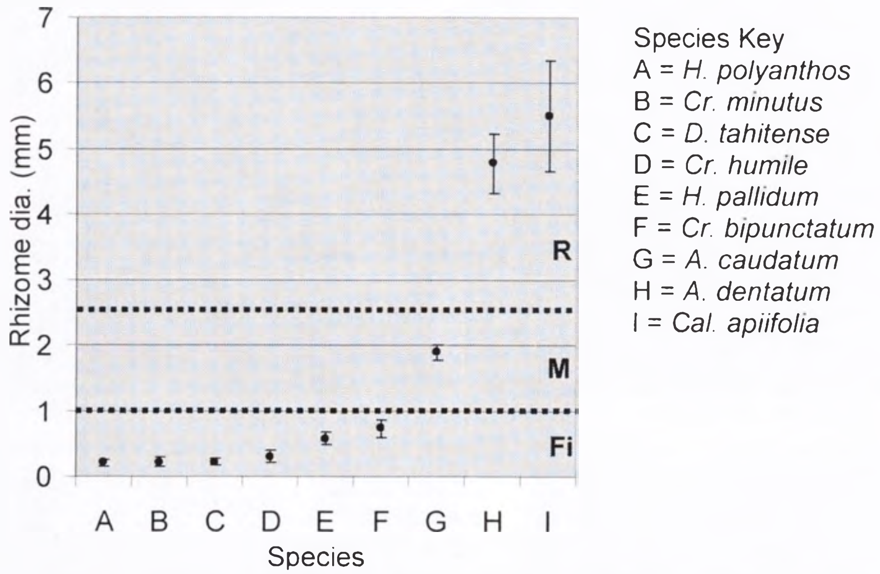


FIG. 11. Rhizome diameter data and 95% confidence intervals. Rhizomes from 9 of the 12 Moorea species were measured. Rhizomes sorted into 3 size classes, separated in the figure by dashed lines and labeled with letters. Fi = "filiform," M = "medium," R = "robust."

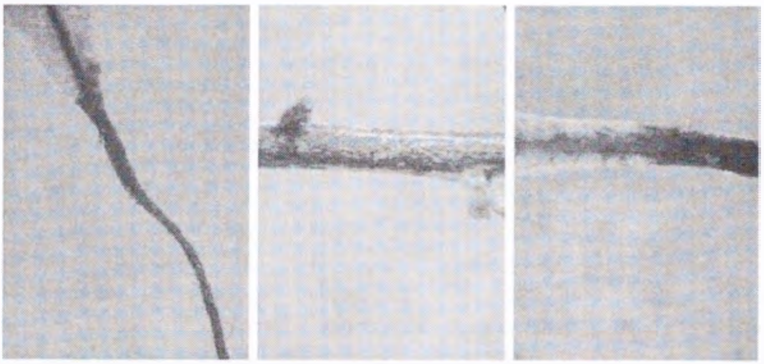


FIG. 12. Examples of wingless and winged stipes. *A. digitatum* (left) lacks any wing on the stipe. *A caudatum* (middle) has a stipe with a very narrow wing. *Cr. humile* (right) has a stipe with a wide wing. All magnification 20x.

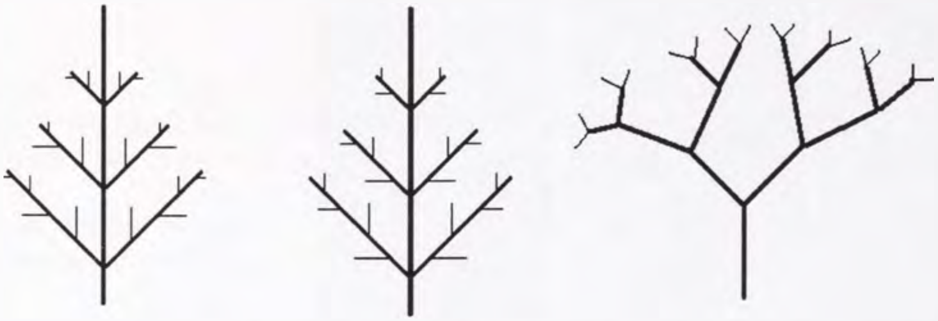


FIG. 13. Schematic diagram of vein types. In anadromous venation (left), the first vein of each costa points towards the tip of the frond. In catadromous venetion (middle), the first vein of each costa points towards the base of the frond. In dichotomous venetion (right), there is no central rachis or costa, and the vein divides in two at each junction.

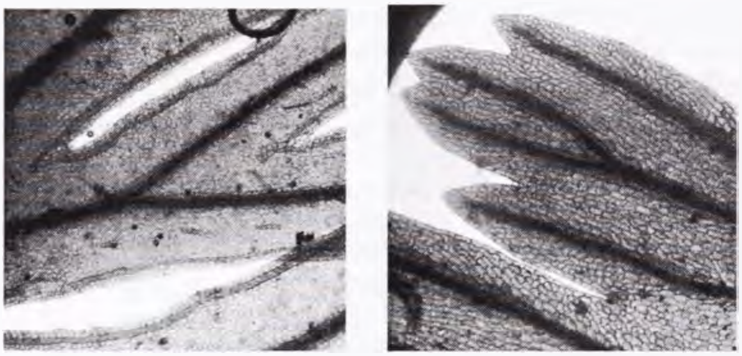


FIG. 14. Examples presence and absence of a sub-marginal false vein. *Cr. bipunctatum* (left) has a discontinuous false vein beneath the margin. *A. dentatum* (right) lacks any such specialized cells beneath the margin. All magnification 40x.

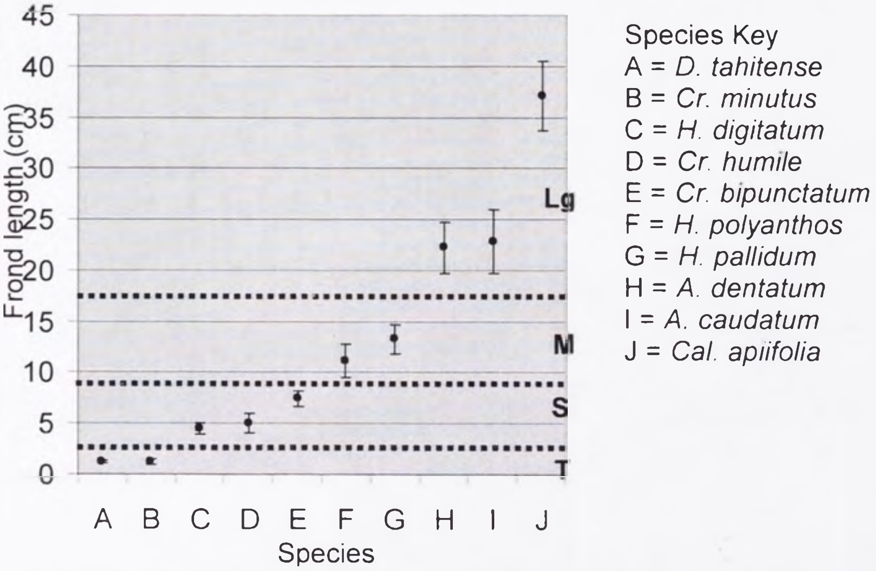


FIG. 15. Frond length data and 95% confidence intervals. Fronds from 10 of the 12 Moorea filmy fern species were measured. Fronds sorted into 4 size classes, separated in the figure by dashed lines and labeled with letters. T = "tiny," S = "small," M = "medium," Lg = "large."

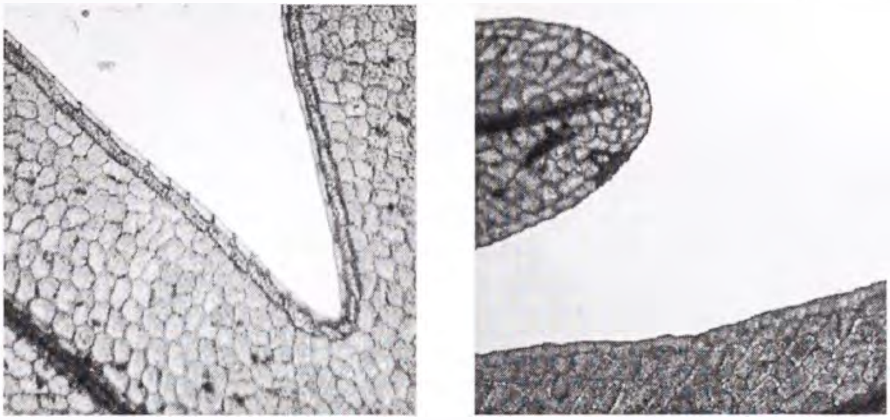


FIG. 16. Examples of specialized marginal cells. *Cr. humile* (left) has a double row of elongated marginal cells. *A. caudatum* (right) has distinct marginal cells that are the same length as other lamina cells. All magnification 40x.

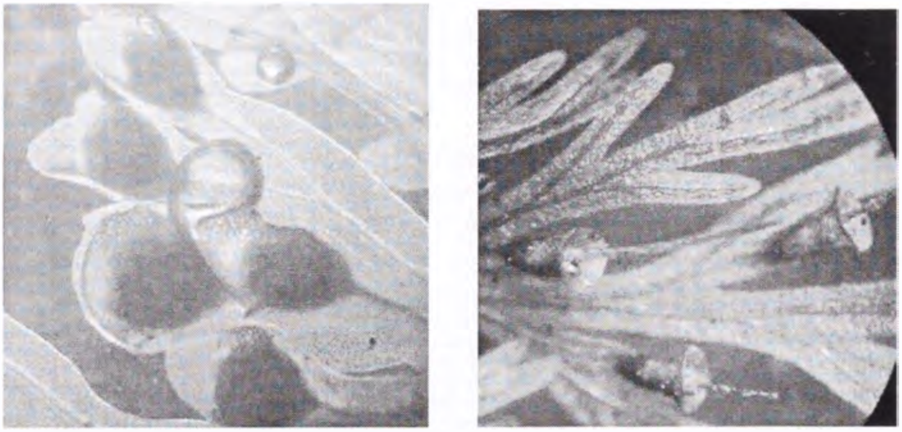


FIG. 17. Examples of involucre variation. *H. polyanthos* (left) has bivalve involucres with an included receptacle (not visible). *A. caudatum* (right) has tubular involucres with an exserted receptacle.

	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
A. caudatum	2	1	2	1	?	1	1	1	0	?	0	2	0	0	1	0	1	0	0	1	?	0	1	1	0
A. dentatum	0	1	2	0	?	2	0	0	0	1	0	2	0	0	0	0	0	0	0	0	1	0	1	1	0
Ca. apiifolia	0	1	2	1	?	2	0	1	?	1	1	2	0	0	0	0	0	0	0	1	1	0	0	0	0
Cr. bipunctatum	2	0	2	?	1	0	2	0	1	1	1	2	1	0	0	1	0	1	0	1	1	0	1	1	0
Cr. humile	2	0	2	?	1	0	2	1	0	1	1	1	0	0	2	1	1	1	0	1	1	0	1	1	0
Cr. minutus	2	0	2	?	1	0	0	0	?	2	1	1	0	0	1	2	0	1	0	0	0	0	1	1	0
D. tahitense	2	0	1	?	1	0	?	?	?	?	0	0	0	1	0	2	2	0	0	0	0	0	1	1	1
H. digitatum	2	0	0	?	1	0	0	0	?	2	1	1	0	0	0	2	1	0	1	1	0	0	1	1	0
H. polyanthos	2	0	0	?	1	?	0	0	?	1	1	2	0	0	0	1	0	0	0	1	0	0	0	0	0
H. pallidum	2	0	1	?	1	?	0	0	?	1	0	2	0	0	1	1	1	1	0	1	1	0	1	1	0
Cr. sp. 1	2	0	1	?	1	0	0	1	?	2	1	1	1	0	0	3	0	1	0	1	0	0	1	1	0
Cr. sp. 2	2	0	1	?	1	?	1	0	0	1	1	2	0	0	0	1	1	1	0	1	1	0	1	1	0
H. flabellatum	2	0	1	?	1	?	0	?	?	1	1	2	0	0	0	0	1	0	0	?	0	0	0	0	0
A. asae-grayi	0	1	1	1	?	2	1	?	0	1	?	2	0	0	0	0	0	1	0	0	1	1	1	1	0
H. dilatatum	2	?	0	?	?	1	2	0	0	1	?	2	0	0	0	0	?	0	0	1	0	0	0	0	0
H. imbricatum	2	?	0	?	?	1	2	0	0	1	?	2	0	0	0	0	?	0	0	1	0	0	0	0	0
H. tunbrigense	2	?	0	?	?	1	1	0	0	1	?	2	0	0	0	1	?	0	0	1	0	0	0	0	0
V. davallioides	2	1	2	1	?	1	2	1	0	1	?	2	0	0	0	0	?	1	0	1	1	1	1	1	0
Cr. intermedium	0	1	2	1	?	2	1	0	0	1	?	2	0	0	0	0	?	1	0	0	1	0	1	1	0
V. maximum	1	1	2	0	?	1	2	1	0	1	?	2	0	0	0	0	?	1	0	1	1	0	1	1	0

TABLE 2. Character matrix used to infer phylogeny.

APPENDIX B: REVISED KEY TO THE FILMY FERNS OF MOOREA, WITH DESCRIPTION OF EACH

1a. Rhizome long-creeping, growing on rocks or trees..2
1b. Rhizome erect, growing terrestrially..3
2a. Rhizome of moderate thickness (ca. 2 mm dia.), epiphytic on tree ferns...*Abrodictyum caudatum*
2b. Rhizome filiform (less than 1 mm dia.)..4
3a. Long reddish hairs present on stipe..*Callistopteris apiifolia*
3b. Stipe lacks hairs. Fronds dark green... ....*Abrodictyum dentatum*
4a. Sub-marginal false vein (ca. 3 cell rows beneath margin of lamina) present..5
4b. Sub-marginal false vein absent. 6
5a. Sub-marginal false vein continuous, fronds < 1 cm long... ....*Crepidomanes sp. 1*
5b. Sub-marginal false vein discontinuous, fronds 3 – 8 cm long..*Crepidomanes bipunctatum*
6a. Specialized marginal cells (ca. 3x longer than others) present..*Crepidomanes humile*
6b. Specialized marginal cells absent.. 7
7a. Fronds simple or 1-pinnatifid..8
7b. Fronds 3-pinnatifid or greater. 9
8a. Fronds peltate, tightly adpressed to substrate, stipe absent..*Didymoglossum tahitense*
8b. Fronds not as above... 10
9a. Lamina light green, stipe and rachis black. Involucres distinctly bivalve....*Hymenophyllum polyanthos*
9b. Rachis color not different from lamina. Involucres more tubular than bivalve..11
10a. Fronds with wavey margins, margin hairs present..*Hymenophyllum digitatum*
10b. Fronds fan-shaped, margin hairs absent, < 2 cm long..*Crepidomanes minutus*
11a. Frond covered in fine white hairs, resulting in white sheen.. *Hymenophyllum pallidum*
11b. Frond lacks white hairs, costa often elongated with short branching along length...*Crepidomanes sp. 2*

Abrodictyum dentatum

Large (average frond length 22 cm) 3-pinnatifid fronds on a short-creeping, erect rhizome. Fronds dark green, with tough texture. Involucres occur in series of two parallel rows along pinnae. Occurs terrestrially at moderate to high elevations. May occur on open forest floor or under high amount of cover, often in groups of 4 – 5 individuals or more. Most common terrestrial species.

Abrodictyum caudatum

Large (average frond length 22 cm) 3-pinnatifid fronds on a medium (2 cm diameter) long-creeping rhizome. Rhizome of this species is larger than other long-creeping species. Fronds light green, delicate. Occurs epiphytically at moderate to high elevations. This species grows exclusively on tree ferns. The roots are often deeply intertwined with the scales of the tree fern rhizome, making it difficult to remove.

Crepidomanes humile

Small (average frond length 5 cm), 2-pinnatifid fronds on a long-creeping rhizome. Laminae have specialized row of elongated marginal cells visible with a hand-lens. Fronds ovate-lanceolate, light green. In favorable conditions, will grow in a mat with interweaving rhizomes on the substrate. Common at low (30 m) to middle (450 m) elevations in forests with at least moderate (70%) cover, growing on tree trunks or rocks. Often occurs with *Cr. bipunctatum* at lower elevations.

Crepidomanes bipunctatum

Small (average frond length 7 cm), 3-pinnatifid fronds on a long-creeping rhizome. Laminae have a discontinuous sub-marginal false vein visible with a hand lens. Fronds lancelote (overall shape resembles a Christmas tree). Fronds are dark green in color, but may vary with state of desiccation. In favorable conditions, will grow in a mat with interweaving rhizomes on the substrate. Common at low elevations in forests with at least moderate cover, growing on tree trunks or rocks (occurs more often on rocks than trees). Often occurs with *Cr. humile* at lower elevations.

Crepidomanes sp. 1

Tiny (average frond length 1 cm), 1-pinnatifid fronds on a long-creeping rhizome. Laminae have a continuous sub-marginal false vein. Fronds ovate-lanceolate, light green. In favorable conditions, will grow in a mat with interweaving rhizomes on the substrate. Rare at low elevations. Only grows on rocks, often on boulders next to stream beds. Easily confused with juvenile *Cr. humile*, but the two may be differentiated by size and specialized margin and vein structures.

Crepidomanes sp. 2

Gross morphology similar to *Cr. humile* or *Cr. bipunctatum*. Small fronds on a long-creeping rhizome. Occasionally a single costa will be elongated, with short pinnae along its length. Rare at high elevations. I only found this once in the field, growing epiphytically with mosses and *H. digitatum*.

Crepidomanes minutus

Tiny (average frond length 1.5 cm), fan-shaped fronds on a long-creeping rhizome. Veins split dichotomously. Stipe occasionally proliferous, producing new fronds. In favorable conditions, will grow in a mat with interweaving rhizomes on the substrate. Occurs at high elevations (> 450 m) on tree trunks and branches, often with dense moss or *H. polyanthos*.

Callistoperis apiifolia

Large (average frond length 37 cm) 3-pinnatifid fronds on an erect, short-creeping rhizome. Stipe and rachis covered with long, reddish hairs. Occurs terrestrially at moderate to high elevations. Much less abundant than *A. dentatum*, usually only found in populations of a single individual. Fertile fronds often absent in small specimens.

Didymoglossum tahitense

Small (average frond width 1.5 cm) peltate fronds lacking a stipe on a filiform, long-creeping rhizome. Fronds are adpressed to substrate, often difficult to remove. Rhizoids on underside of leaf help anchor to substrate. Fronds may overlap slightly so that rhizome is hidden from view. At first glance may appear to resemble a liverwort more closely than a fern. Does not grow in a mat like other low elevation species, but will form a line of peltate fronds as the rhizome grows along the substrate. Found on tree trunks (especially *Inocarpus fagiferus*) at low elevations.

Hymenophyllum digitatum

Delicate in appearance, with very fine, glabrous stipe and rhizome. Small (average length 4.5 cm) fronds hang lax from the substrate, often in a dense mat. Frond veins split dichotomously, up to 10 times. Fronds have characteristic wavey edges, with small hairs along margin. Fronds light green, with dark brown to black veins. Laminae turn black when severely desiccated; blackened individuals often present within populations. Involucre bivalve at ends of veins. Occurs epiphytically at mid- to high- elevations, often on underside or recessed areas of trunks or branches.

Hymenophyllum pallidum

A moderately sized fern (average frond length 13 cm), characterized by fine, whitish hairs covering the frond. Hairs are usually concentrated towards the tips and margins, and are most conspicuous on young fronds. Another unusual morphological feature is a double layer of cells 4 cells wide on either side of the veins. Frond is three times pinnatifid, oblong to ovate in shape. Growth habit similar to other epiphytes (long-creeping rhizome which forms a mat). Occurs as a rare epiphyte at high elevations.

Hymenophyllum polyanthos

This species contains a high degree of morphological variation. Fronds vary in length from small (5 cm) in some populations to large (15 cm) in others; light green laminae on a dark-brown to black stipe. Frond shape may be long and narrow with limited branching, or short and wide with long branching. Involucres are distinctively bivalve, with rounded wings and an included receptacle. A common epiphyte at high elevations, often growing on *Metrosideros collina*. Similar growth habit to *Cr. humile* or *Cr. bipunctatum*, with long-creeping rhizomes forming an interwoven mat.

INSECT BIODIVERSITY AND ASSESSMENT OF HERBIVORY IN NATIVE AND NON-NATIVE PLANTS IN MO'OREA, FRENCH POLYNESIA

ERIN M. PRADO

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

Abstract. The objective of this study was to determine if the distribution of insect species and presence of herbivory differed between native and non-native plants in the coastal region of Mo'orea, French Polynesia. Therefore, four native plant species (*Barringtonia asiatica, Hibiscus tiliaceus, Terminalia catappa, Thespesia populnea)* and four non-native plant species *(Carica papaya, Mangifera spp., Morinda citrifolia, Musa spp.)* were sampled. Each collected insect was tested for herbivory, and placed in a cup with a 2X1in. piece of undamaged leaf from the tree it was found and frequently checked for damage. Significantly greater insect species abundance was found on native plants compared to non-native plants (p=0.0431). No significant difference was found in richness (p=0.6409) or diversity (p=0.8451) between native and non-native plants. Significantly more herbivory damage was observed on the whole tree in native plants (p=0.0001). The herbivory trials found more cases of herbivory damage in non-native plants compared to native plants, 14 cases and 10 cases respectively, but more total area damaged in native plants compared to non-native plants, with 5.015% and 4.18% damage respectively. No significant differences were found between abundance and height of sampling, richness and height of sampling, or diversity and height of sampling (p=0.1108, 0.0933, and 0.07695). No significant differences were found between abundance and tree height, richness and tree height, or diversity and tree height (p=0.5305, 0.6156, 0.7805). The results show that there is more insect abundance and more herbivory damage in native plants, suggesting that generalist herbivores are feeding on non-native plants while specialist and generalist herbivores are feeding on native plants.

Key words: insects; community structure, herbivory, French Polynesia, Barringtonia asiatica, Carica papaya, Hibiscus tiliaceus, Mangifera spp., Morinda citrifolia, Musa spp., Terminalia catappa, Thespesia populnea.

INTRODUCTION

Whether or not intended, increased trade and transportation has resulted in more plants being introduced to foreign areas of the world. Many non-native plant species have been found to exhibit greater abundance in new environments, causing serious environmental and economic concern (Bossdorf 2005; Pimentel 2005). Most notably, non-native species are considered the second most common cause for loss of native biodiversity (Wilcove et al. Al. 1998). The success of non-native plants may be explained by the Enemy Release Hypothesis.

The Enemy Release Hypothesis predicts that in the absence of coevolved specialist herbivores and pathogens, plants will achieve greater growth, reproduction, and size than in their native range. Introduced plants are able to do better because the native plants that they are competing with are not free of their respective coevolved herbivores (Keane and Crawley 2002). Several studies have found support for the Enemy Release Hypothesis. For example, in a comparison of leaf herbivory between native and invasive woody plants on the tropical island of Mahé, the percentage of leaves affected by herbivores was significantly higher on native species than on invasive species due to specialist herbivores feeding on native plant species, with 50% and 27% leaf damage respectively (Hansjorn 2004). However, there are cases that contradict these results, with more herbivory damage observed in non-native plant species, making this controversial hypothesis worth testing (Agrawal 2003).

The differences in biodiversity and herbivore damage between native and non-native plant species have not yet been studied in the rich coastal region of Mo'orea, French Polynesia. Previous investigations in insect distribution have looked at pest populations on specific species of plants or assessed the total distribution of specific insect species (Lehr 2004; Tang 1999). One study looked at the distribution of invasive insect species across different habitats and found the Glassy-winged sharpshooter, *Homalodisca vitripennis*, to be the most abundant insect on all plant species, native and introduced alike (Weiss 2004). Since this study, a biocontrol protocol has been introduced and the Glassy-winged sharpshooter population has fallen by 90% as of August 2006 (Petit 10/02/06). Results from my study will update the information on insect species distribution in this region.

The purpose of this study is to evaluate the insect species composition, herbivory damage, and major herbivore species across native and introduced plant species in the coastal region of Mo'orea. I will observe the differences between four native plant species: *Thespesia populnea (*Miro*), Barringtonia asiatica (*Hutu*), Terminalia catappa (*Autaraa Maohi*), and Hibiscus tiliaceus (*Purau*)*, and on four non-native plant species that co-exist in the same coastal habitat, including *Mangifera spp (*Vi Popaa: Mango*), Carica papaya (*Iita: Papaya*), Musa spp. (*Meia: Banana*)*, and *Morinda citrifolia* (Nono*)*. I hypothesize that there will be a difference in species composition of insect communities and presence of herbivory between native and non-native plants, with more insect biodiversity and herbivory on native plant species. The results of this study will reveal the insect species distribution in this region, and provide a case study to support or reject the Enemy Release Hypothesis.

METHODS

Study site

Data assessing species composition of insect communities and presence of herbivory was collected from eight plant species in the coastal region of Moorea, French Polynesia (Fig. 1) located at approximately 17°29'18" S and 149°43'38" W between the dates of October 10th and November 17th, 2006. Data was compared between four native plant species and four introduced plant species (see introduction) that have been cultivated in the same coastal region. Two sites were used in this study, including a stretch of coastal strand south of the UCB Gump Research Station located in the northwest corner of Cook's Bay (Fig. 2), and a plot of land belonging to Marimari Kellum located in the southeast corner of Opunohu Bay (Fig. 3). The habitat of the two sites varied slightly with Site 2 having more canopy cover than Site 1, however both are characteristic of Moorea's coastal region with rich soil and a varied composition of native and introduced plant species.

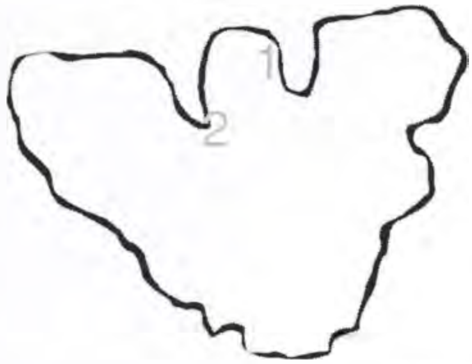


FIG.1. Sites sampled in this study. Site 1: Northwest corner of Cook's Bay, Site 2: Southeast corner of Opunohu Bay.

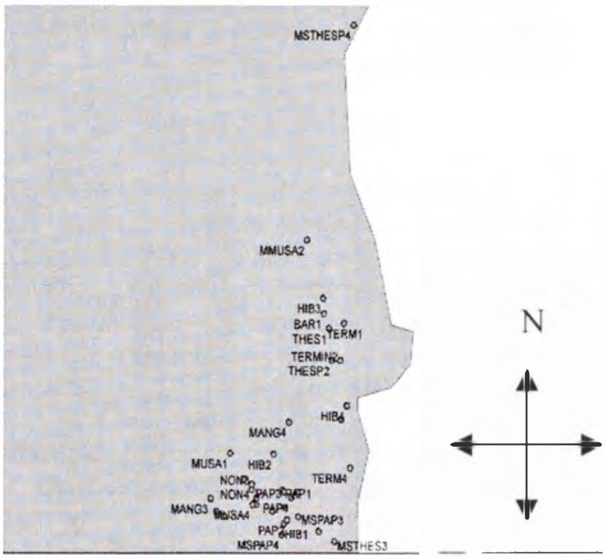


FIG. 2. Site 1: NW corner of Cook's Bay, property of Richard B. Gump Research Station.

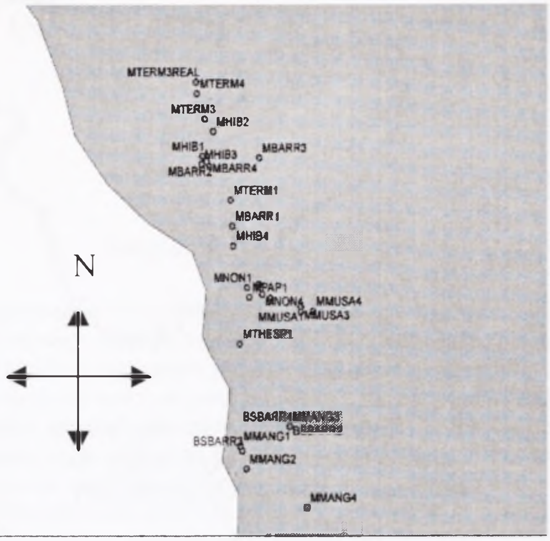


FIG.3. Site 2: SW corner of Opunohu Bay, property of Marimari Kellum.

Experimental design

A preliminary survey was conducted along the coast of Mo'orea to observe locations with multiple native and introduced plant species coexisting in the same habitat. Eight plant species were chosen due to their abundance along the coastal strand and similarity in height and leaf size. Two sites were selected based on the presence of at least four individuals of each plant species of interest to this study. Individuals were chosen to sample in order to have the shortest distance between the two vegetation types, native and introduced.

Sampling method

Each individual tree (n=64) was sampled once for ten minutes with a sweep net. Insects were collected from low and high vegetation in the tree and separated into these categories. Five minutes were allotted for sampling the low vegetation (0-6ft) with ten seconds spent sampling each branch. Five minutes were spent sampling the high vegetation (6ft-+) with ten seconds spent sampling each branch. If a tree had no low vegetation, that is, it's lowest branches were above 6ft, ten minutes were designated for sampling the vegetation and the insects collected were placed into the high category only. Insects were separated into different compartments in a Petri dish and observed under a light microscope for identification. Johnson's sixth edition (1989) was used to identify insects to family, while Renaud (2003) was used to identify insects to species. The diameter at breast height (DBH) and height of each tree was measured. DBH was measured in centimeters with measuring tape. The height of each tree was measured in centimeters by taking a picture with a scale of a known height next to the tree, and using Digimizer (MedCalc 2005) to measure the tree relative to the scale.

Data on the presence of herbivory were collected from each sampled tree. Total visible herbivore damage (ie. holes) was quantified and placed into one of four categories: 1:0-25%, 2:26-50%, 3:51-75%, and 4:76-100% herbivory damage. Herbivory trials were conducted as well, with each live insect collected from an individual tree being placed into a clear cup with a perforated lid. A 2X1 inch portion of a fresh, undamaged leaf from the tree the insect was collected was placed in the cup with the insect, and the leaf was continuously checked for herbivory damage and other forms of damage, such as tearing. The damaged area on the leaf, if any, was measured as a percentage of total area using Adobe Photoshop. Using the wand tool, the color of the leaf was separated from the white backdrop. The leaf without the damaged area was highlighted and a histogram was produced, showing the number of pixels the leaf was after damage. Then the holes were filled in, and another histogram was made, showing the number of pixels the leaf was before damage. The number of pixels after damage was divided by the number of pixels before damage to yield a percentage of total area damaged.

Data analysis

The Shannon-Weiner index of biodiversity was used to quantify the insect diversity of each tree, testing both insect richness and evenness (Shannon, C; Weiner 1953). Differences in insect species abundance, richness, and diversity between native and introduced plants were tested for significance. The data failed a test for normality and equal variance, so a nonparametric test was used to assess significance in difference. A Wilcoxon rank sum test was run to test for differences in insect diversity, richness, and abundance between native and non-native trees. A Kruskal-Wallace test was performed to test for differences between tree species. A Tukey HSD multiple comparison test was used to identify significantly different groups. A linear regression test was used to test correlations between insect abundance, richness, and diversity and herbivory damage category. All statistical analyses were performed using JMP 5.1 (SAS Institute 2003). A p-value of less than 0.05 showed a significant difference between vegetation types. A p-value of less than 0.00178, determined by Bonferroni's correction was needed to show a significance difference between tree species.

RESULTS

Insect Biodiversity

Abundance- A greater insect abundance was found on native vegetation compared to non-native vegetation with 243 individuals found on non-native vegetation and 619 individuals found on native vegetation. Insect abundance was significantly different between vegetation types (p=0.0431). Insect abundance was significantly different between tree species (p= 0.0351; Appendix B). *C. papaya* had the lowest insect abundance with 37 individuals, while *H. tiliaceus* had the highest insect abundance, with 238 individuals (Fig. 4).

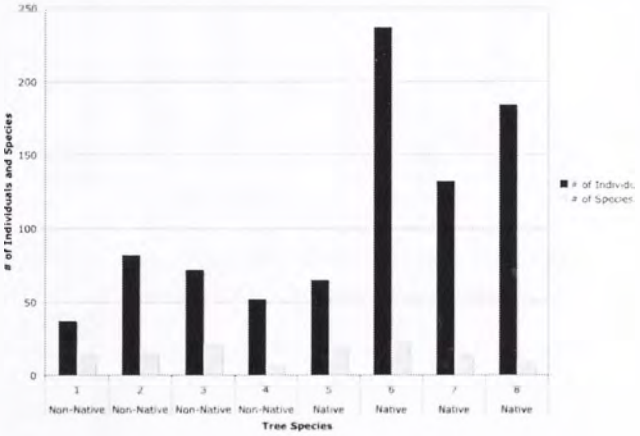


FIG. 4. Insect abundance and richness: the number of individuals and number of species in each tree species. 1: *Carica papaya*, 2: *Mangifera spp.*, 3:*Morinda citrifolia*, 4: *Musa spp.*, 5: *Barringtonia asiatica*, 6: *Hibiscus tiliaceus*, 7: *Terminalia catappa*, 8: *Thespesia populnea*.

Richness- A greater number of insect species was found on native plants, 44 of the total 60, while 37 of the total 60 insect species were found on non-native plants. 16 insect species were only found on native plants, 17 insect species were only found on non-native plants, and 27 insect species were shared between the two vegetation types (Fig. 4). Using the Wilcoxon rank sum test, the difference between vegetation types was deemed insignificant (p=0.6409). Richness between tree species was also insignificant (p=0.1951). *H. tiliaceus* had the highest insect species richness with 24 insect species, *M. citrifolia* had 21 insect species, and *B. asiatica* had 19 insect species. *Musa spp.* had the lowest insect species richness, with a total of 7 insect species (Appendix C).

Diversity- Using the Shannon-Wiener index for diversity in a test for richness and evenness of insect species, no difference was found between native and non-native vegetation (p= 0.8451) or between tree species (p= 0.1068) (Fig. 5). *M. citrifolia* had the highest average diversity (H'= 0.5910), followed by *Mangifera spp.* (H'= 0.5488), and *B. asiatica* (H= 0.5314). *Musa spp.* had the lowest average diversity (H= 0.3802).

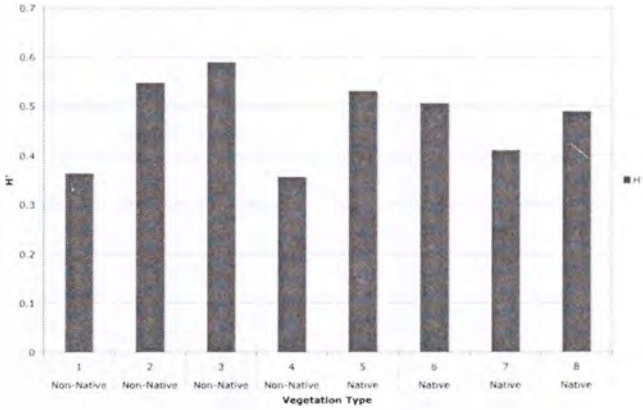


FIG. 5. Shannon-Weiner diversity index averages for each tree species. 1: *Carica papaya*, 2: *Mangifera spp.*, 3: *Morinda citrifolia*, 4: *Musa spp.*, 5: *Barringtonia asiatica*, 6: *Hibiscus tiliaceus*, 7: *Terminalia catappa*, 8: *Thespesia populnea*.

Insect Communities- 20 herbivore species, 10 predator species, 3 parasitoid species, 4 sap sucker species, 1 detritivore species, 1 granivore species, and 16 spider morphospecies were collected from native vegetation (Fig. 6). Non-native vegetation had 16 herbivore species, 7 predator species, 3 parasitoid species, 4 sap sucker species, 2 detritivore species, 1 granivore species, and 6 spider morphospecies (Fig 6). Native vegetation had 267 herbivores, 39 predators, 3 parasitoids, 290 sap suckers, 5 granivores, 1 detritivore, and 40 spiders (Fig. 7). Non-native vegetation had 82 herbivores, 69 predators, 12 parasitoids, 66 sap suckers, 9 granivores, 2 detritivores, and 11 spiders (Fig. 7, Appendix C).

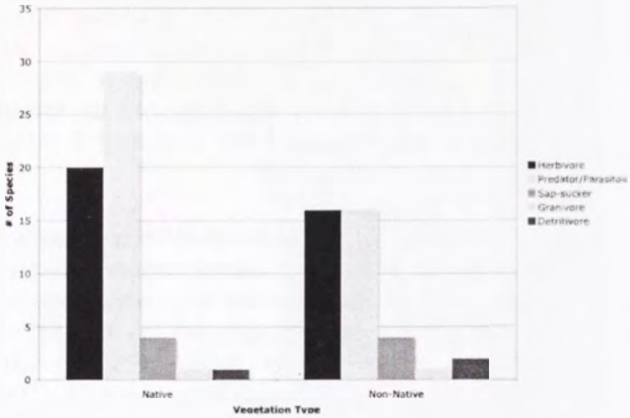


FIG.6. The number of herbivore, predator and parasitoid, sap sucker, granivore, and detritivore species by vegetation type.

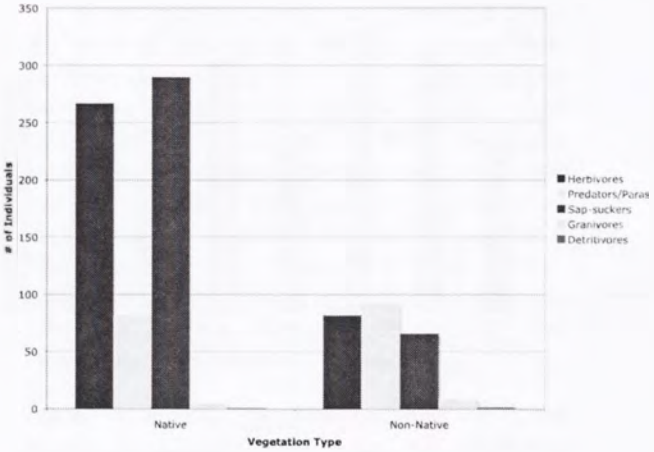


FIG.7. The number of herbivore, predator and parasitoid, granivore, and detritivore individuals by vegetation type.

High vs. Low

No differences were found between abundance and height of sampling (p=0.1108), richness and height of sampling (p=0.0933), or diversity and height of sampling (p=0.07695).

Tree height

No differences were found between abundance and tree height (p=0.5303), richness and tree height (p=0.6156), or diversity and tree height (0.7805).

Herbivory

Tree damage- More herbivory damage was observed on native plants with an average damage category of 1.76 for non-natives, and 2.56 for natives (Fig. 9). Using the Wilcoxon rank sum test, this difference was determined to be significant (p=0.001). *H. tiliaceus* had the highest average damage category of 3.25, while *C. papaya* had the lowest average damage category of 1.5. Herbivory damage between tree species was significant (p=0.0001). Using linear regression, a positive correlation between abundance of insects and tree damage was observed (p-value 0.0001; Fig.8). A similar positive correlation was found between number of insect species and tree damage (p-value 0.0054).

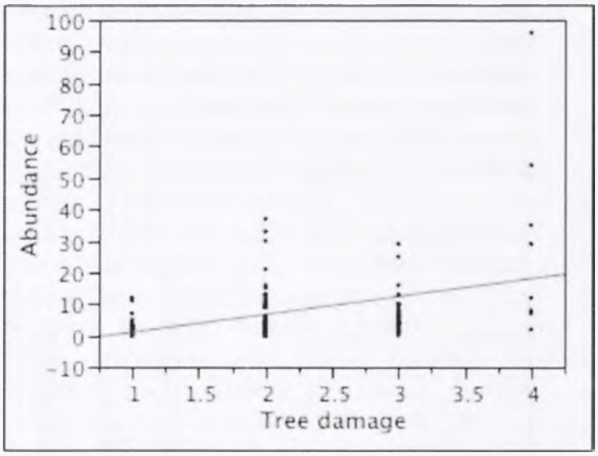


FIG.8. A positive correlation between tree damage and abundance of insects.

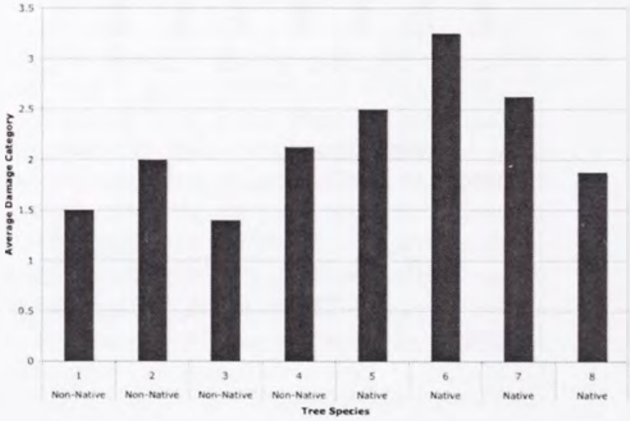


FIG.9. The average damage category for each tree species. Damage categories (Y-axis): 1=0-25% damage, 2=25-50% damage, 3=50-75% damage, 4=75-100% damage. Tree species (X-axis): 1: *Carica papaya*, 2: *Mangifera spp.*, 3: *Morinda citrifolia*, 4: *Musa spp.*, 5:

Barringtonia asiatica, 6: *Hibiscus tiliaceus*, 7: *Terminalia catappa*, 8: *Thespesia populnea*.

Herbivory trials- Contrary to the observed herbivory damage on native plant species, more damage cases were observed in non-native plant species. However, 6 of these cases were by non-herbivores. Herbivory trials resulted in more non-native plants being damaged, with 10 herbivory cases and 14 non-native cases respectively (Fig.10). The total percentage of leaf damage was greater in native plant species, with an average 15.13% leaf damage in native plant species and 12.48% leaf damage in non-native plant species. The standardized percentage of leaf damage (standardized by the number of days until damage was observed) was also greater in native plant species with an average 5.02% damage on natives, and 4.20% damage on non-natives (Appendix A).

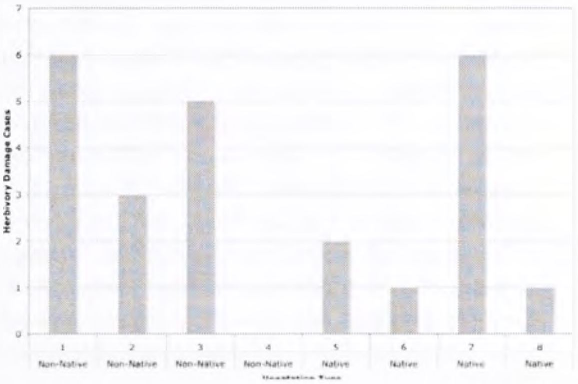


FIG.10. The number of herbivory damage cases by trees species. 1: *Carica papaya*, 2: *Mangifera spp.*, 3: *Morinda citrifolia*, 4: *Musa spp.*, 5: *Barringtonia asiatica*, 6: *Hibiscus tiliaceus*, 7: *Terminalia catappa*, 8: *Thespesia populnea*.

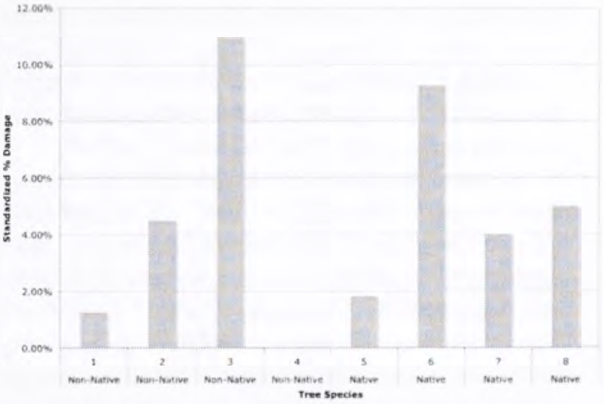


FIG.11. Standardized herbivory damage (total % damage/ # of days for insect to eat leaf). 1: *Carica papaya*, 2: *Mangifera spp.*, 3: *Morinda citrifolia*, 4: *Musa spp.*, 5: *Barringtonia asiatica*, 6: *Hibiscus tiliaceus*, 7: *Terminalia catappa*, 8: *Thespesia populnea*.

DISCUSSION

Insect Biodiversity

Abundance- Consistent with previous findings (Hansjorg 2004, Wolfe 2002), the results of this study indicate a significant difference in insect abundance between native and non-native vegetation, with native plants supporting a greater number of individuals. This difference was predicted by my hypothesis, which was based on a previous study in Mo'orea that found native vegetation in high elevations to support both native specialist insects and non-native generalist insects (Weiss 2004). The Enemy Release Hypothesis supports this phenomenon by explaining that non-native plants do better in terms of growth and reproduction compared to native plants because they lose contact with their co-evolved specialist herbivores. Specialist herbivores are those that feed on one or a few closely related plant species, and generalist herbivores are those which feed on several non-related plant species (Joshi 2005). With significantly more insects residing on native plants compared to non-native plants, 619 to 243, it is likely that co-evolved specialist insects as well as introduced generalist insects make up this large population.

Richness- A higher number of insect species on native plants was expected based on the Enemy Release Hypothesis which expects native plants to support both co-evolved specialist herbivores as well as generalist invasive herbivores while non-native plants only support generalist herbivores. However, no significant differences in richness were found. No significant difference in diversity was found either, as determined by the Shannon-Weiner test for diversity. This result is not surprising since richness is used to calculate diversity, and diversity failed the test for significance as well. A possible explanation for these results is that the introduced plants have evolved a decreased defense against specialist herbivores, which are scarce in the introduced range, and a higher protection against generalist herbivores. It is possible that the native co-evolved specialists have started to move over to the native plant species, which are not well defended against them. This sequence of events led to the invasive success of *Senecio jacobaea* in Australia, New Zealand, and North America (Joshi 2005). However, richness alone may not be a good indicator of differences between native and non-native plants or very relevant to the Enemy Release Hypothesis if there is only one individual for each insect species on the non-native vegetation and five individuals for every insect species on the native vegetation. In determining if the Enemy Release Hypothesis supports what is seen in the coastal region of Mo'orea, it is more important to look at insect communities grouped by diet.

Insect communities- An equal number of predator/parasitoid species and herbivore species were found on non-native vegetation, while more predator/ parasitoid species and more herbivore species were found on native vegetation. Significantly more individual herbivores were found on native vegetation compared to predator/ parasitoids, while an equal number of predator/parasitoids and herbivores were found on non-native vegetation. Perhaps more herbivory occurred on native trees not because more specialist herbivores were found on native trees but because there were as many predators and parasitoids there to kill the herbivores on the non-native trees. A similar case was observed in central Germany, where herbivory levels were correlated negatively with spider abundances (Unsicker 2006). However, other data support the correlation between number of herbivore species and herbivory damage, such as the positive correlation found between species richness and overall tree damage.

High vs. Low

I expected there to be a difference in insect abundance and richness between high and low vegetation due to differences in abiotic factors such as canopy cover and moisture. A case study from the Ozarks in Missouri supports this hypothesis, finding that insect density and richness increased in the shady areas, and drastically decreased in the sunnier areas of the tree (Jeffries 2006). However, no differences were found between sampling levels in this study. Similarly, I expected there to be a difference in abundance and richness of insects between taller and shorter trees due to differences in abiotic factors. A study from Brazil on *Anadenanthera macrocarpa* (Mimosaceae) found a significant increase in both abundance and species richness of ants and herbivorous insects as tree height increased. This correlation was attributed to the microclimate gradient between the understory and canopy not being very steep (Campos 2006). However, no significant differences were found.

Herbivory

I expected more herbivory damage on native trees based on the prediction of the Enemy Release Hypothesis. My findings from quantifying the damage of each tree support my hypothesis, with more herbivory damage on native trees as a whole. Positive correlations between average damage category for each tree species and abundance, as well as average damage category and richness lead me to believe that native trees exhibit more herbivory due to more herbivore species and individuals. However, in the herbivory trials, more insects fed on non-native plants. Similar results of more insect herbivory exhibited on exotic plants compared to native plants have been found in a few studies. This phenomenon has been explained by the theory that native plants are better adapted to the local herbivore fauna compared to non-natives (Agrawal 2003). Another explanation is offered by the Evolution of Increased Ability Hypothesis, which suggests that non-native plants are more palatable, that is, they taste better because they have reallocated their resources from defense to growth (Genton 2004).

Despite more herbivory damage cases in non-native plants, the resulting damaged area on native leaves, both real values and standardized values, were greater on native plants. This suggests that specialist herbivores are feeding on native plant species, while generalist herbivores are feeding on non-native plant species. These results are consistent with other findings that specialist herbivores cause more damage compared to generalist herbivores in total damage to the plant (Joshi 2005).

Cosmopterigidae 1 may be an example of a co-evolved specialist for *T. catappa.* Cosmopterigidae 1 proved to cause the most damage on *T. catappa* out of the two herbivores that attacked it. The insect ate *T. catappa's* leaves five out of seven times in the herbivory trials and caused an average of 4.2% damage, with as high as 31% total area damage on one 2X1 inch leaf. The individual Cosmopterigidae 1 in these trials came from category three and four damaged trees, suggesting that they are the cause for severely damaging these trees.

Cerambycidae 2 is another example of a possible co-evolved specialist herbivore, having caused significant damage on two native plant species that belong to the same plant family, Malvaceae: *Hibiscus tiliaceus* with and average of 9.25% standardized damage and *Thespesia populnea* with an average of 5% standardized damage. The differences in amount of herbivory between native and non-native vegetation were not found to be significant by the Wilcoxon rank sum test, however, based on biodiversity and field herbivory results, I find it to be representative of what is happening overall.

CONCLUSION

I conclude, based on more visible herbivore damage on the whole tree, more insect abundance, more herbivore species, more herbivore individuals, and fewer cases of damage, yet a higher average percent damage in native plant species, that the Enemy Release Hypothesis can explain the biodiversity and herbivory in the coastal region of Mo'orea, French Polynesia.

ACKNOWLEDGMENTS

I thank the GSIs for their support throughout the semester, Pete Oboyski for his help(!) identifying insects, and the Mo'orea class of 2006 for their assistance in the field.

LITERATURE CITED

Agrawal A.A., P.M. Kotanen. 2003. Herbivores and the Success of Exotic Plants: A Phylogenetically Controlled Experiment. Ecology Letters **6**: 712-715.

Ambrose, H.W. III, K.P. Ambrose. 2002. A Handbook of Biological Investigation. Hunter Textbooks Inc. Winston-Salem, NC.

Anonymous. 1998. Medicinal Plants of the South Pacific, Information on 102 commonly used medicinal plans in the South Pacific. WHO Regional Publications Western Pacific Series **19**: 97.

Bossdorf, O., H. Auge, L. Lafuma, W.E. Rogers, E. Siemann, D. Prati. 2005. Phenotypic and Genetic Differentiation Between Native and Introduced Plant Populations. Oecologia **144**: 1-11.

Campos R.I., H.L. Vasconcelos, S.P. Ribeiro, F.S. Neves, J.P. Soares. 2006. Relationship between tree size and insect assemblages associated with *Anadenanthera macrocarpa*. Ecology **29**: 442-450.

Genton B.J., P.M. Kotanen, P.O. Cheptou, C. Adolphe, J.A. Shykoff. 2004. Enemy release but no evolutionary loss of defense in a plant invasion: an intercontinental reciprocal transplant experiment. Oecologia **146**:404-414.

Hansjorg D., L.R. Wirth, H. Buschmann. 2004. Variation in herbivore damage to invasive and native woody plant species in open forest vegetation on Mahe ́, Seychelles. Biological Invasions **6**: 511-521.

Jeffries J.M., R.J. Marquis, R.E. Forkner. 2006. Forest Age Influences Oak Insect Herbivore Community Structure, Richness, and Density. Ecological Applications **16**: 901-912.

Joshi J., K. Vrieling. 2005. The enemy release and EICA hypothesis revisited; incorporated the fundamental difference between specialist and generalist herbivores. Ecology Letters **8**: 704-714.

Keane, R.M., M.J. Crawley. 2005. Exotic plant invasions and the enemy release hypothesis. Trends in Ecology and Evolution 17:164-170.

Lau J.A., S.Y. Strauss. 2005. Insect Herbivores Drive Important Indirect Effects of Exotic Plants on Native Communities. Ecology **86**: 2990-2997.

Lehr, H. 2004. Insect-Crop Relationships: Community Variation by Taro Variety & Invasive Aphid Feeding Preference. Biology and Geomorphology of Tropical Islands : 87-95.

Mitchell C.E., A.A. Agrawal, J.D. Bever, G.S. Gilber, R.A. Hufbauer, J.N. Klironomos, J.L. Maron, W.F. Morris, I.M. Parker, A.G. Power, E.W. Seabloom, M.E. Torchin, D.P. Vazquez. 2006. Biotic and Plant Invasions. Ecology Letters **9**: 726-740.

Nowicki J., J. Haun, L. Peng et al.10.30.05. Antimutagenic activity of Morinda citrifolia (Noni) fruit juice in ICR mice. 4th Annual Conference on Frontiers in Cancer Prevention Research. Cancer Epidemiology Biomarkers & Prevention 14. Baltimore, MD, USA.

Petit,J.N.(10.02.06)Glassy-winged sharpshooters. UCB Gump Research Station.

Pimentel D., R. Zuniga, D. Morrison. 2005. Update on the Environmental and Economic Costs Associated with Alien-Invasive

Species in the United States. Ecological Economics **52**: 273-288.

Shannon, C. E., and W. Weiner. 1953. The Mathematical Theory of Communication. University of Illinois Press, Urbana, USA.

Tang, A. 1999. Banana cultivation: The agronomic impact of the insect pest *Cosmopolites sordidus* in Moorea, French Polynesia.

Unsicker, S.B., N. Baer, A. Kahmen, M. Wagner, N. Buchmann, W.W. Weisser. 2006. Invertebrate herbivory along a gradient of plant species diversity in extensively managed grasslands. Oecologia 150 (**2**): 233-246.

Weiss, K. 2004. Host relationships among Endemic and Invasive Leafhoppers (Homoptera: Cicadellidae) and Planthoppers (Homoptera: Fulgoroidea) on the Island of Moorea, French Polynesia: 189-198.

Wilcove D.S., D. Rothstein, J. Dubow, A. Phillips, E. Losos. 1998. Quantifying threats to imperiled species in the United States. Bioscience 48 **8**: 607-615.

Wolfe, L.M. 2002. Why Alien Invaders Succeed: Support for the Escape-from-Enemy Hypothesis. The American Naturalist **160**: 705-711.

APPENDIX A

Table 1. Summary of insect species, diet, percent damage of leaf (% damage), standardization of damage (% Damage / # of days to consume leaf), and species of tree damaged (Tree species).

Insect Species	Diet	% Damage	Standardized	Tree Species
Acicnemis variegatus	Herbivore	5%	1.25	*Carica p.*
Adoretus vestitus	Herbivore	24%	24%	*Morinda c.*
Anthribidae	Herbivore	4% 5% 8% 13%	1% 1.25% 0.80% 13%	*Carica p.* *Morinda c.*
Bruchidae	Granivore	1% 2	0.25% 0.50%	*Musa spp.* *Carica p.*
Cerambycidae 1	Herbivore	24% 18%	1.5% 9.25%	*Carica p.*
Cerambycidae 2	Herbivore	37% 5%	9.25% 5%	*Hibiscus t.* *Thespesia p.*
Ceresium unicolor	Herbivore	1%	0.14%	*Barringtonia a.*
Cixiidae	Sap sucker	2% 1%	0.40% 1%	*Musa sp.* *Morinda c.*
Coccinellidae	Insectivore	1%	0.20%	*Musa spp.*
Cosmopterigidae 1	Herbivore	31% 10% 7% 5% 10%	10.33% 3.33% 2.33% 1.66% 3.33%	*Terminalia p.*
Geometridae	Herbivore	3%	3%	*Terminalia p.*
Machilidae	Herbivore	14% 6% 49%	3.50% 1% 12.25%	*Barringtonia a.* *Mangifera spp.* *Mangifera spp.*
Mantidae	Predator	1%	1%	*Morinda c.*
Diplopida (Milipede)	Detritivore	1%	0.33%	*Musa spp.*
Sphingidae	Herbivore	48%	16%	*Morinda c.*
Tettigoniidae 1	Herbivore	3%	0.75%	*Carica p.*
Tettigoniidae 2	Herbivore	1% 1%	0.20% 1%	*Mangifera spp.* *Morinda c.*

APPENDIX B

Table 2. Summary of comparisons in abundance, richness, and diversity between native and non-native vegetation, high and low vegetation, height of trees, tree damage categories, and tree species.

Comparison	Variable	Result	p-value
Native vs. Introduced	Abundance	Greater Abundance in Native Vegetation	0.0431
	Richness	No difference	0.6409
	Diversity	No difference	0.8451
	Herbivory on tree	More herbivory on Native trees	0.001
	Variable	Result	Nominal value
	Real % Damage on leaf	More herbivory damage on Native	12.48% damage on native 15.13% damage on non-native
	Standardized % damage on leaf	More Herbivory damage on Native	5.02% damage on native 4.20% damage on non-native
	Number of Herbivory Incidents	More Herbivory Cases in Non-Native Insects	10 Native 14 Non-Native
			p-value
High vs. Low	Abundance	No difference	0.1108
	Richness	No difference	0.0935
	Diversity	No difference	0.7695
Height	Abundance	No difference	0.5303
	Richness	No difference	0.6156
	Diversity	No difference	0.7805
Tree damage	Abundance	More insects in highly damaged trees	0.0001
	Richness	More insect species in highly damaged trees	0.0054
	Diversity	No correlation between diversity and tree damage	0.6895
Tree Species	Abundance	Difference between tree species	0.0351
	Richness	No difference	0.1951
	Diversity	No difference	0.1068

APPENDIX C

Table 3. Insect species distribution by tree species in real numbers.

	Carica papaya	*Mangifera spp.*	*Morinda citrifolia*	*Musa spp.*	*Barringtonia asiatica*	*Hibiscus tiliaceus*	*Terminalia catappa*	*Thespesia populnea*
Pheidole fervus	1	9	12	39	4	1	0	13
Machilidae 1	1	19	12	4	2	2	0	0
Coccinellidae 1	0	0	0	2	2	0	0	0
Cixiidae 1	6	30	20	3	31	83	29	143
Bruchidae 1	6	0	1	2	3	0	0	2
Muscidae 1	0	0	0	1	1	0	0	0
Tettigoniidae 1	1	0	0	0	0	0	0	0
Tettigoniidae 2	0	5	3	0	2	1	1	4
Blattellidae 1	1	0	0	0	0	0	0	0
Anthribidae 1	6	2	3	0	2	2	1	12
Acicnemis variegetus	1	2	0	0	0	0	0	0
Miridae 1	0	0	0	0	0	3	0	0
Miridae 2	0	0	1	0	1	5	3	0
Miridae 3	1	0	0	0	0	0	4	0
Miridae 4	0	0	0	0	0	1	0	0
Cerambycidae 1	2	1	0	0	0	0	0	0
Cerambycidae 2	1	1	0	0	0	1	0	1
Braconidae 1	7	0	3	0	0	0	0	0
Braconidae 2	0	0	0	0	1	0	0	0
Empicoris 1	1	0	0	0	0	0	0	0
Dermastidae 1	0	0	1	0	0	0	0	0
Dermaptera 1	0	0	0	0	0	1	0	0
Drosophilidae 1	0	0	1	0	0	0	0	0
Drosophilidae 2	0	1	0	0	0	1	1	0
Drosophilidae 3	0	0	0	0	0	0	0	1
Drosophilidae 4	0	0	1	0	0	3	0	0
Neriidae 1	0	0	1	0	0	0	0	0
Coagulata viyripennis	1	0	4	0	1	0	0	1
Adoretus vestitus	0	0	1	0	0	0	1	0
Blattidae 1	0	0	1	0	0	2	0	0
Chalcidoidea 1	0	1	0	0	0	1	0	0
Chalcidoidea 2	0	1	0	0	0	0	0	0
Chalcidoidea 3	0	0	0	0	0	1	0	0
Hemiptera 1	0	1	0	0	0	1	0	0
Pseudoscorpionida	0	1	0	0	3	0	0	0
Nueroptera 1	0	0	0	0	2	0	0	0
Ceresium unicolor	0	0	0	0	2	0	0	0
Cosmopterigidae 1	0	0	0	0	1	0	81	0
Tingidae 1	0	0	0	0	0	0	2	0
Mantidae 1	0	0	2	0	1	1	2	1
Reduviidae 1	0	0	0	0	0	0	1	0
Tineidae 1	0	1	0	0	0	0	0	0
Phyllocnistidae 1	0	0	0	0	0	0	1	0
Coleoptera 1	0	0	0	0	0	0	1	0
Geometridae 1	0	0	0	0	0	0	2	0
Crambinae 1	0	0	0	0	0	0	2	0
Chrysopidae 1	0	0	1	0	0	1	0	0
Psyllidae 1	1	6	0	0	4	1	0	0
Psyllidae 2	0	0	0	0	0	113	0	6
Anthrocoridae 1	0	0	0	0	0	7	0	0
Entomobryidae 1	0	0	0	0	0	1	0	0
Aphididae 1	0	0	0	0	0	3	0	0
Oedomeridae 1	0	0	0	1	0	0	0	0
Sphingidae 1	0	0	1	0	0	0	0	0
Nitidulidae 1	0	0	1	0	0	0	0	0
Dictyopharidae 1	0	0	1	0	0	0	0	0
Pseudococcidae 1	0	0	1	0	0	0	0	0

Formicidae 1	0	0	0	0	1	0	0	0
Formicidae 2	0	1	0	0	0	0	0	0
Neuroptera 1	0	0	0	0	1	0	0	0
Plataspidae 1	0	0	0	0	0	1	0	0
Blattidae 2	0	0	0	0	0	1	0	0

COMPARISON OF FOOD PREFERENCE AND BEHAVIOR OF TWO WATERSTRIDERS *HALOBATES HAWAIIENSIS* AND *LIMNOGONUS LUCTUOSUS* (HEMIPTERA: GERRIDAE) IN MOOREA, FRENCH POLYNESIA

MELISSA K. RILEY

Environmental Sciences, University of California, Berkeley, California, 94720 USA, mkriley@berkeley.edu

Abstract. While water striders (Hemiptera: Gerridae) have a global distribution different species have adapted to very different habitat types. Freshwater water striders such as *Limnogonus luctuosus* live in areas along streams and rivers with little to no flow. Marine water striders, such as coastal species *Halobates hawaiiensis,* have adapted to life on the surface of the ocean. Since these types of water striders live in such different habitats, and face different environmental factors their food preference and behavior can be quite different. In this study, average density of *L. luctuosus* individuals was measured along the Opunohu River. Also, food preference, behavior, and the effects of increased density were tested in the laboratory for both *L. luctuosus* and *H. hawaiiensis.* Response time and frequency of approach to mobile and immobile prey items were recorded for *H. hawaiiensis* and *L. luctuosus*. *H. hawaiiensis* preferred immobile prey while *L. luctuosus* preferred mobile prey. Frequency of several behaviors (i.e. movements, moving away from others, approached by others, approaching others, attacking, being attacked, jumping, and cleaning) were compared between species, and within species at increasing densities. There were differences between species in the frequency of movements, approaching others, being approached, jumping, and cleaning. Density affected movements, moving away from others, jumping, and cleaning for *H. hawaiiensis.* Density affected movements, moving away from others, and cleaning behaviors for *L. luctuosus*. The different ecology of these two species can be used to explain why differences exist in both food preference and frequency of behaviors.

Keywords. Water striders; Moorea; French Polynesia; Hemiptera, Heteroptera, Gerridae; Halobates hawaiiensis; Limnogonus luctuosus, *food preference, behavior, density*

INTRODUCTION

Water striders (Hemiptera: Gerridae) are widely dispersed in both marine and freshwater environments due to their ability to adapt to different habitats (Cheng 1985, Foster and Treherne 1980). According to Spence and Andersen (1994) there are about 1500 species of gerromorpha that have adapted to life on the surfaces of various bodies of water. Adapting to life on the surface of water allows many advantages for dispersal; however it also comes with many challenges especially concerning movement, reproduction, and the ability to find food (Spence and Andersen 1994). Water striders have learned to cope with these challenges, but can be affected greatly by environmental factors.

One of the main environmental factors that affect water striders is food availability. Both freshwater and marine water striders are scavengers and predators (Foster and Treherne 1980, Spence and Andersen 1994, Cheng 1985) that feed on floating insects.

Usually this consists of terrestrial insects, such as arthropods, which become stuck on the surface of the water (Cheng 1985, Spence and Andersen 1994, Sih and Watters 2005). According to Cheng (1985), *Halobates sp.* that live near the coast are able to feed on terrestrial insects that have been taken out to sea. Water striders feed as individuals as well as in groups by using sucking mouthparts and using their front legs to grasp prey items (Cheng 1985, Spence and Andersen 1994). Although water striders are not very specific in food preference, location and abundance of food items influences their distribution and abundance (Cheng 1985).

Another environmental factor that affects water striders is group dynamics and behavioral types. The number of individuals and the types of individuals within a group of water striders can have an affect on how a single individual responds to their surroundings (Sih and Watters 2005). Sih and Watters (2005) also explain how group composition can affect the aggressiveness of individuals, frequency of mating, activity level, and feeding behavior.

The objectives of this study were: 1) to explore the prey type preference of two water strider species of Moorea, *Halobates hawaiiensis* (Montousier 1864) and *Limnogonus luctuosus* (Usinger). 2) To examine the affect of varying densities of individuals on behavior, and 3) to determine the difference in frequency of behaviors between *H. hawaiiensis* and *L. luctuosus*.

METHODS

Field sites

Each field site (n=4) was visited periodically in order to collect individual water striders of each species, and to conduct field observations. The field sites were located on the island of Moorea in French Polynesia (Fig. 1). Sites 1 and 2 were marine sites while sites 3 and 4 were freshwater sites.

Site 1: The Gump Station Dock- The Gump Station is located on the western side of Cook's Bay. A light was used to attract marine water striders to the dock at night.

Site 2: Cook's Bay- The western edge of Cook's Bay was surveyed for the presence of marine water striders by kayaking about 100 meters from shore, out toward the reef crest.

Site 3: Opunohu River- The mouth of the Opunohu River is located at the base of Opunohu Bay. The study site was located at a mid reach of the river near Marae Tetiiroa. The substrate was predominately fine sediment and small rocks. The bottoms of many pools were also covered in leaves, and debris from the overhanging *Inocarpus* sp. trees.

Site 4: Vaioro River- The Vaioro River is located on the eastern side of the island. A mid-low reach was surveyed for freshwater water striders. The banks of the stream were densely vegetated, and the water was extremely turbid.

FIGURE 1: Map of Moorea, French Polynesia (17º30'S, 149º50'W). See figure 2 for expanded view of study sites.

Study organisms

H. hawaiiensis (Appendix Fig. 7) is a widely distributed organism which ranges from Hawaii to the Society Islands (Cheng 1985). A typical life cycle from egg to adult lasts around 60 to 70 days (Cheng 1985, Tsoukatou et al. 2001) although little is known about the complete life history of this organism. Individuals are wingless and have a maximum body length of about 6.5 mm according to Cheng (1985 and 1989). Males and females are very similar in body size and shape. Special adaptations include front legs that are able to hold prey, and for males to grab females during mating (Cheng 1985). Also according to Cheng (1985), *Halobates sp.* have developed good eye sight to aide in hunting and predator defense against birds and fish.

L. luctuosus is also widely distributed. *L. luctuosus* is especially wide spread in the southeastern Pacific at varying altitudes and habitats such as lakes, streams, and pools of water (Andersen 1971). *L. luctuosus* has a distinct pattern (Appendix Fig. 6) on its back that is yellow (Andersen 1971). *L. luctuosus* can also be wingless, and it may use ripples as a form of communication and prey location (Cheng 1989, Spence and Anderson 1994, Wilcox 1972). Fish and birds are common predators for water striders in freshwater environments.

Collection

Both species of water strider were collected using aquarium nets at each of the four study sites. Individuals of *L. luctuosus* were transported from the field in dry plastic bags, and placed in a container with a diameter of 36 cm and covered in mesh.

H. hawaiiensis individuals were attracted to the Gump Station dock using a 60 watt lamp that was placed about 1 meter above the water level around sunset (from about 5pm to 12am). Individuals that were attracted to the light were also collected using aquarium nets, and were stored in a 36cm diameter tub covered in mesh. Individuals were also collected by kayaking on the western side of Cook's Bay out toward the reef crest, around sunset. Individuals were collected by paddling into large aggregations of waters striders and using an aquarium net.

Field observations

Group size and location of marine water striders were observed in Cook's Bay.

Average density of water striders along the Opunohu River was estimated by measuring the length and width of fifteen pools along the river. Number of individuals, water depth, temperature, and type of substrate were also recorded.

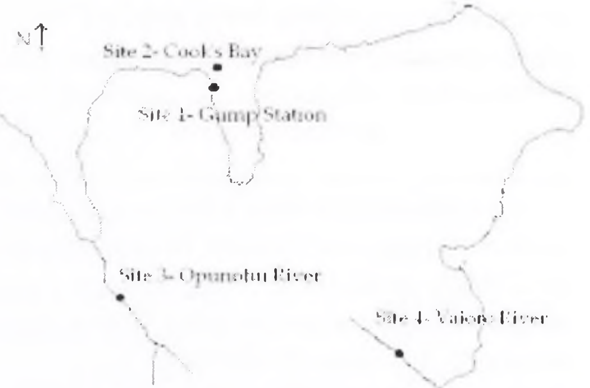


FIGURE 2: Expanded view of study sites on Moorea.

Laboratory experiment- food preference

Individuals were placed in an observation tank (0.33 meters x 0.33 meters), and allowed to acclimate for at least five minutes. After five minutes one mobile and one immobile ant was dropped in the observation tank at equal distances away from the individual being evaluated. The time that it took the individual to approach a prey item, and the number of times individuals approached each prey item were recorded. Twenty-five individuals (males and females) of *L. luctuosus* and twenty individuals (males and females) of *H. hawaiiensis* were evaluated. The experiment was repeated with two immobile prey items. Response time and frequency of approach were recorded.

Laboratory experiment- density and behavior

Individuals were observed and frequency of behaviors was recorded in groups of different sizes for five minutes at a time in the observation tank (0.33 meters x 0.33 meters.) Ten individuals of each species were evaluated individually, in pairs, and in groups of three. Ten individuals of *L. luctuosus* were evaluated in groups of four and five individuals of *Halobates hawaiiensis* were evaluated in groups of four. Behavioral categories included: number of movements, approaching other individuals, being approached by others, moving away from others, attacking others, being attacked by others, jumping, feeding, and cleaning.

Statistical analysis

Food preference observations were evaluated using Chi Squared Test (Ambrose et. al 2002). In addition, behaviors were compared between groups and within groups at varying densities. First the data was checked for normal distributions. If the data did not show a normal distribution, it was log transformed and rechecked for a normal distribution. If the distribution was normal then ANOVA and student's t-test were used in JMP 5.1 (SAS Institute Inc. 2004.) If data were not normal after log transformation Wilcoxon and Tukey-Kramer tests were used in JMP 5.1 (SAS Institute Inc. 2004.

RESULTS

Field observations

Large aggregations (thousands of individuals) of *H. hawaiiensis* were encountered at study Site 4 (Fig. 2) in Cook's Bay. The most common time that individuals were observed was around dusk. However, under windy conditions sightings of large aggregations and single individuals were rare. At Site 3 the light was successful at attracting individuals of *H hawaiiensis.* Individuals were most common around 7pm, and when individuals were observed at the light, there was an average of three individuals per night.

Average surface area of pools along the Opunohu River was 1.67 meters squared. The average number of individuals in each pool surveyed was 4.4. Average density was 3.297 individuals per square meter. Temperatures of pools ranged from 23 degrees Celsius and 25 degrees Celsius. The substrate was primarily small rocks and fine sediments with fallen leaves from overhanging trees.

Laboratory experiment- food preference

The total Chi Squared value between *H. hawaiiensis* and *L. luctuosus* for food prey preference was above the critical value (Ambrose et al., 2002) indicating that there was a difference in prey preference between species. *H. hawaiiensis* tended to approach immobile prey most often (Fig. 3). Only three individuals of *H. hawaiiensis* approached mobile prey first, while eight individuals did not approach prey at all. The average time for *H. hawaiiensis* to approach a prey item was 60.91 seconds (values were only counted if the individual approached a prey item). *L. luctuosus* was more likely to approach mobile prey (Fig. 3) and only two individuals choose not to approach prey. The average response time for *L. luctuosus* was much less at 36.65 seconds. Figure 4 examines the response *H. hawaiiensis* and *L. luctuosus* when both prey items were immobile. Nine individuals of *L. luctuosus* approached prey while only six *H. hawaiiensis* approached the prey. Average response time in this experiment for *H. hawaiiensis* was 10.9 seconds and *L. luctuosus* on average took 98 seconds to approach prey items.

Laboratory experiment- density and behavior

Tables 1 and 2 summarize the results of ANOVA and Wilcoxon tests. Table 1 compares the frequency of the eight behaviors between species. *H. hawaiiensis* individuals were approached by others, approached others, jumped and moved more frequently than *L. luctuosus.* However *L. luctuosus* cleaned more frequently than *H. hawaiiensis.* All other behaviors remained the same.

Table 2 explores how the frequency of behaviors changes with increasing densities of individuals in both species of water strider studied. For *L. luctuosus* movements and moving away from others increased infrequency while the amount of cleaning decreased. *H. hawaiiensis* moved away from others, jumped, and cleaned more frequently while all other behaviors remained the same.

The mean frequency of interactions (i.e. approaching others and being approached) and its relationship to increasing densities is illustrated in Figure 4. For *L. luctuosus*, there is a fairly steady increase in the frequency of interactions as density increases (L1-L4). *H. hawaiiensis* also increases frequency of interactions overall (H1-H4), however there is not as clear of a pattern as with *L. luctuosus*.

TABLE 1. Comparison of the frequency of behaviors between H. hawaiiensis (Marine) and L. luctuosus (Fresh Water). For tests with significant p-values, the species that preformed the behavior most frequently is indicated.

Test	Result	p-value
Frequency of Movements	Marine> Fresh Water	.0015
Moving Away from Other	No difference in Frequency	.3194
Approached by Other	Marine> Fresh Water	.0005
Approaching Other	Marine>Fresh Water	.0043
Attacking Other	No difference in Frequency	.9485
Being Attacked	No difference in Frequency	.7644
Jumping	Marine> Fresh Water	.0002
Cleaning	Fresh Water> Marine	.0250

Note: Significant values are in bold.

TABLE 2. The frequency of behaviors within species at different densities. Significant values are those with p≥ 0.05, and (+) indicates an increase in frequency of behavior with increased density and (-) indicates a decrease in frequency.

Test (Frequency of) Organism	L. luctuosus	H. hawaiiensis
Movements	+	n/a
Moving Away from Other	+	+
Approached by Other	No change	No change
Approaching Other	No change	No change
Attacking Other	No change	No change
Being Attacked	No change	No change
Jumping	No change	+
Cleaning	-	+

Note: Significant values ($p \leq 0.05$) are highlighted. Frequency of movements for H. hawaiiensis were unable to be counted in groups of two, there, and four individuals because they were too frequent.

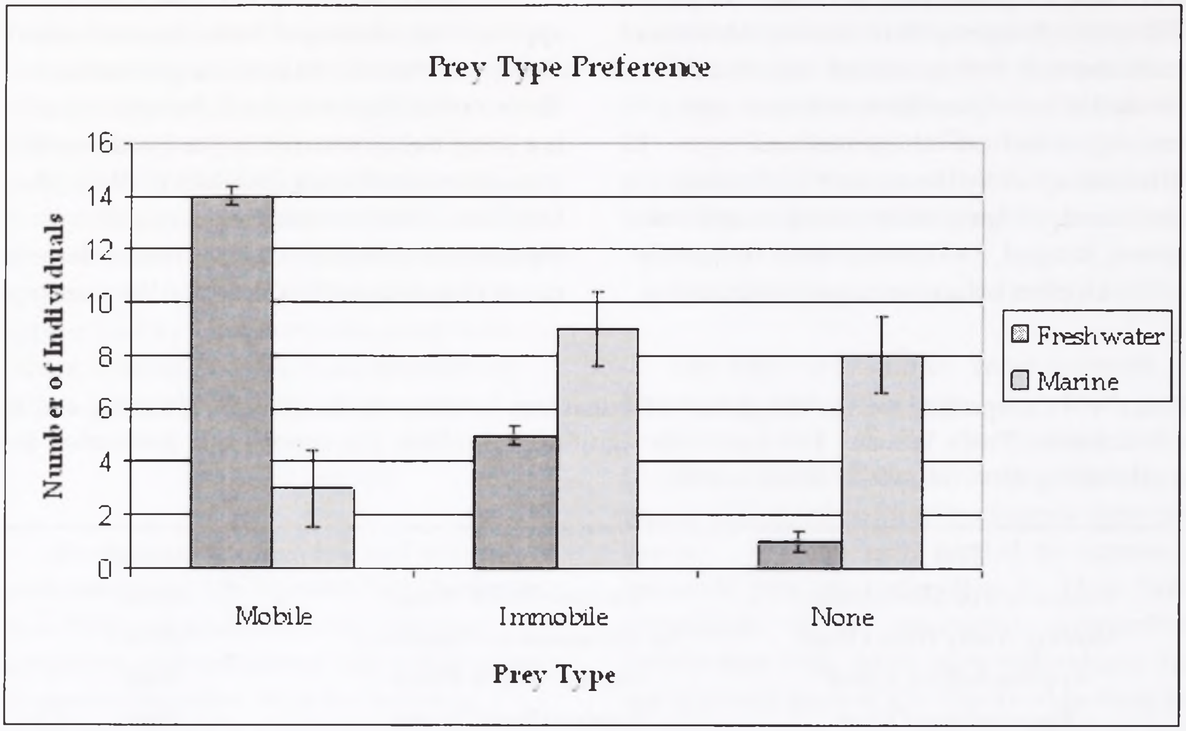


FIGURE 3. Summary of prey type preference experiment for both H. hawaiiensis and L. luctuosus. Figure includes the number of individuals approaching each prey type (immobile or mobile) and the number of individuals that did not approach prey items after a five minute observation period.

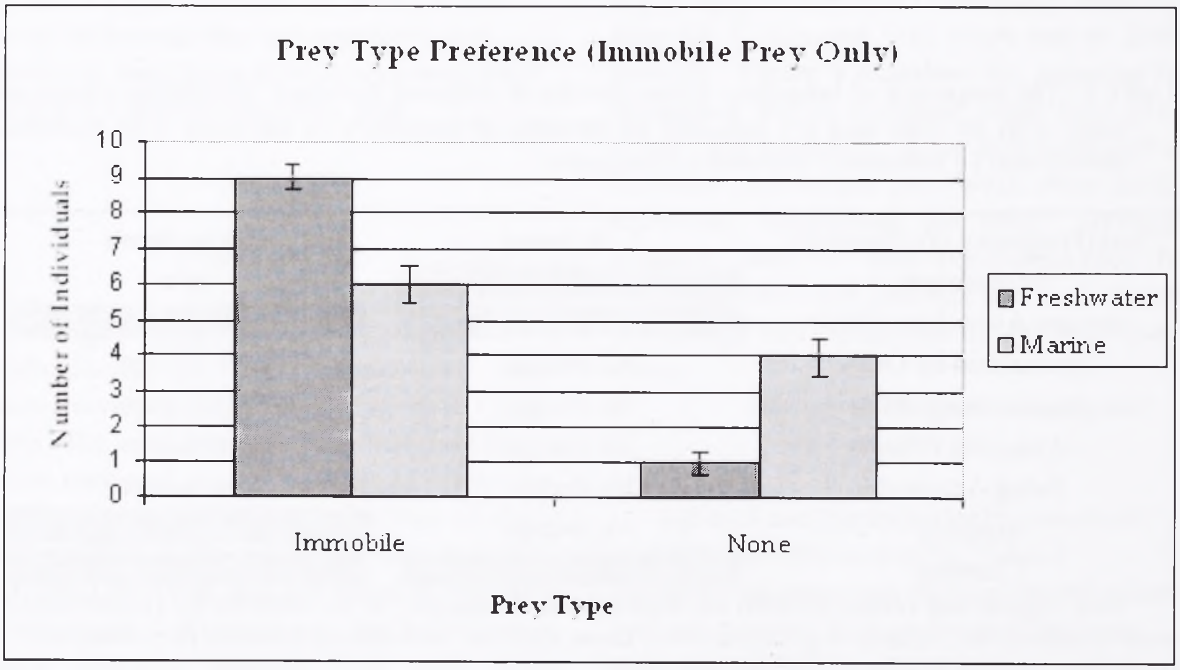


FIGURE 4. Summary of H. hawaiiensis and L. luctuosus prey type preference experiment using two immobile prey items; includes number of individuals the approached prey and number of individuals that did not approach prey items within a five minute observation period.

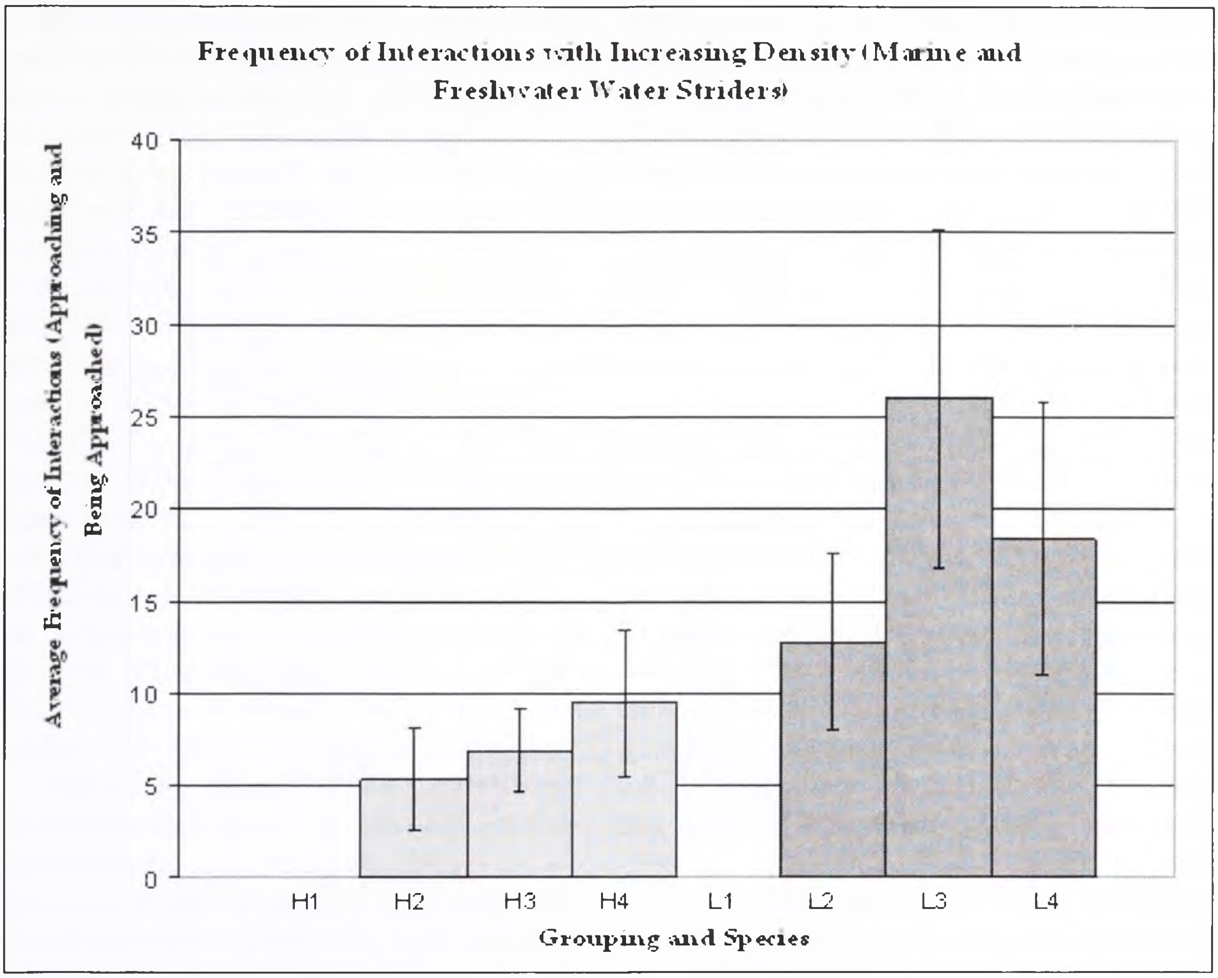


FIGURE 5. Mean Frequency of interactions (approaching others and being approached) with increasing density in two water strider species, *H. hawaiiensis* and *L. luctuosus*. H1 to H4 represent *H. hawaiiensis* alone (H1), in pairs (H2), in groups of three (H3), and in groups of four (H4). L1-L4 represent *L. luctuosus* alone (L1), in pairs (L2), in groups of three (L3), and in groups of four (L4). Error bars represent standard deviations from the mean.

DISCUSSION

The findings of this study indicate that both *H.* hawaiiensis and *L. luctuosus* have a prey type preference. *L. luctuosus* went to mobile prey most often, and had a shorter response time than *H. hawaiiensis* when given the choice between different prey types (Figure 3). When both prey items were immobile, *L. luctuosus* still approached prey items nine times out of ten (Figure 4). Other studies have shown that freshwater gerrids are more partial to prey that is still mobile (Jamieson and Scudder 1977, 1979). There is a relationship between the stillness of water, and prey type preference. Foster and Treherne 1980 explain that "gerrids on still water show a marked preference for moving prey." This could be due to their ability to use ripples on the surface of water as a means of communication.

In contrast, *H. hawaiiensis* approached immobile prey more often (Figure 3). The genus *Halobates* have been shown to be sensitive to prey movements (Foster and Treherne 1980). Even though they will approach moving and non-moving prey items, if prey starts to struggle they will move away quickly (Foster and Treherne 1980, M. Riley unpublished data 2006). In the field, *Halobates sp* were most often found with non-moving prey that had recently died, and was not decomposing (Foster and Treherne 1980). When given the choice of two prey items that were immobile, *H. hawiiensis* approached prey items six times out of ten (Figure 4). While Cheng (1985) found that two other species of *Halobates* (*H. sobrinus* and *H. sericeus*) will approached struggling prey more often than prey that is not struggling, Foster and Treherne (1980) showed that *H. robustus* (a marine water strider from the Galapagos) avoided struggling prey. By approaching only immobile prey, *Halobates* may be avoiding predators (Cheng 1985) by reducing the amount of movement and disturbance on the surface of the water. Other possible explanations could be body size, or availability of food (Foster and Treherne 1980). In rough seas *Halobates* may not have a choice as to what type of prey it encounters, and it may not be able to make the distinction between mobile and immobile prey items (Jamieson and Scudder 1979, Foster and Treherne 1980).

Also, there are some differences in behavior between *H. hawaiiensis* and *L. luctuosus*. *H. hawaiiensis* are more active than *L. luctuosus*; as a result they approach others and are approached more frequently. *H. hawaiiensis* also jumps more frequently than *L. luctuosus* as a way to avoid others. These differences can be explained by the different habitats that these species live in. *H. hawaiiensis* lives on faster flowing water with more disturbance, while *L. luctuosus* was found in areas with slow moving water or in pools without flow (M. Riley unpublished data 2006). Marine water striders are more commonly found in large aggregations which provide protection against predators (Foster and Treherne 1980, Cheng 1985). In these aggregations they may approach and come into contact with other individuals more often than *L. luctuosus*.

Increased density also has some affect on the frequency of behaviors for both *H. hawaiiensis* and *L. luctuosus*. As density increases, both species become more active, and the mean number of interactions of individuals shows an increase (Figure 5). As density increases from one individual to groups of four, *L. luctuosus* shows a steady increase in its interactions with others. This could be a result of trying to avoid other individuals. It also explains the greater frequency of each species to move away from others. As *H. hawaiiensis* increases in the density of individuals, it shows a rise in the mean frequency of interactions with other individuals, (Fig. 4) but there is not as clear of a pattern as with *L. luctuosus*. Since *H. hawaiiensis* is most often found in larger aggregations, individuals may not be as affected by increasing densities as individuals that are normally found in smaller groups like *L. luctuosus*. However *H. hawaiiensis* may express a need to avoid others by increasing the frequency of behaviors like jumping (Table 2). In addition, cleaning behaviors make an interesting change as density increases. *H. hawaiiensis* increases this behavior with increased density, and *L. luctuosus* decreases this behavior with increased density. *L. luctuosus* may decrease this behavior because it is coming into contact with more individuals, and is disturbed more frequently.

Environmental factors greatly impact water strider species like *H. hawaiiensis* and *L. luctuosus*. Due to their different habitats these two species show a significant difference in both food type preference, and the frequency of behaviors. However, there is still much to learn about water striders and the environmental factors that affect their behaviors and distributions. While frequency of behaviors increase with higher densities of individuals, it would be interesting to look at the composition of individuals that make up these groups. It would also be interesting to study marine water striders more closely, and follow there day to day distributions looking at different factors that may have an influence them like wind speed or currents and water flow. Another area that could be explored is the attraction of marine water striders to light, to see if this attraction relates to navigation or other factors. *H. hawaiiensis* and *L. luctuosus* are both fascinating organisms that still offer much to learn about the behaviors and environmental factors that influence water striders in different areas of the world.

ACKNOWLEDGEMENTS

I would like to thank the UC Berkeley Richard B. Gump Research Facility for the use of lab space. In addition, I would also like to thank all of the professors, graduate student instructors, and students of the IB 158 class (especially Sarah Chinn, April Dobbs, Natalie Valencia, and Tom Bell) for all of their hard work, help, and guidance during research and while writing this paper.

LITERATURE CITED

Ambrose, H. W., K. P. Ambrose, D. J. Emlen, and K. L. Bright, 2002, *A Handbook of Biological Investigation: Sixth edition*. North Carolina: Hunter Textbooks.

Andersen, N. M., 1975, The *Limnogonus* and *Neogerris* of the Old World with Character Analysis and a Reclassification of the Gerrinae (Hemiptera: Gerridae). *Entomologica Scandinavia Supplement*. **7**:1-96.

Cheng, L., 1985, Biology of Halobates (Heteroptera: Gerridae). *Annual Review of Entomology*. **30**: 111-35.

Cheng, L., 1989, Factors Limiting the distribution of *Halobates* species. *European Marine Biology Symposium*. **23**:5-9.

Foster, W. A., and J. E. Treherne, 1980, Feeding, predation, and aggregation behaviour in a marine insect, *Halobates robustus* Barber (Hemiptera: Gerridae), in the Galapagos Islands. *Proceedings if the Royal Society of London*. **209**:539-53.

Foster, W. A., and J. E. Treherne, 1982, Group size and Anti-predator Strategies in a Marine Insect, *Animal Behavior*. **32**:536-542.

Foster, W. A., and J. E. Treherne, 1986, The Ecology and Behavior of a marine insect, *Halobates fijiensis* (Hemiptera: Gerridae). *Zoological Journal of the Linnean Society*. **86**:394-412.

Jamieson, G. S., and G. C. E. Scudder, 1977, Food Consumption in *Gerris* (Hemiptera). *Oecologia*. **30**:23-41.

Jamieson, G. S., and G. C. E. Scudder, 1979, Predation in *Gerris* (Hemiptera): reactive distances and locomotor rates. *Oecologia*. **44**:13-20.

Sih, A., and J. V. Watters, 2005, The mix matters: behaviorual types and group dynamics in water striders. *Behaviour*. **142**:1417-1431.

Spence, J. R., and N. M. Adersen, 1994, Biology of Water Striders: Interactions Between Systematics and Ecology. *Annual Review of Entomology*. **39**: 101-28.

Tsokatou, M., L. Cheng, V. Constantinos, and V. Roussis, 2001, Chemical Composition and Behavioral Responses of the Marine Insect *Halobates hawaiiensis* (Heteroptera: Gerridae). *Z. Naturforsch* **56c**: 597-602.

Wilcox, R. S., 1972, Communication by Surface Waves: Mating behavior of a Water Strider (Gerridae). *Journal of Comparative Physiology*. **80**:255-266.

FIGURE 6. Photograph of *L. luctuosus*.

FIGURE 7. Photograph of *H. hawaiiensis*.

CHRISTMAS COLORS: COLORMORPH DISTRIBUTION OF SPIROBRANCHUS GIGANTEUS PALLAS 1766 ON MOOREA, FRENCH POLYNESIA

DANIEL S. SONG

Environmental Sciences, University of California, Berkeley 94720 USA

Abstract. *Spirobranchus giganteus* Pallas 1766 is an obligate associate of coral. This study focused on the distribution of five branchial crown colormorphs (Blue, Brown, Marigold, Purple, and White) on eight coral species (Acropora I, Acropora II, Porites I, Porites II, Porites III, Porites IV, Porites V, Porites VI) by quadrat sampling method. White was the most abundant colormorph, representing 24.0% of the total. Blue was the least abundant colormorph at 9.5% of the total. There were no significant differences in Shannon-Weiner Diversity Index (H') of colormorphs between coral species. Also, relative colormorph abundance did not differ significantly between coral species or between the Front and Back positions. Only Blue and Marigold differed significantly in relative abundance between Top, Midde, and Bottom positions. Findings support a colormorph distribution of colormorphs. There are two possible explanations: 1) mortality and selection effects on distribution and 2) phenotypic plasticity, a combination of genetic and environmental factors contributing to the occurrence of certain phenotypes.

Key words: density; coral species; suspension feeding; phenotype; branchial crown; Polychaeta

INTRODUCTION

Coral reefs are complex systems that provide a multitude of habitats for associates, which use the tissue and skeleton as substrate (Frank et al. 1995). Risk et al. (2001) define "associate" as sessile invertebrates that live in or on the coral. *Spirobranchus giganteus* Pallas 1766 is one of the most conspicuous and colorful of coral associates.

Spirobranchus giganteus is an obligate associate of coral and found in tropical and sub tropical waters (Marsden and Meeuwig 1990). Hove (1970) divided *S. giganteus* into two sub-species: *Spirobranchus giganteus corniculatus* Grube 1862 in the Pacific and *Spirobranchus giganteus giganteus* Pallas 1766 in the Atlantic and Caribbean.

Spirobranchus giganteus is sequentially hermaphroditic and is a broadcast spawner (Kurpiyanova et al. 2001). The pelagic larvae stage lasts between 9 and 12 days (Marsden 1987). The adult worm can live between 10 - 35 years (Smith 1985) with a few surviving over 40 years (Nishi and Nishihira 1996, Nishi and Kikuchi 1996). The species does not burrow, instead it builds a calcareous tube on the surface and the coral colony grows around it (Smith 1984).

As active suspension feeding adults, the paired branchial crowns protrude from the margins of the tube. Each crown is composed of successively smaller tentacle whorls layered with 4 - 6 spiraling about a central stalk (Fig. 1). Tentacles consist of radioles with cilia laden pinnules. The cilia serve to whip water in between the pinnules (Strathmann et al. 1984).

Branchial crowns appear in a wide variety of colormorphs (e.g. blue, brown, marigold,

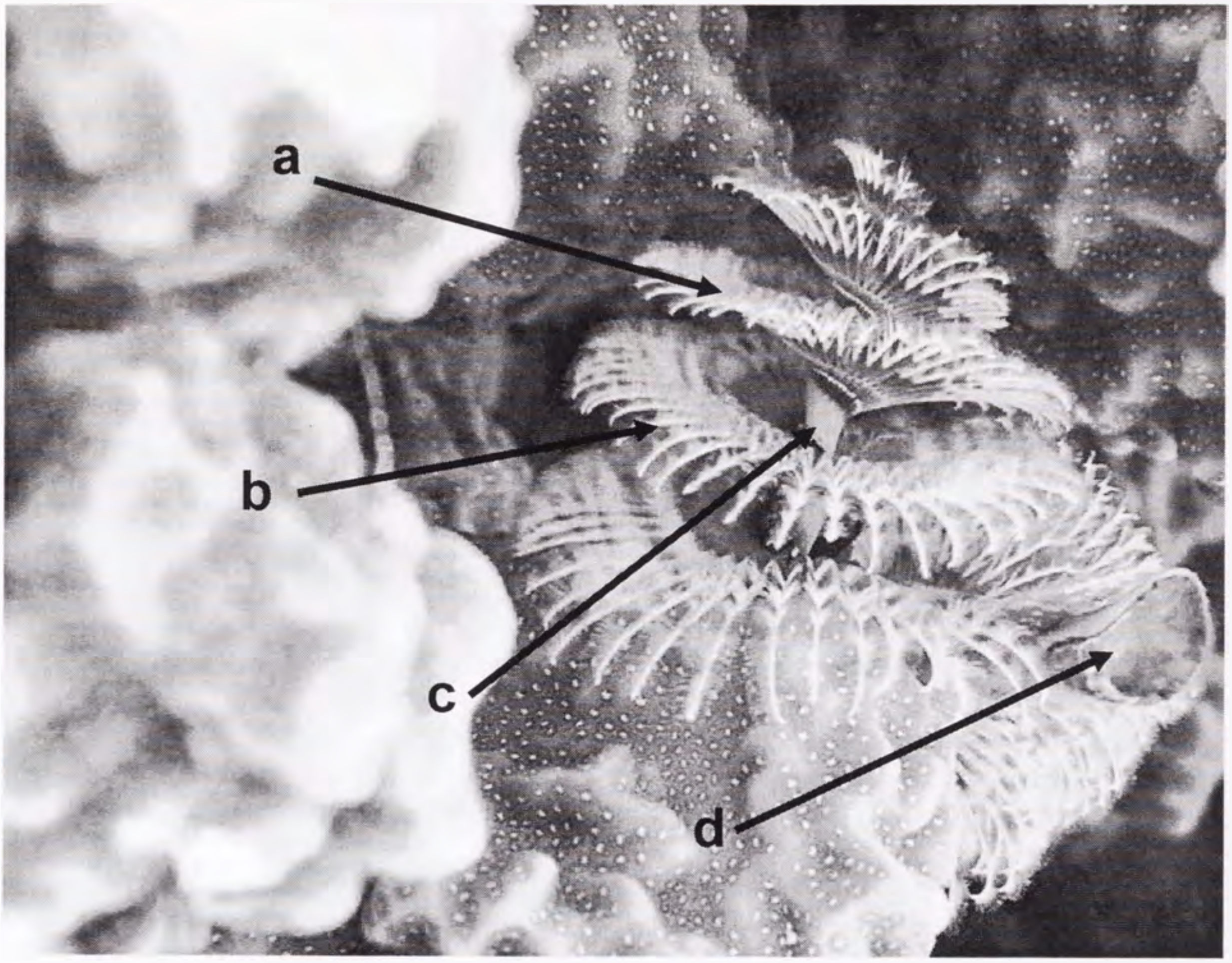


FIG. 1. Picture of a Blue *S. giganteus* colormorph. a) spiraling whorl, b) Individual tentacles with radioles and cilia, c) central stalk and d) operculum.

purple, and white). The downward slope and colorful appearance may be the source of its common name,"Christmas Tree worm".

The presence of branchial crowns and tubes facilitate the flow of water and excrements about the coral surface (Mokady et al. 1998). *Spirobranchus giganteus* also protects coral against predation by irritating the underside of starfish *Acanthaster planci* Linnaeus 1758, inducing it to move (Devantier et al. 1986).

Ben-Tzvi et al. (2006) found that 1) *S. giganteus* colonized area was kept alive and continued to grow while areas adjacent to colonization died and 2) that coral tissue surrounding *S. giganteus* showed no predatory damage and the colonies recovered.

Previous distribution studies of *S. giganteus* show that distribution among coral is non-random and worm densities are higher on certain coral species (Hunte et al. 1990a, Marsden 1990, Marsden and Meeuwig 1990).

Distribution patterns of sessile invertebrates on coral may be affected by mortality rates (Connell 1985). *Spirobranchus giganteus* tends to cluster with increasing number of worms per colony (Dai and Yang 1995).

Hunte et al. (1990b) found that the more heavily colonized the coral, the larger the worm; larger worms were found to possess increased gamete production. The density of *S. giganteus* were found to increase with depth (Floros et al. 2005).

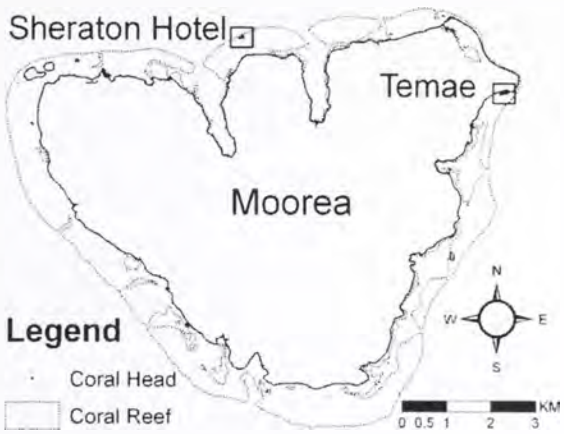


FIG. 2. Sheraton Hotel and Temae public beach, Moorea, French Polynesia.

Colormorphs of *S. giganteus* have not been extensively studied as in the case of other organisms, such as snails. Snail shell colormorphs were found to vary with abiotic environmental factors such as nutrients (Slotow and Ward 1997) and stresses, such as high temperatures (Etter 1988).

One approach to clarifying the occurrence of different *Spirobranchus giganteus* phenotypes is to investigate the distribution of branchial crown colormorphs. The primary purpose of this study is to study the distribution of five branchial crown colormorphs. This paper focuses on the abundance and diversity of colormorphs at different coral positions and species.

METHODS

Site description

The distribution of branchial crown colormorphs was studied on Moorea, French Polynesia at two different sties: Temae public beach (Temae) and near the Sheraton Hotel (Sheraton; Fig. 2). The survey was conducted during October and November 2006.

Temae beach (-17°27'56"N, -151°30'34"W) is a government protected public beach, within the reef crest. The depth varied between 1 - 3 m.

The Sheraton (-17°28′49″N, -149°50′50″W) is within the Plan de Gestion de l'Espace Maritime (PGEM) Marine Protected Area (MPA). Sampling took place between 100 - 140 m from the Sheraton Hotel and depths varied between 2 - 4 m.

Sampling

Quadrats were 3 x 3 m in size and marked off with weighted flags. The quadrats were placed randomly at Temae Beach by kayak, inside the reef crest and at the Sheraton Hotel site by motorboat. To standardize quadrats, the bottom-right corner was set first and both the bottom and top edges were lined up as closely parallel to the reef crest as possible.

Coral-host

Eight different coral species were examined. Coral colonies were included if more than half the coral was present within the quadrat. They were only recorded if live worms were present. Individual coral colonies were determined as separate if they were not visibly attached at the base.

The water temperature at the base, the largest diameter, the tallest height, and GPS location (Garmin 76Cx) of each coral were recorded. Corals were identified to genus offsite with digital photographs and a field guide (Allen and Steen 1994, Vernon 2000). Photographs were taken with a Casio X-Slim and a Casio underwater housing unit.

Colormorph count

Worm presence was determined by visual evidence of living *S. giganteus* inside their tube. Branchial crown colormorphs were only recorded once, only when at least one crown was exposed.

When possible, I positioned myself down-current to reduce turbulence. Positions on corals were standardized by three equal horizontal and two equal vertical planes (Top, Middle, Bottom; Front, Back). The Front of the coral was determined as the plane facing the reef crest.

Colormorph designation

A preliminary survey of branchial crown colormorphs at Temae Beach determined the five colormorphs to study: Blue, Brown, Marigold, Purple, and White (Fig. 3). Colormorphs were designated visually, on-site, by the majority color. Each worm was only counted once and in only one colormorph category.

Variations in colormorph patterns were great. I observed instances where colormorphs were not homogenous. Crowns occurred in various shades and tints (i.e. light purple, dark yellow, tan) and patterns (i.e. banded, striped, dotted).

Statistical analysis

My question was aimed toward patterns on the island as a whole, thus I did not compare sites on the island.

Surface area of coral colonies was estimated to = 2 π r^2, under the assumption that the shape of coral colonies are approximately hemispherical (Dahl 1973). The radius was an average of the height and

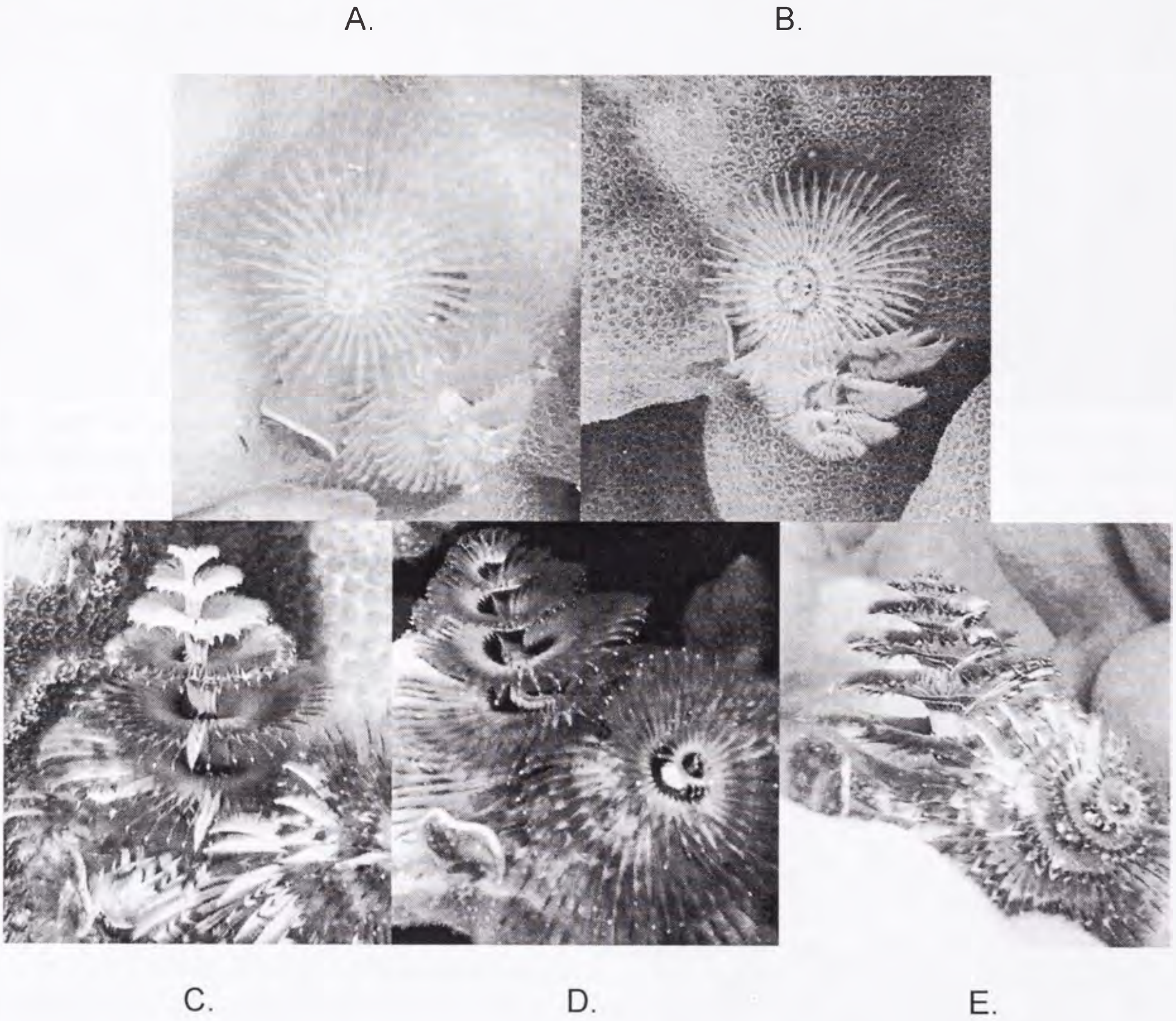


FIG. 3. Colormorph categories: A) Marigold, B) White C) Brown D) Blue and E) Purple.

TABLE 1. Summary of worms and coral surveyed: coral species abundance (n), total surface area (SA, m^2), and *S. giganteus* abundance

Species	*n*	SA	Colormorph				
			White	Brown	Purple	Marigold	Blue
Acropora I	1	9.12	7	4	1	18	3
Acropora II	2	2.47	9	13	6	8	3
Porites I	4	5.17	38	28	32	28	13
Porites II	8	16.67	46	42	36	39	14
Porites III	5	7.13	23	14	33	39	19
Porites IV	6	17.12	83	88	59	54	27
Porites V	5	10.79	53	49	47	75	24
Porites VI	4	9.14	59	53	51	53	23
Total	35	77.61	318	291	265	314	126

of half the diameter.

Shannon-Weiner (Shannon and Weiner 1953) Diversity Index, H′, was calculated for colormorphs at several levels: individual corals; the front and back; and the top, middle, and bottom. The Shapiro-Wilk W-statistic was used to test the worm and coral surface area data for normality.

Parametric analyses were not possible as the data were non-normally distributed and could not be normalized through transformations.

Kruskal-Wallis (K-W) test was used to calculate significant differences in mean values to determine: if H′ of individual corals differed between species; if the relative abundances differed between positions (Front & Back; Top, Middle, & Bottom); if the relative abundance of colormorphs and abundance of worms differed between positions (Front-Back; Top-Middle-Bottom); and if the relative colormorph abundance for each corals differed between coral species.

If significant differences were found, Tukey's test was used to detect where the differences occurred.

Spearman's rank correlation coefficient analyses were used to determine whether surface area correlated with relative abundance of colormorphs.

Kruskal-Wallis tests, Tukey's tests, and Spearman's correlation analyses were performed using JMP 5.1 (SAS Institute 2003).

RESULTS

Distribution of S. giganteus

A total of 1,314 *S. giganteus* were recorded on 8 species of coral (Table 1). The distribution of worms was not normal (S-W: $P < 0.001$). White (318 individuals) was most abundant and Blue (126) was least abundant. Porites I had the highest, 26.9 (worms / m^2) and Acropora I had the lowest overall density, 3.62 (worms / m^2).

Coral species

There were no significant differences in relative abundances of colormorphs between coral species (K-W: $P > 0.24$). There were no significant differences in H′ of colormorphs between coral species ($P = 0.09$; Appendix A).

Distribution of coral surface area was not normal (S-W: $P < 0.001$). Using Spearman's rank correlation coefficient, Brown was the only colormorph not significantly correlated with surface area (Brown r_s=0.13, P = 0.460; Table 2).

TABLE 2. Spearman's rank correlation between colormorph abundance and coral surface area, Rho and probability values (P).

Colormorph	Spearman's rank correlation	
	Rho	P
Blue	0.3891	0.021
Brown	0.1292	0.460
Marigold	0.4257	0.012
Purple	0.4144	0.013
White	0.5485	0.001

Front-Back Position

The relative worm abundance differed significantly between the front and back positions (K-W: $P < 0.001$; Table 3). The front was colonized more ($\bar{x} = 0.59$) than the Back ($\bar{x} = 0.41$). The relative abundances of each colormorph did not differ significantly between the Front and Back (White, $P = 0.84$; Brown, $P = 0.56$; Marigold, $P = 0.84$; Purple, $P = 0.633$; Blue, $P = 0.96$).

Top-Middle-Bottom Position

Relative abundance of worms differed significantly Top to Bottom (K-W: $P < 0.001$). The Top position was colonized the most followed by the Middle and both were different from the Bottom position, the least colonized ($\bar{x} = 0.42$, $\bar{x} = 0.38$, & $\bar{x} = 0.20$, respectively).

The relative abundances of Blue and Marigold ($P = 0.002$ and $P = 0.008$ respectively) differed significantly from Top to Bottom, both colormorphs favoring the Top and Middle positions over the Bottom. The relative abundances of the other colormorphs were not significantly different at each position (Fig. 4).

DISCUSSION

In summary, this study found that there were no differences 1) in H' between coral species and 2) in relative colormorph abundance.

The findings of this study suggest that there are no differences in colormorph diversity as well as abundance of colormorphs on coral.

Mortality and phenotypic plasticity may explain this study's findings of colormorph distribution.

Death is a possible explanation for the apparent random distribution of colormorphs. Mortality by density-independent means, such as predation and habitat constraints, may affect distribution of marine sessile invertebrates (Connell 1985).

Like the polymorphic *Littorina sp.*, a Mangrove snail, which was found to change in colormorph frequencies over time (Hughes and Mather 1985, Johannesson and Ekendahl 2002) a similar change in distribution also studied in (*Argiope keyserlingi*), the St.

TABLE 3. Probability values (P) for the K-W test to determine significant differences in relative colormorph abundances between positions (mean ± SE).

Color	P	Position	
		Front	Back
Blue	0.957	0.076 ± 0.063	0.112 ± 0.182
Brown	0.559	0.232 ± 0.162	0.212 ± 0.188
Marigold	0.841	0.196 ± 0.140	0.185 ± 0.136
Purple	0.633	0.230 ± 0.191	0.211 ± 0.198
White	0.837	0.266 ± 0.190	0.251 ± 0.166

TABLE 4. Probability values (*P*) for K-W test to determine which colormorphs differed between coral positions (Top, Middle, and Bottom).

Colormorph	*P*	Position		
		Top	Middle	Bottom
Blue	0.002	0.086 ± 0.010	0.101± 0.091	0.037± 0.076
Brown	0.879	0.218 ± 0.171	0.200 ± 0.167	0.225 ± 0.260
Marigold	0.008	0.262 ± 0.219	0.199 ± 0.176	0.135 ± 0.176
Purple	0.900	0.212 ± 0.195	0.196 ± 0.154	0.240 ± 0.234
White	0.567	0.223 ± 0.169	0.276 ± 0.208	0.278 ± 0.262

Andrew's Cross spider (Hoese et al. 2006). In both cases, distributions of colormorphs were attributed to predation.

S. giganteus predation has not been well documented. On a few occasions, I did observe damaged branchial crowns as well as completely missing or partially broken opercula. This could indicate possible attempts at predation by.

The abiotic aspects of the environment may also have an effect on colormorph distribution. Sessile invertebrates were found to influence the flow of water around coral surface areas, thus affecting nutrition available to the worms (Ben-Tzvi et al. 2006).

This may lead to the accessibility of certain nutrients, such as n the case of a desert landsnail (*Trochoidea simulata*). Slotow and Ward (1997) found that $CaCO_3$ abundance was a predictor to the frequency of shell colormorphs in *T. simulata*.

Environmental stress is also a potential factor influencing polymorphism. In the case of *Nucella lapillus*, an intertidal snail, high energy wave action and temperature was related to increased polymorphism, where as sheltered snails exhibited a higher frequency of white shells (Etter 1988).

The depths of the coral colonies in both Temae and Sheraton were relatively low in comparison to some worms found in excess of 18 m (Floros et al. 2005). The close proximity to the surface of the water may influence the fauna composition as well as subject coral and worms to abiotic factors such as sun light, current speeds, and current direction.

The consideration of both environmental and genetic factors may also be a possible explanation for. Hadfield et al. (2006) found that phenotypes were poor proxies for insight in genetic patterns for color of *Parus caeruleus* (Tit bird) but found presence of common environmental factors to be important.

Phenotypic plasticity may help explain the dichotomy between the environment and genetics. Phenotypic plasticity is the consideration of both nature (genetics) and nurture (environment) to explain variations in phenotype (Pigliucci 2001).

In the example of *Daphnia magna*, depending on the abundance of food, *D. magna* produced varying clutch sizes, responding to environmental stresses. *D. magna* would produce larger clutch sizes when food was scarce and smaller clutch sizes for when food was abundant (Ebert et al. 1993).

As it relates to S. giganteus, there is the possibility that colormorphs may not be any one single factor influencing phenotype. It could be influenced by a polymorphic genotype, environmental factors, or even both.

The possibility exists that the cause of branchial crown colormorphs could be dictated by a combination of abiotic and genetic factors beyond the scope of this study.

The findings of this study calls prompts a meaningful question in regards to phenotype distributions: are colormorphs heritable?

A more proximal study could be conducted to determine spatial distribution patterns at the individual coral level (i.e. nearest neighbor method) done similarly with worm-density studies (Dai and Yang 1995).

The next step in uncovering the pattern of colormorph distribution would be to investigate the genetic factors. This would shed light as to the mechanisms that may be responsible for S. giganteus branchial crown colormorphs and for their distribution.

ACKNOWLEDGEMENTS

I would like to thank:

Jere Lipps and Carole Hickman for their constant availability, Vince Resh for a source of endless enthusiasm, Jamie Bartolome for his statistical prowess, and Brent Mishler for his encouragement.

Elena Kupriyanova for graciously offering her help for a curious undergraduate.

Liz Perotti, Alison Purcell, and Erica Spotswood for all their help.

Carol Chang and Tom Hata for their valued company and assistance throughout.

And the rest of my colleagues for their help and lifetime supply of memories.

LITERATURE CITED

Allen, G. R., and R. Steen. 1994. Indo-Pacific Coral Reef Field Guide. Tropical Reef Research, Singapore.

Ben-Tzvi, O., S. Einbinder, and E. Brokovich. 2006. A beneficial association between a polychaete worm and a scleractinian coral? Coral Reefs **25**:98.

Connell, J. H. 1985. The consequences of variation in initial settlement vs. post-settlement mortality in rocky intertidal communities. Journal of Experimental Marine Biology and Ecology **93**:11-45.

Dahl, A. L. 1973. Surface area in ecological analysis, quantification of benthic coral reef algae. Marine Biology **23**:239-249.

Dai, C.-F., and H.-P. Yang. 1995. Distribution of Spirobranchus giganteus corniculatus (Hove) on the coral reefs of southern Taiwan. Zoological Studies **34**:117-125.

Devantier, L. M., R. E. Reichelt, and R. H. Bradbury. 1986. Does Spirobranchus giganteus protect host Porites from predation by Acanthaster planci: predator pressure as a mechanism of coevolution. Marine Ecology Progress Serie **32**:307-310.

Ebert, D., L. Yampolsky, and S. C. Stearns. 1993. Genetics of life history in Daphnia magna. I. herbitatbilities at two food levelss. Heredity **70**:335-343.

Etter, R. J. 1988. Physiological Stress and Color Polymorphism in the Intertidal snail Nucella Lapillus. Evolution **42**:660-680.

Floros, C. D., M. G. Samways, and B. Armstrong. 2005. Polychaete (Spirobranchus giganteus) loading on South African corals. Aquatic Conservation **15**:289-298.

Frank, U., I. Brickner, B. Rinkevich, Y. Loya, R. P. M. Bak, Y. Achituv, and M. Ilan. 1995. Allogeneic and xenogeneic interactions in reef-building corals may induce tissue growth without calcification. Marine Ecology Progress Series **124**:181-188.

Hadfield, J. D., A. Nutall, D. Osorio, and I. P. F. Owens. 2006. Testing the phenotypic gambit: phenotypic, genetic and environmental correlations of colour. Journal of Evolutionary Biology:1-9.

Hoese , F. J., E. A. J. Law, D. Rao, and M. E. Herberstein1). 2006. Distinctive yellow bands on a sit-and-wait predator: prey attractant or camouflage? Behavior **143**:763-781.

Hove, H. A. 1970. Serpulinae (Polychaeta) from the Caribbean. 1. The genus Spirobranehus. Studies of Fauna of Curacao and Other Caribbean Islands **32**:1-57.

Hughes, J. M., and P. B. Mather. 1985. Evidence for Predation as a Factor in Determining Shell Color Frequencies in a Mangrove Snail Littorina SP. (Prosobranchia: Littorinidae. Evolution **40**:68-77.

Hunte, W., B. E. Conlin, and J. R. Marsden. 1990a. Habitat selection in the tropical polychaete Spirobranchus giganteus . 1. Distribution on corals. Marine Biology **104**:87-92.

Hunte, W., J. R. Marsden, and B. E. Conlin. 1990b. Habitat selection in the tropical polychaete Spirobranchus giganteus . 3. Effects of coral species on body size and body proportions. Marine Biology **104**:101-107.

Johannesson, K., and A. Ekendahl. 2002. Selective predation favouring cryptic individuals of marine snails (Littorina). Biological Journal of the Linnean Society **76**: 134-144

Kurpiyanova, E., E. Nishi, H. A. t. Hove, and A. V. Rzhavsky. 2001. Life-history patterns in serpulimorph polychaetes: ecological and evolutionary perspectives. Oceanography and Marine Biology **39**:1-102.

Marsden, J. R. 1987. Coral preference behaviour by planktotrophic larvae of Spirobranchus giganteus corniculatus (Serpulidae: Polychaeta). Coral Reefs **6**:71-74.

Marsden, J. R., B. E. Conlin, and W. Hunte. 1990. Habitat selection in the tropical polychaete Spirobranchus giganteus . 2. Larval preferences for corals. Marine Biology **104**:93-99.

Marsden, J. R., and J. Meeuwig. 1990. Preferences of planktotrophic larvae of the tropical serpulid Spirobranchus-giganteus pallas for exudates of corals from a Barbados West Indies reef. Journal of Experimental Marine Biology and Ecology **137**:95-104.

Mokady, O., Y. Loya, and B. Lazar. 1998. Ammonium contribution from boring bivalves to their coral host - a mutualistic symbiosis? Marine Ecology Progress Series **169**:295-301.

Nishi, E., and T. Kikuchi. 1996. Preliminary observation of the tropical serpulid Spirobranchus giganteus corniculatus Pallas. Publications from the Amakusa Marine Biological Laboratory, Kyushu University **12**:45-54.

Nishi, E., and M. Nishihira. 1996. Age-estimation of the Christmas tree worm Spirobranchus giganteus (Polychaeta, Serpulidae) living buried in the coral skeleton from the coral-growth band of the host coral. Fisheries Science **62**:400-403.

Pigliucci, M. 2001. Phenotypic Plasticity. John Hopkins University Press, Baltimore, MD.

Risk, M. J., J. M. Heikoop, E. N. Edinger, and M. V. Erdmann. 2001. The assessment 'toolbox': Community-based reef evaluation methods coupled with geochemical techniques to identify sources of stress. Bulletin of Marine Science **69**:443-458.

Shannon, C. E., and W. Weiner. 1953. The Mathematical Theory of Communication. University of Ilinois Press, Urbana, USA.

Slotow, R., and D. Ward. 1997. Habitat Constraints on Shell-Colour Variation of a Desert Landsnail, Trochoidea Simulata. The Journal of Molluscan Studies **63**:197-205.

Smith, R. 1984. Development and settling of Spirobranchus-giganteus polychaeta serpulidae. Pages 461-483 *in* P. A. Hutchings, editor. First International Polychaete Conference. The Linnean Society of New South Wales, Sydney.

Smith, R. 1985. Photoreceptors of serpulid polychaetes. James Cook University of North Queensland, Townsville, Australia.

Strathmann, R. R., R. A. Cameron, and M. F. Strathmann. 1984. Spirobranchus-giganteus breaks a rule for suspension feeders. Journal of Experimental Marine Biology and Ecology **79**:245-249.

Vernon, J. E. N. 2000. Corals of the World. Australian Institute of Marine Science, Townsville, Australia.

APPENDIX A

Abundance of each coral species (n), mean H′ value for total coral species with standard deviation.

Species	n	Mean	Standard deviation
Acropora I	1	1.178	*
Acropora II	2	1.360	0.148
Porites I	4	1.200	0.407
Porites II	8	1.392	0.0959
Porites III	5	1.127	0.358
Porites IV	6	1.403	0.119
Porites V	5	1.497	0.032
Porites VI	4	1.540	0.019

*only 1 individual

APPENDIX B

Abundance of each coral species (n), mean H′ value (Mean) for total coral species with standard deviation.

Colormorph	K-W	Tukey's Test Results							
		Acropora I	Acropora II	Porites I	Porites II	Porites III	Porites IV	Porites V	Porites VI
Blue	0.6657	No Significant Differences Found							
Brown	0.2450								
Marigold	0.2395								
Purple	0.5402								
White	0.7258								

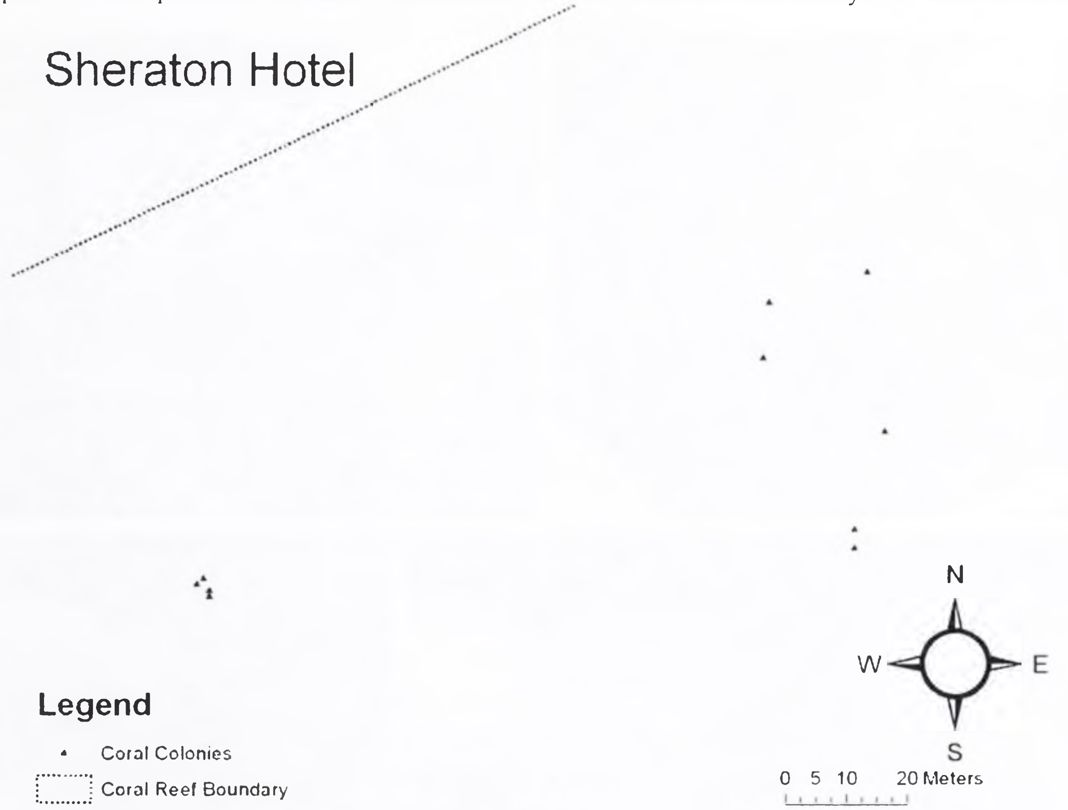
APPENDIX C

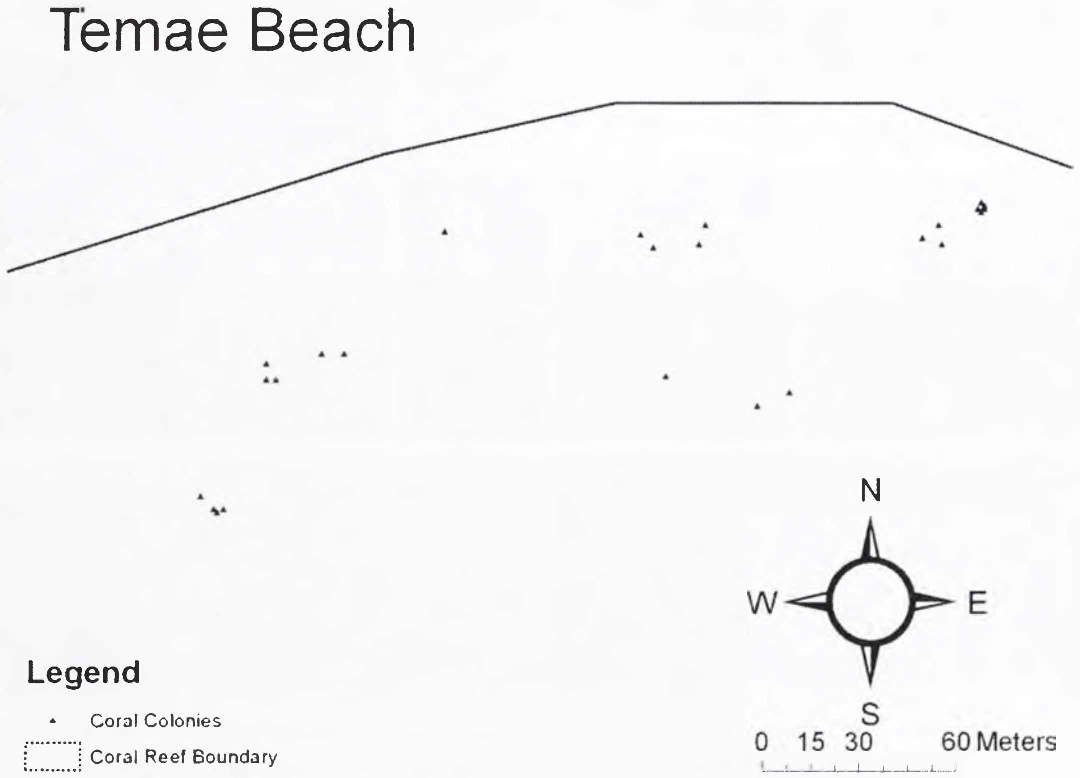
Abundance for each colormorph (n) by position.

Colormorph	Position				
	Front	Back	Top	Middle	Bottom
White	175	143	98	134	86
Brown	156	135	115	124	52
Marigold	148	117	111	108	46
Purple	211	103	108	133	73
Blue	64	62	41	69	16
Total	754	560	473	568	273

APPENDIX D

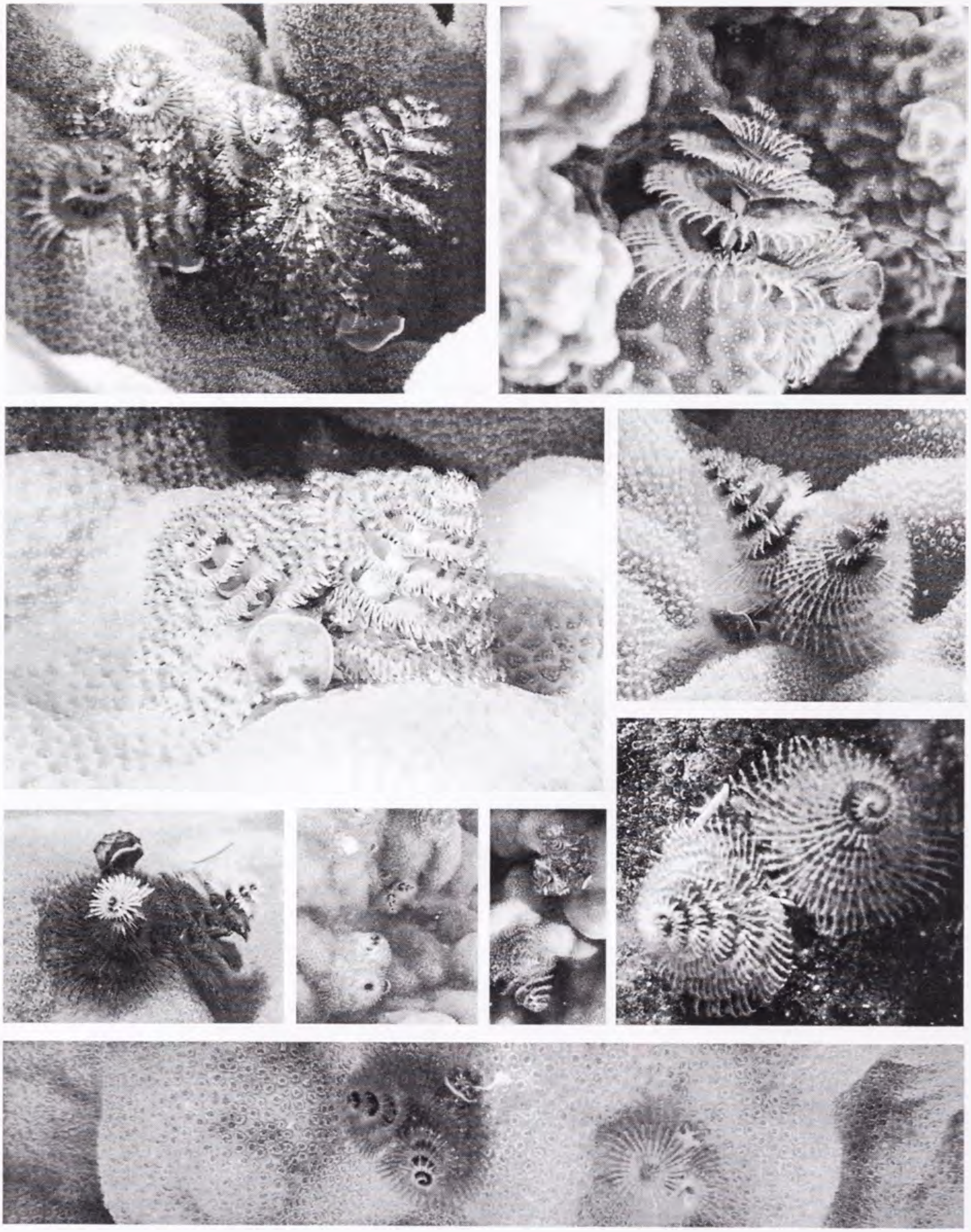
Site maps of Temae public beach and Sheraton Hotel with coral reef boundary and individual coral.





APPENDIX E

Various colormorphs of *S. giganteus corniculatus*.



DIFFERENCES IN THE DIUNRAL AND NOCTURNAL DEFENSE MECHANISMS OF *OCTOPUS BOCKI* (ADAMS, 1941)

NATALIE VALENCIA

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

Abstract. Octopuses are known for the advanced behaviors and elaborate displays used in predator avoidance. Although studies have provided anecdotal evidence on the defense mechanisms of these animals, whether these behaviors vary under light and dark conditions is unknown. This study investigated the diurnal and nocturnal predator defense mechanism s of *Octopus bocki* (Adams, 1941) in Moorea, French Polynesia. Seven behaviors were identified as primary defense mechanisms for protection from fish predators during daylight and nighttime hours. Rates of occurrence and durations for defense behaviors significantly differed between diurnal and nocturnal conditions, as *O. bocki* frequently Crawled during the daylight hours , but sat still and Curled during the nighttime hours. Results indicate that *O. bocki* modifies predator defense behaviors for survival under light and dark conditions.

Key words: octopus signal transmission, antipredator defense mechanisms, diurnal and nocturnal behaviors, predation

INTRODUCTION

Animals evolve antipredator defenses that account for biotic and abiotic factors that may inhibit successful signal transmission to the predator (Bradbury and Vehrencamp, 1998). Biotic factors include the predator's sensory system, while abiotic factors can include phenomena such as ambient lighting. (Bradbury and Vehrencamp, 1998)

Octopuses are known for their elaborate visual displays used to deter predators. Some of the best-studied antipredator displays include crypsis, aposematic coloration, mimicry, and polyphenism (Hanlon and Messenger, 1996, Hanlon, et al., 1999). Although these defenses are well known, it is not known whether they are used during both daylight and nighttime hours.

Octopus bocki is a pygmy octopus found on Moorea, French Polynesia. Individuals are nocturnal and inhabit dens in dead coral rubble along coral reef crests. Past studies on the species have looked at their learning abilities (Lebensohn, 1999) sensory cues (Johnson, 1995), as well as intraspecific den defense (Logan, 2001). Although some of their defenses have been studied, the identification and usage of their antipredator defenses remains to be investigated.

This study investigated the antipredator defense mechanisms of *Octopus bocki* under diurnal and nocturnal conditions. I tested three hypotheses: 1) octopuses would adjust their defense mechanisms for daylight and nighttime hours; 2) octopuses would ink and jet away more frequently during the daylight hours, as ambient lighting would increase the predators' detection

MATERIALS AND METHODS

Biology of Octopus bocki

Octopus bocki is a nocturnal species of pygmy octopus found in Moorea, French Polynesia (Cheng, 1996, Caldwell, 2005). It inhabits cavities in coral rubble distributed along coral ridge crests throughout the island (Cheng, 1996). Juveniles are transparent to light brown in coloration and flash red chromatophores, while adults are a solid dark brown but turn transparent or speckled when disturbed (Valencia, pers. obs.). Males with 5mm mantle length (ML) or greater can be distinguished from females by the presence of a hectocotylus, a modified third right arm used in the transfer of spermatophores (Cheng, 1996).

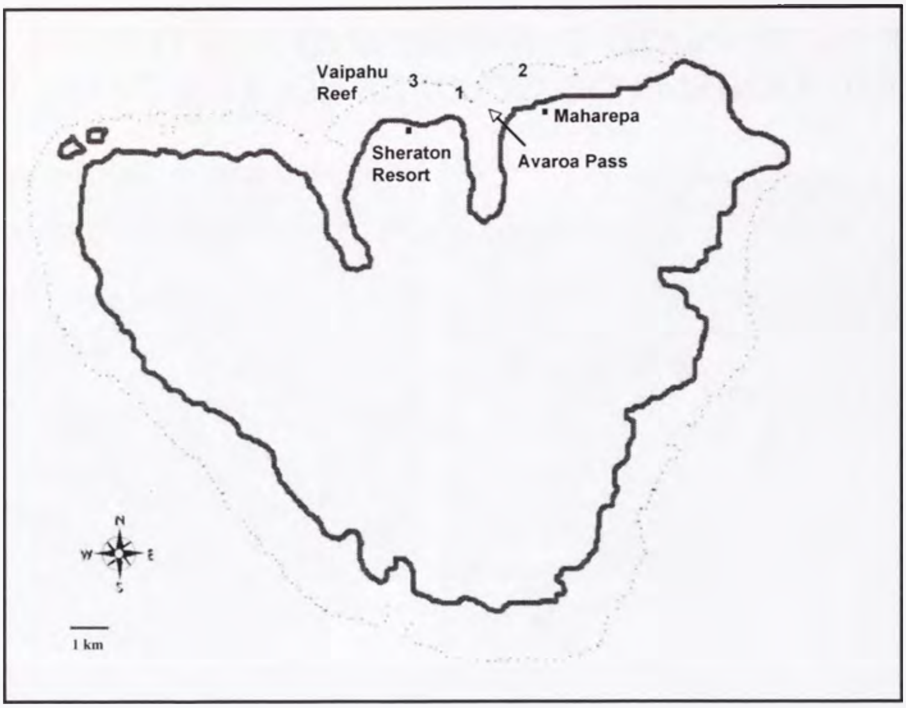


Figure 1. Map of Moorea showing sites from which octopuses were collected. Animals were collected from three sites: 1) Vaipahu Reef near Cook's Bay; 2) a reef located 1 km east of Avaroa Pass; 3) Vaipahu Reef across from the Sheraton Resort. (Adapted from Cheng, 1996.)

Collection and Maintenance Individuals were collected from three sites around Moorea: (1) northwest of Cook's Bay on Vaipahu Reef,; (2)1 km east of the Avaroa Pass across from Maharepa; (3) across from the Sheraton Resort on Individuals were collected from three sites a around Moorea: (1) northwest of Cook's Vaipahu Reef (Fig. 1; Table 1). A boat was necessary to reach sites 2 and 3 however, a kayak was sufficient to reach site. 1. Large bins containing one brick each were taken out to the crests where they were filled with porous coral rubble collected from areas with strong wave action (Fig. 2). The bins were left undisturbed for at least 30 min, allowing water to drain from the rocks. After sitting for at least 30 min, the bottoms of the bins were searched for octopuses (Lebensohn, 1999). Twenty-one octopuses were collected and placed in plastic cups with lids and were transported to the University of California, Berkeley, Richard B. Gump Research Station. Each animal was kept in individual plastic containers at the wet lab, which provided running seawater for daily water changes. In keeping with the octopuses' nocturnal habits, feedings took place every night after testing was completed. A red light was used to feed the animals in the dark to prevent disturbance of their circadian rhythms. Juveniles were provided with freshly- collected marine zooplankton.

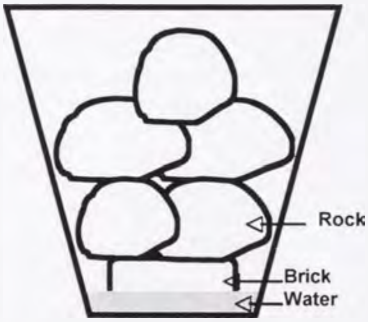


Figure 2. Sample bin used for collecting octopuses. Bins were taken out to reef crests, where they were filled with porous rocks. They were then left undisturbed for 30 min, emptied and searched for octopuses.

Table 1. GPS coordinates, water depth, and description of habitat for sites from which octopuses were collected. GPS coordinates are given in UTM. Coordinates for location 2 were not taken.

Site	Location	GPS Coordinates (utm)	Water Depth (m)	Habitat Description
1	Vaipahu Reef, northwest of Cook's Bay	WGS 1984 Datum 0199980 6South8064842	0-1	Barrier reef crest; frequent wave action; sparse dead rubble
2	1 km east of Avaroa Pass, across from Maharepa		0-4	Reef flat; strong wave action; large coral heads; dense patches of dead coral rubble
3	Vaipahu Reef across from Sheraton Resort	WGS 1984 Datum0197595 6South8065043	0-4	Reef flat; strong wave action; large coral heads; dense patches of dead coral rubble

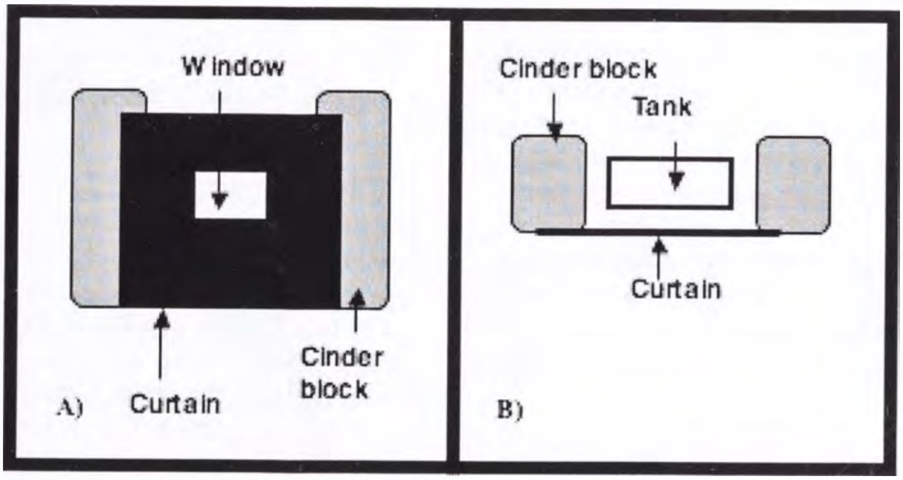


Figure 3. Experimental design. A) Front view; B) Top view. A tank (arena) was placed between two cinder blocks. The front wall of the tank was covered by a curtain attached to both blocks. A small window on the curtain allowed the researcher to observe interactions between fish and octopus.

Twenty-one octopuses were collected and placed in plastic cups with lids and were transported to the University of California, Berkeley, Richard B. Gump Research Station. Each animal was kept in individual plastic containers at the wet lab, which provided running seawater for daily water changes. In keeping with the octopuses' nocturnal habits, feedings took place every night after testing was completed. A red light was used to feed the animals in the dark to prevent disturbance of their circadian rhythms. Juveniles were provided with marine zooplankton collected fresh every night, while adults were fed live crustaceans collected throughout the day.

Testing for Differences Between Diurnal and Nocturnal Predator Defense

Fish were collected off the station dock to serve as predators during testing. A baited fish line with a small hook and sinker was used to catch the fish with little injury to the animals. After collection, the fish were identified to species before being placed in a tank with flowing seawater. Each fish was immediately returned to it's collection site after testing, and no fish was kept for more than 4 day

Testing was done twice a day during daylight and nighttime hours. One *O. bocki* and one fish were randomly chosen for each test using an EXCEL random number generator. An arena was set up outside for natural lighting. The arena consisted of a tank sitting between two cinder blocks. The front wall of the tank was covered by a curtain on one side to minimize the effects of the behavior (Fig 3). Tests conducted at night were visualized under red lighting, and all tests lasted for 5 min. This same procedure was done using an empty arena to control researcher's presence on the animals' for the effects of human handling on the animals' behavior. Twenty tests were run for both diurnal and nocturnal conditions; ten controls were run for both treatments.

Preliminary Tests

Preliminary tests were done using the experimental design described above.
Tests were used to identify *O. bocki* defensive postures and body movements relevant to the study (see Results). As it was difficult to assess body coloration and texture at night under red lighting, these behavioral parameters were excluded from the study.

Statistical Analysis

Rate data for defense mechanisms were not normally distributed and were, therefore, square root transformed prior to statistical testing. Similarly, duration data for all mechanisms were log transformed prior to analysis. T-tests were done using JMP statistical software to test for differences between the mean rates and durations of diurnal and nocturnal defense mechanisms. As treatments consisted of 20 replicates and controls consisted of 10 replicated, these data could not be compared for differences between means.

RESULTS

Octopus bocki Predator Defense

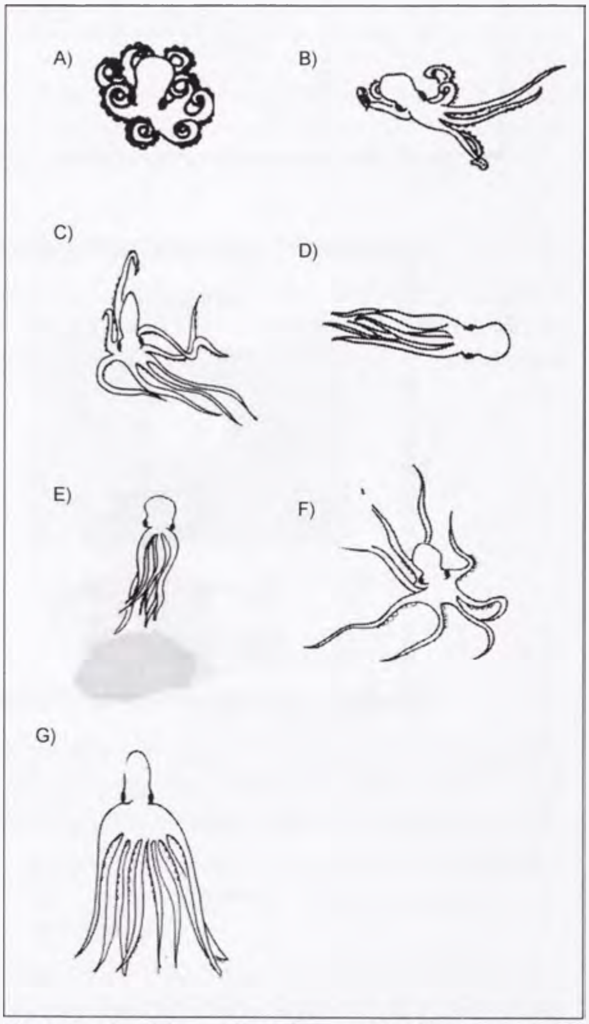


Figure 4. Line drawings of *Octopus bocki* defense mechanisms used for diurnal and nocturnal treatments. See text next page.

Seven defense strategies were identified for both diurnal and nocturnal tests (See Fig 4A-G):

A. Curl: Sitting still with arms held (tightly or slightly extended) against the body and curled around the mantle.

B. Crawl: Walking with arms extended.

C. Mantle Erect: Mantle is stretched and narrow while crawling or sitting still.

D. Jet Propulsion: Expulsion of water via the funnel, resulting in rapid swimming (Hanlon and Messenger, 1996).

E. Spread: Sitting still with the body flat against the substrate and all arms extended.

F. Ink: Release of black ink.

G. Ballooning: The web, head, and mantle are lifted with arms held against the substrate.

Assessing Differences Between Diurnal and Nocturnal Predator Defense

Octopuses were mobile in the presence of a predator during the daylight hours, but were less mobile at night. Crawling had the highest rate of occurrence (18.98±7.36 defense/sec) followed by Spread (10.91± 5.07 defense/sec) and Curl (9.15± 4.54 defense/sec) (Fig. 5A, Table 2). There were significant differences in the mean rates of Crawl (*t*-test, P<0.0001) and Curl (*t*-test, P<0.0001) between diurnal and nocturnal treatments (Table 2). All other defenses were rarely observed during the day (Fig. 5A, Table 2). Inking, in particular, was very rare, occurring in only 3 out of 40 tests, when the fish was 0 to 1 cm away from the octopus (Table 3) The most frequent antipredator defense used at night was Spread (8.87±3.71 defense/sec). There were no significant differences in the mean rates of Spread between treatments (*t*-test, P=0.1477).

Mean durations for defenses differed between diurnal and nocturnal conditions. Curls were held the longest during both daylight (1.23±0.54 sec) and nighttime (1.47±0.94 sec) (Fig. 5B, Table 2). Spread had the second longest durations during the day (0.90±0.50 sec) and night (1.41±0.62 sec) (Fig. 5B, Table 2). Mean durations for Spread significantly differed between daylight and nighttime hours (*t*-test, P<0.0001) (Table 2).

DISCUSSION

Octopus bocki Predator Defense

Data suggest that the type of defense mechanism does not depend on time of day. *Octopus bocki* used the same seven antipredator defense mechanism s for diurnal and nocturnal treatments. The horned lizard, *Phrynosoma cornutum*, elicits blood-squirting in the presence of a predator (Middendorf and Sherbrooke, 1992). Frequencies of blood-squirting did not significantly differ between diurnal and nocturnal hours, but were affected by predator type (Middendorf and Sherbrooke, 1992). Similarly, *O. bocki* did not change the type of antipredator mechanisms to account for the time of day, but mechanisms varied in rates of occurrence and durations.

Assessing Differences Between Diurnal and Nocturnal Predator Defense

Crawling appears to be a primary antipredator behavior during light hours.

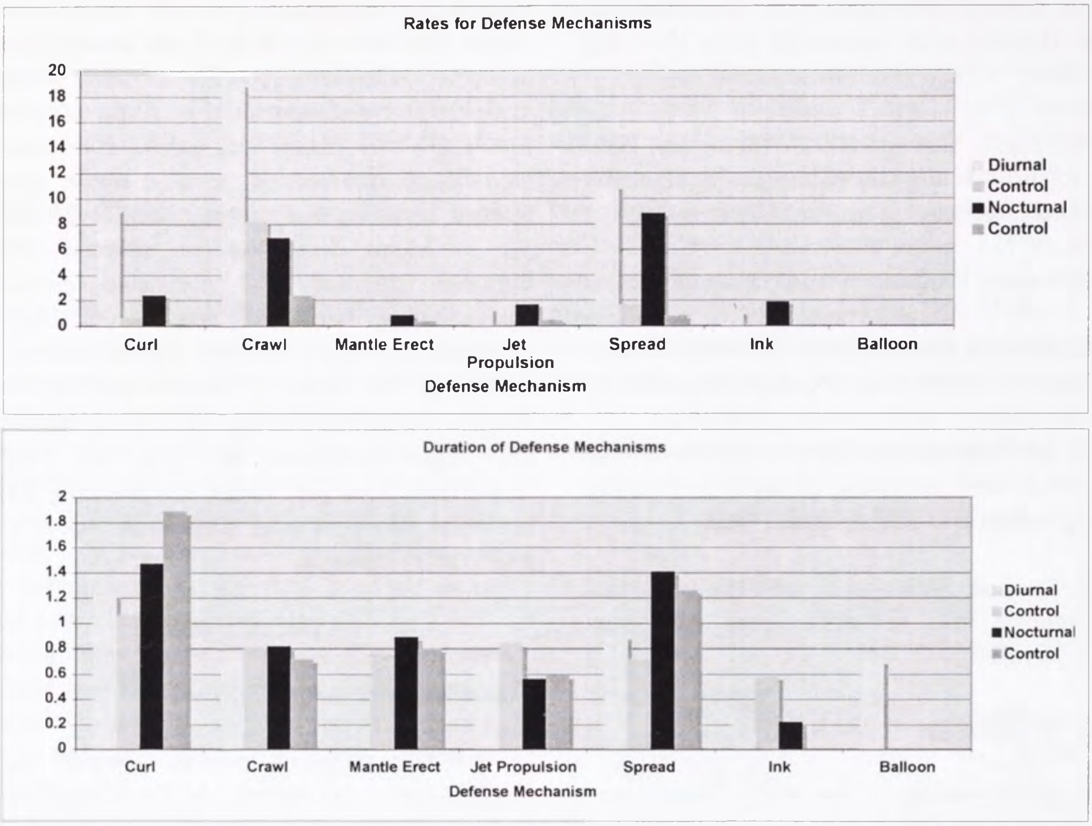


Figure 5. A) Mean rates of defense behaviors for diurnal (N=20) and nocturnal treatments (N=20), and controls (N=20). Crawl had the highest rate of occurrence under diurnal conditions (18.98±7.36 defense/sec), while Spread was the most common behavior under nocturnal conditions (8.87±3.71 defense/sec). Crawl was the most common defense behavior for both diurnal (8.18±4.61 defense/sec) and nocturnal (2.87 defense/sec±2.38) controls; B) Mean durations of defense behaviors for diurnal and nocturnal treatments, and controls. Curls were held the longest during diurnal (1.23±0.55 sec) and nocturnal (1.47 ±0.94 sec) treatments, as well as for diurnal (1.06±0.5 sec) and nocturnal (1.89±0.74 sec) controls. Blue bars indicate results for diurnal treatments; red bars indicate results for diurnal controls; yellow bars indicate results for nocturnal treatments; green bars indicate results for nocturnal controls.

Results revealed that Crawling was used more than any other behavior during diurnal treatments. Daylight hours provide more sunlight and increase a predator's ability to detect prey. Easy detection by the fish may have induced a flee response in the octopuses, resulting in frequent crawling to escape the predator. This hypothesis, however, does not explain the infrequent occurrence of jet propulsion. If the octopuses had indeed attempted to rapidly escape the predator, jet propulsion would have occurred frequently, as it is a rapid escape response in cephalopods (Anderson, 2001, Hanlon and Messenger, 1996).
Alternatively, *O. bocki* may actively seek for dens for protection during the day.

Nocturnal octopuses are known to spend the day in their dens, leaving them only at night to forage (Hanlon and Messenger, 1996). It is likely that *O. bocki*, a nocturnal pygmy octopus, spends most of the day hidden from predators in its den, and, therefore, searches for cavities in response to predation during the daylight hours.

Immobility may be an effective antipredator defense at night, increasing crypticity when visibility is difficult for the predator. Curling and Spreading, both resting states, had the highest rate of occurrences at night. Cryptic animal s resemble their surroundings so that predators cannot distinguish them from their background environment (Bradbury and Vehrencamp, 1998).

Table 2. Mean rates and durations of defense mechanisms for diurnal and nocturnal treatment. Results for t-test testing for significant differences between diurnal and nocturnal means are also given. Crawl had the highest rate of any other behavior (18.98±7.36 defense/sec, Mean±S.D.) under diurnal conditions. Spread occurred at a higher rate (8.87±3.71 defense/sec, Mean± S.D.) under nocturnal conditions. There were significant differences between the diurnal and nocturnal rates of Curl and Crawl ($p<0.0001$). The duration of Curl was highest under diurnal (1.23±0.54 sec, Mean±S.D.) and nocturnal (1.47±0.94 sec, Mean±S.D.) conditions. Mean durations of Spreads significantly differed between treatments ($p<0.0001$).

Defense	Mean Rate±S.D. (defense/sec)		_P_-value*	Mean Duration±S.D. (sec)		_P_-value*
	Diurnal (N=20)	Nocturnal (N=20)		Diurnal (N=20)	Nocturnal (N=20)	
Curl	9.15±4.54	2.42±3.10	**<0.0001**	1.23±0.54	1.47±0.94	0.4478
Crawl	18.98±7.36	6.92±5.81	**<0.0001**	0.78±0.31	0.82±0.50	0.6098
Mantle Erect	0.29±1.23	0.87±2.12	0.4252678	0	0.89±0.27	n/a
Jet Propulsion	1.37±2.92	1.68±3.05	0.7552266	0.69±0.46	0.56±0.66	0.64
Ink	1.08±2.75	1.91±3.93	0.3863901	0.30±0.55	0.22±0.94	0.83
Ballooning	0.57±1.78	0	0.1624	0.71±0.67	0	n/a
Spread	10.91±5.07	8.87±3.71	0.1477078	0.90±0.50	1.41±0.62	**<0.0001**

* Significant values ($p<0.05$) are highlighted in bold.

As crypticity involves a match to the surroundings, immobility is crucial to its effectiveness (Hanlon, and Messenger, 1996, Bradbury and Vehrencamp, 1998) Shallow-water cephalopods are known to exploit this phenomenon; *Octopus cyanea*, a common nocturnal species in Tahiti, spends most of it's life hidden in it's den and lies in a vigilant position at the entrance of its home, cryptically mimicking its surroundings and remaining still as it monitors its surroundings (Hanlon and Messenger, 1996). In addition, ambient lighting influences the effectiveness of camouflage; lower light levels increase survival via camouflage, as the predator's vision is restricted (Hanlon and Messenger, 1996, Bradbury and Vehrencamp, 1998). It has been suggested that one function of the vertical migration in cephalopods is to keep the animal in water with low-light levels to protect it from predators (Hanlon and Messenger, 1996). As cryptic behavior is a common primary defense in cephalopods, it is not surprising that it was used frequently by *O. bocki* during test trials (Hanlon and Messenger, 1996). The combination of *O. bocki*'s dark brown coloration along with immobility, may make it difficult for a fish to detect the animals at night.

Inking was surprisingly rare in *O. bocki*, refuting the hypothesis that inking would occur at high frequencies during daylight hours. Inking behavior in *O. bocki* was observed in only 3 out of a total of 40 tests. Energy costs associated with inking may limit the frequency of inking in cephalopods. Nolen and Johnson (2001) showed that that seahare, *Aplysia* spp., required at least three days between ink extractions to replenish ink supplies. Individuals in this study were given at least 5 days to rest between tests, however, data showed that no octopus inked in more than one test. Thus, inking may have been rare in *O. bocki* because individuals require several days to replenish their ink sacs. Moreover, inking was elicited when the fish was at close distances for ingestion of the octopus (between 0 and 1 cm), suggesting that the octopuses conserve ink for extreme situations.

Table 3. Raw data showing occurrences of inking. Inking occurred in 3 out of 40 tests, when the fish was between 0 and 1 cm away from the octopus.

Octopus ID	Diurnal		Nocturnal	
	# Ink Puffs Released	Distance from Predator (cm)	# Ink Puffs Released	Distance from Predator (cm)
O.24	3	0	0	n/a
O.30	0	n/a	3	1
O.19	0	n/a	2	0
Total	3		5	

ACKNOWLEDGEMENTS

Thank you to all the IB 158 Instructors for their help in experimental design and execution. Special thanks to Liz Perotti and Erica Spotswood for their help with the statistical analysis. Thank you to Julie Himes, April Dobbs, Maya Almaraz, and Aimee Ellison for helping with animal collection .

LITERATURE CITED

Bradbury, J.W. and S.L. Vehrencamp. 1998. Principles of animal communication. Sinauer Associates, Inc., Sunderland.

Cheng, M.A.W. 1996. The reproductive biology of two species of pygmy octopuses: *Hapalochlaena lunulata* and *Octopus bocki* [dissertation]. Berkeley (Ca): University of California.

Hanlon, R.T., Forsyth, J.W., and D.E. Joneschild. 1999. Crypsis, conspicuousness, mimicry and polyphenism as antipredator defenses of foraging octopuses on Indo-Pacific coral reefs, with a method of quantifying crypsis from video tapes. Biological Journal of the Linnean Society **66**:1-22.

Hanlon, R.T and J.B. Messenger.1996. Cephalopod Behaviour. University Press, Cambridge.

Johnson, A.1995. Comparison of sensory cues in *Octopus bocki* by introduction of predator and prey organisms. The Biology and Geomorphology of Tropical Islands, Student Research Papers. University of California, Berkeley, 44-51.

Lebensohn, M.A.1999. Learning capabilities of different size *Octopus bocki*. The Biology and Geomorphology of Tropical Islands, Student Research Papers. University of California, Berkeley, 80-86.

Logan, C.A.2001. Den defense and intraspecific behavior in the tropical pacific pygmy octopus, *Octopus bocki* (Adam, 1941). The Biology and Geomorphology of Tropical Islands, Student Research Papers, University of California, Berkeley, 43-51.

Nolan, T.G. and P.M. Johnson. 2001. Defensive inking in Aplysia spp.: multiple episodes of ink secretion and the adaptive use of a limited chemical resource. The Journal of Experimental Biology **204**: 1257-1268

Meglitsch, P.A. 1972. Invertebrate Zoology. Second Edition. Oxford University Press Inc., Oxford.

MUTINY ON THE BOUNTY OR BOUNTIFUL MUTANTS? DIVERSITY AND COMPOSITION OF WOOD-DECAYING MACROFUNGI ON HIBISCUS TILIACEUS WOOD IN FRENCH POLYNESIA

FELICIA WHEATON

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

Abstract. Wood-decaying macrofungi are an important component of forest ecosystems because they are the major decomposers of dead woody debris and are crucial for nutrient cycling. This is especially true in the tropics where biomass is high. However, most studies to date have focused on temperate forests in the northern hemisphere. Little is known about wood-inhabiting fungi in French Polynesia. In fact, no identification materials exist. The following study seeks to fill this gap in knowledge. First, a general survey was done of the wood-fungi occurring in the mountains of Moorea. Next, in a pilot study, all Hibiscus tiliaceus dead wood (>1cm) was measured and surveyed for fungi using 10, 25-meter line transects. The aim of this phase was to determine if wood with fungi has different characteristics than wood without. Fungi were found on 61% of wood surveyed, but larger logs, and wood of intermediate decay were more likely to have at least one species. An additional 20 transects focused only on wood with fungi. A total of 114 species were found on 644 pieces of Hibiscus tiliaceus wood. However, 36.8% species were found only once and most wood had only 1 or 2 species. There were a few very abundant species, and the others were rare. When common species were examined individually, it was evident that many had preferences for certain wood sizes and decomposition. Species richness was found to positively correlate with average diameter, and wood of intermediate decay was also found to have greater species richness. In general, the results of this study were found to support much of the research conducted in temperate forests. Although the details differ, the underlying trends of diversity and succession are surprisingly similar.

Key words: fungi, species richness, diversity, succession, species composition, decomposition, tropical forest.

INTRODUCTION

Wood-decaying macrofungi are an important, but often overlooked and understudied component of forest ecosystems. They play a major role in decomposition and nutrient cycling (Boddy and Watkinson 1995; Cromack and Caldwell 1992). Yet not all "wood-decaying macrofungi" are created equal. Instead, this umbrella term disguises the diversity of different morphologies, decay capabilities and niches exhibited by this group. Urcelay and Robledo (2004) have proposed to divide species into functional guilds. Many fungi decay wood of a certain size and level of decomposition (Renvall 1995).

The extent of decomposition has been demonstrated to be one of the most important factors determining fungi species presence and diversity (Kuffer and Senn-Irlet 2005; Tofts and Orton 1998). Wood in intermediate to advanced stages of decay has the highest species richness (Niemala et al. 1995; Bader et al. 1995; Renvall 1995; Crites and Dale 1998). Wood is not only a substrate upon which fungi live, but it is also an energy/nutrient source that is transformed by the fungi (Allen et al. 2000; Stewart and Burrows 1994; Boddy and Watkinson 1995). Colonizing fungi can alter the moisture, physical structure, acidity, and nutrient content of the wood, making way for other species. For example, dead wood often contains resins, alkaloids, and phenols that are toxic to fungi (Holmer et al. 1997). Several studies have shown that there are patterns of species succession; as wood decays, initial colonizers are replaced by other species (Niemela et al. 1995; Renvall 1995; Holmer et al. 1997, etc). This is not deterministic however, and there may be many alternative pathways for succession, even when other factors are the same (Boddy 1992).

Species composition and diversity are also correlated to wood size. To date, the majority of studies on wood-decaying fungi have focused on coarse woody debris (CWD) (Edman et al. 2004) that is defined as wood with diameters of >10cm, such as fallen trees, downed branches, etc (Waddell 2002). Species richness tends to be higher on larger logs, which is partially due to greater surface area and volume (Edman et al. 2004; Bader et al. 1995; Kruys et al. 1999). Additionally, the decay rate varies even on the same log, resulting in heterogeneous microhabitats (Crites and Dale 1998). Several studies have been carried out in northern Europe in disturbed or managed forests where CWD is unnaturally scarce (Lindhe et al. 2004; Heilmann-Claussen and Christensen 2003; Kuffer and Senn-Irlet 2005, etc). They emphasize the threat this poses to fungi biodiversity. Recently, fine woody debris (FWD, diam 5-9cm) and very fine woody debris (VFWD, diam <5cm) have also been found to contribute to overall species richness and should not be overlooked (Kuffer and Senn-Irlet 2005). If the same surface areas are examined, FWD has the same diversity as CWD, and diversity is greater on FWD when equal volumes are compared (Kruys and Jonsson 1999). Branches and twigs have a greater surface-area-to-volume ratio and smaller wood pieces are likely to harbor different species. However very little research has been done to determine if these patterns hold true in tropical forest ecosystems.

The purpose of this study was to see if these trends found in temperate forests in the Northern hemisphere could also predict species richness and composition the tropical environment of Moorea, French Polynesia. It is known that wood biomass is far greater in tropical forests, and both the rate of decomposition and activity of wood-decaying fungi are higher (Ferrer and Gilbert 2002). Specifically I investigated the following questions: 1) How much/what type of wood is available and what percentage of has fungi? 2) Is species richness correlated to the physical characteristics of the wood (i.e. size or decomposition)? 3) Does species composition vary depending on the physical properties of the wood? I chose to examine only Hibiscus tiliaceus woody debris because I wanted to compare the physical variables of the wood and not differences that could be due to the tree specie or possible host specificity of certain fungi. Thus, they are not included in the scope of this study.

METHODS

Study site

Moorea is a tropical oceanic island in the Society Islands. The average temperature is 26° C, although it is warmer and wetter during the winter rainy season (Meyer 1996). The Belvedere trail on Moorea, French Polynesia was the primary field site for this study (FIG. 1). It is located at 17°32'29.0" S, 149°49'25.6W and the elevation is approximately 250m (Ranker et al. 2005). The forest is composed of primarily Hibiscus tiliaceus, Metrosideros polymorpha, Angiopteris evecta, and Inocarpus fagifer.

FIG. 2. Map of Moorea, French Polynesia. The Belvedere field site is indicated by the star

Survey of Moorea fungi

Initially, it was necessary to conduct a general survey of fungi that occur in Moorea. This was necessitated by the absence of any identification materials. Thus, the period from October 3, 2006 through October 19, 2006 was spent collecting various species at the Belvedere and at the Gump Station (Cooks Bay). Each sample was assigned a species number and was photographed, described and dried. Spore prints and permanent spore slides were made when possible. This datum was used to create a field key for the second phase of the study. Additional new species of fungi encountered during the sampling phase were also added. Each specimen was identified to the most specific taxonomic level possible. Specimens will be deposited in the Jepson Herbarium at the University of California, Berkeley.

Sampling methods

Field sampling began October 23, 2006 and ended on November 11, 2006. A total of thirty, 25-meter line transects were laid out at 200 pace intervals (0 paces corresponded to the Belvedere lookout/parking lot). At each interval, a transect was placed 10 paces from either side of the trail and parallel to it due to the dense vegetation and often steep incline. Every piece of dead Hibiscus tiliaceus wood greater than 1cm intercepting the line was examined including stumps, logs, branches and twigs. This tree species was chosen because it is common at this site, fallen wood is abundant on the forest floor, and it is fairly easy to identify. Each qualifying piece was examined for presence or absence of fungal fruiting bodies. Although this method leads to an underestimate of fungi, it has been widely used in comparable studies (Norden and Paltto 2001). Searching for mycelia would have been impractical because it requires destroying the wood, a huge amount of wood was examined, and not enough is known about the species occurring in the area to identify species from the mycelium alone. Physical data was taken on length, large and small end diameters, % cover (visually estimated), and decomposition stage. The decomposition stage was measured using a qualitative scale ranging from 1-5 (Renvall 1995; Waddell 2002) that was modified for Hibiscus wood (Table 1; Appendix A). If fungi were present, each species was noted as present or absent. If the identity of a fungus was questionable, a sample was taken back to the lab and compared to previously collected vouchers to either confirm its identity or to give it a unique species number.

Pilot study

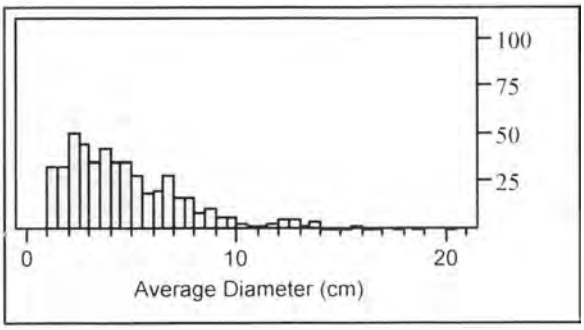


FIG. 2. Distribution of all 505 pieces of wood with and without fungi in the pilot study by average diameter (cm).

For 10 out of 30 transects, every qualifying piece of dead Hibiscus wood intercepting the line was recorded and measured whether or not fungi were observed. All dead wood was recorded in this step to quantify distribution of potential substrates. Another aim was to see what percentage of wood harbored fungi, and if there were certain features that correlated to fungi presence. First, the distribution of all wood was examined by average diameter and decomposition. The frequencies of fungi occurrence were calculated for the five decomposition categories using Microsoft Excel. Diameters and decay were compared graphically for wood with and without fungi using JMP univariate histograms.

Species richness and composition

For the remaining 20 transects, only Hibiscus wood with fungi was measured and recorded. This was to acquire additional data on species diversity and species composition on logs of various sizes and states of decomposition. The percentage of wood with different numbers of species was graphed to determine how diversity was distributed. To assess species richness trends, the average number of species was graphed for average diameter and decomposition stage. The Wilcoxon test in JMP was used to analyze species richness by average diameter and decay stage because the wood did not display a normal distribution. To analyze species composition, the abundances of all species found were graphed, as well as the % of species with a given abundance. Then, species found at least 5 times were examined. For each, the diameter and decay of the wood was averaged and the standard deviation was calculated to determine if it had a substrate preference.

RESULTS

Pilot study

A total of 505 pieces of dead Hibiscus wood were found in the pilot study: 197 without fungi, and 308 with one or more species. Average diameter was not normally distributed, but was positively skewed so that smaller units outnumbered larger logs (FIG. 2). However, wood with fungi had a slightly larger average diameter of 5.33cm, whereas the mean was 4.01cm for wood lacking fungi.

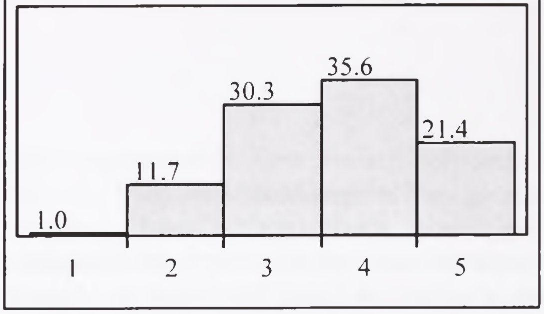


FIG. 3. Distribution of all 505 pieces of wood with and without fungi in the pilot study by decay stage. Values given are % of total wood.

Also, the decomposition stages were not normally distributed, but were negatively skewed so that more decayed wood was found more frequently (FIG. 3).

Overall, fungi were found on 61% of wood encountered, but this rate was different for each decay stage. No fungi were found on wood in the first stage, but fungi were found on 70% of wood in decomposition category 4. However, the frequency of fungi was found to taper off for the 5th stage of decomposition. Decomposition stages 2 and 5 were less likely to have fungi than the average, and stages 3 and 4 had fungi more frequently (FIG. 4)

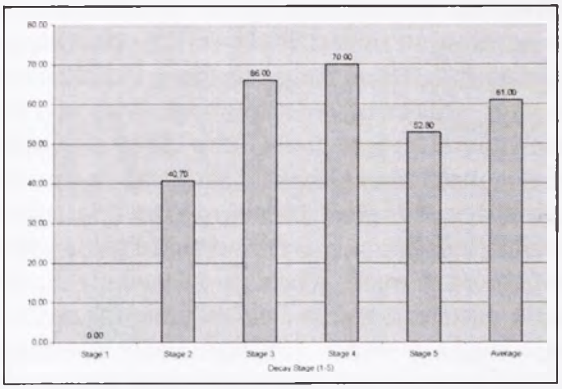


FIG. 4. Percentage of wood surveyed in the pilot study with fungi for each decay stage compared to the average overall.

Species richness

In all 30 transects, a total of 644 pieces of dead Hibiscus wood were found with at least 1 fungi species. Of these, 406 pieces had 1 species (63.04%), 149 had 2 species (23.14%), 56 had 3 species (8.7%), 24 had 4 species (3.73%), 5 had 5 species (0.78%), 4 had 6 species (0.62%), and only 1 piece of wood had 7 species (0.16%). When these percentages were natural log transformed, there was a strong negative relationship between species richness and the percentage of wood found with that number of species [ln(% of wood)=5.1122884-0.9850012(species richness), R^2=.99, P<0.0001] (FIG. 5).

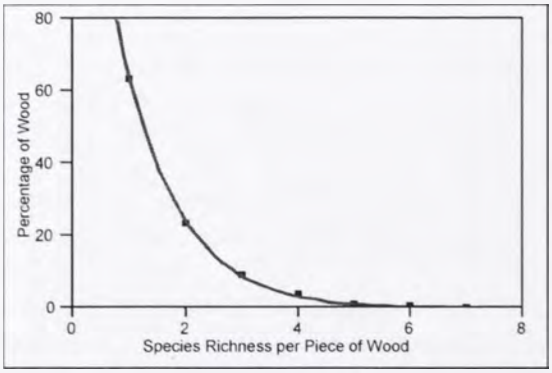


FIG. 5. The percentage of wood found for each species richness. The chance (%) of

finding a given species richness on wood with fungi=e^(5.112-0.99(species richness)

A Wilcoxon non-parametric analysis was done to determine if there were significant differences between the average diameters of wood with different species richness. Then the Tukey-Kramer honestly significant difference test was used to compare means (q*=2.96, Alpha=0.05). There were significant differences between species richness 5 and 1, between 4 and both 1 and 2, and between species richness 3 and 1 (FIG. 6; Appendix B). When the average number of species per piece of wood was plotted against the average diameter, there was a strong positive linear correlation (FIG. 7).

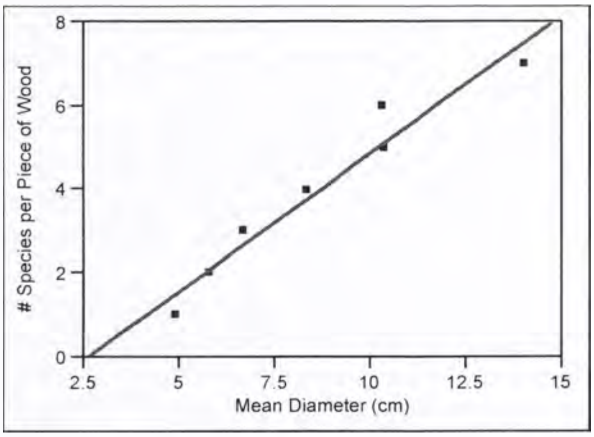


FIG. 7. Linear regression of # species per wood piece as a function of mean diameter (cm). [# species=-1.68+0.66(mean diameter), R^2=0.95, P>0.0002].

The Wilcoxon non-parametric test was used to determine if each decomposition stage was significantly different in terms of species richness. When pairs were compared using the Tukey-Kramer HSD test, statistically significant differences were found between decay stage 3 and both decay stages 2 and 5 (q*=2.58, Alpha=0.05). Decay stage 3 also had the highest mean species richness value of 1.75 species per unit (FIG. 8; Appendix B). The average number of species per piece of wood was graphed against decomposition stages 2-5 (no fungi were found on logs of decay stage 1). Species richness was found to be a quadratic function [#Species=0.74+0.32 (decomposition stage)-0.25(decomposition stage)^2, R^2=0.97, P>0.035] with a peak between stages 3 and 4 (FIG. 9)

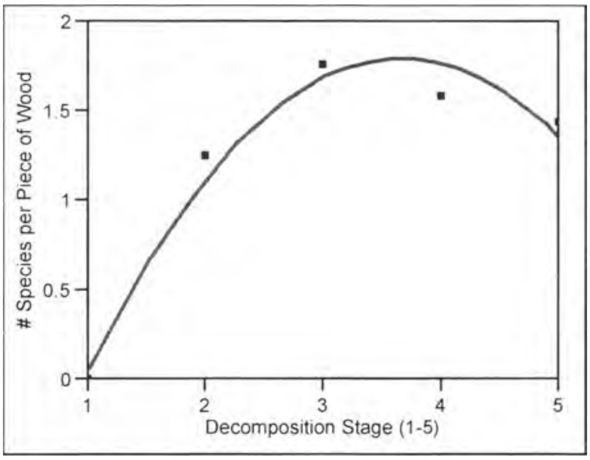


FIG. 9. Species richness per piece of wood as a function of decay stage.

Species composition

A total of 114 species were found, but 36.8% were found only once. 72 species were found 2 or more times, and 56 species were found 3 or more times. The distribution of abundances of each species and analysis with the Shapiro-Wilk W Test revealed that species abundance was not normally distributed. Then, species abundance was graphed against the % of species found at a given abundance. The datum for species abundance was transformed to fit the reciprocal [% species by abundance=-0.86+34.69$(\text{abundance})^{-1}$, R^2=0.96, P<0.0001]

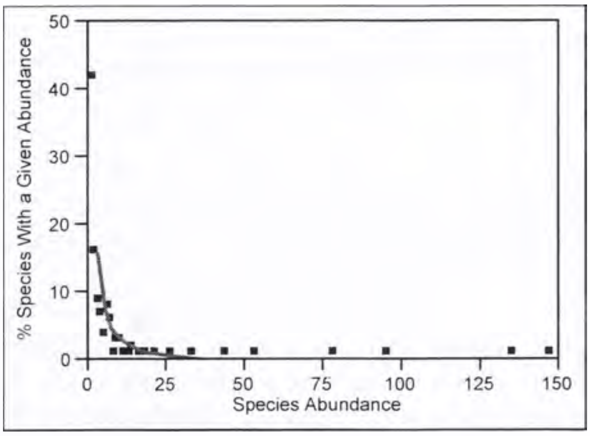


FIG. 10. Percentage of species with a given abundance as a function of species abundance.

Only species found 5 or more times were used to examine fungal communities. The average diameter and decay stage on which each was found and standard deviation were calculated and graphed (FIG. 11 and FIG. 12; Appendix C).

DISCUSSION

Pilot study

The results of the pilot study show general trends in the features of wood with and without fungi. Few studies report datum for wood without fungi, so it is difficult to compare the occurrence of fungi in the tropical island forest ecosystem of Moorea to other studies. Overall, more than half of the wood surveyed had fungi, but the frequency of fungal presence varied depending on the average diameter and decomposition stage of the wood. The likelihood of encountering fungi increased with the average diameter, and was greater for wood of intermediate decay. One striking observation was that no fungi were found on newly fallen wood. This could in part be due to the fact that this category was rarely found overall, which might be attributed to fast rates of decay in the moist, tropical environment. Also, I observed that many Hibiscus limbs began decaying while still attached to the tree, so they may be more decomposed when they do fall (this may explain the high amount of Hibiscus dead woody matter).

Interestingly, there were few significant differences between wood with and without fungi. I was unable to determine why certain wood that appeared suitable lacked fungi. This may have been due to chance and the inability for spores to either reach the wood or to survive on it. Edman et al. (2004) found that local spore sources play a key role in the presence of fungi species. They also point out that establishing mycelium and a fruiting body is a complex process that can be hindered at many points. Another possible explanation is that mycelia were present, but did not exhibit fruiting bodies.

Species richness

Although many species of fungi were surveyed and encountered along the transects, most wood had one species. Some had 2 species, but the chance of finding each additional species decreased exponentially. Thus, most individual logs display little species diversity.

When richness was examined in closer detail, it was found that it increased linearly with average diameter. This is similar to the pattern found in northern temperate forests (Bader et al. 1995; Renvall 1995; Kruys et al. 1999). Edman et al. (2004) found that in Sweden, the size of a log is an important factor in colonization and species diversity over time. This is somewhat intuitive because CWD has larger volume and surface area. As well as providing more substrate, larger wood may also contain more moisture and be more stable (Edman et al. 2004). In addition, larger units of wood may decay at different rates, providing heterogeneous microhabitats (Boddy and Watkinson 1995).

Also, fungi richness was higher on logs in intermediate stages of decay. No fungi were found on newly fallen logs and the frequency of fungi tapered off for very decomposed logs. This is also similar to findings from research done in other parts of the world (Renvall 1995; Linde et al. 2004). It may take some time for fungi to become established and develop fruiting bodies, and older wood may provide fewer energy and nutrients.

Species composition

ACKNOWLEDGEMENTS

I would like to thank the professors of Integrative Biology 158 for their guidance and support: Jamie Bartolome, Carole Hickman, Jere Lipps, Brent Mishler, and Vince Resh. The graduate student instructors Liz Perotti, Alison Purcell-O'Dowd, and Erica Spotswood provided priceless assistance and advice. Maya Almaraz, Tom Bell, April Dobbs, Valerie Howell, Erin Prado, Vince Resh, and Natalie

Valencia are greatly appreciated for their assistance in the field collecting data. Thank you to Tom Bruns, Tanya Chapple, Dennis Desjardin, and Else Vellinga for their help with fungi identification. I would also like to acknowledge my classmates for their support and camaraderie. Finally, thank you to the Richard B. Gump and the class coordinator, Jere Lipps, for making this opportunity possible.

LITERATURE CITED

Allen, R. B., P. K. Buchanan, P. W. Clinton, and A. J. Cone. 2000. Composition and diversity of fungi on decaying logs in a New Zealand temperate beech (Nothofagus) forest. Canadian Journal of Forest Research 30:1025-1033.

Bader, P., S. Jansson, and B. G. Jonsson. 1995. Wood-inhabiting fungi and substratum decline in selectively logged boreal spruce forests. Biological Conservation 72:355-362.

Boddy, L. and S. C. Watkinson. 1995. Wood decomposition, higher fungi, and their role in nutrient redistribution. Canadian Journal of Botany 73:S1377-S1383.

Boddy, L. 2000. Interspecific combative interaction between wood-decaying basidiomycetes. FEMS Microbiology Ecology 31:185-194.

Crites, S., and M. R. T. Dale. 1998. Diversity and abundance of bryophytes, lichens, and fungi in relation to woody substrate and successional stage in aspen mixedwood boreal forests. Canadian Journal of Botany 76:641-651.

Cromack, K. Jr., and B. A. Caldwell. 1992. The role of fungi in litter decomposition and nutrient cycling. Mycology Series 9:653-668.

Edman, M., N. Kruys, and B. G. Jonsson. 2004. Local dispersal sources strongly affect colonization patterns of wood-decaying fungi on spruce logs. Ecological Applications 14:893-901.

Ferrer, A., and G. S. Gilbert. 2003. Effect of tree host species on fungal community composition in a tropical rain forest in Panama. Diversity and Distributions 9:455-468.

Gilbert, G. S., A. Ferrer, and J. Carranza. 2002. Polypore fungal diversity and host density in a moist tropical forest. Biodiversity and Conservation 11:947-957.

Heilmann-Clausen, J., and M. Christensen. 2003. Fungal diversity on decaying beech logs-implications for sustainable forestry. Biodiversity and Conservation 12:953-973.

Holmer, L., P. Renvall, and J. Stenlid. 1997. Selective replacement between species of wood-rotting basidiomycetes, a laboratory study. Mycological Research 101:714-720.

Kruys, N., and B. G. Jonsson. 1999. Fine woody debris is important for species richness on logs in managed boreal spruce forests of northern Sweden. Canadian Journal of Forest Research 29:1295-1299.

Kuffer, N., and B. Senn-Irlet. 2005. Influence of forest management on the species richness and composition of wood-inhabiting basidiomycetes in Swiss forests. Biodiversity and Conservation 14:2419-2435.

Lindhe, A., N. Asenblad, and H. G. Toresson. 2004. Cut logs and high stumps of spruce, birch, aspen and oak-nine years of saproxylic fungi succession. Biological Conservation 119:443-454.

Meyer, J. Y. 1996. Tahiti's native flora endangered by the invasion of Miconia calvescens DC. (Melastomataceae). Journal of Biogeography 23:775-781.

Niemela, T., P. Renvall, and R. Penttila. 1995. Interactions of fungi at late stages of wood decomposition. Annales Botanici Fennici 32:141-152.

Norden, B., and H. Paltto. 2001. Wood-decay fungi in hazel wood species richness correlated to stand age and dead wood features. Biological Conservation 101:1-8.

Ranker, T. A., G. Trapp, A. R. Smith, R. C. Moran, and B. S. Parris. 2005. New records of lycophytes and ferns from Moorea, French Polynesia. American Fern Journal. 95:126-127.

Renvall, P. 1995. Community structure and dynamics of wood-rotting Basidiomycetes on decomposing conifer trunks in northern Finland. Karstenia 35:1-51.

Stewart, G. H., and L. E. Burrows. 1994. Coarse woody debris in old-growth temperate beech (Nothofagus) forest of New Zealand. Canadian Journal of Forest Research 24:1989-1996.

Tofts, R. J., and P. D. Orton. 1998. The species accumulation curve for agarics and boleti from a Caledonian pinewood. Mycologist 12:98-102.

Urcelay, C., and G. Robledo. 2004. Community structure of polypores (Basidiomycota) in Andean alder wood in Argentina: Functional groups among wood-decay fungi? Austral Ecology 29:471-476.

Waddell, K. L. 2002. Sampling coarse woody debris for multiple attributes in extensive resource inventories. Ecological Indicators 1:139-153.

APPENDIX A

Decomposition Stage	Description
1	Sound wood, bark completely intact, original color
2	Sapwood slightly decayed, heartwood sound, bark mostly intact
3	Sapwood somewhat decayed, heartwood mostly sound, bark starting to come off in patches
4	Sapwood decayed and pitted, bark mostly gone, wood is soft, but maintains shape
5	Bark absent, heartwood is very decayed and crumbling, breaks apart when touched

Table 1: Decomposition scale modified from Renvall (1995) and Waddell (2002)

APPENDIX B

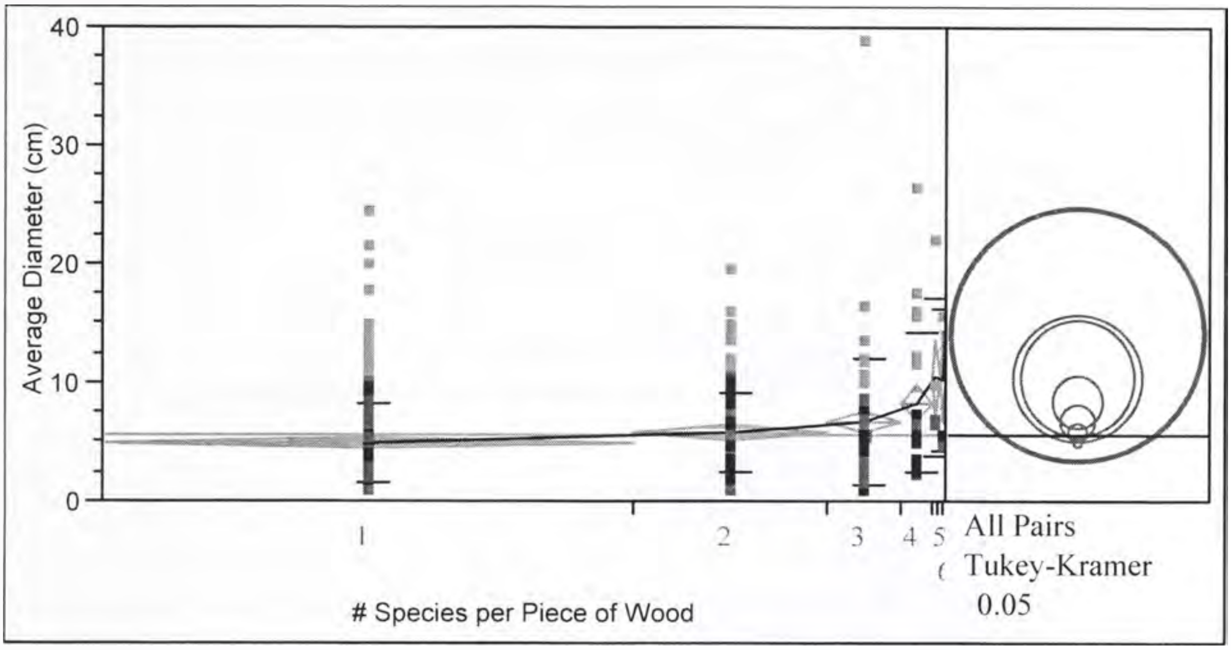


FIG. 9. Results for Wilcoxon Test for differences between species richness if the datum does not display normal distribution. A Tukey-Kramer test was used to compare means (average diameter in cm). The middle of the green diamond represents the mean diameter. Non-overlapping circles are statistically different from each other.

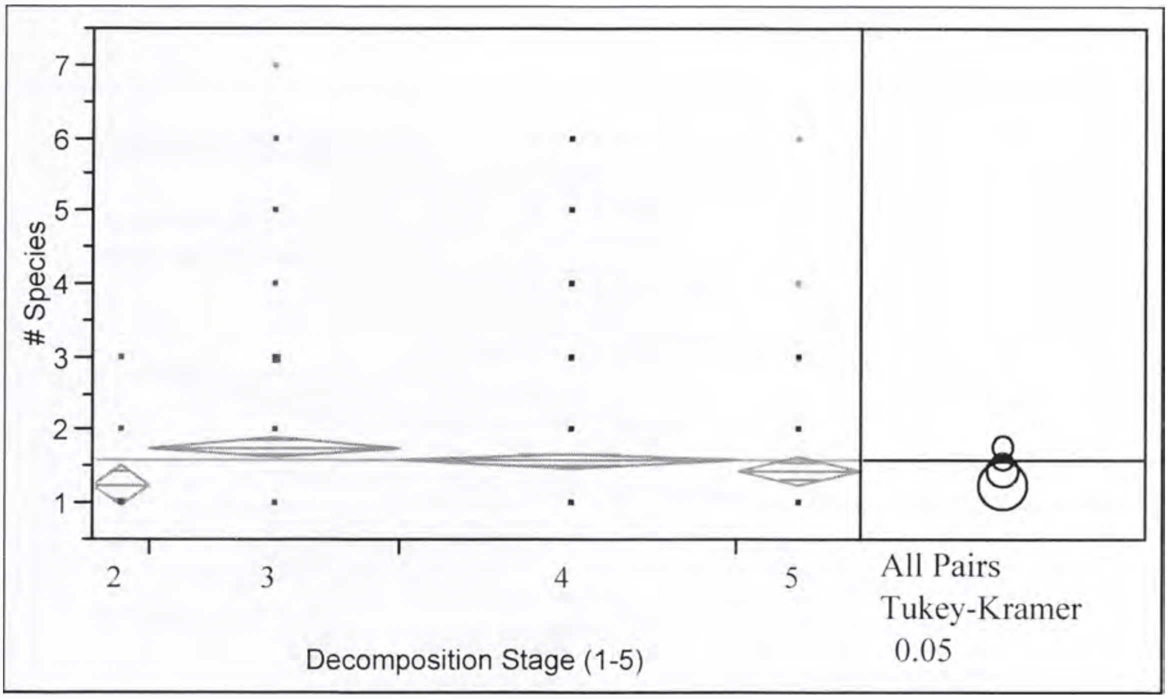


FIG. 8. Results from Wilcoxon Test for differences between decay stage (the datum does not display normal distribution). A Tukey-Kramer test was used to compare means (average number of species). The middle of the green diamond represents the mean species richness. Non-overlapping circles are statistically different from each other.

APPENDIX C

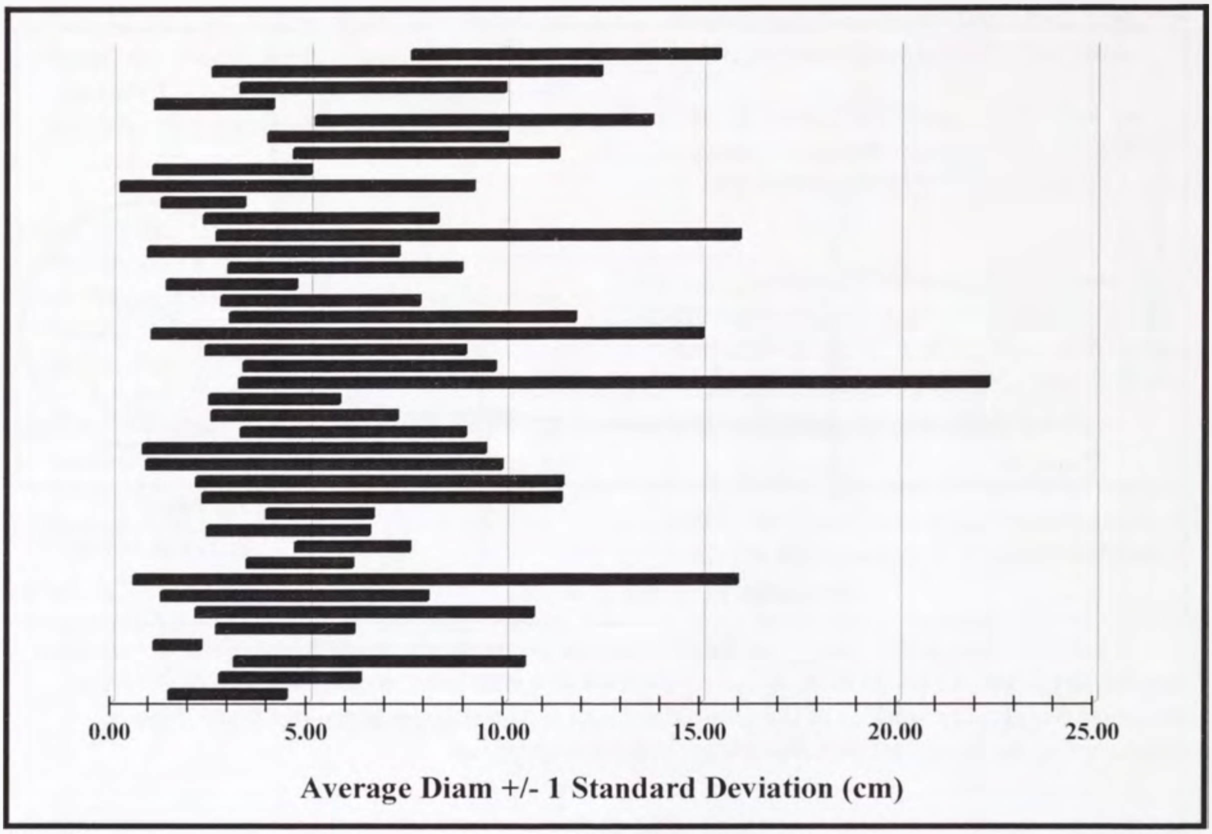

0.00
5.00
10.00
15.00
20.00
25.00
Average Diam +/- 1 Standard Deviation (cm)

FIG. 11.

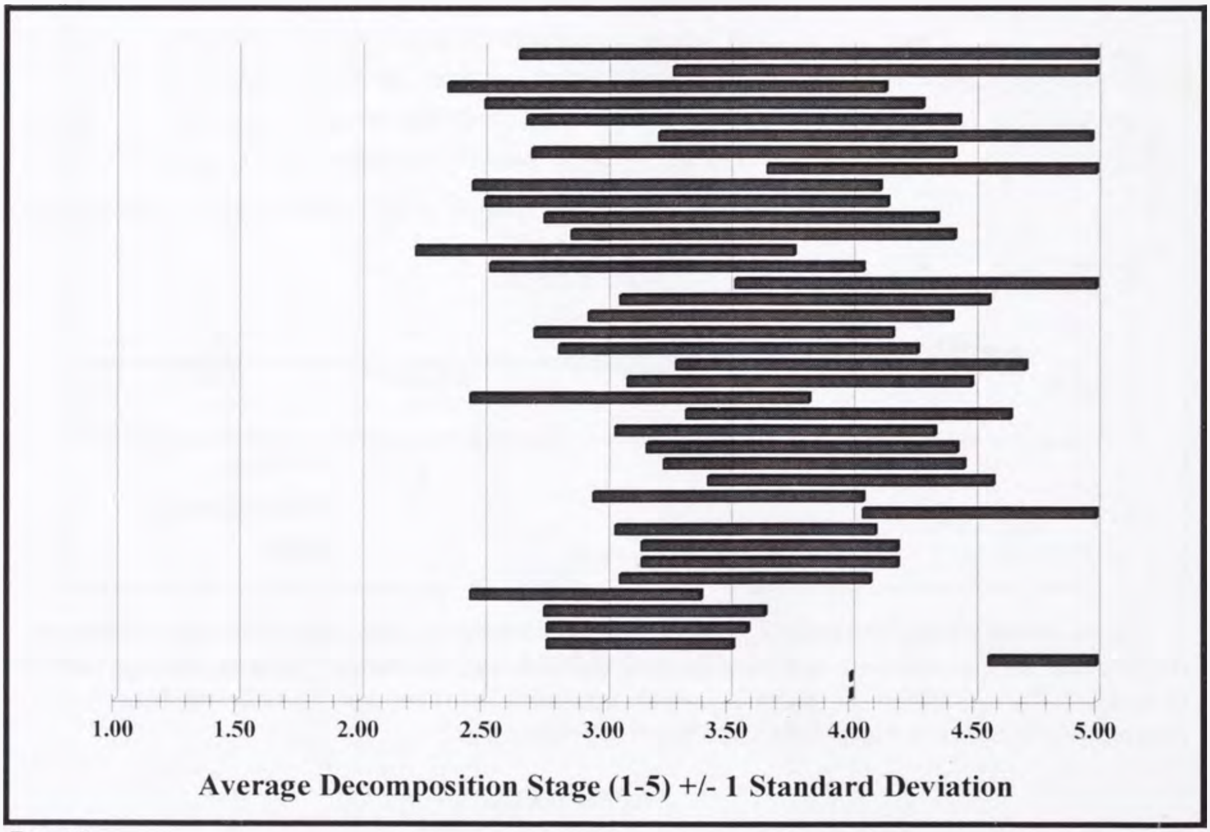

1.00
1.50
2.00
2.50
3.00
3.50
4.00
4.50
5.00
Average Decomposition Stage (1-5) +/- 1 Standard Deviation

FIG. 12.

Moorea, French Polynesia