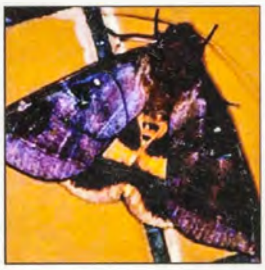
Biology and Geomorphology of Tropical Islands

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

Student Research Papers :: Fall 2001





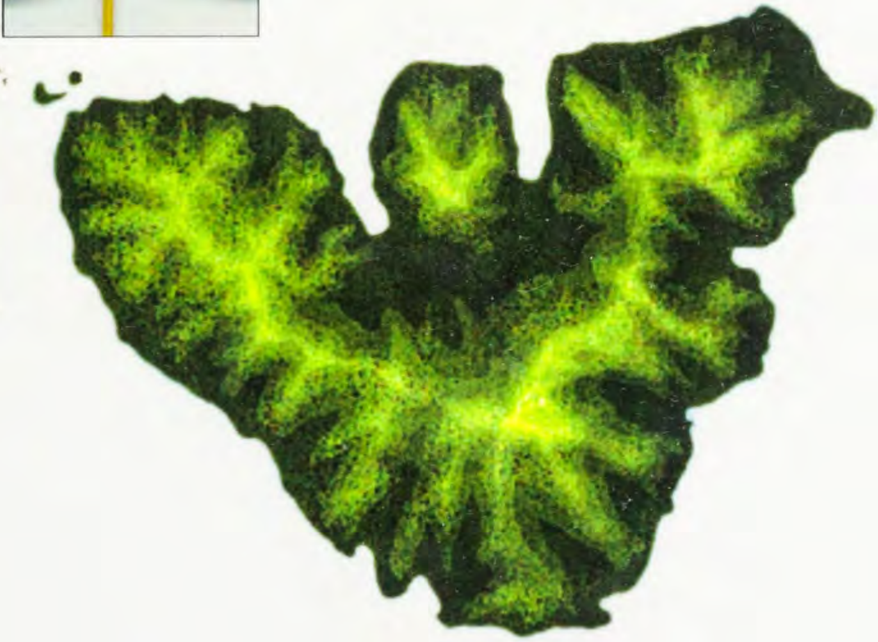










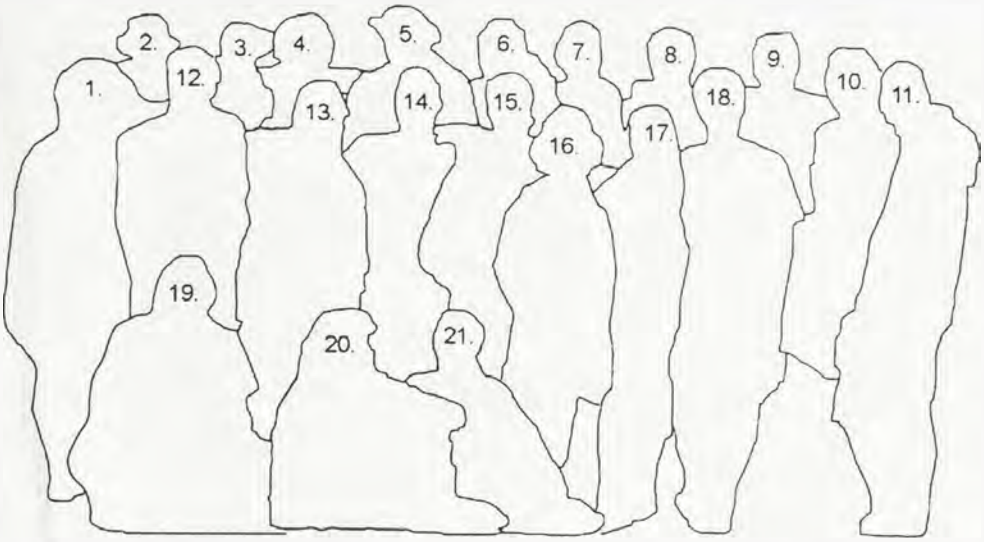




Richard B. Gump South Pacific Biological Research Station :: Moorea, French Polynesia

University of California, Berkeley

The Moorea 2001 Class

Moorea 2001 Class photo:



1. Dakota Betz 2. Brian Forest 3. Hillary Thomas 4. Anna Frumes 5. Michael De Salvo 6. Katie Cochrane 7. Julie Rentner 8. Jonathan Wagner 9. Cheryl Logan 10. Kerry Nickols 11. Ben Elitzur 12. Theo Leung 13. Jane Lee 14. Mathew Glaser 15. Hung Ma 16. Marisa Chung 17. Marie Franc 18. Anthony Darrouzet-Nardi 19. Tyler Kreitz 20. Nathan Garfield 21. Brie Lindsey

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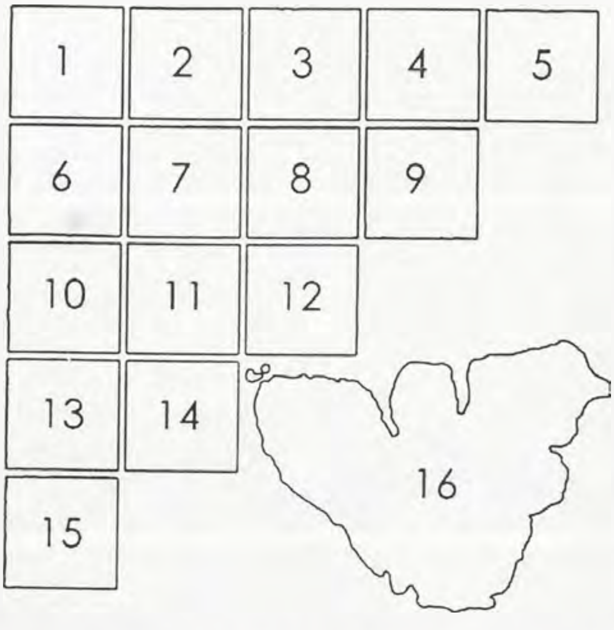

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16. *Topographical relief of Moorea*. Graphic by Sean Askay.

The Moorea 2001 Class

Class at the Belvedere Lookout on Moorea

(Pictures, left to right, top to bottom) Opunohu Bay, class field trip in Le Truck, station fare at the point, sunset at Cook's Bay, Rover ride on Tahiti, class stream survey.

Integrative Biology 158 :: Environmental Science, Policy and Management 107

THE BIOLOGY AND GEOMORPHOLOGY OF TROPICAL ISLANDS

Student Research Papers, Fall 2001

This is a rare course in undergraduate education, an interdisciplinary course open only by application and interview, that occupies one whole semester (13 units), with a student : teacher ratio of about 2:1, featuring all-inclusive research experience and full immersion in the life of science and a foreign culture. The 21 students spend a month in Berkeley for lectures and labs five days a week, then go to the Richard B. Gump South Pacific Biological Research Station on Moorea in French Polynesia for nine weeks to carry out an intensive research project as well as some general educational field trips and group projects, finally returning to Berkeley for two weeks of non-stop data analysis, library research, and writing. Following a round of peer reviews, the students prepare camera-ready copies for their papers and give an oral presentation in a professional-style symposium.

The 2001 class, whose papers follow in this book, set new standards of achievement and formed bonds with each other and the teaching staff that will last a lifetime. The professors (who spent 2-3 week periods each on Moorea) and the Graduate Student Instructors (who spent the whole nine weeks on Moorea) had a unique opportunity to help each student design their project, go with them in the field, observe their data-gathering and work habits, and assist them in the analysis and write-up phase. This close living and working situation allows the transfer of fundamental methods, theories, and even attitudes about science, in addition to the expected transfer of basic information about islands and their biology. It is extremely rewarding for us to see students transform into research scientists in front of our eyes!

Such a course is an expensive and time consuming effort that would not be possible without the help of many people. As lead instructor, I had the privilege of working with several other faculty members, all experts in tropical studies but with a wide spectrum of specialties. In addition to myself, field teaching was done by professors Tom Carlson, Rosie Gillespie, Carole Hickman, and George Roderick, all of whom gave lectures at Berkeley as well. Additional faculty who gave lectures on campus include Roy Caldwell, Carla D'Antonio, Patrick Kirch, Jere Lipps, and Vince Resh.

The three intrepid Graduate Student Instructors: Elin Claridge, Anya Hinkle, and Crissy Huffard deserve special mention. They were responsible for the day-to-day running of the course on Moorea, helping the students in many ways ranging from mundane logistical matters to sophisticated scientific techniques. We literally couldn't have done it without them!

We thank the Executive Director of the Gump Station, Dr. Neil Davies, who resides on Moorea --he helped in all aspects of the course, kept everything working, and was generous with scientific assistance and advice. Thanks also to Frank and Hinano Murphy for organizing trips and sharing knowledge and to Marimari Kellum for opening her wonderful garden to the students. We also are grateful to Gordon and Betty Moore (and the Moore Family Foundation) for their continuing support of laboratory and computation facilities at the Station.

The two departments committed financial support, personnel, equipment, and facilities. In particular we would like to thank Julie Meyers in Integrative Biology for dealing with finances and accounting, and Dorothy Tabron in Integrative Biology for help with supplies and equipment. Don Bain kindly provided help with map making. Jim Hayward gave snorkeling and water safety instruction. A special thanks goes to Sean Askay (Moorea class of 2000!) who provided much technical help for the oral presentations as well as for the preparation of this book.

Brent D. Mishler
Department of Integrative Biology

See also the WWW site at: http://ib.berkeley.edu/moorea/

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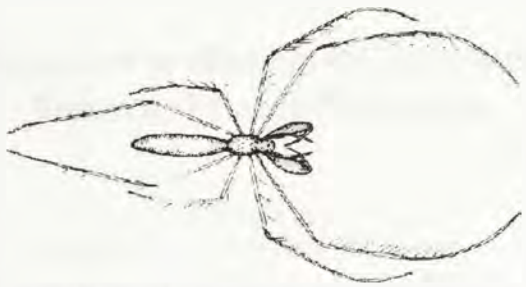

Terrestrial Research:

Salinity Level Contributions to Bleaching and Distribution of Corals in Moorea, French Polynesia

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ABSTRACT. Samples of *Pocillopora verrucosa* and *Pocillopora damicornis* from inside and outside of Pao Pao Bay in Moorea, French Polynesia were tested experimentally for their tolerances of salinity changes. Statistical significance was found for coral sample response to altered salinities, but no statistical significance was found for influence of sample source location on bleaching tolerances was not found to be significant. In addition to experimental study of corals and salinity, a quantitative survey of Pao Pao Bay was performed. The main objective of this survey was to examine the spatial pattern of diversity of corals (*Cnidaria)* between the back (land-end) and mouth (ocean-end) of the bay. A strong gradient in individual frequency and species richness was found between the back, where five of the eight genera examined were absent, and the mouth of the bay where they were abundant. The absence of corals at the back of the bay may be explained by low salinity and high turbidity which occur after heavy rains during the wet season.

Introduction

Coral bleaching is the phenomenon whereby corals lose most or all of their pigmentation through either the loss of their symbiotic algae or a reduction in the algae's chlorophyll concentrations. Bleaching can devastate coral reefs; oftentimes bleached corals fail to recover (Hoegh-Guldberg and Salvat 1995; Huppert and Stone 1998). Reefs particularly affected have suffered up to a 96% reduction of live-coral cover in some areas (Edwards, et al 2001). Over the last 25 years, there has been a dramatic increase in both the number and severity of bleaching events worldwide (Glynn 1993; Goreau and Hayes 1994). Between 1979 and 1990, 60 coral reef bleaching cases were reported, compared with a mere nine recorded between 1960 and 1979, a period of equally zealous coral research (Glynn 1993). And there seems to be no reversal of this trend, which strikes many as being detrimental to these fragile, incredibly diverse ecosystems.

As a result of the large amount of attention the subject has received, a variety of stressors have been identified as possible factors in triggering the events. The major factors that have been predominant in recent literature are increased sea surface temperature (Goreau and Hayes 1994; Gleeson and Strong 1995; Williams and Bunkley-Williams 1990) and increased irradiance, including ultraviolet radiation (Lesser et al. 1990). However, many areas of study have been neglected; a disproportionately small number of studies relating to other factors, such as reduced salinity and bacterial or other infections, have been conducted (Brown 1997).

Salinity

Salinity is among the neglected stressors. A bay is an inlet of a larger body of water. In such a system the potential for mean salinity fluctuation may be significant. For instance, a shallow bay system with poor water circulation that is exposed to prolonged, heavy precipitation or the flood of a nearby freshwater river may experience lowered salinity levels for a short period of time. If the bay is closed off from its communicating body of water, it is possible that evaporation will cause the salinity level of the body to rise. This study will be an effort to understand the effects of heightened or reduced salinity on bay corals.

A small number of previous studies have attempted to observe salinity effects on corals. One such study scored two species of *Porites (P. astreoides* and *P. furcata)* for the presence of mucous sheets as a measure of stress induced by reduced salinity, as well as fine and natural sedimentation (Coffroth 1985). The study found suggestive, but not significant, evidence that mucous sheet formation on corals belonging to the genus *Porites* can be induced by reduced salinity and increased sedimentation. Chamber effects (such as limitation of current flow around the coral) may have been partly responsible for the statistical insignificance. Additionally, *Porites* corals tend to be much more tolerant of environmental changes relative to other species, particularly branched species such as those belonging to the *Pocillopora* genus (Brown 1996).

Coral Distribution

In their paper on coral community biodiversity in Polynesia, Adjeroud and Salvat found a strong gradient in corals and other benthic organisms (including echinoderms) from the mouth of Opunohu Bay toward the back of that bay (1996). Specifically, they stated that there was decreasing trend in species richness and percent cover closer to the freshwater inlet. This trend was attributed to environmental factors related to the freshwater inlet at the land-end of the bay; aspects such as low salinity, submarine groundwater discharge, bottom sediments, and nutrient runoff were suggested as possible distributional influences.

Pao Pao Bay has a very similar geomorphological system to that of Opunohu Bay, located directly to the west. At the land-end of Pao Pao Bay there is a pair of freshwater inlets that release freshwater into the bay after periods of rainfall. If there is a significant effect on corals from slightly reduced salinity levels, it would follow that there would be fewer corals at this end of the bay.

Materials and Methods

Study Site

Observations were made and samples taken on coral reefs in Pao Pao Bay, and outside of the bay, near the barrier reef in Moorea, French Polynesia. Moorea is a high volcanic island, young relative to most of the Society archipelago, located in the eastern Pacific Ocean, north of the Tropic of Capricorn, at 17deg.S, 149deg.W. Annual average rainfall is 325cm. Concerning recent major coral community disturbances, bleaching events have been recorded in 1991, 1994, and 1999. The most recent tropical cyclone occurred in 1997.

Two major bays are located along the northern coast of Moorea: Opunohu Bay and Pao Pao (or Cook's) Bay. Pao Pao Bay is surrounded by steep volcanic mountains on all land sides and by a valley at the south end, where a river flows into the bay.

Inside the bay, the coral ecosystem is comprised of three major reef habitats: the outer reef slope (ocean side), the barrier reef, and the fringing reef. Inside the bays, the fringing reef is comprised of three zones: the reef flat, the reef crest, and the reef wall (Adjeroud and Salvat 1996). Figure 1 illustrates these regions. In this study, the fringing reef of the bay is distinguished from the fringing reef of the lagoon (outside of the bays).

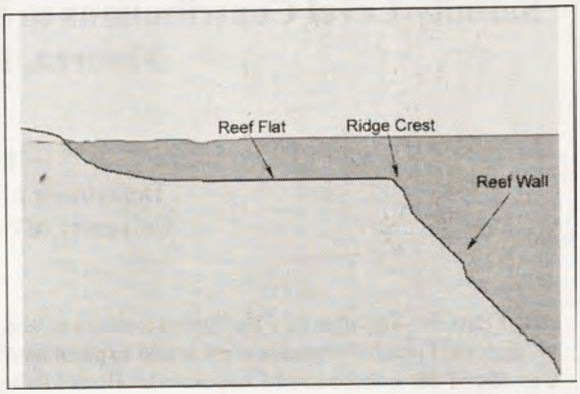

Figure 1. The fringing reef can be described as a composition of three zones: the reef flat, the ridge crest, and the reef wall. For the distributional survey section of this study, transects were laid down parallel to the shoreline along the ridge crest and the middle of the reef flat at each station along the fringing reef.

1. Response to experimentally altered salinity

Experimental Treatments

Corals belonging to the genus *Pocillopora* were used in the experimental portion of the study. These corals were chosen for both their abundance in the area and their reported relative sensitivity to environmental changes. During the months of October and November, 2001 a total of 48 samples were taken: 27 samples from outside Pao Pao Bay and 21 samples from inside the bay. Samples were placed in one of three tanks for treatment: a tank containing water with *in situ* salinity, to control for the effects of sampling, a tank with low treatment-level salinity, or a tank with high treatment-level salinity.

Preliminary measurements of salinity along the bay allowed the author to establish a mean normal level of 36ppt. This value remained relatively constant throughout the duration of the study, with the exception of a reading of 34ppt, taken at the land-end of the bay after a long period of rain. These values were used as a measure of natural salinity variation.

For exposure to reduced salinity conditions, enough freshwater was combined with saltwater in the tanks to make the salinity of the treatment water contain 34ppt, about 94.5% of the normal mean salinity level found in Pao Pao Bay. For exposure to increased salinity levels, the same method was used, with freshwater being replaced by hypersaline water obtained through evaporation of *in situ* water and enough being added until salinity level is 38ppt, about 105.5% of the normal mean level.

Six treatments were run for a minimum of three days. After a three day period with no change in the corals, the treatment was stepped down (and up) from the treatment levels by the same amount as from the *in situ* level and new samples were added at this stage to measure whether sudden changes were more significant than gradual changes. If there was a response in the samples during the first three-day period, but there was not complete bleaching, treatments were handled as previously described. If there was complete bleaching within the first three-day period, the treatment was ended and a new run was began. The corals were monitored and scored daily during treatment, with water hand-pumped through the tank with a large syringe to create water motion around the samples several times each day.

Scoring/Assessment of Corals

Pocillopora is a common branched genus, and can be pink, pale green, or light brown under favorable conditions. Some species of *Pocillopora,* such as *P. damicornis,* may display white growing tips; it is important that these branch tips are not confused with bleaching when assessing stress levels of the study corals.

Corals were scored as stressed if they displayed bleaching (to any degree, including paling pigmentation) or formation of mucous, as mucous formation has been suggested as a response to extreme conditions (Bak and Elgershuizen 1976, and Thompson 1980, cited by Coffroth 1985). The terminology used to describe the level of bleaching was as described in Table 1.

Severity of Bleaching	Terminology Used
Not damaged	"not bleached"
Lightened tips or <10% surface damaged	"slightly bleached"
10-50% surface damaged	"partially bleached"
50-100% surface damaged	"mostly bleached"
100% surface damaged	"completely bleached"

Table 1. Summary of bleaching severity terminology used in the study.

Data Analysis

Chi-squared tests (one-way and two-way) were used to determine statistical significance of experimental data. Two-way tests were used to compare samples from inside the bay and from outside the bay in their responses to the different salinity treatments. A p-value of <0.05 was taken to mean there was statistical significance in the difference between data sets.

2. Distribution of coral species along Pao Pao Bay with respect to distance from freshwater source

Distributional Survey. Along Pao Pao Bay seven stations were established at which coral individual frequency and species type were investigated (Figure 2).

For each station a list of species and number of separate heads was compiled along transects laid down parallel to the shoreline along either the middle of the reef flat or the edge of the reef crest. This orientation was chosen in order to avoid the increasing coral cover gradient from the shore to the ridge crest (personal observation). Two more such stations were established outside of Pao Pao Bay, along the back reef, with each transect laying parallel to the algal ridge. At every station, transects were 15 meters long and corals within one meter of either side of the transect tapes were included in the survey.

Corals were identified to the species as closely as possible, with one exception: all corals in the genus *Porites* were simply labeled "*Porites*" except for *Porites australiensis,* which was more easily identified under water than the rest of the *Porites* genus.

In addition to species surveys, water salinity measurements were taken on a regular basis. Special attention was given to the south end of the bay during and after rainfall events to measure whether there was a significant change in salinity after or during rainfall.

Data Analysis. Chi squared tests (one-way and two-way) were used to determine statistical significance of proposed trends in species frequency, species richness, and species diversity. Simpson's diversity index,

$$1\text{-SUM }(p_i^2),$$

where p_i = (individual species)/(total number of individuals), was used to find species diversity along the distance gradient away from the barrier reef. A p-value of <0.05 was taken to mean

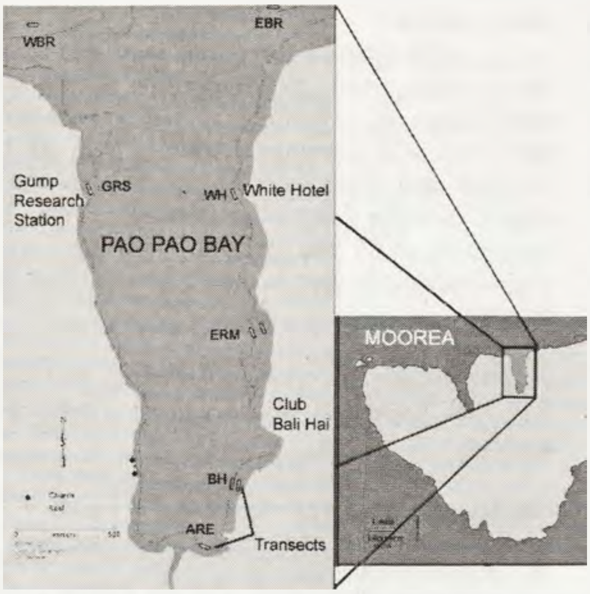

Figure 2. Data from nine transects shown here (small rectangles) along Pao Pao Bay were used to determine species frequency, richness, and diversity. Station names are labeled on the figure (farthest out to closest to back of bay): East Back Reef (EBR); West Back Reef (WBR); White Hotel (WH); Gump Research Station (GRS); East Red Marker (ERM); Bali Hai (BH); ARE Market (ARE).

there was statistical significance in the differences between data sets.

Results

1. Responses to Experimentally Altered Salinities

The experiments revealed statistically significant evidence that there is a correlation between salinity alterations and coral bleaching (low salinity values: chi^2=10.667, df=2, p=0.005; high salinity values: chi^2=12.000, df=2, p=0.002, with variables low treatment and high treatment weighted against control treatment variable in one-way chi-squared tests). Furthermore, of all corals that bleached, those in the second part of each treatment run (where there was a sudden salinity change, as opposed to a gradual salinity change) bleached at a significantly higher rate (chi^2=6.250, df=1, p=0.012) than samples run with a gradual salinity change treatment. Samples in gradual treatments tended to pale over a period of two to four days, while samples in sudden treatments nearly always bleached within the first 24 hours (usually less). Statistical tests were inconclusive concerning whether high salinity treatments illicited more or less of a response from the test corals than low salinity treatments. Responses are summarized in Table 2.

		Low Salinity	High Salinity	*In Situ* (control)
Samples Bleached	Inside Bay	4/4	6/6	0/7
	Outside Bay	5/5	6/6	2/6

Table 2. Sample responses to different salinity treatments, including controls. Responses are reported as number of individuals out of number of samples within each treatment.

As far as severity of bleaching was concerned, there was suggestive, but not significant (p=0.061), evidence that sudden salinity alterations cause more severe initial responses in corals than gradual salinity changes. Samples run in gradual treatments tended to pale over a period of two to four days, while samples run in sudden treatments usually completely bleached within a period of 24 hours or less.

To determine whether sample source location (outside or inside Pao Pao Bay) was a significant factor in the severity of bleaching, two-way chi-squared tests were performed with source location compared to degree of bleaching and treatment type. The results of the tests showed that the difference in source location was not a statistically significant influence (high salinity values: chi^2=2.000 and 6.667, df=2 and 6, p=0.368 and 0.353; low salinity values: chi^2=3.200 and 7.200, df=2 and 6, p=0.202 and 0.303). Tests run to determine whether source location was a significant factor in the rate of bleaching revealed similarly inconclusive results.

2. Distribution of Coral Species along Pao Pao Bay

The total number of species encountered and identified in the seven stations was 25, belonging to a total of eight genera, as presented in Table 3.

The general trend was an increase in species richness beginning with the highest values at the barrier reef near the mouth of the bay and steadily decreasing toward the land-end of the bay (Figures 3 and 4).

Additionally, there was a significant decrease (chi^2=181.677, df=5, p<0.001) in frequency of individuals toward the back of the bay (Figure 5). There was no significant gradient in species evenness or diversity found along the bay. In fact, species diversity appeared to be relatively even along the bay, with the exception of a few outliers (EBR with a Simpson's Diversity index value of 0.90 and ARE with a SDI value of 0.0). See Figure 6.

GENUS /species	Total Number of Heads in Transect at Each Station: EBR	WBR	WH Crest	GRS Flat	ERM Crest	ERM Flat	BH Crest	BH Flat	ARE
ACROPORA	14	8	0	0	0	0	0	0	0
A. austera	2	2	0	0	0	0	0	0	0
A. cytherea	3	3	0	0	0	0	0	0	0
A. elseyi	0	1	0	0	0	0	0	0	0
A. hyacinthus	0	1	0	0	0	0	0	0	0
A. nobilis	0	1	0	0	0	0	0	0	0
A. valida	9	0	0	0	0	0	0	0	0
FUNGIA	0	0	8	0	22	0	0	0	0
F. concinna	0	0	1	0	0	0	0	0	0
F. danai	0	0	5	0	11	0	0	0	0
F. paumotensis	0	0	1	0	0	0	0	0	0
F repanda	0	0	1	0	11	0	0	0	0
LEPTASTREA	4	2	0	0	1	0	1	0	0
L. transversa	4	2	0	0	1	0	1	0	0
MONTIPORA	26	26	24	0	8	0	1	0	0
M. digitata	16	0	17	0	3	0	0	0	0
M. efflorescens	7	26	7	0	5	0	1	0	0
M foliosa	1	0	0	0	0	0	0	0	0
M. monasteriata	2	0	0	0	0	0	0	0	0
PAVONA	4	7	7	0	3	0	0	0	0
P. cactus	0	0	3	0	2	0	0	0	0
P. varians	4	7	4	0	1	0	0	0	0
POCILLOPORA	42	12	10	0	1	0	0	0	0
P. damicornis	0	0	4	0	0	0	0	0	0
P. eydouxi	9	2	0	0	0	0	0	0	0
P. meandrina	10	5	0	0	0	0	0	0	0
P. verrucosa	23	5	6	0	1	0	0	0	0
PORITES	24	79	62	12	25	0	2	0	0
P. australiensis	3	6	0	4	0	0	0	0	0
P. lobata/lutea	17	51	14	8	10	0	2	0	0
P. rus	4	22	48	0	15	0	0	0	0
PSAMMOCORA	6	7	1	2	0	0	0	0	0
P. profundacella	6	7	1	2	0	0	0	0	0
Total # of individual colonies	120	139	112	14	60	0	4	0	0

Table 3. All species encountered and described in most transects are listed here. For the sake of simplicity, transects performed at the CH station were omitted. Notice that *Porites lobata* and *Porites lutea* are listed in the same row. The reason for the grouping was the difficulty of identifying these two species under water. They were counted together along each transect at every station. Also notice that there were significantly fewer (chi^2=11.313, df=1, p=0.008) coral colonies along the reef flat than the ridge crest.

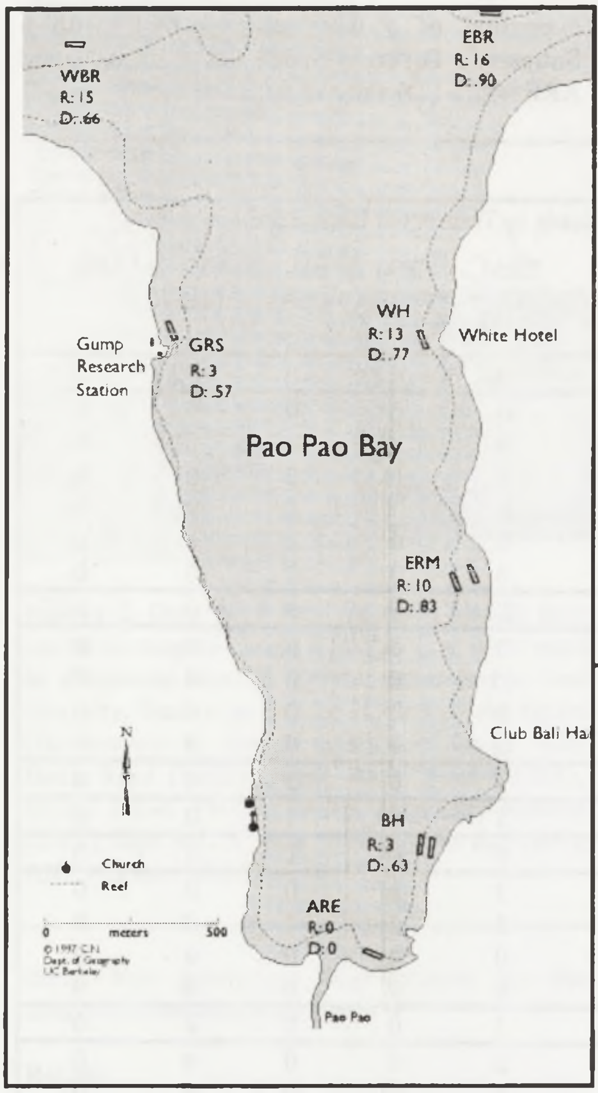

Figure 3. Map of Pao Pao Bay with transects outlined (small rectangles) and species Richness (R) and Diversity (D) reported for each station. The GRS station is the only place where diversity and richness data are reported in the figure. All other numbers refer to ridge crest data.

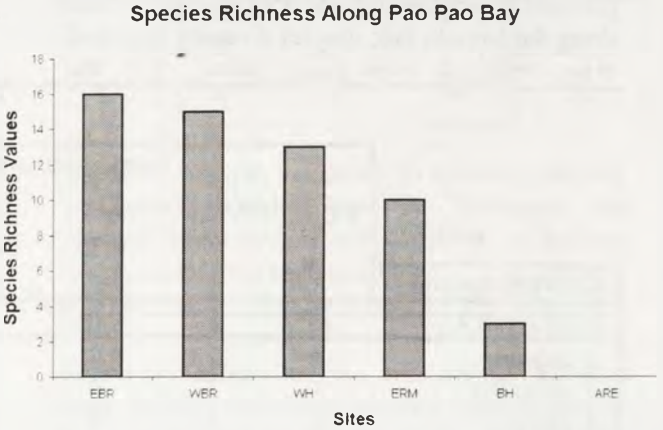

Figure 4. Species richness by station along Pao Pao Bay. There is a significant decrease in richness toward the back of the bay. All numbers refer to data taken from ridge crest, not from reef flat.

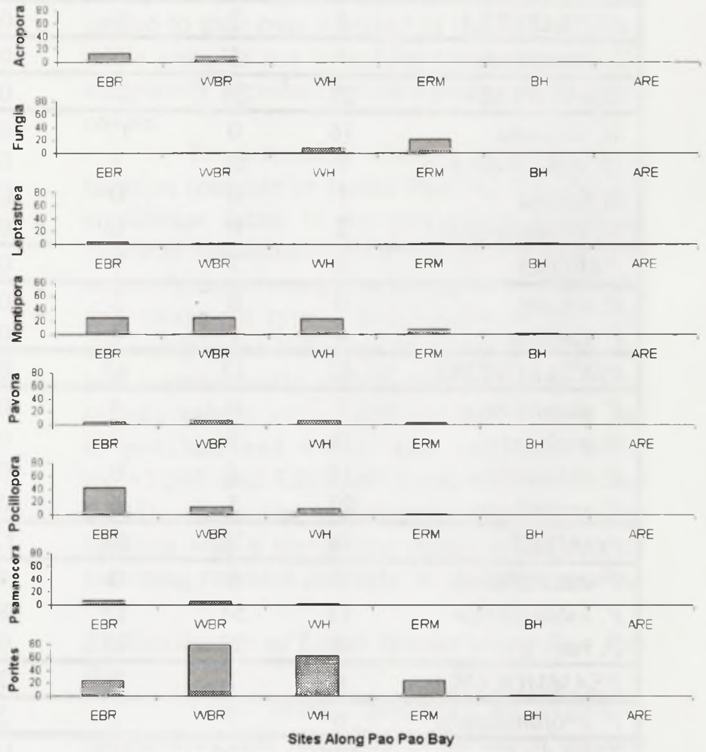

Figure 5. Frequency distribution chart of the eight coral genera identified within Pao Pao Bay by station, from mouth of bay to back. All numbers in this figure refer to data taken along the ridge crest, not the reef flat.

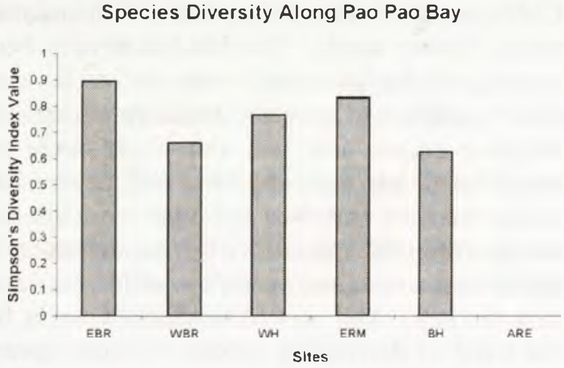

Figure 6. Diversity of coral species in Pao Pao Bay by station (mouth to back of bay). Diversity appears to be approximately even throughout the majority of the bay. Outliers are ARE with a value of 0.0 and EBR with a value of 0.90.

The only genera found in the portion south of the ERM station were *Leptastrea, Montipora, and Porites.* No corals occurred south of the BH and CH stations.

Discussion

1. Response to experimentally altered salinity

The results of this experiment suggest that corals are extremely sensitive to salinity changes, even slight ones (only a 5% change, in some instances) . Furthermore, corals seem to be more sensitive to sudden changes than gradual changes, as was evidenced by the rate and severity of bleaching responses of test corals in the different treatments. Coral samples were able to tolerate low salinity levels that were gradually increased (or decreased) for a period of one to four days, while samples placed in already increased (or decreased) treatments virtually always bleached within the first 24 hours of treatment.

These conclusions are not in agreement with some previous studies that indicated that corals can tolerate salinity dilution to 27ppt (Coffroth 1985). There are several reasons both sets of conclusions may be accurate. Coffroth (1985) studied the effects of salinity on *Porites* corals, which form massive colonies. Massives have been shown to be less sensitive to environmental stressors than branched corals, such as those belonging to the *Pocillopora* genus.

Another possibility to consider is the difference between experimental methods. Coffroth used chambers that he placed around the heads of *Porites* corals and filled with water with altered salinity levels. In this study, tips of coral branches were actually removed from their natural habitat and transferred to tanks where they could be monitored more easily. It is possible that the controls did not accurately and completely represent stresses placed on corals removed from their colonies and natural environments. In other words, there is the possibility that the salinity alterations and the actual removal of the animals are compounding factors; the controls may have only shown that removal itself was not a strong enough stressor to bleach a piece of coral, even if salinity may be able to bleach a piece of coral already weakened by the disturbance of removal. Unfortunately, this complication was unavoidable with the resources available and conditions of the study.

Another major indication of the results of this portion of the study is the noninfluence of source location on both the severity and rates of bleaching responses of test corals. Such a suggestion could imply that there are no adaptive differences within species, at least on the local scale. Had there been differences between salinity tolerances of corals inside the bay and outside the bay, a conclusion about adaptation to location could have been drawn. Instead, the salinity tolerances of coral species in the area seem to have influenced distribution patterns, not the other way around.

2. Distribution of coral species along Pao Pao Bay with respect to distance from freshwater source

The most striking and visible distributional trend in the survey conducted in this study was the significant decrease in individual coral colony frequency. The numbers decreased from 120 and 139 individuals in stations EBR and WBR, respectively, to 4 and 0 individuals in stations BH and ARE, respectively. A similar trend of decreasing species richness toward the back of the bay was also observed. This gradient is strongly correlated with distance from the barrier reef beyond the mouth of the bay, and more importantly, with the proximity to the freshwater inlet at the land-end of the bay, where rainwater washes in from the valley after storms.

Although salinity was measured frequently during the duration of the study, few salinity fluctuations were observed. One such fluctuation was observed on October 1, 2001, after a five-day period of rain, when a reading of the salinity was 34ppt at the ARE station. Subsequent readings were made after short periods of rainfall, but there were no fluctuations of more than 0.5ppt. The most probable reason for this is that the study was not conducted during Moorea's wet season, where the island gets substantial amounts of rain.

In a similar study on distribution performed in Opunohu Bay by Adjeroud and Salvat (1996), the lowest salinities of the wet season occurred on March 9, 1992 after three days of heavy rain. On this day, the salinity measured at the back of Opunohu Bay was 2.0ppt, certainly enough to stress a coral colony if there was one there to stress (there are no corals at the base of Opunohu Bay). Adjeroud and Salvat also reported that the land-end stations were generally the only areas affected by freshwater discharge, but that sometimes freshwater influence reached the middle part of Opunohu Bay. Pao Pao Bay is very similar to Opunohu Bay and likely experiences similar fluctuations when there is heavy rain. Thus, the lack of corals at the base of Pao Pao Bay appears to be mainly due to the salinity stress following heavy rain, which could become lethal during cyclonic flashfloods. Another factor in coral distribution, not directly measured in this study, may be sedimentation. After heavy rainfall, fine sediments are washed into the bay along with freshwater. In his study on coral stresses, Coffroth (1985) found that fine sediments may stress *Porites* corals. Since it has already been established that branched corals tend to be even more sensitive to environmental stressors than massive corals, the fact that there were no branched corals near the land end of the bay where there are massives and other more tolerant corals could be explained by the influx of a combination of fine sediment and freshwater into the bay. This would certainly account for the trend of decreasing species richness toward the back of the bay. Serious stressors of this nature would also explain the trend of decreasing individual frequency toward the back of the bay.

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Coral reef composition and patterns of recovery in Moorea, French Polynesia

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ABSTRACT. Coral reefs are important and complex ecosystems which show substantial variation in terms of species diversity, coral cover, and recovery patterns. Because of their importance and slow recovery, it is necessary to monitor coral reefs over long time periods. This study established three 15 meter permanent transects at each of two sites in the fore and back reef environments in Moorea, French Polynesia, which had been studied by previous researchers. Two hundred random points were sampled at each transect and data on substrate type as well as coral and algae species present were recorded. Size and density measurements for *Pocillopora sp.* corals were taken from six, 15 m^2 transects in the back reef and 16, $1m^2$ quadrats in the fore reef. These data were used to identify differences between the fore and back reef environments as well as identify patterns of recovery by comparing result from this study to data from previous studies. The fore and back reef were found to be significantly different from each other. The fore reef had higher total coral coverage, higher species diversity, and larger density and average size of *Pocillopora* corals. When compared to data collected directly after a hurricane and coral bleaching event in 1991, it was found that the reef had recovered significantly. Indicators of this recovery are increased coral cover, larger colony size of *Pocillopora sp.* corals, and lower cover of fleshy algae.

Introduction

Coral reefs are diverse and extremely complex ecosystems which provide a CO_2 sink, protect coastlines from wave energy and provide a home for a myriad of vertebrate and invertebrate organisms (Smith and Buddemeier 1992). Any disturbance to the coral reef ecosystem can have long term effects, many of which are still not well understood. Some of the possible results of disturbance are change in the role of reefs as CO_2 sinks, loss of species diversity and coral cover, and potentially a change to an earlier succession state or different stable state.

Damage to the coral reef ecosystem could result in a loss of this important carbon sink and contribute to a runaway greenhouse scenario in which increases in atmospheric CO_2 levels kill organisms which sequester or process CO_2, and result in even higher temperatures and CO_2 levels (Boering pers.com.). It is important to collect baseline data on species diversity, recovery patterns and the physical characteristics of reefs in order to track the effects of global climate change. Hopefully an understanding of coral reef responses to disturbance will allow appropriate conservation measures to be implemented to preserve the valuable coral reef ecosystem.

Coral population density and diversity are important indicators used in monitoring the health of coral reefs. The "longevity of coral reef communities" makes it necessary to study coral reefs over long time periods (Grigg and Maragos 1974). Data on reefs need to be collected when they are in both disturbed and non-disturbed states in order to examine reef responses to individual disturbance events and long term climate change.

Several environmental factors increase the importance of monitoring coral reefs. Present increases in greenhouse gases are predicted to contribute to a 6 cm/year increase in global sea level, and a 0.2-0.5 °C/decade increase in global temperature. These temperature increases will likely alter coral species distribution due to increased water temperatures at higher latitudes (Smith and Buddemeier 1992). UV irradiance, which is also predicted to increase in the near future, and increased temperatures have been shown to be major causes of coral bleaching which can lead to significant coral mortality (Spencer et al. 2000).

Global warming is also likely to affect CO_2 saturation states and cause changes in amounts of CO_2 in solution. The effects of such a change on corals is unknown (Smith and Buddemeier 1992). Another possible result of global warming is storms of increased frequency and intensity (Druyman et al. 1999). Mechanical damage and extreme tides during storms significantly decrease live coral cover and cause the emergence of an earlier succession state (Loya 1976).

Moorea is an interesting location to study coral reefs because it is an area of low coral diversity due to its small size and isolation from the colonization source (Adjeroud 1997). Lower species diversity makes it easier for researchers to identify corals in Moorea, and isolation from the colonization source ensures that recovery from disturbance will have little outside influence. Several studies have examined the health and diversity of both the fringing and barrier reefs of Moorea, French Polynesia (Done et al. 1991; Gleason 1994). Eight years have lapsed since the last study was conducted. Done et al. (1991) were looking at reef recovery from an outbreak of the coraliverous starfish *Acanthaster planci* in 1982. Gleason (1994) looked at recovery from cyclone Wasa in 1991 and a coral bleaching event later that same year. Another study published in 1997 on spatial patterns of reefs around Moorea provides limited data on coral species (Adjeroud 1997).

In the period since these studies were conducted, there have been two disturbances which may have affected the health of the reef. The first is a cyclone which struck French Polynesia in 1997 but whose effect on the reef of Moorea is uncertain, and the second is a coral bleaching event in 1999 which is described in the literature as "high bleaching and mortality, with great variability at the inter- and intra-island scale" (Salvat 2000). Two years have elapsed since the last disturbance, which is the same amount of time between the last year of Gleason's (1994) study and cyclone Wasa and the 1991 coral bleaching event. This study will determine if the effects of these two most recent disturbances were as significant as those observed by Gleason.

The back and fore reef environments have different physical characteristics. The back reef is shallow, 1-2 meters in depth, and is protected from wave action by the algal ridge. It is also a patchy landscape with many different microhabitats consisting of open areas of sand, rubble, and pavement, as well as topographic diversity created by dead standing coral (Gleason 1994). The fore reef is deeper, and is exposed to greater wave action. The fore reef has less topographic diversity on small (<5 m) scale, but on the larger scale has spur and groove topography which creates grooves similar to valleys running perpendicular to the reef crest (Glynn et al. 1996).

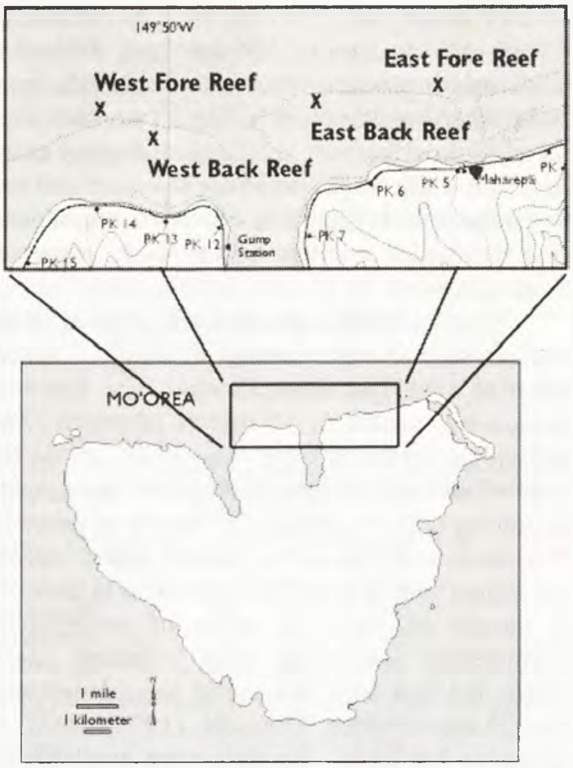

Figure 1. Map of Moorea showing locations of study sites. EBR= east back reef; EFR= east fore reef; WBR= west back reef; WFR= west fore reef

The first part of this study looked at coral cover, species diversity, and *Pocillopora* colony size and density. These data were used to test the hypothesis that the back and fore reef environments were different in terms of coral cover and species diversity.

The second part of this paper compares the findings of this study to previous research on the reefs of Moorea to examine over all reef health and recovery responses to natural disturbance events. The hypothesis above is expanded to state that the fore and back reef are significantly different because of their differing recovery patterns.

Study Site

This study was conducted on Moorea (17°30'S; 149°50'W) in the Society Islands of

French Polynesia. Moorea is a volcanic high island with an area of 134 km^2 and 49 km^2 of reefs and lagoons. Four individual sites were established outside Cook's Bay. Two sites were selected on either side of the boat channel at the entrance to the bay, one in the fore reef and one in the back reef (Figure 1). For GPS coordinates and detailed directions to the study sites see Appendix A.

At all sites, transects ran parallel to the reef crest, and were located at least 15 meters apart to avoid pseudoreplication. The fore reef sites were located in 10 meters of water. The individual transects were established across the tops of spurs in the spur and groove topography, following the 10m contour as closely as possible. The back reef sites were located approximately 50 meters inland from the ridge crest in about 1-2 meters of water in areas of topographic complexity containing dead standing coral. These habitats were chosen in accordance with the characteristics Gleason (1994) used in choosing her sites. No data were available on the exact sites which any of the previous researchers used. These sites were established as close to Gleason's original sites as possible, based on the map in her dissertation and personal communication.

Materials and Methods

At each of the four sites, three 15 meter long permanent transects were established parallel to the reef crest. The two back reef sites were established using snorkeling gear. The two fore reef sites were established using SCUBA. All diving was conducted under the auspices of UC Berkeley and conformed to the protocols of the University of California, Berkeley Diving Safety Board. Depth measurements at the fore reef site were taken using a Suunto Vyper dive computer. GPS measurements were taken with a Garmin eTrex hand held GPS unit.

Voucher specimens from the Centere de Recherches Insulaires et Observatoire de l'Environnement (CRIOBE) station and those left at the Gump Research Station by Gleason were used to identify the coral species. Additional identification information came from Corals of Australia and the Indo-Pacific by J.E.N. Veron (1993).

1 Natural History of the coral reefs of Moorea

Percent Cover and Species Diversity. The sampling procedures followed Gleason (1994). A 15 meter chain composed of 1.5cm links was strung from pieces of rebar marking the starting and ending points of the transect. Samples were obtained at 200 of the links. Five links were randomly chosen within each half meter length of chain. The remaining 50 links were randomly chosen from the whole length of the chain. The type of algae, substrate, or coral was recorded at each random link.

All organisms were identified to the species level with the following exceptions. Crustose algae other than *Lithophyllum flavescens* were categorized as "crustose algae". Fleshy macroalgae were grouped into the category "Other fleshy algae" except for *Turbinaria ornata, Chlorodesmis fastigiata,* and *Asparagopsis taxiformis* which were identified to the species level, and *Sargassum* and *Halimeda* which were identified to the genus level. *Porites lutea* and *lobata* were grouped into a single category due to difficulties distinguishing them in the field. Since many of their distinguishing characteristics arise only after a certain size has been reached, *Pocillopora* corals too small to be identified reliably were placed into a category named "juvenile."

Categories for substrate were sand, rubble, pavement, and Damsel fish territory. Pavement refers to level areas composed of cemented coral debris. Rubble consisted of large pieces of unattached dead coral. Damsel fish territories were areas with a turf of fleshy macroalgae protected by *Stegastes nigricans* (Done et al. 1991).

Data from the east and west side of either environment were pooled for analysis. This was done to achieve a larger sample size and to make the data more representative of the reefs of Moorea as a whole. Data were analyzed with a chi-squared test to determine significance levels.

Species diversity was examined using the Shannon-Weiner index as modified by Loya (1972). $H'_c,=\sum p_i \, ln p_i$ where p_i is the proportion of total cover contributed by the i^{th} species.

Colony Size, Density, and Population Structure of Pocillopora *Corals.* To determine colony density and size, all *Pocillopora* corals within the areas defined below were identified to the species level and measured. For the back reef habitats all corals within 1 meter of the south side of the transect were measured for a total of 45 square meters per site. Because of the higher density of *Pocillopora* corals in the fore reef environment, and keeping with the methods of previous researchers, colony density and size were measured in eight, 1x1 m quadrats, for a total of eight square meters per site. Branching corals were assumed to be a single colony if only one point of attachment could be discerned.

For colony size calculations, colonies were assumed to have elliptical shapes. The longest axis of a colony and the perpendicular axis were used as the two axes of the ellipse. Measurements were taken from branch tips and recorded to the nearest centimeter.

Colony density is the total number of *Pocillopora* colonies at a site divided by the total number of square meters observed for that site. Data from sites within the same habitat was compared using chi squared tests and combined for analysis when not shown to be significantly different.

Population structure was examined by graphing the relative proportions of each species of *Pocillopora* coral measured for the size and density calculations.

All quantitative data were analyzed using a one-way ANOVA, while frequency data was analyzed using chi-squared.

Videotapes were made of all transects and are available on request from the author or the Department of Integrative Biology at UC Berkeley.

2 Recovery Patterns

Data from this survey were compared to that collected by earlier studies on Moorea in order to look at long term changes and recovery patterns for the study area. Trends in reef recovery are shown by graphing back reef data from this study with that collected in 1989 (Done et al. 1991) and 1992 (Gleason 1994).

Results

1 Natural History of the coral reefs of Moorea

Percent Cover and Species Diversity. A total of 24 coral species from 11 genera were identified (Table 1). Eight out of 11 genera were significantly different ($p<0.05$) between the fore and back reef environments, with the exception of *Porites, Montipora,* and *Psammocora*. There were significant differences in coral cover for 17 out of 24 species. With the exception of *M. digitata*, all the species not shown to be significantly different have occurrences at 9 or fewer sample points per environment.

All alga categories were shown to be significantly different with the exception of the encrusting algae *Lipthophyllum flavescens* and the fleshy algae *Chlorodesmis fastigata* (Table 2).

Table 1. Total number of coral species recorded for each environment. Genus names are in capitals and species names are below. In *Pocillopora*, "juvenile" refers to colonies which were to small too be identified to the species level. *Porites lobata* and *P.lutea* were combined for analysis due to difficulties distinguishing between the two. The values in the row with the genus names are the sums of all species found in that genus. Key for the final column: ** $p<0.01$; *$p<0.05$; blank indicates data for that row is not significantly different between environments.

	Back Reef	Fore Reef	p-value	Significance
POCILLOPORA	89	237	2 E-16	**
verrucosa	50	96	1E-04	**
meandrina	19	108	3 E-15	**
damicornis	2	0	0.157	
eydouxi	17	31	0.043	*
juvenile	1	2	0.564	
ACROPORA	8	123	9 E-24	**
austera	4	9	0.166	
cytherea	0	2	0.157	
gemmifera	1	40	1E-09	**
valida	0	63	2 E-15	**
hyacinthus	3	1	0.317	
PORITES	183	214	0.120	
lutea& lobata	164	107	5 E-04	**
rus	17	98	4 E-14	**
australiensis	2	9	0.035	*
MONTIPORA	123	126	0.849	
efflorescens	56	18	9 E-06	**
verrucosa	9	2	0.035	**
monasteriata	39	77	4 E-04	**
digitata	21	29	0.258	
LEPTASTREA	2	12	0.008	**
transversa	2	12	0.008	**
MILLEPORA	13	0	3 E-04	**
Millepora	13	0	3 E-04	**
MONTASTREA	0	10	0.002	**
curta	0	10	0.002	**
CYPHASTREA	0	9	0.003	**
micropthalma	0	9	0.003	**
PSAMMOCORA	2	4	0.414	
profundacella	2	4	0.414	
PAVONA	2	19	2 E-04	**
varians	2	17	2 E-04	**
FUNGIA	0	4	0.046	*
Fungites	0	4	0.046	*
CORAL COVER	424	758	2 E-22	**

Substrate data are reported as the number of random points which were over a certain type of substrate (Table 3). The back reef was found to have a more clustered distribution. On many of the transects, all recorded colonies were close together with large areas of exposed

Table 2. Total number of algal species found for each environment. The last two categories contain all other algae which could not be identified to the species level. **= $p<0.01$; *= $p<0.05$.

	Back Reef	Fore Reef	p-value	Significance
Turbinaria ornata	33	0	9 E-09	**
Sargassum sp.	19	0	1E-05	**
Halimeda sp.	0	3	0.083	
Lithophyllum flavescens	20	18	0.746	
Chlorodesmis fastigiata	2	0	0.157	
Asparagopsis taxiformis	0	18	2 E-05	**
Other encrusting	3	40	2 E-08	**
Other filamentous	0	36	2 E-09	**

Table 3. Number of samples belonging to each substrate type. Pavement is hard, flat, cemented substrate; rubble is uncemented coral debris; dead coral is all coral skeletons still upright and attached to the reef; sand is loose sand; damsel fish territory is an area of macroalgae which is protected from other grazing fish by *S.nigricians*.

	Back Reef	Fore Reef	p-value	Significance
Pavement	289	178	3 E-07	**
Rubble	139	82	1 E-04	**
Dead Coral	153	63	9 E-10	**
Sand	79	4	2 E-16	**
Damsel Fish Territory	39	0	4 E-10	**

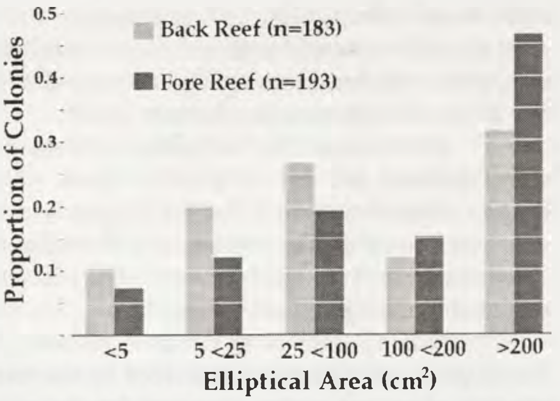

Figure 2. Proportion of colonies in each of five size categories. Notice that all categories less than $100cm^2$ had higher frequencies for the back reef while all categories over $100cm^2$ had higher frequencies for the fore reef.

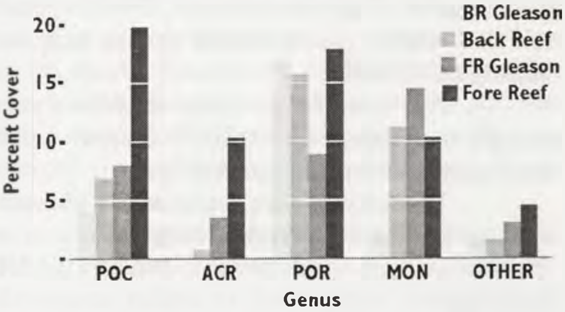

Figure 3. Mean percent cover data for back (BR) and fore (FR) reef sites from this study compared with Gleason's 1992 data. Categories are: POC= *Pociloopora* ACR= *Acropora* POR= *Porites* MON= *Montipora* Other= All other genera not identified by Gleason.

substrate separating them. The higher occurrence of dead coral (Table 3) was observed to give the back reef much higher topographic relief than the fore reef.

Species diversity for corals was $H'=2.08$ for the back reef and $H'=2.52$.

Colony Size, Density, and Population Structure of Pocillopora *corals.* Average colony size of *Pocillopora* corals was similar for the fore and back reef; 222 and 261 cm^2 respectively. Although average size was similar, figure 2 shows that there are large differences in proportion of colonies within each size class. These differences were not found to be statistically significant ($p>0.05$).

Density of corals was 12.1 colonies/m^2 in the fore reef and 2.0 colonies/m^2 in the back reef.

The dominant *Pocillopora* species seems to change with distance from the shore. *P. damicornis* was observed in high concentrations on preliminary surveys on the fringing reef, but was found only in small amounts on the transects (2.8% in the back reef and 0% in the fore reef). *P. verrucosa* was the most prominent *Pocillopora* in the back reef, 62%, but decreases to 32% of all *Pocillopora* corals in the fore reef. *P. meandrina* represented only 20% of the genus total in the back reef, but was the most prominent in the fore reef (54%). *P. eydouxi* was present in similar amounts between the two sites, 16% in the back reef and 11% in the fore reef.

2 Recovery Patterns

Figure 3 compares the data on coral cover for the major genera of coral identified by Gleason to the findings of this paper. All genera showed an increase in percent cover except for *Porites* which decreased by 1.75% in the back reef, and *Montipora* which experienced a 4.26% decrease in the fore reef. Chi-squared tests showed the significant changes to be for *Montipora* in the back reef (p=0.012) and for *Pocillopora* (p=0.023) and total coral cover (p=0.012) in the fore reef.

When the data on colony size were analyzed with a one-way ANOVA, all changes were shown to be non-significant, with p-values greater than 0.05 (Figure 4). In both environments the proportion of smaller colonies exhibited a decrease, while the proportion of larger colonies increased, especially those over 200 cm^2 in area. In the back reef, the proportion of colonies in the under 5 cm^2 and 5-<25 cm^2 categories decreased, while all larger categories proportionally increased. All fore reef colony categories have lower proportion of colonies than was found in 1992 except of the category over 200 cm^2 which increased by more than 100%.

Changes in colony density were opposite for the two sites. This shift was a decrease from 2.8 to 1.8 colonies/m^2 in the back reef, as opposed to an increase from 7.5 to 10.9 colonies/m^2 in the fore reef. These colony densities are different than the ones reported above because they include only colonies which had a diameter greater than 3 cm in accordance with the methods used by Gleason (1994). Proportions of *P. eydouxi* and *P. damicornis* remained relatively constant, but there was a decrease in amount of *P. meandrina* and an increase in *P. verrucosa* in both the back .and fore reef environments (Figure 5). This study found much larger disparities between the proportion of P. *verrucosa* and *P. meandrina* between the back and fore reef sites.

Further trends in reef recovery are shown in figure 6. The significant changes from 1989 to the present include the increase in live coral cover (p<0.01) and the decrease in Damsel Fish territory (p<0.01). Decrease in Damsel Fish territory from 1992 to present was also shown to be significant (p<0.01), but all other changes were not significantly distinct.

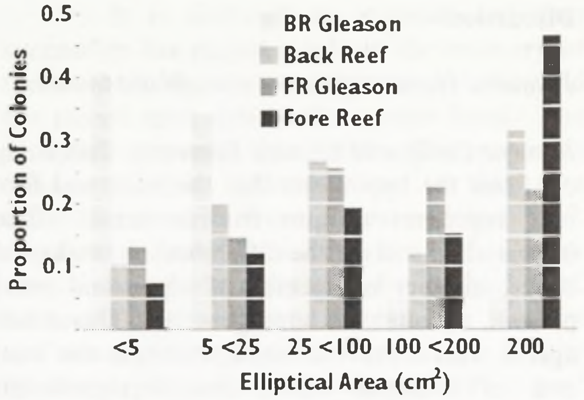

Figure 4. Comparison of colony size with Gleason's 1992 study. Size categories are the same used by Gleason

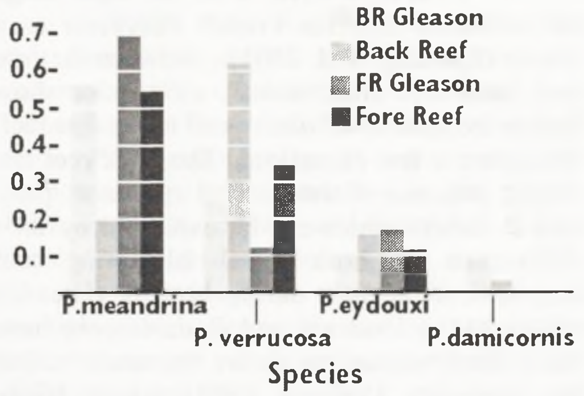

Figure 5. Proportion of total *Pocillopora* colonies measured belonging to each species graphed with Gleason's 1992 data. Omitted are all corals in the category "juvenile".

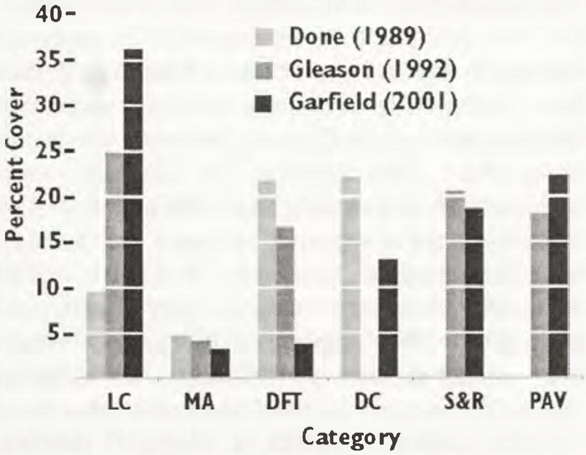

Figure 6. Comparison of back reef data to previous research. The 1989 data was collected 7 years after a major *Acanthaster planci* attack, and the 1992 data was collected 1 year after a cyclone and coral bleaching event. Blank columns represent categories not used by that study. Categories are: LC= Live Coral MA= *Turbinaria* and *Sargassum* DFT= Damsel Fish Territory DC= Dead Coral S&R= Sand and Rubble PAV= Pavement.

Discussion

1 Natural History of the coral reefs of Moorea

Percent Cover and Species Diversity This study confirms the hypothesis that the back and fore reef represent unique environments. The indicators of this are the differences in total coral cover, number of species of algae and coral present, and habitat homogeneity. This result agrees with a previous study done on the west end of Moorea which found significant differences between the back reef and the fore reef environments when examining corals, sponges, and mollusks Adjeroud (1997).

Total coral cover is on the upper range of published data for French Polynesia as a whole (Spalding et al. 2001). Between the fore and back reef sites, almost all species show higher frequencies of occurrence in the fore reef. There are a few exceptions. The back reef has higher presence of the massive species *P. lutea* and *P. lobata* which can be explained by their resistance to both coral bleaching and mechanical damage during storms (Gleason 1994). *M. efflorescens* and *P. damicornis* have both been shown to thrive in more turbid environments (Vernon 1993) which likely explains their high back reef occurrences. *M. millepora* was observed at both environments, and in apparently equal amounts. The lack of any fore reef samples on *M. millepora* is indication of the small sample size of this study.

Although the findings of this study are similar to the data published on French Polynesia as a whole for percent cover and dominant genera, they are much lower in terms of species diversity. Spalding et al. (2001) report approximately 168 coral species and note that *Pocillopora, Porites,* and *Acropora* are the most common genera. Done et al. (1991) found around 150 species during their study, and Adjeroud (1997) reported 79 species. These differences are due to different size sampling areas. The author observed numerous other coral species, such as *A. noblis, A. elseyi, F. concina, F. danai,* and *P. cactus*, which were not encountered by the random sampling method used in this study. Increasing the number of transects would likely increase the number of species identified.

Measurements of species diversity are important for identifying recovery patterns and stable states. Loya (1976) states that "maximal species diversity can be expected just before interspecific competition becomes significant." For this reason it is important to have baseline data on species diversity available for determining when a reef is at a stable state, and when it is still recovering from a disturbance event. It is interesting to note that although the Shannon-Weiner diversity index is widely used in coral ecology studies (Loya 1972; Grigg and Maragos 1974; Adjeroud and Slavat 1996 and others) it is not used by either Done et al. (1991) or Gleason (1994). The area closest to this study with a published diversity index is the fringing reef at the mouth of Opunohu Bay, Moorea (Adjeroud and Salvat 1996). Since these indices come from different environments, they cannot be compared, but they do indicate that the need for additional research on Moorea.

In addition to having lower coral cover and fewer species present, the back reef has several habitats not found in the fore reef. These include areas of non-palatable fleshy algae, *Turbinaria ornata* and *Sargassum sp.*, and Damsel Fish territories. These two habitats likely offset each other in terms of their effects on coral species diversity and percent cover. The fleshy algae areas cannot be eaten by grazing fish and, once established, kill existing corals and prevent colonization of recruits by blocking sunlight and abrading colonies (Gleason 1994). Damsel fish territories have the opposite effect. Done et al. (1991) found that these territories had higher cover and diversity than surrounding areas. The higher cover and diversity is a result of the grazing patterns of the Damsel fish. By defending their territory from other grazing fish, the Damsel fish can "farm" a standing crop of fleshy algae. Coral recruits can settle on the substrate below the algae, and will not be eaten by the Damsel fish, who do not graze the substrate bare, and are protected from grazers who would otherwise eat the recruits while grazing algae (Gleason 1994).

Colony Size, Density and Population Structure of Pocillopora *corals.* The general population structure for *Pocillopora* corals was for larger colonies and higher density in the fore reef. When combined with the above data on coral species diversity and abundance, these data to indicate that the fore reef is more mature and is less susceptible to the effects of bleaching and cyclones.

2 Recovery Patterns

Current reef condition shows significant improvement in the eight year interim since the last study on Moorea was conducted. The main indicators of this are increased total coral cover and increased colony size for *Pocillopora* corals. Coral cover is much higher, and cover of fleshy algae is much lower after the present, 10 year recovery from bleaching and a cyclone than after a seven year recovery from a *Acanthaster planci* outbreak reported by Done (1991). This is likely due to the comparatively small impact the coral bleaching event and cyclone had on the reef. The 1991 bleaching event caused severe damage to *Pocillopora* (17% mortality) and *Acropora* (63% mortality) but only resulted in a 3% decrease in total coral cover. For the entire three year period starting a few months before the bleaching and continuing until two years after cyclone Wasa, there was less than 5% change in percent cover of the major coral genera (Gleason 1994). It appears that the neither the bleaching events of 1991 and 1997 nor the cyclones of 1991 and 1997 had nearly as much impact on the reef ecosystem as the *Acanthaster planci* outbreak of 1982.

Predicted recovery rates vary significantly between different studies. The range is from 2-10 years for limited reef damage (Harmelin-Vivien 1986) to 20-50 years for recovery from complete destruction (Grigg and Maragos 1974). Recovery times tend to be faster when there are nearby live corals to produce larva which can quickly recolonize the area. The presence of coral species which reproduce by brooding and releasing large numbers of larvae as well as those which can reproduce through asexual fragmentation further reduce recovery time (Glynn 1992). This appears to be the case for the rapid (~10 year) recovery the reefs of Moorea have undergone. There was still living coral after the both the 1982 *Acanthaster planci* outbreak and the bleaching event and cyclone in 1991.

There has been considerable debate on whether coral reefs exhibit succession. Rogers (1993) states that "no predictable, orderly progression of species has been demonstrated to date for coral reefs." Researchers who do not see evidence for succession often believe that there are multiple stable states for coral reefs, and that disturbance provides the reef with an opportunity to change to a different stable state (Harmelin-Vivien 1986; Rogers 1993). Other studies have found five stages of succession: dominance, recruitment of coral larvae, growth form differentiation, expansion, and competition between corals (Colgan 1981). No studies dealing specifically with succession of coral reefs have been conducted on Moorea.

It is difficult to evaluate whether succession has played a role in the recovery of the reefs of Moorea since previous studies did not record coral data to the species level. The exception is species data for *Pocillopora* corals (Gleason 1994). Comparison to this data illustrates a shift from *P. meandrina* to *P. verrucosa* in both the back and fore reef. Colony density data shows a decrease for the back reef. Although colony density increased in the fore reef since, present is still lower than the density Gleason reported for 1991. Colgan (1981) used similar findings on decrease in colony density with increase in average colony size to support his theory that reefs exhibited five stages of succession.

While there may be some indication that succession plays a role in the recovery patterns of the coral reefs of Moorea, it is inconclusive. These indicators include the shift from *P. meandrina* to *P. verrucosa*, the proportionally large increases in the genera *Porites* and *Acropora*, and decreased colony density with increased colony size since 1992. There is no evidence to support the theory of multiple stable states for the reef. Future studies will be needed to investigate whether the differences between the back and fore reef identified in this study affect the role of succession or multiple stable states.

It is important to understand which of these theories applies to coral reefs in order to best protect and manage this resource. For example, if multiple stable states are possible and a disturbance caused a large reef ecosystem to change to an algae dominated state there could be serious ecological and environmental consequences. If instead reef ecosystems followed a pattern of succession to a climax state, they would be less susceptible to disturbance, and would not need such diligent monitoring and management. Anthropogenic disturbances can be lessened by decreasing greenhouse gas emissions and trying to keep nutrient and sediment cycles undisturbed. French Polynesia is taking an active step in preserving their reefs by collecting the coraliverous starfish *Acanthaster planci* (Adjeroud 1997) in order to prevent further outbreaks such as the one in 1982.

The overall health of the coral reef ecosystem of Moorea is above average for French Polynesia on a whole in terms of total coral cover. Future studies are needed to see if a dominant species will emerge in the absence of future disturbance, or if the reef has already reached a stable climax state. By understanding reef responses to disturbance, future studies will be able separate short term changes due to disturbance events from long term responses to global climate change. Understanding reef recovery and responses to climate change will allow us to employ conservation techniques which will help preserve this valuable ecological resource.

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Appendices

Appendix A: Directions to sites.

- East Back Reef: GPS: 17°28.598'S; 149°49.252'W

Located in 1.5-2 meters of water, 50 meters from the reef crest. When in position the inner channel marker of the reef pass entrance to Cook's Bay (red) is in line with the first clump of trees on the farthest visible ridge to the west. Also, the outer of the two red markers denoting the boat channel on the east side of the bay lines up with the gap between two portions of a house consisting of two identical rooms, each a square with a pointed roof made of palm leaves. Transects are 15 meters apart in a east west line, parallel to the ridge crest.

- West Back Reef: 17°28.660'S; 149°50.231'W

Located in 1.5-2 meters of water, 50 meters from the reef crest. The site is 300m from the point were the boat channel inside the reef turns from north to east, as denoted by markers black on the bottom, white on top. The second buoy in line starting islandward marking the reef pass to Cook's Bay (green) is in line with the last point of land to the east. A large coconut palm tree is in line with the left side of the Gump House. A tall pointy roof at the Sheraton is on the right side of a large notch in a roof directly behind it. There is a large head of Porites, the top of which is covered with Turbinaria and comes very close to the surface near the start of the second transect. The transects each run parallel to the reef crest and are parallel to each other, separated by 15 meters.

- East Fore Reef: GPS: 17°28.350'S; 149°48.301W

Located directly seaward from the westernmost building of the Bali Hai Hotel in 10 meters of water. Transects are in a line east to west across the tops of spurs in the spur and groove topography, separated by at least 15 meters. There is a large head of Porites at the end of the third transect.

- West Fore Reef: 17°28.572'S; 149°50.584'W

Located in 10m of water almost directly seaward from the Sheraton Hotel. There is a small piece of debris which protrudes from the water directly inside the reef. The hole in the top of Mauaputa lies directly over a basalt exposure on the first ridge to the west of Cook's Bay. Transects are in a line east to west across the tops of spurs in the spur and groove topography, separated by at least 15 meters. There is a large head of Porites coral at the end of the third transect.

Vertical stratification of zooplankton populations in Pao-Pao Bay in Moorea, French Polynesia: a test for diel vertical migration

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ABSTRACT. The diel vertical migration (DVM) of zooplankton populations has been used to describe vertical stratification of zooplankton populations all over the world. I sought to explore whether vertical stratification in Pao-Pao Bay in Moorea, French Polynesia was influenced by diel vertical migrations. The unique properties of the bay make it an interesting testing ground for DVM. Not only is Pao-Pao Bay shallow, it is also protected by a barrier reef. In order to observe DVM, plankton tows were performed over a lunar cycle or 28 days and at two times during the day: at 1000H-1200H (daytime) and 2200H to 2400H (nighttime). During each sampling, a deep tow was done at 15m and a shallow tow at 0-1m. Towing was performed over a 250-300 meter line near the center of the bay. After tows were performed, two 10mL samples were used to extrapolate the total population of zooplankton collected. Of the organisms found, three major groups appeared in nearly all collections: chaetognaths (Phylum *Chaetognatha*), copepods (Order *Calanoida*) and gastropod larvae (Class *Gastropoda*). The amount of each organism group found during each tow (either day or night and either deep or surface) was compared to the total number of that tow to create percentages. These percentages were then compared to see whether greater populations of the organisms resided in deeper waters during the daytime and surface waters during the nighttime as DVM dictates. Copepods remained in deeper waters during the daytime and were randomly dispersed during night. Gastropod larvae remained in shallower waters during daytime and were randomly dispersed at night. Chaetognaths showed no definitive pattern of stratification. These findings can be explained by the presence of predators or the lack thereof, availability of food resources, differences in development and weather patterns. Copepods exhibited DVM at the depths tested while it was unclear whether DVM dictated vertical stratification of the gastropod larvae and chaetognaths.

Introduction

The vertical stratification of zooplankton populations in bodies of water is often attributed to diel vertical migration (DVM). By understanding DVM, one can understand where and when zooplankton populations migrate over a daily period. The general pattern of DVM amongst zooplankton is derived from the movement of plankton upward towards the surface during daytime, and then migrating downwards during nighttime. The DVM of zooplankton and its relevance to the vertical distribution of zooplankton has been well-studied. The approaches to researching DVM and its causes have gone mainly in two directions: one direction investigates the physiological processes that induce the action of diel vertical migration or the 'proximate' causes and another direction that is directly linked to the first, is the factors that influence the evolutionary change of plankton or the 'ultimate' causes (Ramos-Jiliberto and Gonzalez-Olivares 2000).

Each of these causes affect zooplankton differently from species to species. General patterns can be seen among major groups of zooplankton. Zooplankton can be categorized into two major groups: holoplankton and meroplankton. Holoplankton are zooplankton that spend their entire lives as plankton and meroplankton are zooplankton that spend a part of their lives as plankton (e.g. larvae of *Crustacea* are often zooplankton and then undergo metamorphosis to become benthic as adults). These different life processes that meroplankton and holoplankton undergo are one way to view differences in DVM between major groups.

The proximate causes of DVM have been explained with three main factors: light, food and temperature (Han and Straskraba 2000). Depending on the intensity of light, photosensors are triggered in the zooplankton that cause them to either move towards or away from the region of light. Food availability is another factor in controlling DVM. Zooplankton undergo DVM to find areas in the water where food is more plentiful. Finally, temperature is another proximate cause of DVM, as zooplankton tend to migrate to areas that support thermally optimal conditions for their metabolic processes. Traveling down the temperature gradient can adversely affect metabolic processes of zooplankton (Loose and Dawidowicz 1994).

Surface waters tend to be warmer and therefore provide a thermal optimum for zooplankton.

In recent decades, a great amount of attention has been given to the evolutionary significance or 'ultimate' reasons of why zooplankton perform DVM. The predator-avoidance theory has been extensively researched and is regarded as one of the most important hypotheses that caused DVM to evolve. Carsten J. Loose notes the beginnings of this theory from M. Kozhov (1963), T.M. Zaret and J.S. Suffern (1976) and states how chemical exudates from predators help induce movement of plankton (Dodson 1988; Neill 1990; Dawidowicz and Loose 1992, Loose 1993). Rodrigo Ramos-Jiliberto and Eduardo Gonzalez-Olivares (2000) outline the five major points of the 'predator-avoidance' hypothesis: 1) certain pelagic fish that depend on their vision to feed therefore require light to feed, 2) light can be found closer to the surface in pelagic environments during daytime, 3) zooplankton will in turn want to move to darker locations to avoid predators during day, 4) at night, zooplankton are in an environment that is uniformly safe from predators, and 5) zooplankton will reside near the surface during low-predation times to take advantage of food and higher temperatures. Lampert (1993) notes that metabolic theories have been falsified in some cases, while the predator avoidance theory has received great support and a unifying acceptance.

Even though plankton can avoid predators through DVM, they often give up certain qualities such as growth, fecundity and loss of resources for the protection they receive from DVM (Riessen 1992, Sih 1992). Traveling down the temperature gradient can adversely affect metabolic processes of zooplankton (Loose and Dawidowicz 1994). Although these are immediate costs of DVM, the ultimate costs of DVM must benefit the zooplankton in order for them to develop this behavior. The cost of undermining these qualities versus the avoidance of predators has been well-studied (Loose and Dawidowicz 1994, Lampert 1993). At what threshold of predator risk will trigger the plankton to perform DVM? It is generally thought that food resources become more scarce as the organisms travel deeper into water (Williamson et al. 1996). The phenomenon of DVM goes against this though, as zooplankton travel to the surface and possibly away from maximum food resources. All of these factors are costs that the zooplankters must pay when performing diel vertical migration. Specific chemoreceptors provide zooplankton with the information they need (e.g. if they are in a colder or warmer environment, if they are starving, or if they are ready to undergo reproduction) and in turn, determine at what thresholds do these signals induce DVM in order to avoid predators.

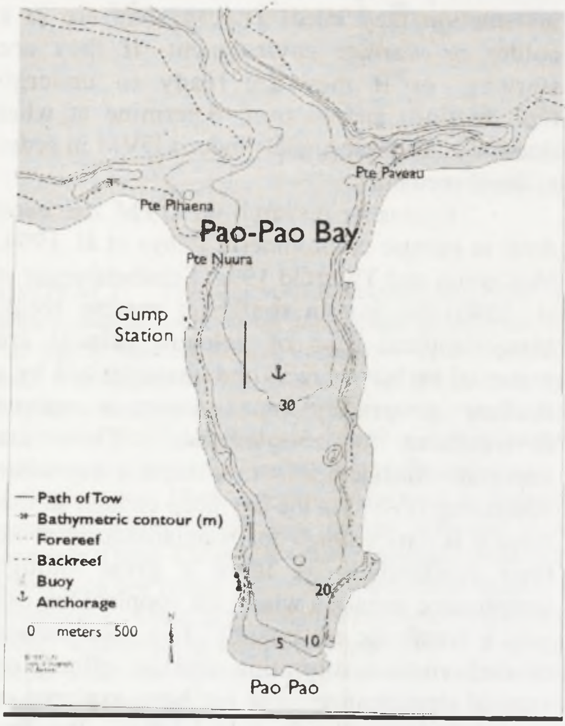
Extensive research on DVM has been done in pelagic environments (Hays et al. 1994, McKinnon and Thorrold 1993, Schabetsberger et al. 2000) but not in shallower marine areas. Many tropical bays of volcanic islands are protected by barrier reefs and characterized by a shallow geography that creates a unique environment for zooplankton. There are important considerations in a tropical bay when addressing DVM. Is the bay deep enough so that there it is dark enough for zooplankton to avoid their predators? Is there a great enough temperature variation where the zooplankton can gain a metabolic advantage? The phenomenon of diel vertical migration and its effects on vertical stratification have not been explored or observed within the Society Islands. Pao-Pao Bay provides an interesting marine environment because it is a shallow tropical bay with a fringing reef bordering a portion of its mouth. A stream, Pao-Pao River, also feeds into the bay. To what extent is DVM utilized by the zooplankton when the bay's deepest point is less than 40 meters? How does the emptying of the river into the bay affect populations? These many questions allude to the significance of DVM in a bay such as Pao-Pao Bay and how zooplankton deal with these possible challenges that a shallow bay offers. Through this experiment, I sought to find whether vertical stratification of zooplankton populations in Pao-Pao Bay are dictated by diel vertical migration. Bo-Ping Han and Milan Straskraba (2001) notes that with the numerous conditions that different bodies of water provide, great variation is to be expected in the way zooplankton will react to each condition. Therefore, this study takes advantage of the environment of Pao-Pao Bay to explore the phenomenon of diel vertical migration in the populations of zooplankton in a classic shallow tropical marine embayment.

Methods, and Materials

Site

Plankton tows were conducted in Pao-Pao Bay in Moorea, French Polynesia. Moorea is located at approximately 149o50'W longitude

Figure 1. Map of Pao-Pao Bay in Moorea, French Polynesia.



and 17o30'S latitude. Pao-Pao Bay can be found towards the east side of the island. The bay is found along the northern shore of Moorea with its mouth facing north. The city of Pao-Pao is situated at the base of the bay. The Pao-Pao River empties into the bay where the city of Pao-Pao lies along the shore. The tow started directly east of the Richard B. Gump Biological Research Station dock and ran 250-300m south along a line that went through a green buoy at the mouth of the bay.

The temperature of the waters was found to be consistently warm during the time of the experiment. During the daytime, the surface waters averaged at a temperature of 27.7°C and at 15.2 meters deep, the average temperature was 27.8°C. At nighttime, the surface waters were at an average 26.3°C and at 15.2 meters deep, the water was an average 26.5°C.

Plankton Tows

Plankton tows were conducted over a lunar cycle (28 days) beginning on October 16, 2001. To observe diel migrations, collections were conducted at two times of the day, six days a week: once at 1000H-1200H and another at 2200H-2400H. A two-person ocean kayak was used and an assistant came along with each tow. During collection times, two tows were done: a deep tow done at an approximate depth of 15.2 meters and a shallow tow done at a depth of 0-1 meter from the surface. A net with 200 micrometer mesh was used in the tows and was attached to a 15.2-meter line. The net measured 98cm in length. The plankton net's opening was a stainless steel ring with an ovular shape measuring 27cm by 24cm or with an area of .005089m^2. The cod attached to the plankton tow held 750mL of liquid. The opening of the cod measured 9cm in diameter. To ensure the tow would sink to the desired depth during the deep tow, a 4 kg weight was attached to the ring of the net to help sink the plankton net. Weather conditions were recorded as accurately as possible. Each tow was conducted along a 250-300 meter line that ran north and south along the center of the bay. The amount of water displaced by the net (not taking current into consideration) was calculated to be approximately 1.27m^3 to 1.53m^3.

The deep tow was done going south or into the bay and the shallow tow was done going north or out of the bay. After a tow was completed, the plankton net was brought up and thoroughly rinsed before being emptied into properly labeled containers. Samples were emptied into Nalgene bottles and stored if necessary in a refrigerator for no longer than 12 hours.

To analyze the samples, each tow's samples were transferred to glass beakers. Each sample collected was mixed thoroughly and then two 10mL samples were withdrawn with a pipet and then placed into petri dishes. The petri dishes were lined with All-Weather graph paper with .25in squares to help guide counting. To each sample, approximately 1mL of 95% ethanol was added to kill the zooplankton. The samples were then counted under a dissecting microscope. Each organism was distinguished under major groups found in *A Guide to Marine Coastal Plankton and Marine Invertebrate Larvae* (Smith and Johnson 1996) and counted. For instance, all calanoid copepods found were counted under the group of copepods by order *Calanoida*. Each species was categorized with major organism groups and if they were not able to be distinguished, they were stored as voucher samples for later identification. Voucher specimens were stored in 95% ethanol.

22 different organism types were found amongst the samples. Species that were consistently found throughout the 28-day period were copepods (order *Calanoida*), chaetognaths (phylum *Chaetognatha*) and gastropod larvae (class *Gastropoda*) (see Appendix A for numbers found from each tow). Copepods and

chaetognaths are both examples of holoplankton and gastropod larvae are examples of meroplankton Their consistent presence provides a good focusing point in observing DVM in the bay.

For each time of day and depth a tow was performed, the number of either copepods, chaetognaths, or gastropod larvae were compared to the total number of zooplankton found in that tow to create a percentage (see Appendix B for sample calculation). The percentages were than plotted on graphs for each species at each time of day. Deep and surface percentages were presented on the same graph pertaining to each species and each time of day.

Hazardous weather and water conditions prevented some days of sampling. Days 2, 15, 16, 17, and 21 are missing either daytime data or nighttime data or both. Therefore, during daytime hours, 24 samples were taken over the 28-day period, and during nighttime hours, 21 samples were taken over the lunar cycle.

Chi-square tests were performed to determine statistical differences in the species collected. Two days were randomly selected out of the four weeks tested. The first chi-square test was done for each of those eight days for each species to compare differences between organisms found during the day and night. The second chi-square test was done to determine statistical significance between the numbers of organisms found either deep or surface waters. P-values less than 5% were considered statistically significant.

Results

Figures 2-7 each plot the percentage of each group found at each depth during the days collected. Nearly all percentages of copepods found in deeper waters were greater than those found in surface waters. 21 out of the 24 samples taken during the daytime and 18 out of 21 samples taken during the nighttime had percentages that were greater in deeper waters (Figure 3). Chi-sqaure values from table 1 further indicate that during the daytime, more copepods were found deeper in the bay. However, during the nighttime, the p-values indicate that only half of the eight randomly selected days were significantly greater in deeper waters than surface waters. Chi-square values from table 4 show that 6 out of the 8 days randomly selected had significant differences in copepods found during the day versus night. Of those six days, four days had significantly greater numbers found during daytime. Chaetognath percentages followed similar trends to the copepod populations. Chaetognaths were found in significant numbers during the 28 days of testing. They comprised approximately 8% of the total plankton found from all tows. Chaetognath populations were found in greater percentages in deeper waters during both daytime and nighttime hours (Figure 4). During the daytime, 19 out of the 24 samples had greater percentages of chaetognaths found in deeper waters versus shallow waters. At nighttime hours, 18 of the 21 samples showed percentages of chaetognaths greater in deeper waters than in shallow waters. Chi-square values in table 2 show that four samples each from day and night were significantly greater at 15.2 meters. Chi-square values in table 4 show all eight days having significant differences in chaetognaths found in day versus night, with five of those days being significantly greater during the day.

Figure 2. Percentage of copepods found in daytime hours in Pao-Pao Bay in Moorea, French Polynesia.

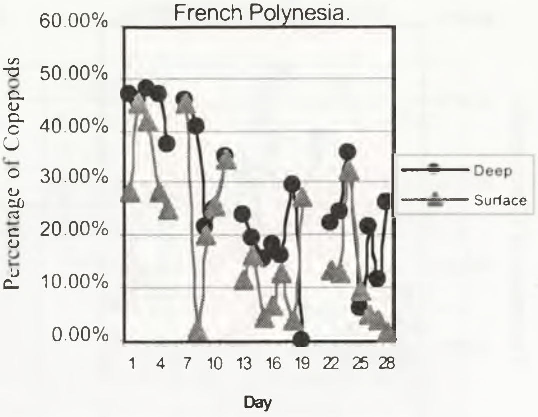


Figure 3. Percentage of copepods found in nighttime hours in Pao-Pao Bay in Moorea, French Polynesia.

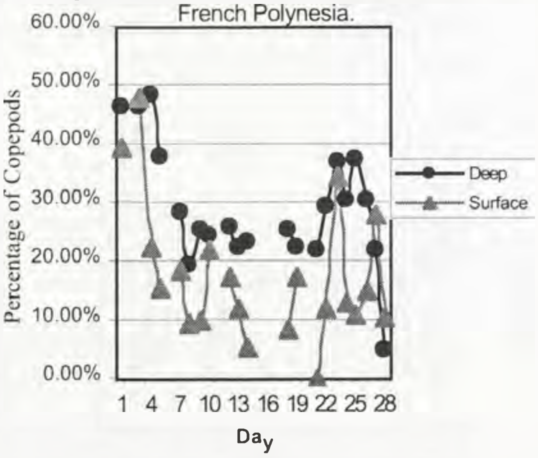


Figure 4. Percentage of chaetognaths found during daytime hours in Pao-Pao Bay in Moorea, French Polynesia.

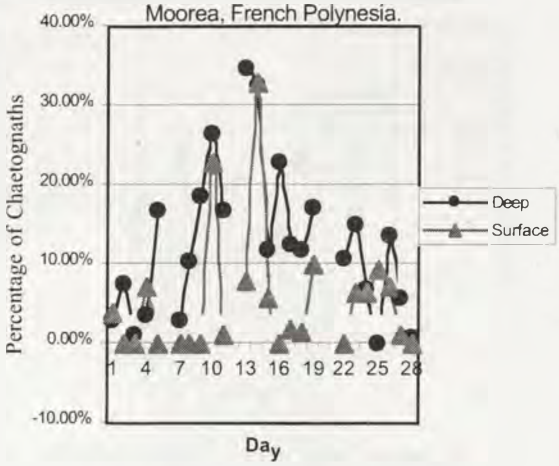


Figure 5. Percentage of chaetognaths found during nighttime hours in Pao-Pao Bay in Moorea, French Polynesia.

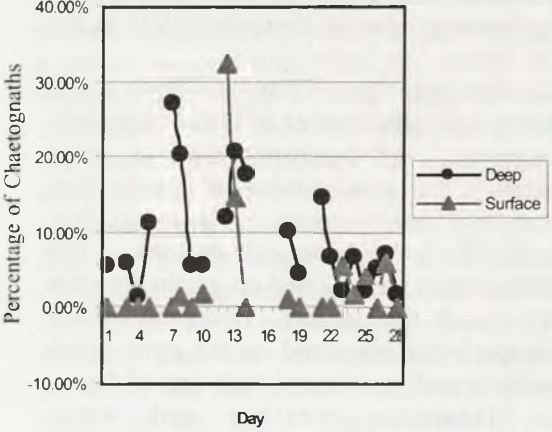


Figure 6. Percentage of gastropod larvae found during daytime hours in Pao-Pao Bay in Moorea, French Polynesia.

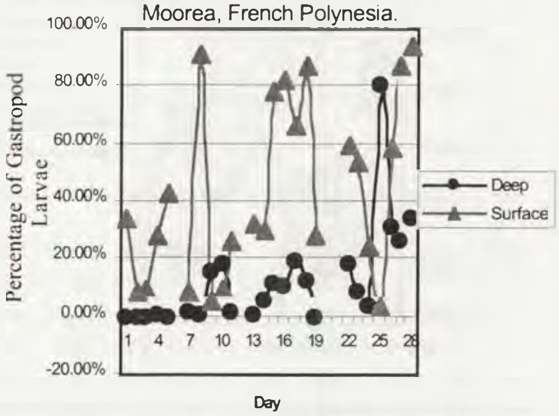
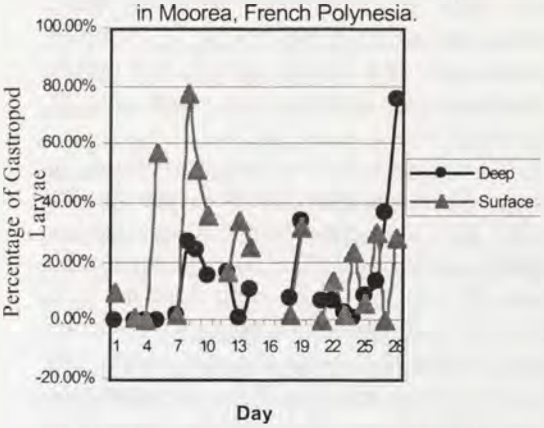


Figure 7. Percentage of gastropod larvae found during nighttime hours in Pao-Pao Bay in Moorea, French Polynesia.



Gastropod larvae populations showed opposite trends to those of the copepods and chaetognaths. Instead of finding more organisms in deeper waters, gastropod larvae were found in shallower waters. In addition, gastropod larvae comprised 27% of total plankton found in all tows, the greatest percentage of all zooplankton types found. During the daytime hours, 21 of the 24 samples showed greater percentages in the surface waters than the deeper waters. The samples taken during the nighttime provide different results then the daytime. Of the 21 samples collected, 12 of the samples showed percentages of gastropod larvae in greater numbers in surface waters. That leaves a little less than half of the remaining samples with greater percentages of larvae in deeper waters. Table 4 shows that at daytime, 6 out of the 8 randomly selected days were had numbers of gastropod larvae significantly greater in surface waters. At nighttime, 3 out of the eight days had numbers of gastropod larvae significantly greater in surface than deep waters.

Discussion

Different trends are observed between the holoplankton and meroplankton analyzed. Although each group exhibited its own vertical stratification during the daytime, all three types of plankton analyzed were randomly distributed during the nighttime. The factors that influence their distribution and migration are specific to each organism group.

Copepods were found to stay deeper during the daytime and had a random distribution during the nighttime, suggesting the migrations of the copepods follow the pattern of diel vertical migration. The implications of remaining at the deeper depth can result from predator avoidance. The random distribution at night means they are free to roam the waters without the risk of being seen by predators.

Table 1. Chi-square values (n=1) and probabilities of tests between depth of copepods found in Pao-Pao Bay in Moorea, French Polynesia during eight randomly selected days (values shaded highlight p-value<.050).

	Day			Night		
	Greater Value	X^2 value	P value	Greater Value	X^2 value	P value
Day 3	Deep	2.036	.154	Deep	8.442	.004
Day 7	Deep	11.854	.001	Deep	10.939	.001
Day 13	Deep	19.105	<.001	Surface	1.059	.303
Day 14	Deep	5.453	.020	Deep	2.689	.101
Day 18	Deep	34.887	<.001	Surface	.667	.414
Day 19	Surface	16.500	<.001	Deep	13.714	<.001
Day 24	Deep	38.534	<.001	Deep	16.000	<.001
Day 27	Surface	.000	1.000	Surface	.750	.386

Table 2. Chi-square values (n=1) and probabilities of tests between depth of chaetognaths found in Pao-Pao Bay in Moorea, French Polynesia during eight randomly selected days (values shaded highlight p-value<.050).

	Day			Night		
	Greater Value	X^2 value	P value	Greater Value	X^2 value	P value
Day 3	Deep	.500	.480	Deep	5.500	.019
Day 7	Deep	1.500	.221	Deep	12.500	<.001
Day 13	Deep	44.507	<.001	Surface	3.270	.071
Day 14	Deep	5.149	.023	Deep	10.500	.001
Day 18	Deep	13.762	<.001	Deep	.667	.414
Day 19	Surface	2.882	.090	Deep	3.500	.061
Day 24	Deep	7.200	.007	Deep	4.500	.034
Day 27	Deep	.333	.564	Deep	.077	.782

Table 3. Chi-square values (n=1) and probabilities of tests between depth of gastropod larvae found in Pao-Pao Bay in Moorea, French Polynesia during eight randomly selected days (values shaded highlight p-value<.050).

	Day			Night		
	Greater Value	X^2 value	P value	Greater Value	X^2 value	P value
Day 3	Surface	4.000	.046	Surface	.500	.480
Day 7	Surface	.667	.414	Deep	.333	.564
Day 13	Surface	26.471	<.001	Surface	53.070	<.001
Day 14	Surface	12.302	<.001	Surface	49.191	<.001
Day 18	Surface	70.511	<.001	---	.000	1.000
Day 19	Surface	16.500	<.001	Deep	16.254	<.001
Day 24	Surface	1.500	.221	Surface	8.333	.004
Day 27	Surface	70.146	<.001	Deep	17.500	<.001

Table 4. Chi-square test values (n=1) of day and night comparisons and probabilities of eight randomly selected days of zooplankton collection in Pao-Pao Bay in Moorea, French Polynesia.

	Chaetognaths			Copepods			Gastropod Larvae		
	Greater Value	X^2 value	P value	Greater Value	X^2 value	P value	Greater Value	X^2 value	P value
Day 3	Night	9.981	.002	Night	8.333	.004	Day	5.444	.020
Day 7	Day	13.885	.000	Night	17.286	.000	Day	1.000	.317
Day 13	Day	5.183	.016	Day	11.782	.001	Night	5.813	.016
Day 14	Day	.653	.419	Day	46.339	.000	Night	18.985	.000
Day 18	Day	10.922	.001	Day	8.333	.004	Day	121.993	.000
Day 19	Night	1.080	.299	Day	4.167	.041	Night	11.560	.001
Day 24	Day	32.295	.000	Day	5.143	.023	Day	4.000	.046
Day 27	Night	28.571	.000	Night	6.250	.012	Day	33.507	.000

Likewise, a similar trend of vertical migration has been observed in many copepods in the ocean (Hays et al. 1994). Although copepods showed a tendency to have a greater presence at 15.2 meters in the water, it is not known if their presence is greater deeper in the bay. Their populations could possibly increase as darkness increases with decreasing depth.

Chaetognaths showed a more random distribution during both the daytime and nighttime and at varying depths. The vertical stratification of chaetognaths therefore, is described less easily. Although Figures 4 and 5 show nearly all daytime percentages to be greater in deeper waters, the statistical analysis points to a more random distribution in the depths tested (Table 2). However, their random distribution does not dismiss the performance of DVM by the chaetognaths in the bay. The diel vertical migrations of chaetognaths may occur at a greater amplitude than 15.2 meters. Chaetognaths could also aggregate in larger percentages than at depths not tested. One reason for the random distribution can be a low threat of predation. Food resources could also be randomly dispersed and provide greater freedom of movement for the chaetognaths without loss or decrease in food concentration. All these factors suggest that chaetognaths may not be performing diel vertical migrations in Pao-Pao Bay over the depths tested. The conditions in the bay could provide an environment that allows the chaetognaths to still acquire resources while having a low threat of predation.

Gastropod larvae show a different migration pattern than the copepods and chaetognaths over the depths tested. Instead of going into deeper waters during the daytime hours, they remained near the surface during the day and a random distribution at night. Table 4 is inconclusive in showing if larger populations are present during the daytime and nighttime. Predator avoidance is probably the main reason for these findings. Their predators may not rely on sight as their means of acquiring food like larger predators, therefore the brighter surface may not be the area of water the gastropods are trying to avoid. Therefore, the gastropod larvae observed reveal a contradiction to the general pattern of DVM that was expected to be observed. The gastropod larvae could remain near the surface in order to avoid predation by copepods, chaetognaths and other zooplankton residing lower in the water. Hickman (1999) discovered that the larva of the gastropod, *Nassarius paupertus* found in Oahu Island, Hawaii were preyed upon by other zooplankton. The similar environments of Hawaii and Moorea make the possibility of zooplankton predation on gastropod larvae a strong possibility. Furthermore, a chi-square test performed between total zooplankton found in deep waters and surface waters revealed a significantly greater number of zooplankton found in the deeper waters (p-value=.029, see Appendix C). These findings further reinforce the strong possibility that gastropod larvae are avoiding predators by remaining in shallower waters.

The results show that a lack in variation in temperature does not affect the observed migration over the scale of depth tested. Temperature is a significant factor in influencing DVM. Loose and Dawidowicz (1992) found that zooplankton suffered a >50% decrease in growth due to low hypolimnion temperatures. Without a significant change in temperature, it would be advantageous for the zooplankton in this study to remain anywhere along the surface to 15m range.

Temperature is therefore not a good explanation of the influences of DVM at the scale tested. Instead, predators, food resources, and light intensity would act as better indicators.

Eating habits of each species are important indicators to their migratory patterns. Chaetognaths are carnivores that are known to be big consumers of copepods. 30% of their biomass is made up of copepods (Casanova 1999). Calanoid copepods are carnivores that utilize their mandibles with strongly chitinized gnathobase to consume their prey (Bradford-Grieve et al. 1999). Once again, the findings may be greatly affected by predators of the chaetognaths and copepods. Chaetognaths are predators to copepods, but do they provide a sufficient threat to the copepods to induce migration? The random dispersal of chaetognaths and the concentration of copepods in lower waters suggest that the copepods could be migrating lower to avoid their predator.

The gastropod larvae are harder to categorize as a whole as some are carnivorous and others are herbivorous. However, the herbivorous snail larvae feed often upon phytoplankton and detritus (Todd et al. 1999). Therefore, it would be advantageous as the findings show for gastropods to remain near the surface where they can find phytoplankton and detritus floating around.

The lifestyles of the zooplankton analyzed are an important factor to consider. The differences in lifestyle between the holoplankton and meroplankton can affect DVM as well. For instance, gastropod larvae are found along the surface layers during mostly all times tested. The development of the gastropods are dependent on their ability to find the right substrate they can metamorphose upon (Hickman, pers. con.). Gastropod larvae contain chemosensors that can detect when a larva has floated over its desired substrate for metamorphosis. If the larva has accumulated enough energy and is metabolically ready to carry out metamorphosis, it will descend and land on the substrate to develop into an adult. Therefore, it would be advantageous for gastropod larvae to float in shallower waters, depending on the average depth their substrate desired is found.

Rains during days 13, 14 and 27 are a possible reason to the large increases in gastropod larvae percentages following those days. Nearly all the species of gastropods found on land in Moorea, French Polynesia are amphidromous (Canepa 1996); the gastropods live as adults in the freshwater streams and release their larvae into the streams that empty into the oceans, in order to develop. The Pao-Pao River empties directly into the bay where the city of Pao-Pao meets the shore. Rains on the mountains and on lands near the bay wash down large amounts of freshwater and with them, snail larvae from adult snails. The river provides the gastropod larvae of the river and other tributaries a direct route to travel in order to metamorphose. Therefore, it is likely the large increases in snail larvae come from those rains. Chi-square tests (p-values for both periods of rain $<.001$, see Appendix D) show significant increases between the days of rain, and those that followed, further strengthening the rain as the source of gastropod larvae increases.

The potential roles of predators, temperature and food all must be considered in explanations of how DVM is induced in Pao-Pao Bay. How do we know when one has greater influence over the other? Huntley and Brooks (1982) concluded that *Calanus pacificus* follow the rule of "better dead then unfed," which suggests the copepod would rather risk the threat of predation then starve. Experimentation done with *Daphnia* (Haney and Buchanan 1980) showed that even with a high concentration of food at a certain level in water, the *Daphnia* almost immediately migrated away from this area when their predator was introduced suggesting their chemoreceptors act more readily against predators than a lack of resources. Temperature has been shown to dictate how low *D. carinata* will migrate in the water depending on how cold those depths are (Haney 1993). All these theories and factors play specialized roles in the DVM of each species of zooplankton done in previous studies. Categorizing zooplankton as a whole and generalizing their migrations is difficult to do. Although this experiment seeks out to find a general pattern of migration in major groups, a species to species study can only fully answer how and why each species migrates the way they do.

Conclusion

While copepods showed the more generally accepted pattern of DVM for zooplankton, chaetognaths and gastropod larvae were exceptions to the rule. Gastropod larvae followed a modification to the predator avoidance theory and the general pattern of DVM since its predators may have been the zooplankton. Therefore, during the daytime, the gastropod larvae migrated opposite to its zooplankton predators. Finally, chaetognaths were randomly dispersed throughout the depths tested and require more experimentation at deeper depths and observation of its predation and food resources to better understand its migration.

There are various studies of zooplankton in Pao-Pao Bay and diel vertical migration that can be done in the future. Research can be done over greater depths to see if the vertical distance traveled by some zooplankton is greater than what was observed in this experiment. Also, it is not known who are planktivores in the bay. Both migrations of zooplankton and larger organisms such as fish and crustaceans can be researched to better understand the predator-prey interaction. The migrations of zooplankton as well as those predators can provide insight on the effects of the predator avoidance theory. Furthermore, vertical stratification of food eaten by zooplankton could provide interesting results as well. Understanding the vertical stratification of zooplankton populations in Pao-Pao Bay is only the first step in fully understanding the influence of diel vertical migration within the bay.

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Appendix A. Numbers of chaetognaths, copepods, gastropod larvae and total zooplankton found in Pao-Pao Bay in Moorea, French Polynesia

Day	Time of Day	Chaetognaths		Copepods		Gastropod Larvae		Total Zooplankton*	
		Deep	Surface	Deep	Surface	Deep	Surface	Deep	Surface
1	Day	9	3	142	22	0	26	303	77
1	Night	22	0	176	24	1	6	378	61
2	Day	37	0	226	136	0	25	493	298
2	Night	---	---	---	---	---	---	---	---
3	Day	1	0	48	35	0	8	99	83
3	Night	11	0	81	48	0	1	174	100
4	Day	39	1	502	4	3	4	1061	14
4	Night	2	0	57	5	0	0	118	22
5	Day	19	0	43	15	0	26	114	60
5	Night	8	0	27	4	0	15	71	26
6	Day	---	---	---	---	---	---	---	---
6	Night	---	---	---	---	---	---	---	---
7	Day	3	0	50	21	2	4	109	46
7	Night	25	0	26	7	2	1	92	38
8	Day	21	0	84	1	1	42	206	46
8	Night	27	7	26	39	37	311	133	401
9	Day	35	0	41	12	30	3	189	59
9	Night	11	0	50	16	47	84	195	163
10	Day	54	39	51	44	38	16	203	171
10	Night	8	2	35	21	22	34	142	95
11	Day	11	1	23	36	1	27	65	103
11	Night	---	---	---	---	---	---	---	---
12	Day	---	---	---	---	---	---	---	---
12	Night	7	131	15	70	10	68	58	403
13	Day	65	8	45	12	2	32	188	101
13	Night	13	24	14	20	1	56	62	164
14	Day	58	36	35	18	10	33	179	109
14	Night	21	0	28	17	13	81	119	312
15	Day	10	30	13	24	10	403	84	513
15	Night	---	---	---	---	---	---	---	---
16	Day	11	0	9	15	5	180	48	218
16	Night	---	---	---	---	---	---	---	---
17	Day	13	24	17	162	20	826	105	1234
17	Night	---	---	---	---	---	---	---	---
18	Day	19	2	48	5	20	119	161	136
18	Night	4	2	10	14	3	3	39	163
19	Day	5	12	0	33	0	33	29	119
19	Night	7	0	33	9	50	17	148	52
20	Day	---	---	---	---	---	---	---	---
20	Night	---	---	---	---	---	---	---	---
21	Day	---	---	---	---	---	---	---	---
21	Night	16	0	24	0	8	0	108	3
22	Day	8	0	17	7	14	31	75	52
22	Night	10	0	42	6	10	7	142	49
23	Day	13	4	21	8	7	34	86	63
23	Night	2	9	31	53	3	4	84	153
24	Day	16	4	83	20	9	15	230	62
24	Night	7	1	30	6	1	11	99	46
25	Day	0	3	4	3	52	1	65	32
25	Night	4	2	66	5	16	3	177	46
26	Day	19	7	31	5	44	58	140	99
26	Night	2	0	11	8	5	16	36	53
27	Day	2	1	4	4	9	94	34	108
27	Night	7	6	21	27	35	0	95	97
28	Day	1	0	35	5	46	242	134	256
28	Night	2	0	5	22	73	58	96	205
Total		685	359	2380	1068	660	3058	6966	6711
Percentage of Total Zooplankton		5.01%	2.62%	17.40%	7.81%	4.83%	22.36%	50.93%	49.07%

*Note "Total Zooplankton" refers to all zooplankton found during a tow, not only organism groups evaluated during experiment (i.e. copepods, chaetognaths, gastropod larvae).

Appendiz B. Sample percentage calculation for each zooplankton organism group evaluated.

Chaetognath percentages of total zooplankton found on every tow from day 1:

Deep, day tow: 9/303 = 2.97%
Surface, day tow: 3/77 = 3.90%
Deep, night tow: 22/378 = 5.82%
Surface, night tow: 0/61 = 0.00%

Each value was the plotted on corresponding graphs (Figures 2-7).

Appendix C. Pearson chi-square tests (n=1) between number of gastropod larvae collected in daytime plankton tows found between days of rain (days 14 and 27) and days that followed in Pao-Pao Bay in Moorea, French Polynesia.

	Day 14	Day 15
Gastropod larvae found in tows (daytime tows only)	33	403
Chi-square value	313.991	
P-value	<.001	

	Day 27	Day 28
Gastropod larvae found in tows (daytime tows only)	94	242
Chi-square value	65.190	
P-value	<.001	

Appendix D. Pearson chi-square tests (n=1) between total number of zooplankton found in all deep tows and all shallow tows in Pao-Pao Bay in Moorea, French Polynesia.

	Deep	Surface
Number of zooplankton found in tow	6966	6711
Chi-square value	4.754	
P-value	.029	

The recognition and learning abilities of two stomatopods, *Gonodactylus childi* (Manning 1971) and *Raoulserenea pygmaea* (Caldwell and Manning 2000), in Moorea, French Polynesia

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ABSTRACT. The stomatopods *Gonodactylus childi* and *Raoulserenea pygmaea* were tested between September and November, 2001 on the island of Moorea, French Polynesia to see if they are able to chemically recognize the odors of each other and the ink of their octopus competitors, *Octopus bocki* and *Octopus cyanea*. The ability to learn to recognize the odor and ink was also explored. Individuals of each species were exposed to either the other species of stomatopod or *O. bocki* prior to placement into a test arena that included an artificial cavity. Exposures functioned to classify individuals as "naïve" or "experienced." Upon entry into the arena, behavior was recorded until the stomatopod entered the cavity. It was found that *G. childi* takes longer to enter a cavity containing the odor of *R. pygmaea* than a cavity containing fresh seawater. *R. pygmaea* entered cavities containing their own water quicker than those with *G. childi* odor. Behavioral data was used to supplement the statistical tests. Neither species showed recognition abilities for the octopus ink. *Gonodactylus childi* appeared to learn to recognize the odor of *R. pygmaea*. The results are discussed in the context of the evolution of chemosensory abilities, the natural histories of *G. childi* and *R. pygmaea*, the ecological interactions occurring between *G. childi* and *R. pygmaea*, and the ecological interactions present between the stomatopods and the octopuses.

Introduction

Stomatopod crustaceans are present in shallow marine habitats throughout the world's tropical and subtropical seas. "Stomatopod" is Latin for "mouth-foot," which stems from the presence of a pair of raptorial appendages that unfold to attack prey and defend resources. Stomatopods can be placed into two functional groups based on the morphology of the raptorial appendage, the spearers and the smashers. Spearers unfold their raptorial appendage to spear soft-bodied prey such as worms or fish. Most spearers dig their burrows in sand. Smashers do not unfold their raptorial appendage. Instead, the base of the first segment is blunted and is used to kill armored prey such as snails or crabs (Caldwell and Dingle 1976; Caldwell 1987).

Most smashers live in naturally occurring cavities in live coral or dead coral rubble (Caldwell and Dingle 1976; Caldwell 1987). Other marine invertebrates such as shrimps, crabs, polychaete worms, brittle stars, bivalves, and octopuses also dwell in similar cavities in the coral rubble. As a result, stomatopods and the other rubble residents may compete with each other for shelter (Caldwell and Lamp 1981).

Shelter is absolutely vital to the survival of a stomatopod because shelter provides a safe environment in which the stomatopod can molt, mate, brood eggs, attack prey, and avoid fish predators (Caldwell 1979; Reaka 1980). The importance of shelter is evidenced by the observation that most aggressive competition between stomatopods is centered around the acquisition of shelter (Caldwell 1987). Competition for shelter has been documented between two species of *Gonodactylus* (Steger 1987), and between a spearer and a smasher (Kinzie 1968). Since competitive interactions between stomatopods involve potentially fatal raptorial strikes, those with the ability to avoid aggressive interactions have a fitness advantage. Thus, stomatopods have evolved beneficial chemosensory abilities (Caldwell 1979).

The chemical recognition abilities of stomatopods have been thoroughly researched. Caldwell (1979) demonstrated that individuals of *Gonodactylus festae* are able to recognize the odor of conspecifics that they have previously fought. *G. festae* is also capable of distinguishing between the odors of stomatopods that they have beaten and those that have beaten them. This suggests that *G. festae* is capable of individual recognition (Caldwell 1985). Individuals of *G.*

bredini use chemical cues to identify former mates, which is another example of individual recognition (Caldwell 1992). Caldwell and Lamp (1981) showed that *Gonodactylus bredini* can chemically recognize a cavity containing an individual or the odor of its octopus competitor *O. joubini*. Recognition only occurs if the competitors have fought before. Therefore, the ability is learned.

Learning, the ability to alter responses to stimuli as a result of experience (Dyal and Corning 1973), among Crustaceans is a well-documented ability. Crabs, lobster, and shrimps, as well as stomatopods, have been shown to demonstrate high powers of associative learning (Krasne 1973). Reaka (1980) demonstrated how the stomatopod *G. falcatus* learns to recognize characteristics of its burrow within five trials. Individuals were also shown to have variable learning abilities. Other examples involve spearers learning to avoid cavities containing smasher residents (Caldwell 1988), and smashers learning to break open snail shells more efficiently over repeated attempts (Caldwell 1976).

Because stomatopods compete with others for necessary shelter, and because they have variable abilities in learning to recognize odors of competitors, chemical recognition abilities have evolved (Caldwell 1979). It is to a stomatopod's advantage to choose resources that can be obtained without confrontation. In Moorea, French Polynesia a smasher species, *G. childi*, finds shelter in the cavities eroded and bored into dead coral rubble in less than one meter of water in front of UC Berkeley's Richard B. Gump South Pacific Biological Research Station. *Raoulserenea pygmaea*, a spearing species, *Octopus bocki*, a pygmy octopus, and juveniles of *Octopus cyanea*, a larger octopus species, also inhabit similar cavities.

Octopus ink has long been considered a defense mechanism against predation by paralyzing the predator's olfactory senses (MacGinitie and MacGinitie 1968), by acting as an olfactory distraction (Fox 1974), or by acting as a "smokescreen" (Hanlon and Messenger 1996).

Individuals of *G. childi* and *R. pygmaea* were tested to see if they are able to recognize waters containing the odor of the competing species of stomatopod, and waters containing the ink of the octopus competitors *O. bocki* and *O. cyanea*. The stomatopods were also tested to see if a previous encounter with the competitor enhanced their recognition abilities.

This study is important because *G. childi* and *R. pygmaea* have never been used as test subjects. If recognition is demonstrated by these two species, then ecological inferences can be made about the levels of competition occurring between them. Octopus-stomatopod interaction is not thoroughly studied, nor is the effect of octopus ink on stomatopods. Results from this study will allow hypotheses to be made about the ecological interactions occurring between stomatopods and octopuses. This study, thereby, lays a foundation for future projects to explore the natural histories and chemosensory abilities of stomatopods and their competitors.

I hypothesized that *R. pygmaea* and *G. childi* would display more defensive/investigative behavior when allowed to enter cavities containing octopus ink or the odor of the other stomatopod species, than when allowed to enter cavities containing their own odor or fresh seawater. I also expected to see that stomatopods with prior exposure to either the other stomatopod species or *O. bocki* would display different behavior from that demonstrated by "naïve" individuals.

Methods

Site

Stomatopods and octopuses were found by snorkeling at study sites around Cook's Bay on the island of Moorea, French Polynesia (17°30' S and 149°50' W). Most were collected to the left of the dock directly in front of the Richard B. Gump South Pacific Biological Research Station. Others were found at the reef crest near the pass into Cook's Bay, at the public beach near Temae, and at the fringing reef near the white hotel on the east side of Cook's Bay (see Figure 1).

Collection and Maintenance

Suitable rubble was taken from the sea floor and placed into a tub (d=55 cm; h=38 cm) containing a cinder block resting on the bottom. Suitable rubble was defined by the following criteria: presence of circular cavities, lift ability, and absence of living corals. The rubble was placed on the cinder block, and subsequent rubble was piled on top. No rubble touched the bottom of the tub. When the tub was full of rubble or when the tub became unstable, it was brought to shore. The rubble then dried in the tub for fifteen to thirty minutes. As water drained out of the porous rubble, stomatopods and octopuses left their cavities and congregated in

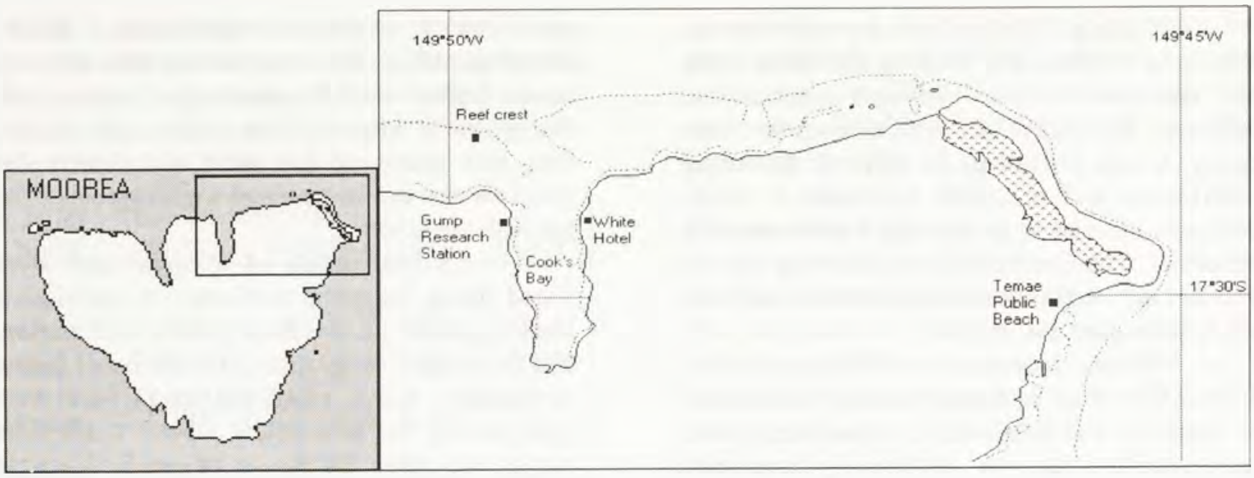

Figure 1. Map of Moorea, French Polynesia (17°30' South latitude and 149°50' West longitude). The island is situated 25 km northwest of Tahiti in the Society Archipelago. Specimens were collected mainly on the northeast side of the island around Cook's Bay (Gump Research Station, White Hotel, and reef crest near Cook's Bay pass). A few stomatopods were collected on the east side of the island at the Temae public beach. Maps courtesy of the UCB Geography Department Cartography lab.

the water at the bottom of the tub. Additional stomatopods were collected by breaking the rubble into pieces with a rock hammer

Stomatopods and octopuses were measured (length), identified, numbered and placed into small plastic containers (d=70 mm; h=65 mm; V=150 ml) where they lived for the duration of the testing period. A piece of PVC pipe was added to function as a cavity. Octopus water was changed daily; stomatopod water, every two days. Octopuses were fed a small crab every two days; stomatopod smashers, a small crab every 3 days. Spearing stomatopods were fed plankton and dead crabs.

Ink was obtained by dissecting individuals of *O. bocki* and *O. cyanea* under a dissecting microscope. Ink sacs were extracted and placed into a plastic vial. Before conducting trials, the ink sacs were diluted with 2 mL of seawater producing a non-viscous black liquid.

Behavioral experiments

Preliminary experiments were run between 11 October and 24 October 2001. During this period of time individuals of *G. childi* and *R. pygmaea* were tested for recognition of each other's odors.

The test arena was a cleaned out ice cream container (l=17 cm; w=17 cm; h=10cm; V=2 L) or a smaller, rectangular container (l=17 cm; w=12 cm; h=5.5 cm; V=750 ml). A hole of diameter 14 mm was cut out in the wall of the arena. The screw-on-top of one small glass vial (h=35 mm; d=14 mm) was glued into the hole using aquarium sealant. The top of the screw-on-top was cut away, and the vial was covered with electrical tape. The stomatopods used the vial as a cavity when placed into the test arena, and the cavity could be easily screwed in and out of the test arena.

Stomatopods were placed into an arena and their behavior was recorded until they entered the cavity, or for a maximum of 12 minutes. After a 24 hour acclimation period, each stomatopod was subjected to three different waters: their own (O-H2O), the other species' (*R. pygmaea* P-H2O, *G. childi* G-H2O), and clean/fresh seawater (C-H2O). The sequence was randomized, and the three trials occurred one after the other. The stomatopod was evicted from the glass vial; the vial was cleaned with bleach and rinsed in seawater for 5 minutes; the vial was filled with one of the three test waters; the vial was screwed back into the arena; and the stomatopod was reintroduced into the arena. During trials the following behaviors were noted along with the time they occurred: coils (posture common during aggressive encounters between stomatopods where the body is essentially rolled into a ball), charges (any contact with the cavity), attenulation (an investigative behavior involving the attenules), in/out (entry followed by rapid exit), partial entry, and complete entry. 22 individuals of *G. childi* were tested in this fashion. I attempted to test 8 individuals of *R. pygmaea*, but most refused to enter the cavity.

During these preliminary experiments, it became evident that running the three trials one after another was adding a confounding variable. The stress of being evicted from the cavity, which proved to be difficult for larger individuals, and the rapid repetition of trials, definitely seemed to be altering the stomatopods behavior. The octopus ink and learning aspects still had not been incorporated into the study, so a new procedure was created.

From 1 November to 13 November data were collected for 25 individuals of *G. childi* and 16 individuals of *R. pygmaea*. Experiments were conducted using the following improved procedure.

12 individuals of *G. childi* were acclimated for 24 hours in individual arenas. Before being placed into the test arena for acclimation, each of the 12 stomatopods was allowed to interact with an individual of *R. pygmaea* for 5 minutes in a circular container (d=6 cm; V=1.9 L). These 12 *G. childi* were "naïve" to octopus ink and "experienced" with the odor of *R. pygmaea*. Six different individuals of *R. pygmaea* were used during the staged encounters. An individual of *R. pygmaea* was placed in the container first along with its own PVC pipe cavity. The following interactions were recorded once the *G. childi* was added: attenulation, strikes, coils, lunges, and any other notable signs of aggression. After 5 minutes *G. childi* was removed and placed in one of twelve arenas to begin acclimation. After roughly 24 hours, the stomatopods underwent the first of four trials. The sequence of test waters (O-H2O, C-H2O, P-H2O, and I-H2O) was randomized using playing cards. For the P-H2O trial, the individual whose water was taken was randomly selected. I-H2O was fresh seawater with 2 drops of octopus ink. The octopus ink was a mix of 2 mL of seawater and the ink sacs of 4 individuals of *O. bocki* and one individual of *O. cyanea*. Trials were conducted in the same fashion as the preliminary trials, except only 2 trials were performed each day with each stomatopod, and the trials were conducted at least 2 hours apart.

After the stomatopods underwent the 4 trials, they were placed back into their home containers and the arenas were cleaned with bleach and rinsed with seawater. 12 more individuals of *G. childi* were tested in the same manner, except before acclimation they were allowed to interact with *O. bocki* (in a PVC cavity) for 5 minutes. These 12 *G. childi* were "naïve" to *R. pygmaea* odor and "experienced" with octopus ink. 2 individuals of *O. bocki* were used during exposure experiments. Coils, contacts, partial and complete entries, and any escape behavior of the stomatopod were noted. During the 5 minutes if the octopus did not ink, then two drops of ink were added with the intention that the stomatopod would associate the ink with the octopus.

12 individuals of *R. pygmaea* were tested using the same methods. 6 individuals were exposed to *G. childi* before acclimation, and the other 6 were exposed to *O. bocki* before acclimation. Six *G. childi* and two *O. bocki* were used during the encounters. Four trials were conducted after 24 hours of acclimation in individual arenas. The trials were the same as those used in testing *G. childi,* except G-H2O was substituted for P-H2O.

Following the 12 *R. pygmaea*, the remaining 9 stomatopods were tested. Of the 9, 2 were *G. childi*; one was exposed to an octopus and the other to *R. pygmaea*. Of the 7 remaining individuals of *R. pygmaea*, 4 were exposed to *O. bocki* (2 different octopuses were used), and 3 were exposed to *G. childi* (3 different individuals were used).

Results were analyzed statistically using Microsoft Excel (mean, standard deviation, median), SYSTAT (Friedman Two-Way Analysis of Variance and Wilcoxon Matched Pairs test), and StatXact-4 (Friedman Two-Way Analysis of Variance). The above non-parametric statistical tests were used because times of entry data were being analyzed. This type of data typically is not normally distributed. Behavioral data were analyzed by tallying defensive and investigative behaviors displayed by a species during each of the four trials, and by using the Friedman test. Recognition was suggested by greater time of entry and different defensive/investigative behavioral displays in the I-H2O and P/G-H2O treatments than in the C-H2O and O-H2O trials (controls). Learning was determined based on differential time of entry and behavioral data between the naïve and experienced groups

Results

General

A total of 53 stomatopods were collected between 23 September and 5 November 2001. 28 were *G. childi* and 25 were *R. pygmaea*. The mean length of all *G. childi* was 19.61±3.45mm, and the mean length of all *R. pygmaea* was 20±0.96mm. Three of the *G. childi* were not tested due to their small size

Table 1. Descriptive statistics for time of entry (sec) for 24 individuals of *G. childi* during the four trials. In the O- and P-H2O trials, the range value in parentheses is the time of entry next highest behind 720 seconds (no entry).

G. childi	Time of entry (sec)			
N=24	C-H2O	O-H2O	I-H2O	P-H2O
Mean	73.79	96.33	77.46	102.42
SD	108.65	169.79	113.67	171.85
Range	1-375	1-720 (468)	4-388	2-720 (524)
Median	29	28.5	31.5	42.5

Table 2. Tally of behaviors displayed by 24 individuals of *G. childi* during 4 trials. For the charge column, the value is the number of individuals with more than one charge during a certain trial. In/out is entry followed by rapid exit. Significant differences were found between C-H2O and P-H2O (Friedman test, T=5, p=0.025), and between O-H2O and P-H2O (T=5, p=0.025).

Behavior	C-H2O	O-H2O	I-H2O	P-H2O
Charge	8	8	11	14
Coil	0	0	0	1
Partial entry	13	6	10	14
In/Out	0	1	3	2
Attenulation	2	2	2	4

Table 3. Statistical test results for time of entry data for 23 individuals of *G. childi.*

Wilcoxon Signed Ranks Test (N=23)				
p-values	C-H2O	O-H20	I-H20	P-H20
C-H2O	1			
O-H2O	0.749	1		
I-H2O	0.796	0.429	1	
P-H2O	0.088	0.412	0.346	1
Friedman Two-Way Analysis of Variance				
(N=23)	C-H20 vs. P-H2O			
Test Stat	7.348			
p-value	0.007	based on Chi-square distribution with 1 df		

(length<13mm). Seven of the *R. pygmaea* died prior to testing, and two were not tested because entry never occurred during acclimation. Thus data were obtained for 25 *G. childi* and 16 *R. pygmaea*.

Gonodactylus childi

Descriptive statistics (Table 1) showed that *G. childi* may be capable of recognition. The mean and the median times of entry for C-H2O were lower than those for I-H2O and P-H2O, and the same was true for O-H2O and P-H2O. Behavioral data supported some of the above trends. More stomatopods displayed defensive and/or investigative behavior in the P-H2O trials than in the C-H2O and O-H2O trials (Table 2). Friedman tests, using the behaviors scored in Table 2, between C-H2O and P-H2O (T=5, p=0.025), and between O-H2O and P-H2O (T=5, p=0.025) were significant.

Friedman and Wilcoxon tests were used to find any significant relationships between all of the trials (Table 3). The Wilcoxon test p-values were all greater than 0.3, except for the C-H2O and P-H2O comparison. Statistical analysis between C-H2O and P-H2O was also performed using a Friedman Two-Way Analysis of Variance (N=23, T=7.348, p=0.0067 based on Chi-square distribution with 1 df). Figure 2 is a line graph illustrating how in the C-H2O trial a greater number of individuals had entered the cavity in a shorter amount of time than in the P-H2O trial. It must be specified that comparisons between the control trials and I-H2O were not statistically significant (p>0.05) with time and behavioral data.

Times of entry data for the naïve individuals were not statistically different than that of the experienced *G. childi* for C-H2O vs. I-H2O, and O-H2O vs. both I- and P-H2O (Friedman and Wilcoxon p>0.1). The C-H2O vs. P-H2O comparison, however, does seem to suggest learning abilities. For the naïve 12 stomatopods, p>0.05, and for the experienced 12, p<0.05. Table 4 shows the evidence for *G. childi's* ability to learn to recognize the odor of R. *pygmaea*. Behavioral data in Table 4 show that the experienced group is less investigative than the naïve group.

Raoulserenea pygmaea

Many individuals did not enter the cavity during trials. Consequently, their data was omitted in time of entry statistical tests. Descriptive statistics (Table 5) show that R. pygmaea may be capable of recognition. The

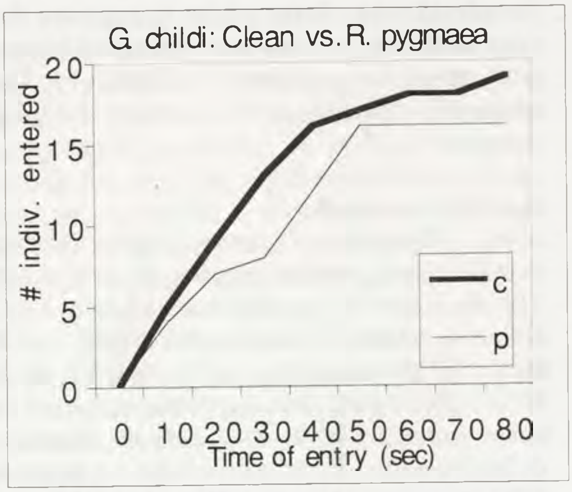

Figure 2. Comparison of C-H2O and P-H2O trials with respect to number of individuals of *G. childi* entered into the cavity over time in seconds. Beyond 80 seconds, the curves converge. These two trials were significantly different (Friedman test, p=0.0067).

Table 4. Evidence of learning and recognition in *G. childi*. Mean time of entry is in seconds. The first two columns are behavioral data, and the last two columns are time of entry statistics.

	# indiv. w/ >1 charge	# of part. entries	mean time of entry	Friedman p-value
Naïve				
C-H2O	4	8	90.67	
P-H2O	8	8	143.42	0.0833
Exper.				
C-H2O	4	5	56.92	
P-H2O	6	6	61.42	0.0348

Table 5. Descriptive statistics for 9 individuals of *R. pygmaea*. In the C, I, and G-H2O trials, the range value in parentheses is the time of entry next highest behind 720 seconds (no entry).

Time of entry (sec) *R. pygmaea* N=9	C-H2O	O-H2O	I-H2O	G-H2O
Mean	300.56	233.33	226.33	375.33
SD	301.23	186.57	246.45	290.61
Range	3-720 (514)	13-518	10-720 (455)	1-720 (598)
Median	158	179	141	400

Table 6. Tally of behaviors displayed by 16 individuals of *R. pygmaea* during the 4 trials. For the charge column, the value is the number of individuals with more than one charge. In/out is entry followed by rapid exit.

Behavior	C-H2O	O-H2O	I-H2O	G-H2O
Charge	8	11	8	10
Coil	0	0	2	1
Partial entry	5	7	6	7
No entry	7	5	4	4
In/Out	0	1	0	1
Attenulation	7	2	5	9

mean and median values of the time of entry in the C-H2O and O-H2O trials are lower than the mean and median values in the G-H2O trial. Behavioral data (Table 6) does not seem to show any significant change in behavior between the controls and the ink or the odor of *G. childi.* Friedman tests on these relationships are also insignificant.

Wilcoxon p-values for all six comparisons between the four treatments were all >0.1. The lowest p-value was between O-H20 and G-H2O at 0.139. When conducting a Friedman test for these two trials, p=0.0956 (Table 7). Figure 3 illustrates how at least for the first 400 seconds, a higher percentage of individuals entered the cavity in a shorter amount of time.

Learning to recognize ink and the odor of *G. childi* by *R. pygmaea* was not suggested by the data. The experienced group showed no difference in behavior or time of entry in comparison with the naïve group.

G. childi vs. R. pygmaea

While conducting trials it became evident that these two species displayed strikingly different behaviors. With respect to times of entry, *G. childi* entered much faster than *R. pygmaea* in all 4 trials (compare Tables 1 and 5). There were only two trials in which *G. childi* did not enter the cavity within 12 minutes. That is opposed to the 20 trials in which *R. pygmaea* refused to enter within 12 minutes. *Raoulserenea pygmaea* also attenulated, coiled, and charged more often than *G. childi* (compare Tables 2 and 6). 36% of trials involved attenulation by *R. pygmaea*, whereas, only 10% of trials involved

Table 7. Statistical test results for time of entry data for 9 individuals of *R. pygmaea.*

Wilcoxon Signed Ranks Test (N=9)				
	C-H2O	O-H2O	I-H20	G-H20
C-H2O	1			
O-H2O	0.314	1		
I-H2O	0.263	0.767	1	
G-H2O	0.735	0.139	0.327	1
Friedman Two-Way Analysis of Variance (N=9)	O-H2O vs. G-H2O			
Test stat	2.778			
p-value	0.096	based on Chi-square distribution with 1 df		

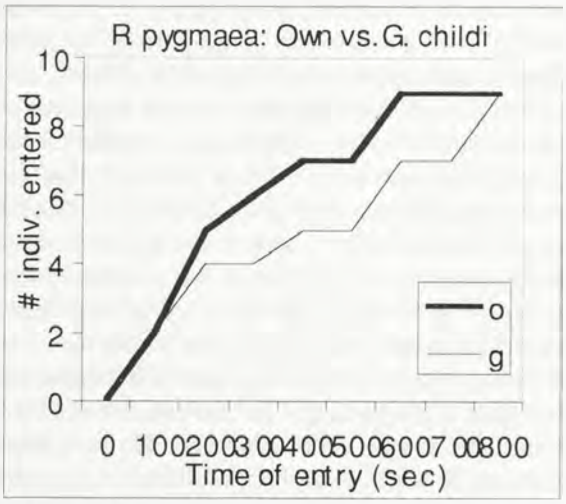

Figure 3. Comparison of O-H2O and G-H2O trials with respect to number of individuals of *R. pygmaea* entered into the cavity over time (seconds). The curves represent 9 data points. Recognition is evident between 200 and 700 seconds. Statistical tests yielded p=0.096.

attenulation by *G. childi.* 58% of trials involved *R. pygmaea* charging the cavity more than once, whereas, 43% of trials involved *G. childi* charging the cavity more than once.

Discussion

This study has done much to elucidate the interactions between *G. childi* and *R. pygmaea* and between the stomatopods and their octopus competitors. It can be reasonably inferred that *G. childi* is capable of recognizing cavities containing the odor of *R. pygmaea.* The opposite, though, that *R. pygmaea* is able to detect cavities with the odor of *G. childi*, is not as solidly confirmed. The possibility that either species is able to detect the ink of an octopus is not supported by the data. The data suggest that *G. childi* may be able to learn to recognize the odor of *R. pygmaea*, but beyond that learning has not been supported by my results. I have concluded with certainty that *G. childi* and *R. pygmaea* behave in ways highly distinct from each other.

The comparison of C-H2O and P-H2O for 23 individuals of *G. childi* was the most significant outcome of this study. *Gonodactylus childi* took longer to enter and investigated more thoroughly cavities with the odor of *R.* pygmaea than cavities with fresh seawater. This suggests two possibilities: 1) *G. childi* detected the odor in the cavity, recognized it as the odor of *R. pygmaea*, and investigated before entering; or 2) *G. childi* detected the odor in the cavity, recognized it as a foreign or "non-self" odor, and investigated before entering. With more experience the individual may learn to associate the odor with their competitor.

The possibility that *G. childi* recognized the odor as belonging to *R. pygmaea* is viable for the following reasons. Past research on *Gonodactylus* has demonstrated how certain species are able to recognize conspecifics (Caldwell 1979; 1985; 1992) and octopus competitors (Caldwell and Lamp 1981). In all cases, the researchers were able to eliminate possibility 2 because they were able to show that associative learning was occurring. Associative learning is shown when detection is accompanied by an appropriate behavioral response. Given that the smasher's strike can inflict more damage than the spearer's strike (Caldwell 1976; 1988), recognition of *R. pygmaea* odor by *G. childi* may not facilitate hesitation and investigation. Instead, *G. childi* might be incited to action and aggression in trying to evict the resident. This is supported here. Experienced individuals (encountered *R. pygmaea* 1 to 2 days prior to the P-H2O trial) entered the cavity faster then naïve individuals (no encounter with *R. pygmaea* for at least three weeks). The experienced group also charged and partially entered the cavity less than the naïve group (Table 4). In all of the staged five-minute encounters between the two species, either *G. childi* was the aggressor or both were equally passive. Out of 22 battles, only 7 involved a *G. childi* that showed no aggression. Most encounters involved *G. childi* lunging and chasing after *R. pygmaea* and then striking.

Gonodactylus childi was definitely the stronger aggressor; its willingness to engage in aggressive interactions has been shown.

The learning experiment, though it suggests that *G. childi* is capable of learning to recognize *R. pygmaea* odor, may have been more conclusive if more individuals were tested over successive trials. But since detection included a different behavioral response, it remains a possibility that *G. childi* was recognizing the odor as that of *R. pygmaea*.

Based on the above findings regarding the detection and recognition of a competitor's odor, ecological inferences can be made. It seems that the two species are definitely competing for shelter. I collected both species at the same sites and in the same type of rubble. Caldwell and Manning (2000) state that *R. pygmaea* is most often found co-occurring with *G. childi*. It seems that individuals of *G. childi* encounter *R. pygmaea* often enough to facilitate detection, if not recognition of *R. pygmaea* odor. Distribution and abundance data are needed to discern directly the level of competition. If it were found that the two species had high densities and overlapping ranges, then it would be reasonable to state that *G. childi* could learn to recognize the odor of *R. pygmaea* in a lifetime, given *G. childi* has a high learning curve.

The comparison between O-H2O and G-H2O for *R. pygmaea* was very close to being significant. It is very likely that this species does have chemical recognition abilities. This might not have been shown by my data due to the sluggish, inconsistent behavior of most *R. pygmaea* individuals. Since *R. pygmaea* is usually the weaker combatant in a battle with *G. childi*, one would assume that evolution would select for those individuals with the ability to avoid cavities containing the aggressive *G. childi*. Indeed, Caldwell (1988) documented a situation in Hawaii where a spearing species learned to avoid cavities containing smasher residents. If more individuals are tested and they are given more time to acclimate to the arena, then I think that future research on *R. pygmaea* will find that this species is capable of detecting and maybe recognizing the odor of *G. childi*. If we assume that *R. pygmaea* is able to recognize the odor of *G. childi*, then more credence is given to the hypothesis that these two species compete enough so that repeated encounters facilitate the development of harm-reducing chemical recognition abilities. However, if the opposite is assumed, then it seems that *G. childi* has a more powerful chemosensory system.

The difference in the performance of both species during trials is an interesting discussion topic. *G. childi* was very decisive and aggressive. Most individuals entered the cavity soon after being reintroduced into the arena. On the other hand, *R. pygmaea* commonly refused entry for the 12 minutes. Only 2 individuals showed an intense need to be sheltered.

The ecology of *G. childi* and *R. pygmaea* must be explored to discern any reasons for the above behavioral observations. *G. childi* truly is "home-cavity" oriented. This species is known to inhabit cavities for extended periods of time (Caldwell pers. com.). During this time frequent foraging missions are undertaken, and the individual returns to the same cavity (Caldwell pers. com.). *R. pygmaea*, on the other hand, is less "home-cavity" oriented. The species is known to inhabit the interstices of rubble rather than formal cavities and has not been shown to be faithful to a certain shelter (Caldwell pers. com.). A possibility that also must be addressed is that the behavior of *R. pygmaea* was altered due to the added stress of being captive. *R. pygmaea* does seem to be a sensitive species; 7 individuals died before they could be tested. *G. childi* also could have been stressed due to captivity, but individuals still behaved appropriately. There is evidence that *G. childi* is a tough, robust species. No individuals died while captive, and this species is typical of the genus *Gonodactylus* – species are capable of thriving in harsh, intertidal habitats (Caldwell pers. com.).

It was very intriguing to find that both species were unable to recognize octopus ink. For an explanation we must address why octopuses ink. Most research has pointed toward ink expulsion acting to deter predation (MacGinitie and MacGinitie 1968; Fox 1974; Hanlon and Messenger 1996). There is even biochemical evidence that tyrosinase found in octopus ink could account for the olfactory paralysis agent (Prota et al. 1981). So if octopuses only ink when threatened, then it may be the case that *G. childi* and *R. pygmaea* do not have enough experience with ink to recognize it as octopus-originated. Both species are too small to prey upon adult *O. bocki*, but preying upon juvenile *O. bocki* cannot be ruled out. There is no documentation of stomatopods feeding on octopuses. During the staged encounters, no stomatopods ever struck the octopuses, and the octopuses never inked. Most contact ensued when the stomatopod would get close to the octopus, which caused the octopus to attempt to grab the stomatopod. No octopuses fed on a stomatopod during the 5-minute encounters; the stomatopods were able to escape the grip of the octopus. I added ink during the five minutes hoping the stomatopod would associate the added ink with the octopus present in the battle arena. It is likely, though, that adding the ink at any arbitrary time did not resemble a natural situation.

Since *O. bocki* is not threatened by *G. childi* and *R. pygmaea,* it is unlikely that these stomatopods would be exposed to ink under natural conditions. Thus, an ability to associate ink with an individual of *O. bocki* would not be expected. Instead, it would be more likely for these stomatopods to be able to recognize the odor of *O. bocki*. The stomatopod *G. bredini* can chemically recognize the odor of *O. joubini* (Caldwell and Lamp 1981). Selection would once again favor those individuals that could detect the octopus odor and then use that information to avoid the occupied cavity. Detection of odor is possible because reduced circulation in the cavity would allow for the concentration of odors (Caldwell and Lamp 1981).

The study presented here provides a framework for other studies to be conducted. To minimize behavior alterations due to captive stress it is ideal to test the individuals immediately after capture, and allow longer than one day for acclimation to be safe. Learning studies with *G. childi* will be important in distinguishing whether the species is recognizing the odor or merely detecting it. A learning study would also serve to discern the level of interaction between the two stomatopods. It would be very interesting to see if the stomatopods could be trained to associate ink with octopus. These studies need to incorporate repetitive identical trials similar to Reaka (1980). Natural history data on *G. childi* and *R. pygmaea* are needed to confirm the ecological inferences made after detection was found to be an ability possessed by *G. childi* and possibly *R. pygmaea*.

Conclusions

This study presents the first attempt to assess the recognition abilities of two species of small stomatopod. This is also the first time recognition of octopus ink by stomatopods has been studied. Thus, a basis for studies exploring the competitive interactions between the stomatopods and the octopuses has been established.

G. childi is capable of detecting the odor of its stomatopod competitor *R. pygmaea*. Recognition, shown by associative learning, is suggested by this study. A methodology involving repetitive trials would serve to elucidate the learning abilities of both stomatopod species. It seems that these species do compete against each other for cavities. Natural history data would illustrate a better picture of the ecological interactions occurring between the species. Both species were found to be unable to detect the octopus ink, leading to the conclusion that the stomatopods do not present a threat to the octopuses. Lastly, *R. pygmaea* needs to be studied so that ecological interactions, and even evolutionary interactions, between *R. pygmaea* and *G. childi* can be more clearly established.

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Den Defense and Intraspecific Behavior in the Tropical Pacific Pygmy Octopus, *Octopus bocki* (Adams, 1941)

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ABSTRACT. This study focuses on *Octopus bocki*, a nocturnal pygmy octopus that lives in the tropical Indo-Pacific. (1) *Den Defense*: Does *O. bocki* defend its den from conspecifics of the same sex and if so, how do size, sex and den residency influence which octopus dominates the interaction? (2) *Social Interaction*: What kind of visual and tactile communication occurs when two conspecifics of the same sex come into close vicinity and which forms of these can be categorized as displays of dominance? An experiment determined if the den resident dominated over an intruding conspecific of smaller, larger or equal size. The results from den defense trials demonstrate that size difference between resident and intruder helped determine which octopus dominates control of the den. Den residency and sex showed no influence in this determination. Same-sex physical interaction and evasion types in *O. bocki* have been diagramed and ranked according to dominance. Results lead to further questions regarding the intraspecific interaction in *O. bocki*.

Introduction

Octopus behavior, particularly intraspecific interaction, is poorly understood (Messenger 1995). Territoriality has not been observed in the most commonly studied octopus species (*O. vulgaris*, Altman 1967; *O. rubenscens* Dorsey 1976; *O. cyanea*, Van Heukelem 1977). However, past studies indicate that dominance relationships based on size occur in some octopus species (*O. rubenscens*, Dorsey 1976; *O. cyanea*, Yarnall 1977, *O. vulgaris* Boyle 1980). *Octopus bimaculoides* creates size-based dominance hierarchies that determine den use when dens are in limited quantity (Cigliano 1993). In *O. dofleini*, den dominance studies revealed that although the dominant octopus was usually the larger octopus, octopuses that were 30-50% of the weight of the larger octopus were also legitimate competitors (Iribarne, unpublished data). A further study focusing on dominance in *O. cyanea*, revealed that a resident octopus in its den has considerable advantage over an intruder (Yarnall, 1969).

Octopuses use different methods of communication to display dominance over conspecifics (Cigliano 1993). Visual, tactile, and chemical signals can all be used as means of communication in cephalopods (Moynihan & Rodaniche 1977). Location, posture and movement are all important visual signals. Physical interaction, including fighting, mating, cannibalism, and the use of ink are some examples of tactile and chemical signals (Moynihan & Rodaniche 1977, Boal & Golden 1999). Past studies have used behaviors such as den eviction, the use of arms to "web" over another octopus (where one octopus uses its arms and mantle to enclose another octopus) and chasing after a conspecific as displays of dominance (Cigliano 1993, Yarnall 1977). Interaction types have been ranked according to 'intensity of response' in determining dominance relationships (Cigliano 1993). Interestingly, other signs of communication, such as inking and change in ventilation rate, have not previously been used as indicators of dominance (Boal & Golden 1999). Ventilation rate has been directly related to the behavioral state of arousal and has been used to measure the effect of both chemical and visual stimuli on cephalopods (Boyle, 1983).

This study focuses on conspecific interaction in the species *Octopus bocki* Adams (1941). *O. bocki* is a nocturnal pygmy octopus that occurs throughout the tropical Indo-Pacific (Cheng 1996). It is a relatively easy species to collect and work with due to its small size (less than 30 mm in mantle length) and abundance in Moorea, French Polynesia (Cheng 1996). A number of studies completed in Moorea have focused on the behavior of this species, including work on their learning abilities (Lebenson 1999), sexual discrimination, reproductive behavior, activity patterns and den occupancy (Cheng 1996), prey detection (Johnson 1995), and prey preference (Pearson 1994). Little is known about its conspecific interaction besides its precopulatory behavior.

This experimental study address two questions:

(1) Den Defense: Does *O. bocki* defend its den from conspecifics of the same sex and if so, how do size, sex and den resident influence which octopus dominates the interaction? The den resident is hypothesized to have an advantage over the intruder, the larger octopus to have an advantage over the smaller and male-male and female-female dominance interactions to be the same.

(2) *Intraspecific Interaction*: What types of physical, visual and/or chemical communication occurs when two conspecifics of the same sex come into close proximity and which of these forms of communication can be categorized or ranked according to dominance? Behaviors such as inking, webbing and cannibalism are hypothesized to indicate dominance.

Methods and Materials

Collection and maintenance of organisms

Throughout a seven-week experimental period in October and November of 2001, sixteen individuals of *O. bocki* were collected. Individuals collected at four sites on the reef flat of Cook's Bay in Moorea, French Polynesia (17°30'S, 149°50'W) (Figure 1).

Snorkeling and free diving were used to collect pieces of coral rubble 10-40 cm in diameter. Rubble was placed in a 40 L bucket and balanced on top of a non-porous cinder block placed at the bottom of the bucket, which ensured that rubble did not contact the water when collected at the bottom of the bucket (Figure 2). Buckets were left in the sun for a minimum of 15 minutes and a maximum of 1 hour, during which time octopuses crawled out of the dried rubble into the water below.

Octopuses were housed in 200 ml clear plastic cups filled with unfiltered seawater. A 5 cm piece of PVC piping was placed in each cup as a den for the octopus. Individuals were sexed and measured upon collection. Males were discriminated from females by the presence of a groove running along the posterior margin of the third right arm and enlarged suckers on this arm and the third left arm. Individuals were measured from the midpoint between their eyes to the posterior end of their mantle. Missing arms and other unique features were recorded for individual identification during interaction experiments. Octopuses were given the

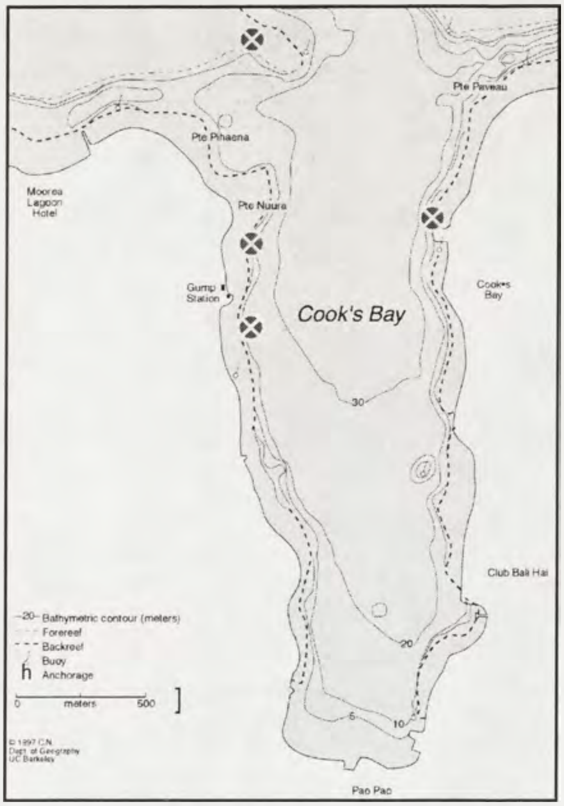

Figure 1. Map of *Octopus bocki* collection sites in Moorea, French Polynesia. The four locations marked with an X refer to four collection sites on the reef flat.

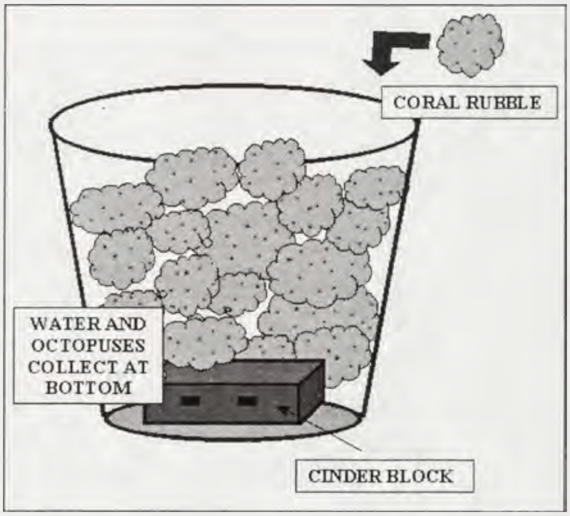

Figure 2. *Octopus bocki* collection method in Moorea, French Polynesia. Pieces of coral rubble were piled in a 40 L bucket and balanced on top of a non-porous cinder block so that rubble did not contact the water when collected at the bottom of the bucket. Buckets were left in the sun between 15 minutes and 1 hour, during which time octopuses crawled out of the dried rubble into the water below.

opportunity to eat one small crab daily so they would be satiated during trials. Unfiltered seawater was changed daily.

Preliminary Observations

More than twenty-five hours of observation was recorded for more than twenty preliminary interactions. Conspecific interaction type, evasion methods and body postures were categorized as indicators of either dominance or submission. These were later given scores so that octopuses could be ranked according to dominance in the den defense experiment.

Interaction. Six different types of same-sex conspecific interaction were observed and classified in this study.

(1) One octopus **touches** another octopus with its arm.
(2) One octopus uses multiple arms to **entangle** with the arms another octopus.
(3) One octopus **pounces** on top of another octopus using its mantle and arms.
(4) One octopus uses only its arms to make a **partial web** over the mantle of another octopus.
(5) One octopus uses its mantle and its arms to make a **full web**, enveloping another octopus.
(6) Octopuses **copulate** with each other. One octopus inserts its hectocotylus into the mantle of another octopus. This occurred in male-male interactions, but never in female-female interactions.
(7) One octopus **cannibalizes** another octopus.

The octopus that initiated one of these physical interactions, the aggressor, was recognized as dominant. The flow of events during any interaction is illustrated in Figure 3. As the interaction proceeds towards full web, cannibalism (female-female only) or copulation (male-male only), the dominance of the aggressor increased.

Evasion. The recipient of one of these interactions used one of three methods to evade the aggressor. These evasion methods were also ranked according to dominance.

(1) The recipient octopus escapes using jet propulsion and inking one or more times.
(2) The recipient octopus escapes using jet propulsion but does not ink.
(3) The recipient octopus moves away with the use of arm locomotion.

Body Posture. The various body postures that an octopus exhibits in the presence of a conspecific are illustrated in Figure 4. Although some postures might indicate dominance, these postures were not later used in the ranking system. All positions were assumed against the bottom or the side of the tub, except for position B, which occurred at the surface of the water. Positions A and B were resting positions, commonly displayed by the submissive octopus when no interaction occurred. The dominant octopus often assumed the other positions prior to interaction. Position C was a momentary position assumed prior to either position A or F. Positions D and E indicate that the octopus was about to move or jet away. Positions F and G are "standby positions." An octopus might have taken on either of these positions before it was about to move or it may

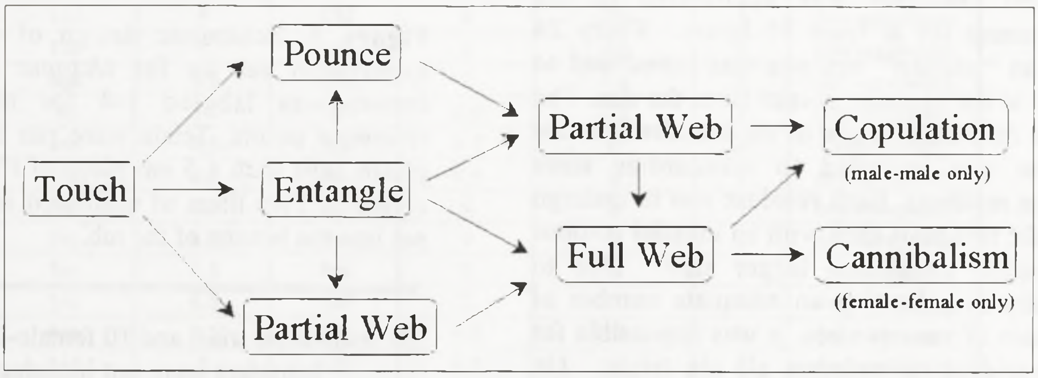

Figure 3. Flow chart of same-sex interactions in *Octopus bocki* preliminary observations. Not all interactions terminate at Cannibalism or Copulation. At any point in the interaction an octopus may evade the aggressor using jet propulsion or moving away with its arms.

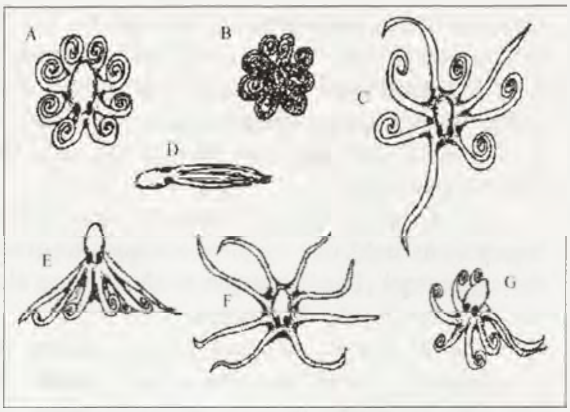

Figure 4. Standard body positions displayed by *Octopus bocki* during preliminary experiments for same-sex interactions. Position B illustrates an upside-down octopus floating at the surface of the water. All other positions were observed against the side or bottom of the tub.

have waited in these positions.

Interaction type and evasion type were ranked according to intensity of dominance (Table 1). At the end of the trial each octopus is given a score according to the number of dominant interactions it initiates and the number of evasion types it elicits. The octopus with the highest score is dominant. The octopus with the lowest score is submissive.

Den defense experiment

Trials were run in 4 L rectangular white plastic tubs with a 5 cm piece of black PVC piping in corner 1 to serve as a den (Figure 5). Two liters of unfiltered seawater was put into the tub. Three identical tubs were used so that multiple trials could be run per night. A "resident" octopus was acclimated to the environment for at least 24 hours. Every 24 hours an "intruder" octopus was introduced to the tub at the opposite corner from the den. The percent difference in size of the intruder from the resident was recorded to standardize sizes between residents. Each resident was to undergo six trials; two trials each with an intruder octopus of smaller, equal and larger size. Due to difficulty in collecting an adequate number of octopuses of various sizes, it was impossible for each resident to undergo all six trials. On average, each octopus underwent 3 trials.

Experiments were performed between 2100h-0600 h. Because *O. bocki* is a nocturnal species, trials were run under a red lamp to reduce the amount of light seen by the octopuses (Cheng 1996). Twenty trials were performed, 10 male-male trials and 10 female-female trials.

Table 1. Dominance ranking in *Octopus bocki* same-sex interactions. Only interaction and evasion type are used to rank dominance. At the end of the trial each octopus is given a score according to the number of dominant interactions it initiates and the number of evasion types it elicits. This ranking system was used in the den defense experiments when den occupancy alone could not determine dominance.

Interaction Type (by aggressor)	**Dominance Score**
(1) Touch	1
(2) Entangle	2
(3) Pounce	2
(4) Partial Web	2
(5) Full Web	3
(6) Copulation	5
(7) Cannibalism	5
Evasion Type (by recipient octopus)	
(1) Jet away and ink	4
(2) Jet away (no ink)	3
(3) Move away with arms (no ink)	2

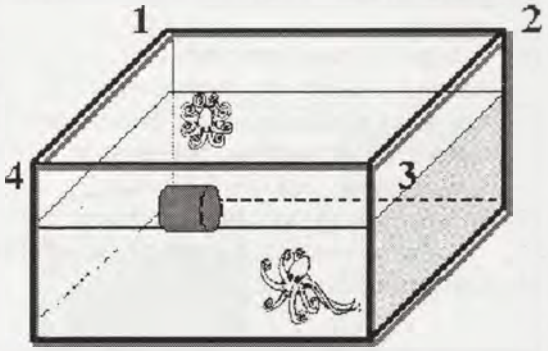

Figure 5. Schematic design of den defense experiment set up for *Octopus bocki*. Each corner was labeled 1-4 for observational reference points. Trials were run in 4 L white plastic tubs with a 5 cm piece of PVC piping in corner 1. Two liters of unfiltered seawater were put into the bottom of the tub.

Intruders were not introduced unless the resident was inside the den. Behavioral observations, including interaction type, evasion type and body posture, were recorded continuously for both octopuses for 40 minutes. The ventilation rate of the resident was recorded once every 5 minutes during this time. This was measured by counting the number of mantle contractions for one minute.

A dominant and a submissive octopus were declared at the end of each trial. Dominance was defined by occupancy in the den and dominance indicators previously defined in the preliminary observations. Submission was defined by den avoidance and evasion from the dominant octopus. If one octopus held residency in the den for more than 15 minutes with no movement from either octopus, the octopus in the den was automatically deemed dominant.

Results

Den defense experiment

The results of the den defense experiment are summarized in Table 2. The dominant octopus was determined in each trial (R=resident, and I=intruder) except for trial #2, where dominance could not be established. This trial was not used in statistical analysis.

Den occupancy, physical interaction, body position and movement were all used in establishing dominance and submission. This determination was unique in each trial, but den occupancy was the most important factor.

In thirteen out of twenty trials, dominance was declared after one octopus had maintained occupancy in the den for 15 minutes with no movement by either octopus. In trials 1 and 16, dominance was determined by cannibalistic means. Octopus 13f and 4f were eaten alive. Both trials were female-female interactions. In trials 8 and 19, dominance was determined by den occupancy off and on throughout the trial, though never for 15 continuous minutes. In trial 12, copulation occurred in a male-male interaction. The dominant octopus in this trial was the octopus that inserted his hectocotylus into the mantle of the other octopus.

Statistical analyses were performed to test the null hypotheses (i.e. that the dominant octopus in each trial was random) (Table 3). Chi squared tests showed that there was no significant difference in the overall results between resident and intruder (χ_, p=.48). Chi squared tests were also used to show that males and females showed no significant difference in determining dominance (χ_, p=.53). In nineteen trials, the resident was dominant eight times and the intruder was dominant eleven times.

Table 2. Results of den defense trials for *Octopus bocki*. The trial number (dominant octopus in bold), octopus identification number, sex (m=male, f=female) and size are given on the left. The percent size difference of the intruder from the resident, the dominant octopus and means of dominance determination is highlighted on the right. Each trial had a dominant octopus (R=resident, and I= intruder) except for trial 2, where dominance could not be determined.

Trial	Resident (R)	Size (mm)	Intruder (I)	Size (mm)	% Difference	Winner	Determination
1	**9f**	10	13f	4	-60	R	cannibalism
2	10m	9.5	15m	7	-36	?	standoff
3	**4f**	8.5	14f	6.5	-24	R	den occupancy
4	11f	5	**12f**	4	-20	I	den occupancy
5	**11f**	5	13f	4	-20	R	den occupancy
6	**6m**	8.5	15m	7	-18	R	den occupancy
7	9f	10	**4f**	8.5	-15	I	den occupancy
8	**6m**	8.5	3m	8.5	0	R	mostly in den
9	10m	8.5	**6m**	8.5	0	I	den occupancy
10	6m	8.5	**8m**	9	6	I	den occupancy
11	3m	8.5	**6m**	9	6	I	den occupancy
12	3m	8.5	**8m**	9	6	I	copulation
13	14f	6.5	**16f**	7	7	I	den occupancy
14	**6m**	8.5	10m	9.5	12	R	den occupancy
15	**11f**	5	14f	6.5	15	R	den occupancy
16	4f	8.5	**9f**	10	15	I	cannibalism
17	11f	5	**14f**	6.5	25	I	den occupancy
18	15m	7	**8m**	9	29	I	den occupancy
19	**11f**	5	15m	7	40	R	mostly in den
20	11f	5	**16f**	7	40	I	den occupancy

Table 3. Actual results and expected results of *Octopus bocki* den dominance results categorically tabulated by sex (R=resident, I=intruder). Chi squared tests showed that there was no significant difference in the overall results between resident and intruder (χ_, p=.48).

Actual Dominance			*Expected Dominance (null Ho)*		
trials	R	I	trials	R	I
all	**8**	**11**	**all**	**10**	**10**
male	4	5	male	5	5
female	4	6	female	5	5

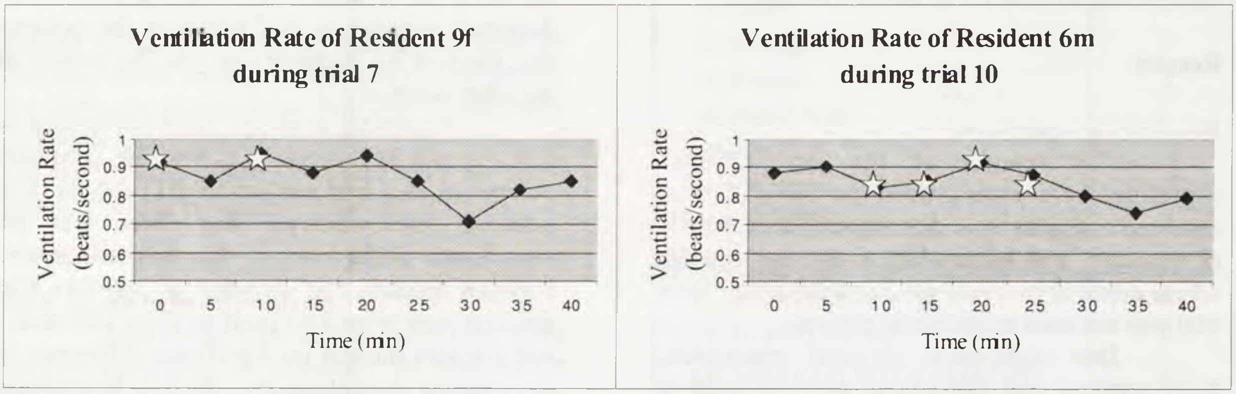

Figure 6. Change in ventilation rate of the resident octopus in two trials during den defense experiments on *Octopus bocki*. Ventilation rate was measured once every 5 minutes during 40-minute trials. Stars represent physical interaction between the resident and the intruder during that time interval.

In the nine male-male trials used in the analyses, the resident was dominant four times, and the intruder was dominant five times. In ten female-female trials, the resident was dominant four times and the intruder was dominant six times (Table 3).

A paired t-test was used to show that the percent size difference between the resident and the intruder was significantly correlated with the dominant octopus of the trial ($t=2.208$, $df=18$, $p < 0.040$).

In general, ventilation rate increased when octopuses were interacting and remained baseline when octopuses were not moving for a few minutes. Although due to octopus movement in and out of the den, making it impossible to measure ventilation rate throughout the entire trial. Trials 7 and 10 were the only trials with complete data for resident ventilation rate for the entire time period (Figure 6).

The stars in Figure 6 represent physical interaction during that 5-minute interval. No statistical analyses could be performed due to insufficient data for most trials. The trend shows an increase in ventilation rate during interaction, but it is shown that this increase is not due exclusively to physical interaction.

Additional interaction observations

Most conspecific communication and interaction in *O. bocki* was defined in the preliminary observations. Additional interaction observations were recorded during the den defense experiment. "Ink feeling" appeared to be another means of interaction. Inking was a response used by the submissive octopus during physical and non-physical interaction. On six different occasions, the dominant octopus was observed to reach out an arm and move it back and forth in the cloud of suspended ink produced by the submissive octopus. Another behavior, called "spying," was often used to detect a conspecific. In eight trials, the octopus inside the den would poke its eyes out, looking in the direction of the other octopus.

Discussion

Intraspecific Interaction

Body posture, same-sex interaction and evasion types in *Octopus bocki* were observed and categorized into dominant and submissive activities. Although dominance determination is mostly based on den occupancy during the den defense trials, the ranking system was used only secondarily as an additional indicator of dominance. Further experiments can make use of this ranking system in understanding the nature of intraspecific and possibly even interspecific interaction. It would be especially interesting to compare the interactions observed in this study with those that occur in the field.

Additional observations were found in this study that could not be categorized as dominant or submissive indicators. One of these is the use of "ink feeling" as a possible means of conspecific recognition. Six instances were recorded in which one octopus waved an arm in the suspended ink released by the other octopus. Previous studies have shown that inking is a defense mechanism whereby predators are distracted by the ink and/or a camouflage that hides the octopus (Siuda 1974, Moynihan & Rodaniche 1977). There is as much evidence that ink is used as a means of conspecific recognition (Moynihan & Rodaniche 1977). Further investigation would be useful for a complete understanding of ink usage in octopuses.

Den occupants would often poke their eyes out, "spying" on the other octopus. Previous studies indicate that *O. bocki* cannot visually discriminate between sexes (Cheng 1996), so this observation was probably not related to sexual discrimination. *O. bocki* have been shown to visually discriminate between the sizes of conspecifics (Cheng 1996). Thus "spying" may be a method of determining the size of the other octopus, perhaps helpful in establishing its dominance position. Additonally, "spying" also allows the den occupant to be protected, yet aware of the location and position of the other octopus. Similar use of visual assessment in size-based rank determination has been seen in dominance studies on *Octopus bimaculoides* (Cigliano 1993).

Den Defense

The results from the den defense trials show that size difference between resident and intruder is the only investigated factor that determines which octopus dominates the den. Size dominance was expected and observed in other octopus species as well (*O. rubenscens*, Dorsey 1976; *O. cyanea,* Yarnall 1977, *O. vulgaris* Boyle 1980; *O. bimaculoides* Cigliano 1993).

Unexpectedly, the data from this study do not favor the resident octopus over the intruder octopus. Yarnall showed that residency was an important factor in determining den dominance for *O. cyanea* (Yarnall 1969). The average den residency period for *O. cyanea*, a species up to ten times the size of *O. bocki*, is at least one month (Yarnall 1969, Van Heukelem, 1969). The average den residency of *O. bocki* is 1.36 days for males and 1.5 days for females (Cheng 1996). Perhaps the significantly shorter den residency period indicates that they do not consider their den a permanent residence and thus have no need to defend it from and intruder.

No previous studies are known to have explored the difference between the way males and females establish dominance. Although the quantitative results were not different between male-male and female-female den defense trials, sexual differences were noted in the determination of dominance. Male-male interaction was terminated in copulation in one trial, but was never seen in female-female trials. This observation is supported by previous work on *O. bocki* (Cheng 1996). The establishment of male-male copulation is at least one difference between sexes in den defense. In two female-female trials, dominance was established by cannibalism. There are not enough data to support the idea that cannibalism is unique to female-female *O. bocki* interaction. Cannibalism has been seen in male-male interactions in other species (*O. maorum,* Anderson 1999; Hanlon & Messenger 1996; *O. mim*us, Cortez et al. 1995). If cannibalism is a unique difference and can be substantiated in further research, it might be yet another difference between male-male and female-female interactions in den defense.

The small sample size and the low number of trials indicate the need to augment this study with more individuals and more trials. Octopuses are known to learn during behavioral experiments (*O. bimaculoides,* Boal 1991; Cigliano 1993; *O. vulgaris,* Fiorito & Scotto 1992), and it is possible this may have caused error in the data since octopuses were used for more than one trial. Cigliano recognized that individual recognition occurs quickly in *O. bimaculoides* and played a factor in the results of his dominance and den defense study (Cigliano 1993). In this study, residents and intruders did encounter each other more than once. In the future, the additional collection of individuals could permit no octopus to encounter a conspecific more than once, removing this as a possible error.

Ventilation rate is closely related to the behavioral state of arousal, and can be used to quantify the effect of both chemical and visual stimuli on cephalopods (Boyle, 1983). Preliminary results from this study show this to be true for *O. bocki* as well. Unfortunately, the data here are not complete enough to accurately quantify these changes. It would be interesting to measure ventilation rate with respect to dominant activities for *O. bocki* in future studies.

Conclusion

Understanding den use and intraspecific behavior of octopuses in studies like this one further our understanding marine ecology. This study provides evidence that *Octopus bocki* forms same-sex dominance relationships in den defense studies. Various interactions and body positions have been ranked by dominance intensity in intraspecific interactions. Future studies might include additional den defense experiments with increased sample size and replication and the investigation of ventilation rate with respect to dominance interactions. The occurrence of cannibalism in same-sex interactions and the use of ink as a means of conspecific recognition are two other areas of interest for additional research.

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On the feeding biology and behavior of *Dendropoma Maxima* in the bays and lagoons of Moorea, French Polynesia

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ABSTRACT. This study of the feeding mechanisms of *Dendropoma maxima* took place in Moorea, French Polynesia. Initial tests were focused on the contents of mucus net hauls. Microscope analysis revealed a haul predominately benthic in nature. Subsequent substrate and water column comparisons, along with observations of flow properties in regards to net reactions, revealed that mucus nets were capturing organisms off of the substrate. This in turn led into the study of *D. maxima* reactions to heavily saturated or disturbed mucus nets. In the end a sampling study was completed in high flow and low flow areas to look for variance in population density. Culmination of these 5 distinct sections offered these new insights into the feeding behavior of this unique organism:

1. Organisms caught in the mucus nets of *D. maxima* are primarily of benthic origin.
2. Samples of water and substrate support the hypothesis that net hauls have a predominately benthic origin.
3. Observations and visualizations of flow show a physical process by which the mucus net traps particles it has dislodged from the substrate.
4. Responses to mucus net saturation or disturbance were uniform, with the net being hauled in immediately.
5. Population densities are highest in high flow areas, which supports the idea that water flow is playing a significant role in the dislodging of particles off the substrate, thus producing a food source for *D. maxima.*

More importantly however, this study provided a base on which further studies can be built.

Introduction

Dendropoma maxima (Vermitidae, Mollusca) is a large, sessile, suspension feeding meso-gastropod that inhabits the tropical reefs of Moorea, French Polynesia. As a member of the family Vermitidae they are distinguished by their uncoiled shell, simple disc-like or domed operculum, absence of aperatural canal, reduced foot and the presence of a large mucus gland (Wells 1998). Their life is spent as a sessile organism after a short larval, crawling phase. Upon settling they cement themselves to the hard substrate of coral reefs (Wells 1998, Schiaparelli et al. 1999). Growth is then linear with the coral so as not to be overgrown or have access to feeding compromised (Smalley 1984).

Populations of *D. maxima* have been found to be most dense in high flow areas on coral reef formations (Hughes & Lewis 1974, Hadfield et al. 1972, Taylor & Reid 1984, Zuschin & Piller 1997). Due to its embedment in the coral *D. maxima* is able to remain stationary amongst the rough currents characteristic of this environment.

However, turbid waters have been found to be a necessary ingredient for successful habitation by *D. maxima*. This is due to the feeding mechanism displayed by this animal. Feeding is performed by emitting a mucus net over the substrate in which it is embedded, creating an almost spider web like layer that is attached to the reef head or substrate. The net however, cannot spread over the substrate in which it lies unless the water current aids in spreading of the mucus threads (Hughes & Lewis 1974). Webs that subsequently form then passively capture particles before being hauled in and digested by *D. maxima.* Kappner et al. (2000), have discovered that in *D. maxima* specimens taken from the Red Sea, the mucus web is hauled in and digested at regular intervals during the day and night. The intervals at which the mucus nets are hauled in changes from day to night with nighttime hauls being more frequent. Content of the net hauls varied from day to night with most organisms being captured and digested in the nocturnal hours.

The contents of what these nets capture have been microscopically examined before (Kappner et al. 2000) and shown a diet rich in benthic organisms as well as phtyo and zooplankton. Beyond this basic knowledge little is known about what is in *D. maxima* net hauls. Some reasons for this are that most studies of *D. maxima* have focused on the feeding methods or distributional patterns of this unusual organism.

This study aims to look at the entire feeding biology of *D. maxima* and how this effects the organismal distribution.

Initial tests involved a miscroscopal investigation of feeding nets. These results were compared to a subsequent study of substrate and water samples surrounding the feeding nets. A following study of flow regimes and net disturbances around *D. maxima* at various locations was then performed to garner insight into possible feeding methods. The final aspect of the study was a sampling of populations at areas of high and low flow tidal regimes.

From the outset of this project the goal was to find out how this animal feeds and if this affects it's general ecology. Specifically whether *D. maxima* were sifting the water column or capturing prey off of the substrate, and how flow regimes may influence these processes. In the end the culmination of these studies allowed a possible insight into the biology and distribution of *D. maxima*. More importantly, however they set the table for future studies on this animal in French Polynesia.

Methods

Feeding Net Composition

A simple approach was used to determine what the *D. maxima* were feeding on. While snorkeling I collected a total of 40 random samples of mucus nets at various locations and observed them under a microscope identifying discernable organisms. Times of collection were random at each site save for the distinction between night and day samples. Twenty samples each from nighttime and daytime hours were collected and distinguished as such in the data. Collection of the nets was done with a standard turkey-baster so as to emulate the hauling in process of *D. maxima*. The nets were at times cut at the base of the operculum with a pair of scissors in order to collect as much material as possible. Nets were stored in a 2.0 ml vial of saltwater in the field until return to the Richard B. Gump South Pacific Biological research station. Upon return to the station's dry lab, as much seawater as possible was siphoned off without disturbing the nets. The leftover mucus was then re-suspended with approximately .75 ml of ethanol. A volume of .25 ml was placed on a glass slide and covered. This suspension was then viewed under a microscope at 400X magnification to determine what microorganisms were in the net.

For each mucus sample three slides were observed, giving a total of 120 data trials. The area on the slide to observe was determined by drawing a card from a 52 card playing deck with each suit representing one of four quadrants on the cover slip. When a card was drawn the viewing lens of the microscope was placed at the center of that corresponding quadrant. Viewing was first done with 100X magnification then 400X magnification for final recording of organisms. All discernable organisms within the viewing area were recorded.

Water and Substrate Comparisons

The comparison of water and substrate samples was very similar in method to that of the mucus net analyzation. Pair samples of water and substrate around the operculum of *D. maxima* were collected in the same type of vials as those used for the mucus nets. For the substrate samples a pair of tweezers was used to scrape coral substrate as well as algal patches and place them in a vial. A corresponding water sample was also taken in 2.0 ml quantities around the same *D. maxima*. Subsequent water and substrate samples were then taken back to Gump research station and inspected under the microscope.

Water samples were first centrifuged for two minutes at a level 4 setting so as to draw all organisms to the bottom of the vial. The top layer of water was taken off immediately until .75 to 1 ml was left in the vial. The left over seawater and organisms were applied in roughly .25ml amounts to slides. Three slides were looked at for each sample, with the entire slide rather than one region being observed at 100X magnification. Presence of organisms, rather than number, on the slide was subsequently recorded. With the substrate samples there was no use of the centrifuge before removal of the water. Following steps were the same, however.

Flow Observations

Flow regime properties were first observed in the field without any use of dye so as to watch the mucus nets react to flow around them. Field observations of *D.maxima* took note of the direction of flow in relation to organism and net orientation. After the initial observation, fluorescent dye (from SIGMA Chemicals) was added to a saltwater solution, then released under water by pipette in close proximity to *D. maxima*. Detection of turbulent or laminar flows in relation to the mucus nets was the primary goal. Net activity was recorded in relation to the direction and action of the surrounding flow.

Notes were taken on the dissemination or coagulation of the released dye relative to the mucus net. Specifically whether or not the nature of the flow allowed the dye to interact with the net in a laminar form before being dispersed. This study of flow and mucus net interaction led directly into observations of mucus net capturing of particles and the ensuing haul in of nets.

Particle saturation and disturbance of feeding nets

Particles of food mixed with red culinary dye were deposited into the water surrounding mucus nets in a manner so as to saturate the nets with as much particle matter as possible. The food particle-mixture used was ground down chips with seawater. This was easily ground down into a fine powder that mixed well with the seawater and dye. Deposition of particles into nets was done using a turkey-baster. The out going flow of the particle solution from the turkey-baster would invariably cause a disturbance of the mucus nets and was regulated so as that trials were distinguished between those that deposited food and those that both deposited food and disturbed mucus nets. After release of particles the nets were observed for signs of hauling in of nets.

Any activity showing signs of *D. maxima* hauling in its' mucus nets within 3 minutes of particle release was recorded. Additional observations were recorded regarding the flow at the time of the particle deposition and rejection or ingestion of large particles.

Distribution Study

In the time line of the study, the abundance and distribution of *D. maxima* was the last aspect to be studied. A total of 24 ten meter by one meter samples were completed in 8 different locations. Locations were labeled as either high flow or low flow areas and then compared against each other. A standard transect tape and 1 meter quadrat made of PVC piping were used in each transect. All *D. maxima,* and the coral family in which they were living, were recorded within the transect line.

Field Locations (Figure 1)

All fieldwork was conducted on the northwestern section of Moorea, French Polynesia between the dates of October 1st, 2001 and November 13th, 2001. Mo'orea is located 25 kilometers northwest of Tahiti and lies on the 17°30' South latitude and 149°50' West longitude. It is one of the Windward Islands of the Society Archipelago, along with Tahiti, Maiao, Mehetia and Tetiaroa.

Figure 1 shows the 8 sites that were used for all phases of the study. Five (sites 4-8) areas were designated high flow and three (sites 1-3) were designated low flow based on their physical and hydrologic setting. The five sites that were designated as high flow areas are associated with strong tidal circulation and turbid currents. Flow here is channeled over the lagoon and through passes in the reef during each tidal cycle. The three sites labeled as low flow areas are associated hydrologically with the near shore Cook's Bay habitat and thus the ebbs and flows due to tidal fluctuations are not as severe.

Site 1 was the reef flat located directly in front of the Richard B. Gump South Pacific Biological research station, on the northeastern tip of Cook's Bay. The flat here ends at a somewhat gradual slope into the bay. Site 2 is located directly across Cook's Bay from the research station. It is on the northwestern tip of the bay and is composed of a shallow reef that circles around the bay's opening and drops off into the channel. The reef of Site 2 continues around the point to Site 3, which is located at PK marker 6 and is in the lagoon east of Cooks Bay

It is a true fringing reef habitat as the coral boundary connects with the shore and then slopes gradually out to the inner lagoon. These three sites were designated as Low Flow sites in the population sampling as they were located in the lower energy regime of Cook's Bay.

The following sites were the High Flow sites for the sampling study and all lie in outer lagoon areas. Site 4 is located on the outer reef flat directly east of Cook's Bay channel. This site encompasses the area from 10 meters within the algal crest of the barrier reef to the channel separating the outer reef flat zone from the fringing reef directly connected to the island. Sites 5, 6 and 7 are both located west of Cook's Bay in the outer reef flat zone similar to that of site 4. Sites 5 and 6 are both within 200 meters from the pass with site 5 located 10 to 30 meters in from the algal crest of the barrier reef and site 6 located within the reef flat area close to the lagoons boat channel. Site 7 is further inside the pass than sites 5 and 6, and is situated directly in front of PK 13. It lies within the coral flat area of lagoon. Site 8 is the only area outside of the Cooks Bay area as it lies within the Temae public beach grounds. It extends from 10 meters within the algal ridge through the coral flat area

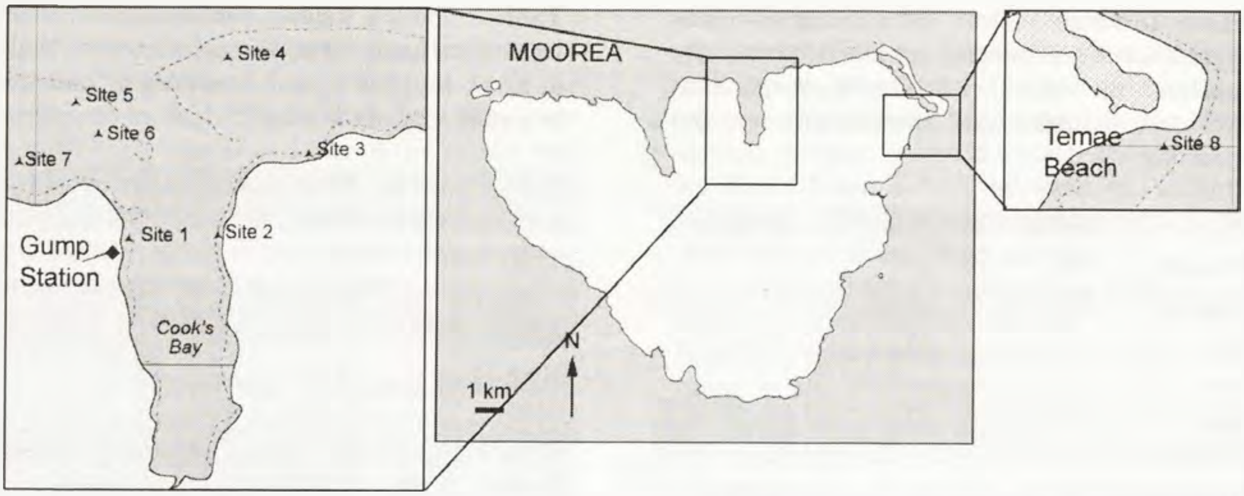

Figure 1. Study sites on the island of Moorea, French Polynesia

of the lagoon 100 meters within. Physical makeup is similar to the previously mentioned High Flow sites. As with all the High Flow sites the water currents are characteristically strong and turbulent.

Statistical analyses

Statistical analyses were done using Systat 7.0 (SSPS Inc.). Pearson Chi squared tests were applied to water and substrate sample results to test for significance among data. Paired t-tests were applied to high flow and low flow sampling data to calculate differences between data sets.

Results

Feeding Net Composition

Microscopic analysis of the mucus nets revealed a higher frequency of blue algal organisms than any other particle (Table 1). Pennate diatoms were quite frequent as well showing in more than half of both day and night sampling. Of the items listed as other, these particles varied and included copepod larvae, naupliar larvae (both in day samples) and an egg case found in the night samples. Centric diatoms were absent from almost all the samples. Overall the contents of the *D. maxima* net samples revealed a high quantity of benthic particles and relatively few planktonic particles.

Table 1. Daytime and Nighttime mucus nets: observed organisms and totals from the 60 day and 60 night samples

	Day Nets	Night Nets
Pennate Diatoms	26	40
Centric Diatoms	1	0
Blue-green algal org.	42	52
Gold-brown algal org.	26	19
Nematodes	2	3
Spicules	4	5
Other	2	1

Water and Substrate Comparisons

Water and Substrate samples results also reflected a benthic composition. As a whole, the organisms found in the water and substrate reflected those found in the mucus nets. However, a majority of these organisms were more prevalent in the substrate samples (Table 2). In turn, the water samples possessed veryfew of the organisms that were evident in the nets. 3 of the seven main organisms were not observed at all in the 30 analyzed water samples, and none of the organisms were present in more than half of the samples. In contrast, 2 organisms were present in all 30 samples of the substrate and a third was present in 25. Only centric diatoms failed to appear in the substrate samples, yet they were also absent in water samples as well. Chi-square analyses revealed that p values for five out of seven samples were below .05 (Table 2).

Table 2. Totals reflect the number of times organisms were present in the 30 trials for substrate or water. P-values correspond to Pearson Chi-square tests between substrate and water totals.

	Substrate	Water	P-value
Pennate	30	0	0
Centric	0	0	1
Blue Green	30	13	0.001
Gold Brown	25	12	0.001
Nematodes	9	1	0.006
Spicules	8	0	0.002
Other	7	2	0.07

Flow Observations

In the field at sites 1 through 7 D. maxima nets appeared to billow and pulse when reacting with the surrounding water. All nets that were observed were attached to the substrate at multiple points and then hovered above forming a taut net like structure. Nets were not blanket like in manner as there was a discernable space between the substrate and the net, nor were the nets free and suspended in the water column, attached only at the *D. maxima* operculum's opening. Nets did not display any sort of elongation as observed by Hughes & Lewis (1974). At each site initial observations saw the product of flow, whether it was strong or weak, to be a billowing of nets rapping to and fro against the substrate in which it presided. In the 15 trials of flow visualization, all tests showed a rapid dissemination of dye as soon as it was released into the water. Water currents and weather conditions varied from calm and clear to turbulent with high winds and heavy cloud coverage. Subsequent results were uniform however, with released dye not yielding any sort of laminar flow.

Particle saturation and disturbance of feeding nets

D. maxima feeding nets were observed at both high and low flow sites in this section of the study. Regardless of flow site retraction of nets was almost uniform with the release of particles and disturbance of the nets. On 10 occasions feeding nets were saturated with food particles without directly disturbing the net with direct hydro propulsion. On 10 other occasions the nets were purposely disturbed by the release of the particles into the nets, with releasing water flow directly aimed at the net. Of the 10 times in which particles were released without disturbing the nets, half were done in high flow areas and half proceeded in low flow areas. The same proportion applied for the disturbed release of particles. Out of the 20 total particle release tests all but 2 nets were hauled in immediately after deposition of particles (Table 3). Nets were from both aggregations and singular organisms but discrepancies in reaction to disturbance or saturation was not observed.

Table 3. Particle Release observations Immediate hauls refers to nets that were hauled in by D. Maxima within 3 minutes of saturation or disturbance of the nets

	Disturbed Nets	Saturated Nets
Total trials	10	10
Low-Flow Sites	5	5
High-Flow Sites	5	5
Immediate Hauls	9	9

Hauling in of the nets by *D. Maxima* gave resemblance to a fisherman hauling in their own catch nets. The jaws of *D. maxima* appear from behind the operculum and rotate to graspthe strands of the net. They then rotate back and haul the net in section-by-section. Net retraction is completed in a half-circular fashion as *D. maxima* works around the operculum opening clearing one side before shifting focus to the other side of the opening.

On all occasions when aggregational nets were observed, one individual would haul nets in after saturation of food particles before the hauling in by others in the aggregation. Left over portions of nets were subsequently hauled in after the initial response by one individual. Nets that were entirely removed from an individual and then placed back onto the operculum were still ingested. Large fragments of food particles were either broken down and ingested with smaller fragments or discarded by the jaws.

Population Sampling

Population comparisons were made between high flow and low flow sites, as mentioned in the Methods. High flow sites were in outer lagoon areas and part of the barrier reef coral table. Random sampling showed a larger sample population in the high flow areas, as well as a higher average *D. maxima* per square meter. T-test of average *D. maxima* per 10-meter plot yielded a t value of –2.841 with probability at .016 (Table 2).

In general high flow sites appeared to have much denser *D. maxima populations*. Every coral head or substrate area capable of settling by larvae was seemingly infested with *D. maxima* populations while those in low flow areas were much more sparsely populated.

Table 4. Sampling data for Low and High Flow Areas

t-value and probability refer to values given by t-test analyses on high flow and low flow data

	High Flow	**Low Flow**
Total D. Max Observed	593	131
Average # per square meter	4.94	1.092
t-value	**-2.841**	
Probability	**0.016**	

Discussion

Initial mucus net composition analysis provided the base on which to build this study. By finding out what was most frequently captured in the *D. maxima* mucus nets, insight was gained into what this organisms diet would consist of. The seven identified organisms were not pre-chosen as key items too identify, but rather were the only organisms that were seen throughout the 120 samples from the day and night time analyses. These organisms in turn became the marked organisms to identify and look for later in the study.

That being said, the relative absence of centric diatoms, and other planktonic organisms that are suspended in the water column, was unexpected. Yet Kappner's (2000) study of mucus net particles revealed that benthic diatoms, nematodes, filamentous algae and macroalgae fragments were among the most easily identified with microscopic analysis. This correlated with observations of benthic blue green and gold brown algal organisms as well as the aforementioned pennate diatoms and nematodes. Thus by looking at what was being captured by the mucus nets I was able to get an idea of what might be floating in the water column or lying on the substrate. In turn, based on what I found in both the water and substrate samples I could theorize as to where *D. maxima* drawing most of their food particles.

Initial observations of the substrate and water samples revealed that almost all of the organisms found in the mucus nets were found in the substrate. Chi square tests subsequently gave p values that, with the exception of centric diatoms, were all below or very close to 0.05. The one category that was above 0.05, 'other', was at 0.07 and could be broken down into 4 different categories of larval organisms found in the substrate and water. If this were done however, 3 out of the 4 organisms would have been present only in the substrate. In addition, the goal of the water and substrate sample analyses was to compare what was in each of the respective samples to what was found in the mucus nets. Thus leaving the larval organisms in the 'other' category corresponded to the 7 organism categories found in the mucus nets.

Therefore, the chi-square tests showed a significant difference in the amount of organisms from the substrate as opposed to the water column. The fact that most of these organisms were from the substrate rather than the water column showed that indeed the mucus nets could be trapping their food off of the substrate, rather than merely filtering the water column.

Many studies (Hughes & Lewis 1974, Hadfield et al. 1972, Taylor & Reid 1984, Zuschin & Piller 1997, etc.) have observed that turbulent, high flow water is necessary for mucus net production. Hadfield (1972), suggests that *D. maxima* feeding is best developed for "exposed situations with continually broken water" while Hughes & Lewis (1974) found that *D. maxima* that are removed from turbulent water and placed in calm surroundings will subsequently fail to produce mucus nets. From the findings of the water and substrate analysis, one can also speculate that settlement in high flow areas is necessary for the capture of food particles by dislodging benthic organisms from the substrate to be captured by the mucus net. While this was not quantitatively tested observed flow dynamics reveal that turbid water causes billowing action of the nets over the substratum. Therefore the action of the water certainly could cause a dislodging of particles to be captured by the mucus nets.

Observed capturing of food particles seemed to cause a stimulation of the haul response system. Kappner (2000) found Net Retraction Frequency to increase with the increased presence of organisms. She thought that this might be an adaptation to minimize prey loss by hauling in the nets as soon as a struggling was sensed. Results from this study do concur with her speculation. 18 out of the 20 trials to disturb the nets in some capacity led directly to net recapture. Thus it is possible that animals caught in the nets trigger a response in *D. maxima* that is similar to that of spiders who find prey caught in their webs.

Due to the seeming reliance on water flow for *D. maxima* feeding that had been observed, it was no surprise to find that higher populations of the animal were found in high flow areas. This discrepancy would have been even higher would it not have been for a rather anomalous random sample in the high flow sites. Site 5 was completely covered in algal organisms thus all recognizable substrate organisms were missing. This was strikingly different from the rest of the high flow sites that were observed, whose density of *D. maxima* populations were quite high. Seemingly every coral head that was come across in the high flow areas was inundated with *D. maxima.* In addition the size of individuals in the high flow areas was substantially larger than those in the low flow areas. Low flow sites shared neither this population density nor organism size. Casual observation outside of recorded sampling saw a much smaller population, both in density and individual size, of *D. maxima* in these areas. Surely this relationship between flow and *D. maxima* size was more than coincidental.

Conclusion

When reviewing results and field notes taken during the course of the study, five aspects regarding the feeding biology of *D. maxima* became readily apparent.

1. Organisms caught in the mucus nets of *D. maxima* are primarily of benthic origin.
2. Samples of water and substrate support the hypothesis that net hauls have a predominately benthic origin.
3. Observations and visualizations of flow show a physical process by which the mucus net traps particles it has dislodged from the substrate.
4. Responses to mucus net saturation or disturbance were uniform, with the net being hauled in immediately.
5. Population densities are highest in high flow areas, which supports the idea that water flow is playing a significant role in the dislodging of particles off the substrate, thus producing a food source for *D. maxima.*

There still as an immense amount of information yet to be discovered about this animal. This study however, has contributed to the small amount of knowledge we do have on *D. maxima.* Thus, although not conclusive, these five factors provide a base on which further studies into this intriguing animal can be built.

Acknowledgments

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The Species Composition and Distribution of the Nudibranchs of Cook's Bay and the Aggregation Behavior of *Glossodoris cincta* (Bergh 1888) in Moorea, French Polynesia

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ABSTRACT. Despite the diversity of the Order Nudibranchia, not much is known about these animals, especially in the South Pacific region. The proposed objectives of this study were to determine the species composition and distribution (population density, species' location, and habitat parameters) of the nudibranchs of Cook's Bay, Moorea, and to determine the reasons behind aggregations of *Glossodoris cincta*. Y-maze trials were conducted to test behavioral preferences. All field and laboratory work was conducted at the Richard B. Gump South Pacific Biological Research Station on the island of Moorea in the Society Islands archipelago. Observations showed that four species are present within Cook's Bay, *Chrormodoris boucheti*, *Risbecia imperalis*, *Glossodoris hikuerensis*, and *Glossodoris cincta*. Nudibranch species were not found in the transects. Chi-squared analysis showed significance for a species' preference for certain habitat parameters (p-value 0.001 and 0.000). T-test and chi-squared analyses showed no significance in the preference trials run in the Y-maze (p-value 0.211 and 0.225). The Y-maze results indicate that nudibranchs show no preference for either the substrate they were found on or a conspecific from their aggregation. However, based on habitat parameter data, and Y-maze results, both substrate and conspecifics are important to a nudibranch.

Introduction

Nudibranchs are marine gastropods that have lost their protective shell. As a result, the Order Nudibranchia demonstrates great morphological and ecological diversity (Todd 1983). However, despite their diversity, basic data such as distribution is not well known. For example, species thought to inhabit only the eastern coast of Africa have been found as far away as the Philippines (Gosliner and Draheim 1996). On subsequent trips to the same sites, some of the newly discovered species were not found again, despite intensive searches. (Gosliner and Draheim 1996). It is not known why or how this shift in species composition occurs.

Further study into the species composition and population dynamics of a region is essential to the study of such a diverse group of animals (Gosliner and Draheim 1996). Before questions pertaining to their behavior can be addressed, it is important to know which species inhabit certain regions and where they can be found within those regions. Indeed, once the distributions of nudibranch species have been established, behavior studies will follow. Since the distribution of many marine invertebrates is mainly determined by their behavior (Meadows and Campbell 1972), the more that is known about their distribution, the more information will be available for behavioral work.

Obtaining such information (behavior and distribution) is worthwhile not only because it pertains to nudibranch ecology, but also because it affects the communities they inhabit. Nudibranchs are important organisms within intertidal and subtidal communities (Todd 1981). Many have close associations with very specific prey items (Todd 1983). Thus, the population dynamics of those prey species can be affected by nudibranch populations.

However, basic information such as species composition and distribution is difficult to come by, as nudibranchs are known for their scarcity (Todd 1983). As a result of this scarcity, very little is known about the nudibranch fauna of the South Pacific (Johnson 1994), especially on the island of Moorea.

It is unclear what species of nudibranchs inhabit the waters of Moorea; let alone what their distribution patterns are like. Brief mention has been made on three of the species present in Moorea and where they are located (Nowell 1995; Wu 1999). However, a concerted effort to determine the species composition and the distribution of the nudibranchs of Moorea has not been done.

This study had three main objectives. The first was to provide an account of the species of nudibranchs that occur in Cook's Bay on the island of Moorea, French Polynesia. The second objective was to look at the distribution of each species within Cook’s Bay. Distribution patterns were studied in three ways by looking at: 1) the population density of each species, 2) the exact part of the reef each species was located on and 3) the habitat parameters of each species. The third objective looked at the aggregation behavior of the nudibranch species *Glossodoris cincta*, which also occurred in Cook’s Bay.

Individuals of *G. cincta* were sometimes observed in the field in groups of 2-3. This behavior was studied because it posed a valuable question. Are these individuals aggregating in order to mate, or are they aggregating for the substrate they were found on? Both have potential value for the animal.

Nudibranchs are simultaneous hermaphrodites and most species reproduce using simultaneous reciprocal copulation (Leonard 1991). Aggregating helps in this breeding process (Meadows and Campbell 1972).

However, most nudibranchs are specialists in the types of prey they consume (Todd 1983). Some prey types include hydrozoans, sponges, and algae. It could also be possible that individuals of *G. cincta* were aggregating for prey items.

The behavior portion of this study hypothesized that individuals of *G. cincta* would exhibit a preference for either a conspecific, or for the substrate that their aggregations were found on. This preference was tested by conducting trials in a Y-maze.

The null hypothesis assumed that individuals would show no preference for either a conspecific, or for the substrate that their aggregations were found on.

Methods and Materials

Study sites

All fieldwork and Y-maze trials were conducted at the Richard B. Gump South Pacific Biological Research Station on Moorea, French Polynesia, between September 2001 and November 2001.

Species composition and distribution studies were conducted at the patch reef in front of the Gump Station (Site 1 on Figure 1), and at the reef wall in front of Cook’s Bay Hotel (Site 2). Site 1 was located at S 17°49, 414’ and W 149°49, 566’, and site 2 was located at S 17°29, 302’ and W 149°49, 133’. All *G. cincta* aggregations for the behavior study were collected from Site 1.

Species composition

Sites 1 and 2 were searched for all nudibranch species by snorkeling. Once a species was found, it was collected and brought back to the Gump Station. There it was measured for length and width and photographed. A description of color was taken, and the nudibranch was then released. All species identifications were done using Dr. William B. Rudman’s Sea Slug Forum website and a journal article also by Dr. William B. Rudman (1986).

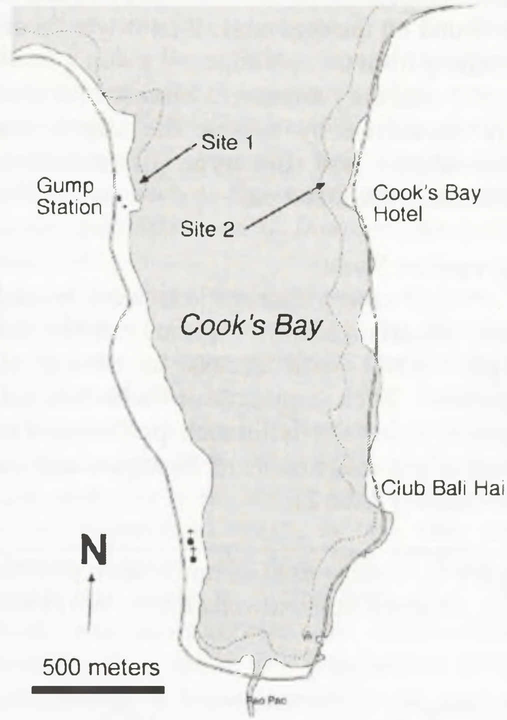


Figure 1. Map of study sites located in Cook’s Bay, Moorea, French Polynesia. Adapted from the UC Berkeley Geography and Cartography Lab.

Distribution Study

Population density. Population densities for all species of nudibranchs found were measured using 10x1m transects. Transects were performed at both sites 1 and 2.

Transects were laid out roughly parallel to the shoreline. Transects would first be done at the reef edge, right where the reef dropped off into Cook’s Bay. A PVC pipe 1m in length would be held over the transect so that 0.5m of the pipe was on one side, and 0.5m of the pipe was on the other side of the tape. Then, whatever substrate fell within the 10x1m area of the transect was searched for nudibranchs. After each transect, the transect tape would then be moved 10m closer to the shore and the procedure would be repeated.

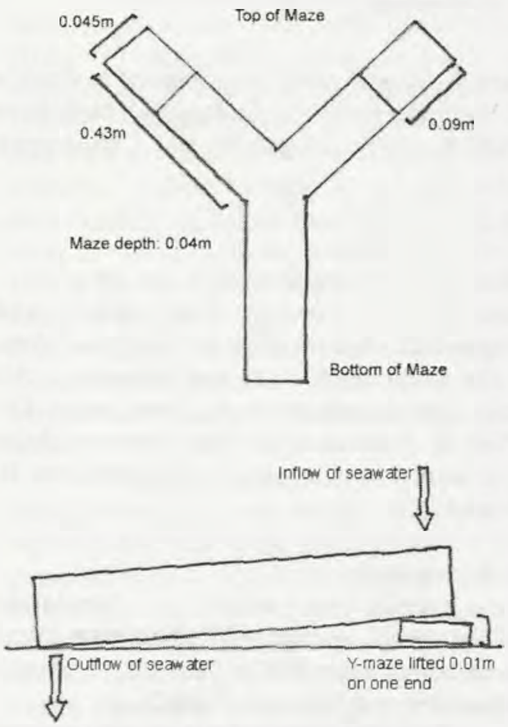
Species location. When a species was found, its location on the reef was recorded. If it was found on the reef edge, then it was recorded as coming from the reef edge.

Habitat parameters. Habitat parameters were measured by noting the depth, water temperature, and the type of substrate a nudibranch was found on.

Aggregation Study

G. cincta aggregations were housed in glass aquaria (length: 0.43m, width: 0.5m, height: 0.4m) with access to natural light regimes. Each aggregation was housed in separate aquaria. Nudibranch preferences were tested in a Y-maze made of Plexiglas and sealed with caulk (Figure 2).

Figure 2. Y-maze used in nudibranch preference trials. Adapted from Nowell (1995); Wu (1999).



Each of the three arms of the maze was 0.43m long, 0.045m wide, and 0.04m high. Green plastic mesh was placed at the top of two of the arms, 0.09m from the end, to prevent a conspecific from traveling the wrong way in the maze. The nudibranch was given a choice between a conspecific from its aggregation or the substrate its aggregation was found on. The top end of the maze was lifted 0.05m to facilitate the flow of water through the Y-maze.

Nudibranch choice and the time it took the nudibranch to choose were recorded during each trial. One trial consisted of when the tested individual was first put into the maze until the point at which it made a choice. A nudibranch was said to have made a choice when it got at least half the way up one of the two arms. Between each trial, all animals and coral rubble were removed the maze and plastic mesh were scrubbed to remove any residual mucus trails. Seawater was the run through the empty maze for 15 minutes to flush out any remaining particles. After 15 minutes, the conspecific and the coral rubble were put in to the tops of the two arms, one in each arm, and water was run through the maze for 5 minutes.

Each member of an aggregation was tested in the Y-maze. Six trials were performed with each individual. Trials were run during midday and in the afternoon between October 22 and November 13, 2001. Each nudibranch was allowed 30 minutes to make a choice. If it was still moving at 30 minutes, it was allowed to continue until its choice was complete. If it was not moving after 30 minutes, it was removed and the results of the trial were noted.

Data analysis

To standardize the time measurements, all measurements were converted to seconds and divided by 3600 seconds (1 hour). If a nudibranch did not make a choice then its time measurements were not included in the analysis.

A chi-squared analysis was performed on the habitat parameter data to test for the significance of preferences shown. A paired T-test was conducted on the time measurements taken while the nudibranchs were in the Y-maze. A chi-squared analysis was conducted on the preference data taken while the nudibranchs were in the Y-maze. Analyses were conducted using Microsoft Excel and Systat.

Results

Species composition

Individuals of four species were observed in the two study sites in Cook's Bay. *Chromodoris boucheti, Risbecia imperalis, Glossodoris hikuerensis,* and *G. cincta* were all observed at site 1. Only *C. boucheti* was observed at site 2. *C. boucheti* populations showed a variation in their color patterns that followed their geographic location in Cook's Bay. For detailed descriptions of each species, see Appendix 1.

Distribution analysis

Population density. Four transects were conducted at site 2. No nudibranchs were found in any of the four transects. Fifteen transects were conducted at site 2. No nudibranchs were found in any of the fifteen transects.

Species location. *C. boucheti* at both sites 1 and 2 were observed at the reef edge and nowhere else in those localities. *R. imperalis* was observed only at site 1, and it was located at the reef edge. However, *R. imperalis* was extremely rare. Between September 2001 and November 2001, only four individuals were observed. As a result of this scarcity, all data pertaining to *R. imperalis* should be observed with caution.

G. hikuerensis was observed at site 1 and also further north along the patch reef in the area where the reef comes to its first point (Figure 1). Individuals of *g hikuerensis* were found either at the reef edge, or 1-2 meters shoreward from the reef edge.

G. cincta was observed only at site 1. Individuals as well as aggregations were located 2-3 meters shoreward from the reef edge.

Habitat Parameters. For a description of the habitat parameters each species was associated with, see Table 1.

Chi-squared analysis for the significance of the substrate preference shown by nudibranch species was only performed on *G. hikuerensis* and *G. cincta*. Due to small sample size, not enough data was available to perform chi-squared analyses for *C. boucheti* and *R. imperalis*. Both *G. hikuerensis* and *G. cincta* showed significance (p-value 0.001 and 0.00 respectively).

Aggregation analysis

The paired T-test analysis for the time data showed no significance (probability of 0.211). The chi-squared analysis for the preference trials showed no significance (p-value of 0.225).

Discussion

Species Composition

When viewed in light of the previous studies conducted on the nudibranchs of Moorea, the species composition of Cook's Bay shows interesting changes.

Previous studies had not indicated that there was a nudibranch population on the west side of Cook's Bay (Johnson 1994; Nowell 1995; Wu 1999). However, during the course of this study, a population consisting of *C. boucheti* individuals was located at site 2, on the western side of Cook's Bay. Since none of the previous studies mentioned searching the area for nudibranchs, it is quite possible that this population of *C. boucheti* was missed. Other explanations such as the migration of part of the founder population of *C. boucheti* are unlikely (Todd 1981).

Watching the evolution of these two populations of *C. boucheti* will be interesting. How did these two populations come to be like this? Could this happen to other nudibranch species, both in Cook's Bay and worldwide? Has this happened to other nudibranch species? There probably is not much gene flow between the two populations seeing as how they are at opposite sides of the bay. Will these two populations begin to differ from each other, either morphologically or behaviorally? Observing how these two populations develop could answer a lot of questions on how new species come about and how long it takes for one species to evolve into two.

Another result that raises more questions deals with the species composition at site 1. Nowell (1995) stated that the nudibranch species *Risbecia tryoni* was found at or near site 1. However, during the course of this study, *R. tryoni* was not found in any of the study sites. It is very possible that individuals of *R. tryoni* were missed during the species search.

However, it is also quite possible that the *R. tryoni* population has undergone a kind of population fluctuation. It is not uncommon for nudibranch populations to experience erratic population dynamics (Beeman 1977). Perhaps the *R. tryoni* population was at a low point during the course of this study. If so, then do the other species that inhabit Cook's Bay undergo something similar? It would be beneficial to monitor this group of nudibranchs to see if similar population dynamics occur in other species.

Distribution

Population density. The lack of nudibranchs within any of the transects indicates a low population density. This is further supported by the fact that only four individuals

Species	Depth (m)	Water Temperature (C)	Substrate found on
Chromodoris boucheti	1.5 - 2	30 - 32	encrusting sponge
Risbecia imperalis	N/A	N/A	N/A
Glossodoris hikuerensis	1.5 - 2	30 - 31	brown algae
Glossodoris cincta	1 - 1.5	30 - 31	brown algae

Table 1. Habitat parameters of all four species that occur in Cook's Bay, Moorea, French Polynesia.

Species	Frequency Observed
Risbecia imperalis	4 individuals (9/01-11/01)
Chromodoris boucheti	10 individuals (9/01-11/01)
Glossodoris cincta	0-5 individuals per observation period
Glossodoris hikuerensis	0-7 individuals per observation period

Table 2. Frequency with which each nudibranch species was observed during the course of the study.

of *R. imperalis* were observed during the course of the entire study (Table 2). It is not known why the population density of all four species was so low.

Predation could be a factor. The six-bar wrasse *Thalassomma hardwickii* and the damselfish are known predators of nudibranchs (Johnson 1994). Both kinds of fish were observed at all study sites. Although the effects of predation by these fish on the nudibranchs are unknown, they could be a factor in the low population density of the nudibranchs of Cook's Bay.

Species location and habitat parameters. All individuals of *C. boucheti* were located on the reef edge at both sites 1 and 2. Since this held true for both populations on either side of the bay, location on the reef could be important to this species. This result could also be tied to the substrate that individuals were found on. Since chi-squared analysis showed significance for the fact that *C. boucheti* was found on encrusting sponges, perhaps the reef edge was the only place with the suitable substrate.

Individuals of *G. cincta* also exhibited the same affinity for location and substrate. Chi-squared analysis showed significance for *G. cincta*'s presence on brown algae. Perhaps its location on the reef was the only suitable place to find a preferred substrate.

Individuals of *G. hikuerensis* showed more range in where they were located on the reef. However, they showed just as strong an affinity for their substrate (p-value 0.001) as individuals of *G. cincta* did. Perhaps *G. hikuerensis* has a wider range of substrates that it can tolerate. Perhaps it is able to exploit a wider range of substrates for resources such as food than either *G. cincta* or *C. boucheti* can.

Although the sample size for *R. imperalis* was too small to perform statistical analyses on which substrate it was found on, brief mention shall be made as to the substrate it was found on. Two individuals were observed on encrusting sponges at the reef edge. Although it is not possible to say whether this shows any significance, if more data can be collected from this species in the future, then perhaps encrusting sponges are a habitat parameter for *R. imperalis*.

Aggregation behavior

Although statistical analyses supported the null hypothesis that no preference would be shown by *G. cincta*, it is still valuable information. Perhaps substrate and conspecifics are equally as important to individuals of *G. cincta*.

Aggregation behavior with the goal of mating is seen in other marine molluscs such as the sea hare *Aplysia californica* (Pennings 1991). When simultaneous hermaphrodites mate in pairs, they experience conflict over which individual will take a particular sexual role (Leonard 1991). By mating in pairs or in larger groups, each individual can simultaneously take both roles (Pennings 1991). Individuals of *G. cincta* could be solving the problem of simultaneous hermaphroditism by aggregating for mating purposes.

Although it is not known for certain what *G. cincta* eats, perhaps they value their prey items as much as they do mating and are willing to share this food with other conspecifics.

Another explanation deals with experimental design. Although the rhinopores of a nudibranch are sites of chemoreception, the oral tentacles, just below the anterior mantle edge, are the main sites of chemoreception (Kohn 1961). In the context of detecting conspecifics, mucus trail following is important (Todd 1981).

Thus, although individuals of *G. cincta* were in the Y-maze with conspecifics, the tested individuals might not have been able to accurately locate those conspecifics due to the lack of a mucus trail. This could result in the lack of preference exhibited by the *G. cincta* individuals in this study.

Should another experiment be designed to address this same issue of aggregation behavior, a different experimental design is suggested. A Y-maze would still be used, but instead of having the nudibranch rely on waterborne chemicals it might or might not be able to detect, an alternative is proposed. Allow the conspecific to lay down a mucus trail before the tested individual is placed into the maze. That way the tested individual will have a less ephemeral means of making a decision. If these nudibranchs are aggregating for conspecifics, then the results of such an experiment should reflect that.

Conclusion

This study looked at three aspects of nudibranch ecology in Cook's Bay, Moorea: 1) what is the species composition of Cook's Bay 2) what are the distribution patterns of those species and 3) why do individuals of *G. cincta* form aggregations? It was hypothesized that *G. cincta* individuals would exhibit a preference for either the substrate they were found on or a conspecific from their aggregation.

Results lent support in favor of the null hypothesis, that *G. cincta* would show no preference for either choice. This could be because both choices were equally important to the nudibranch, or it could be an artifact of the experimental design.

Suggestions for further study include monitoring of *C. boucheti* populations on either side of Cook's Bay. Long-term studies of this phenomenon might lead to more insight on the subjects of speciation and evolution. Monitoring of the species populations at site 1 will also lead to more insight on the population dynamics of a very scarce, yet very important animal.

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Appendix 1

Species Description

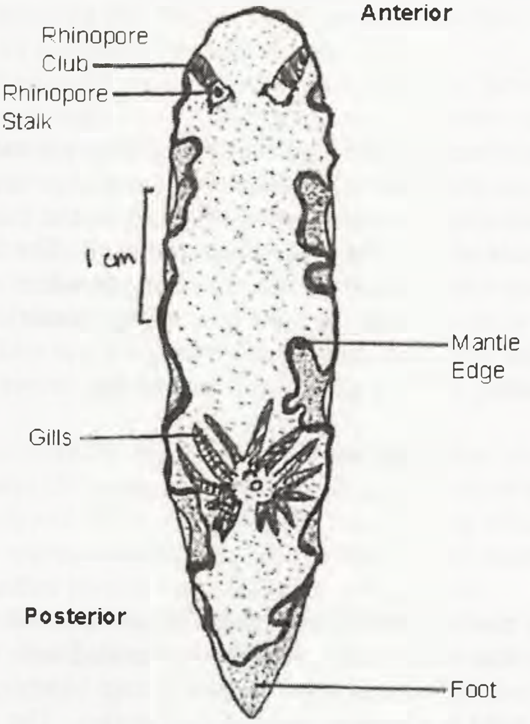


Figure 1A. Basic anatomy of a nudibranch. Drawn and adapted from Thompson (1976)

Chromodoris boucheti (Rudman 1982)

Average length: 1.6cm
Average width: 0.045cm

On the west side of Cook's Bay (site 2), individuals have a base body color of creamy white. Individuals on the east side of Cook's Bay (site 1) have a base body color of light purple-blue. In both localities individuals have a white mantle edge with a black line separating the white mantle from the rest of the body. A black line runs down the midline of the dorsal side of the animal. It starts between the rhinopores at the anterior portion of the animal and ends just before the gills at the posterior portion of the animal. There is also one line on either side of the cluster of gills. The rhinopore stalks are a creamy white while the rhinopore clubs are a bright orange. The gills are similarly colored, with the bottom portion being creamy white and the top half a bright orange.

Risbecia imperalis (Pease 1860)

The base body color is white. Yellow dots that are slightly raised are sprinkled all over the body and the foot. The mantle edge is a dark blue, almost black in color. The yellow dots are also present in this dark border. The rhinopores are a dark blue-black and have fine white dots all over them. One white line runs down the anterior midline of each rhinopore. The gills are white with one blue-black line that runs down their entire length.

Glossodoris hikuerensis (Pruvot-Fol 1954)

Average length: 18.5cm
Average width: 5.75cm

The base body color is brown. The entire body and foot are mottled with very fine white dots. The mantle is comprised of four bands of color. The very outer edge is a thin line of dark brown-black. The next band in from the outer edge is a light gray that fades into the third band, which is blue-teal. The fourth and final band of color is white. The foot is also outlined. The foot outline is comprised of two bands of color. The outermost band of color is an extremely thin line of white. The second band is a thicker band of blue-teal (the same color as the third band of the mantle). The rhinopores are brown with white speckles. There is a thick white line running down the posterior midline of each rhinopore club. The gills are also brown with a mottling of white dots. Solid lines of dark brown define the edges of the gills.

Glossodoris cincta (Bergh 1888)

Average length: 6.6cm
Average width: 2.6cm

This species is easily confused with *Glossodoris hikuerensis*. *Glossodoris cincta* individuals have a base body color of brown. The entire body and foot are mottled with white dots. However, unlike *G. hikuerensis*, *G. cincta* individuals have a mantle comprised of two bands of color. The outermost edge is a dark blue-black. The second band is a broader band of light brown. The rhinopores are brown with white speckles. A white line runs down the posterior midline of each rhinopore club. The gills are also brown with white speckles, outlined in a brown-black line.

Appendix 2

General Behavioral Observations

"Follow the leader"

This behavior was only observed in individuals of *Risbecia imperalis.* When engaged in this behavior, one individual was the leader and the second individual was the follower. In the pair observed, the leader was the larger of the two animals. The follower was positioned so that the right side of the anterior portion of its foot was touching the left side of the posterior portion of the leader's foot. There was actual physical contact, not just an approximation of position.

The leader then moved around the coral head or the aquarium, "towing" the follower along with it. Periodically the leader would stop and extend the front part of its body up off the substrate and out. It would then begin to wave the anterior portion of the mantle fringe up and down. During this head-waving period the follower would not move from its position on the leader's foot. Unless the observer separated the animals, they would continue to stay in physical contact with each other. When not engaged in the "follow the leader" behavior, the smaller individual would place an entire side of its body (left or right) next to the larger animal's body so that they were touching. Even while one individual was laying eggs, the second individual was in physical contact with it.

"Perching"

This behavior was observed in individuals of *Glossodoris hikuerensis* and *Glossodoris cincta.* It was observed both in the field and in the aquarium. "Perching" was characterized by the individual lifting the anterior half of its entire body off of the substrate, and then waving either just the mantle fringe about, or the entire body. When they waved their mantle fringe, they would wave it up and down. When they waved their bodies, they would either wave it up and down or side to side.

Individuals were also observed with the anterior half of their body off of the substratum, but they were completely still. This was usually observed when the animal was situated on a high point on the coral rubble. Sometimes it was the highest point, sometimes not.

Egg laying

This behavior was observed in *r. imperalis, g. hikuerensis,* and *g. cincta.* All three species laid egg masses in the shape of a flattened noodle called an egg ribbon. Egg ribbons from all three species were attached to a substrate on one side (the sides of the aquarium or underneath a rock), so that the rest of the egg ribbon was perpendicular to the substrate. These ribbons were arranged in a spiral. The spiral was oriented so that it spiraled out from the center in a counterclockwise fashion. Egg ribbons produced by *r. imperalis* were a salmon color (light red/dark pink), and egg ribbons produced by *g. hikuerensis* and *g. cincta* were a creamy white color.

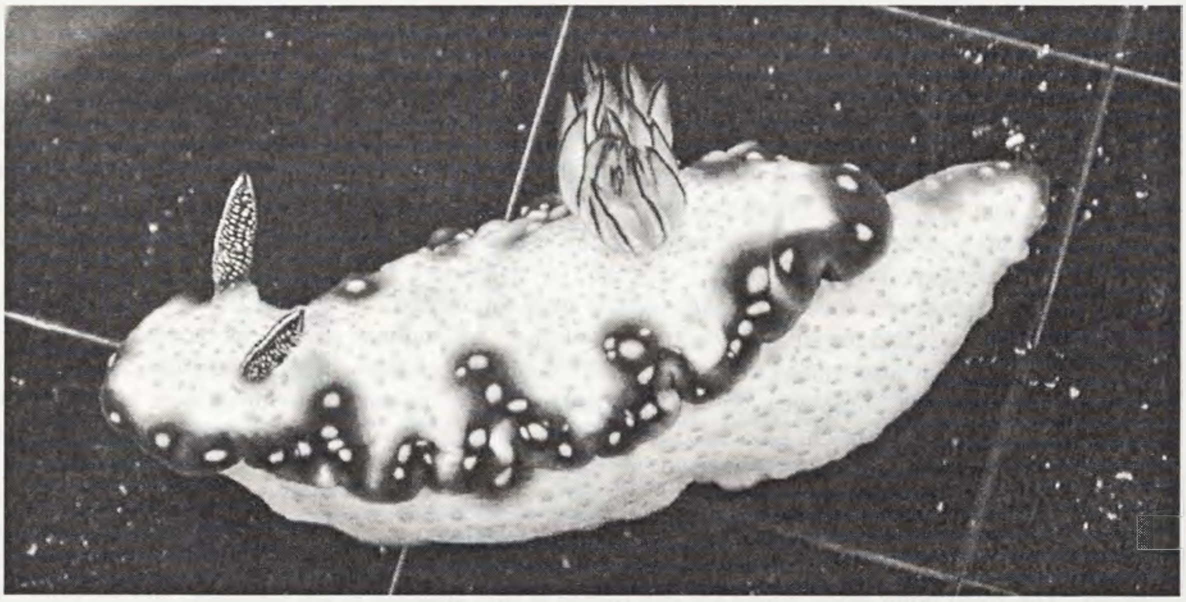
Egg ribbons produced by *g. hikuerensis* were the largest in terms of width. *G. cincta* was next, and then *r.imperalis* had the smallest egg ribbon width. The length of an egg ribbon varied from individual to individual, and it also varied within one individual. Individuals of *g. hikuerensis* and *g. cincta* laid multiple egg ribbons. Some were quite long, others, laid by the same individual, were not. This multiple egg-laying phenomenon might have been a stress response from being collected and put into an aquarium (Johnson, pers. com.).

The egg ribbon would be extruded from the right side of the animal, from an opening located on the anterior portion of the nudibranch. The spiral was generated by "a point having uniform motion around a fixed point, the pole, combined with a uniform motion toward or away from this fixed point." (Costello 1938). The fixed point being where the egg ribbon is first attached to the substrate, and the point "having uniform motion" being the nudibranch as it travels in its spiral path. All direction of travel would be counterclockwise.

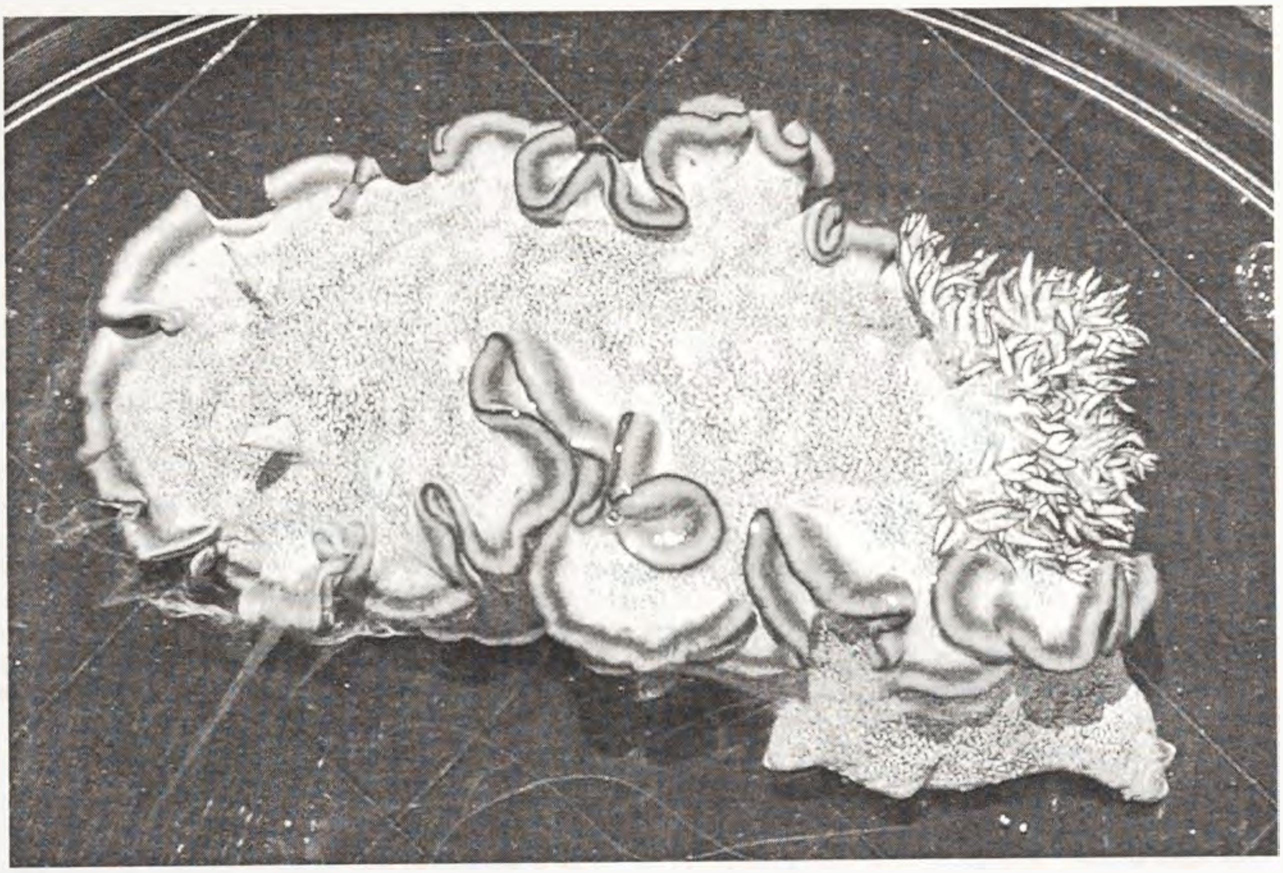
Appendix 3

Species pictures

Chromodoris boucheti



Risbecia imperalis



Glossodoris hikuerensis

Glossodirs cincta (Picture retrieved from Dr. Rudman's Sea Slug Forum Website (http://www.seaslugforum.net))

Behavioral Partitioning by Polynesian Cleaner Wrasses, *Labroides dimidiatus* (Val en Ciennes, 1839) & *Labroides bicolor* (Fowler & Bean, 1928) Along Fringing Reef Walls of Moorea, French Polynesia

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ABSTRACT. The client solicitation behavior employed by cleaner wrasses (*Labroides dimidiatus* & *L. bicolor*) in French Polynesia was studied to determine its role in partitioning the resources available to these specialized microcarnivores. Field observations were made on fringing reef walls of the North & East coasts of Moorea with times recorded for each of four client acquisition behaviors. Cluster analysis of these behaviors revealed two behavioral size classes for each species (Less than 5cm & 5cm and Larger) and these groups were shown to allocate significantly different portions of their time to three of four client solicitation behaviors. Additional results showed a trend towards the enhancement of each species-size class group's preferred behavior in the presence of another cleaner, though insufficient replication failed to reveal significant changes. This study shows that behavioral differences in solicitation behaviors may contribute to the ecological partitioning of clients on Moorea and other situations of high competition.

Introduction

Cleaning symbioses are appreciated as one of the most interesting of interactions between distantly related taxonomic groups. In both the marine and terrestrial realm these groups may not even be of the same order as with birds and large mammals acting as symbionts or crustaceans as cleaners of fish (Losey 1979). By and large the most common of these cleaning symbioses occur between groups of fishes (Losey 1987), some cleaners having highly derived morphologies and behaviors specialized for such interactions (Gorlick et al 1978). Rather than the polarized debate between viewing this interaction as mutualistic or parasitic found in earlier literature (Limbaugh 1961; Losey 1987), the recent work has studied this interaction as a specialized form of miocrocarnivory (Poulin & Grutter 1996). In tropical marine systems these interactions are ubiquitous (Potts 1973) and involve specialized species of fish or crustaceans that remove ectoparasites and infected tissues, along with scales and mucus, from other fish species either resident in or visiting the reef, referred to as clients (Limbaugh 1961).

For the species of cleaners found in Polynesia (*Labroides* Spp) the accepted hypothesis is that they have evolved from an unspecialized ectoparasitic form (Gorlick et al 1978). The evolutionary breakthrough to become specialized as cleaners was the development of a means of stimulating a preexisting tactile reinforcement system believed to be present in most vertebrates (Gorlick et al, 1978). This has been referred to as "host stabilization" and appears to have the function of reducing the probability of the client fish leaving the cleaning station despite the clearly painful stimulus of ectoparasite removal (Losey, 1987). Studies of the ecological significance of cleaners has been debated greatly in the literature since the first account of changed community structure upon the removal of cleaners (Limbaugh, 1961), but the most recent work with *Labroides dimidiatus*, shows no change over the course of six months following their removal (Grutter, 1997). The suggestion is that *Laboroides* cleaners are not essential to the health of reef fishes, but that their own survival depends upon coming into contact with clients to stimulate their tactile reinforcement system or of being noticed by individuals accustomed to the stimulation provided by cleaners.

In Polynesian waters, three species of wrasses (*Labroides bicolor*, *L. dimidiatus*, & *L. rubrisilatus*) coexist performing this function, all of which are fairly conspicuous and have very similar morphology. To coexist, there must be some mechanism by which they partition the resources of the reef (clients). Preliminary studies for this paper found one element of this partitioning involves the limitation of no more than two of the three species in any of the three reef types investigated around the island (forereef, patch reef & fringing wall reef). Often found in close association along fringing wall reefs (versus superdispersed over patch reefs), *L. dimidiatus* & *L. bicolor* were found to clean in identical, or even the same microhabitats with some occupying discrete territories, sometimes advertising their services with displays, and others cruising the reef, actively pursing clients, but with the behavior of an individual ranging anywhere among these behaviors.

Nearly all studies conducted on cleaner fish have shown positive correlations between client size and cleaning preference (Grutter, 1995), ectoparasite load (Grutter, 1994), and frequency and/or duration of inspection (Grutter & Poulin, 1998). Grutter & Poulin's results held only when generic values were treated as independent observations and explained these results as probably reflecting the age and coevolutionary history of their study fish, *L. dimidiatus* and its clients. Considering a cost-benefit model for client behavior proposed by Cote et al (1998), and the presence of a competitive species of cleaner, it is important to consider the cleaning interaction as dependant on the behaviors of both cleaner & client.

This study proposes to determine if there are significant differences in allocation of time to four different solicitation behaviors between the two species of cleaner wrasse as well as between different size classes. If there are behavioral differences, one may expect them to be enhanced by higher densities of competitive congenerics which can be expected to be found at sites on the windward side of an island (found for *L. phthirophagus* and its clients, Youngbluth, 1968). This situation was thus studied to determine if significantly higher proportions of solicitation time were allocated to preferred behaviors in situations of greater competition.

Materials & Methods

Field Site and Study Organisms

This study was performed on fringing wall reefs on the North & East coasts of Moorea (17°30'S, 149°50'W) of the Society Islands, French Polynesia. All sites were coral reef walls with slopes of 75^{O} - 110^{O}, dropping to a sloping sandy lagoon bottom between 10 & 15 meters. The study was conducted at three sites representing locations that were windward, leeward and sheared by the dominant North-East Tradewinds (Fig.1-1). The windward site was on the West side of Cook's Bay at its mouth. The Leeward Site was On the East side of Cooks Bay, slightly inward from the mouth. The sheared site was at the North side of the opening of Vaiare Bay where it curves from South facing to East facing.

The two species of cleaner wrasse studied were *Labroides dimidiatus* (blue-streaked) and *L. bicolor* (bicolored) ranging from 2cm to 9cm which, though similar in body form, are easily distinguishable based on coloration.

Behavioral Observations

Behavioral observations were carried out in all three sites between 1000 &1500 hours (as this has been shown to be the period of most active cleaning, Cote et al, 1998). A total of 111 observations were performed for a total of 164 minutes. Observations were performed from as far away as possible to minimize disturbance, but close enough to differentiate behaviors. This varied with visibility but ranged between 1 & 6 meters. When an individual was encountered, it was identified and measured by sight as well as recording any other cleaners present and their size. For as long as possible (up to 10 minutes) it was followed and times recorded for each of the following behaviors: Stationing (swimming in a restricted area less than 3mX3mX3m without an undulating motion accepted as a client solicitation display), Displaying, Chasing (of clients), Cruising (swimming out of a cleaning station), and Cleaning (including inspection that does not result in the removal of parasites). Data was also taken on attempts made by the fish being observed or other

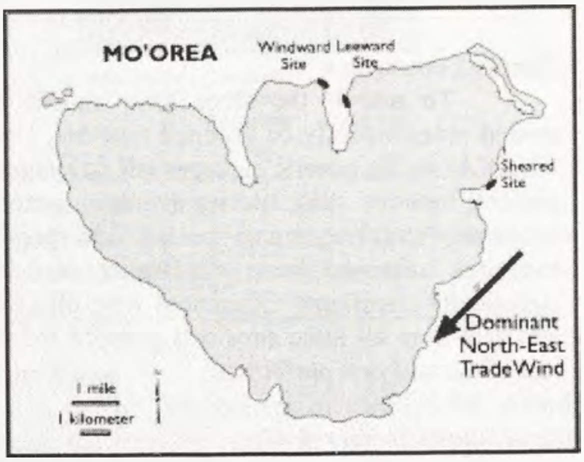

Fig. 1 Location of sites around Moorea representing areas Windward, Leeward & Sheared by the dominant North-East Trade Wind.

L. bicolor	Stationing	Displaying	Chasing	Cruising
3cm	0.464	0.509	0.025	0.00
4cm	0.586	0.255	0.045	0.111
5cm	0.502	0.132	0.058	0.306
6cm	0.469	0.073	0.032	0.424
7cm	0.334	0.090	0.048	0.527
8cm	0.373	0.092	0.066	0.467
9cm	0.0531	0	0.024	0.922
L. dimidiatus	Stationing	Displaying	Chasing	Cruising
2cm	0.00	1.00	0.00	0.00
3cm	0.00	1.00	0.00	0.00
4cm	0.148	0.744	0.037	0.069
5cm	0.379	0.551	0.022	0.046
6cm	0.565	0.248	0.033	0.153
7cm	0.654	0.139	0.035	0.170
8cm	0.573	0.221	0.020	0.185
9cm	0.551	0.435	0.0128	0.00

Table 1 *Percentage of time spent on solicitation behaviors by each centimeter size class of* L. bicolor *&* L. dimidiatus.

cleaners to chase one or the other out of the station.

Comparative Survey

To determine if there were differences in cleaner density between areas on the island, transects of 50m recording the size and identity of all cleaners were performed over the course of 10 minutes at 1000h, 1230h & 1500h at each site and replicated three times.

Statistical Analysis

Density Comparison

To test if the three sites should be treated independently or grouped together, I ran ANOVAs on the generic averages (all *Labroides* present) between sites, species averages, generic time-specific averages and species time-specific averages obtained from the transects. No statistically significant differences were obtained and data from all three sites was grouped for the rest of the analyses performed.

Grouping of Species & Size

Averages were calculated for each behavior for each centimeter size class of each species and transformed into percentages of time spent on behaviors other than cleaning (Fig. 1-1). These four percentages were then used for cluster analysis with SYSTAT 7.0 to determine size class groupings (excluding 9cm *L. bicolor* with only one data point to calculate averages from). For both species the size class cluster analysis demonstrated two groupings- less than 5cm and 5cm and up (referred to from here on as Juvenile & Adult, although pink belly patches, indicative of sexual maturity in females [Grutter, A.S. personal communication], were found on individuals 4cm long).

Group Comparisons

Each individual observation was transformed into percentages of time spent on behaviors other than cleaning. These percentages were grouped into the adult & juvenile size classes of each species (herein referred to as "groups") and each behavior was subject to ANOVA.

Paired Comparisons of Preferred Behaviors

Averages were recalculated for entire Juvenile & Adult size classes of each species and from these values the preferred behavior was determined. Each individual observation was then transformed into percentages and the time allocated to preferred behavior for each was compared with that of all others to determine significant differences.

Behavior Enhancement

For each species-size class, percentage-transformed observations of those individuals observed in the presence of another cleaner were t-tested against those of individuals observed alone. This would allowed us to determine if the preferred behavior was performed for a larger percentage of the solicitation time in the presence of a congeneric.

Results

Group Comparisons

From observation periods averaging 92.2 seconds (SD=73.8), ANOVAs showed significant differences in three of the four behaviors (Table 2), Stationing (p=.00193), Displaying (p=3.6E-13), and Cruising (p=8.71E-8). None was found for the behavior of Chasing (p=.0923). This behavior was used by no group for more than 8% of its time spent soliciting clients (7.55% by Adult *L. bicolor*) and it did not represent a preferred solicitation behavior (though one individual spent equal time on Stationing, Cruising & Chasing).

Behavior	p-Value	f-Ratio	*df*
Stationing	.00193	5.29	3
Displaying	3.6E-13	27.45	3
Cruising	8.71E-8	14.05	3

Table 2 Results from ANOVAs showing significantly different proportions of time allocated to three of the four solicitation behaviors by the four size-species groups.

Preferred Behaviors

Stationing represented the preferred behavior for both Juvenile *L. bicolor* and Adult *L. dimidiatus* comprising 45.4% & 53.7% respectively. Despite this similarity, they had different secondary behaviors with *L. bicolor* secondarily Displaying for 42.1% and *L. dimidiatus* Cruising 14.1% of their solicitation time. Additionally, it has been found that these two groups Station in different microhabitats (Potts 1973) with Juvenile *L. bicolor* Stationing in overhangs and Adult *L. dimidiatus* Stationing above the reef. For Juvenile *L. dimidiatus*, the preferred solicitation behavior was Displaying and was employed 78.6% percent of its time spent not cleaning clients. Cruising was the preferred solicitation behavior of Adult *L. bicolor* and represented 42.2% of its solicitation time.

Behavioral Enhancement

Though no statistically significant changes occurred in the presence of a congeneric, the trend was towards the enhancement of the preferred behavior of each group (Fig 2). The only group to show negative enhancement of the trend was Juvenile *L. dimidiatus* which decreased its preferred behavior of Displaying from 92.9% to 67.8% though this is likely to have been influenced by the uneven distribution of centimeter size classes within this grouping. Within this group all individuals that were found with a congeneric were in the 4cm size class which showed a greater range of behaviors than the smaller size classes (See Table 1). Juvenile *L. bicolor* showed an average of 38.9% of time spent on Stationing in the absence of other cleaners, and 54.0% with a congeneric present. For Adult *L. dimidiatus*, these values were 49.4% without versus 58.3% with a congeneric. Time spent Cruising by Adult *L. bicolor* increased from 40.6% to 44.1% with the presence of a congeneric

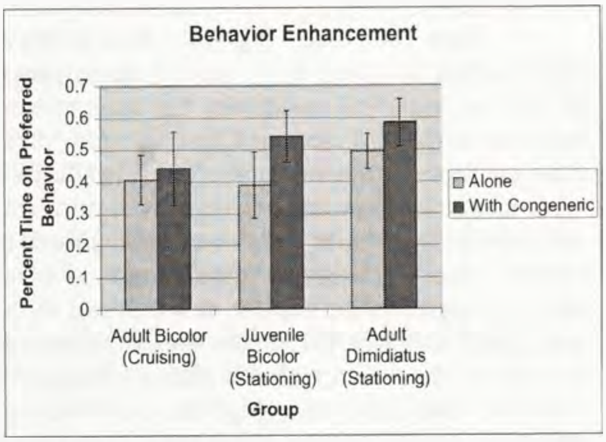

Fig. 2 Change in proportion of time allocated to preferred behavior by cleaner groups.

Discussion

The two species of cleaner fish found along the fringing reef walls of Moorea, French Polynesia were shown to spend significantly different proportions of their time on different behaviors assumed to aid in client acquisition. The literature has shown only anecdotal evidence of such behavioral differences between different developmental stages of *L. dimidiatus* (Potts 1973) and this is the first behavioral study to show such differences for multiple cleaner species found in overlapping habitats.

Potts' (1973) landmark studies on *L. dimidiatus* were conducted (on Aldabra Island) in less human influenced locations than Moorea, where the abundance of potential clients appears to have been much higher. In these situations, cleaners had been shown to defend territories that possessed a focal point to which clients had been trained to return to for cleaning. In this type of situation, the cleaner or cleaners (in pairs or harems) were found to return to their stations day after day. Here in the overfished situation of Moorea's lagoon, this was not the case and individuals could not be expected to be locatable in the same station on subsequent days. Moorea appears to represent a more competitive situation where the need for partitioning is greater. Potts also found that the distribution of cleaners in his study site was strongly influenced by the currents forcing the weak swimming juveniles to accept sheltered locations for their stations. Along the reef walls of Moorea, there was no such strong tidal influence and thus could more accurately demonstrate the effect of behavioral preferences in partitioning the clients of the reef.

Density Comparisons

Due to the lagoon topography (information obtained from station employees) as well as logistical restraints, the comparison between windward, leeward and sheared sites may all represent windward sites (Fig 1) and were unable to show any statistically significant differences in cleaner densities. Yongbluth's (1968) study showed densities of *L. phthirophagus* to be highest at windward sites and thus it is likely that these sites represented the area on the island with the highest density of cleaners thus also the highest partitioning pressures.

Group Comparisons & Behavioral Preference

Potts presents evidence that certain groups of clients are preferred to others by *L. dimidiatus* but that juveniles appear to innately approach potential clients. From the observations made here, it appears as though they also innately swim with an undulating motion that makes their relatively cryptic bodies (more so than all other groups, Potts 1973) more visible to clients. This inference comes from the fact that the smallest size classes were found to do nothing other than this display. In contrast to *L. dimidiatus*, as *L. bicolor* became larger, they became less conspicuous as the yellow of their bodies became a duller shade as well as regressing from anterior dorsal and ventral surfaces. Potts noted the broadening of the blue streaks on *L. dimidiatus* and postulated that as they learned how to approach clients (i.e. avoiding approaching the head of *Lutjanids* first) it would be to their advantage to be more conspicuous allowing them to be approached by clients. Potentially this behavioral niche was already occupied during the evolution of *L. bicolor* and the ones that performed better were those with more cryptic coloration for approaching & stimulating clients without being noticed.

Behavioral Enhancement

In the presence of a congeneric, it seems to be to the advantage of a cleaner to dedicate more time to its prefered behavior though the number of observations of each type was not enough to produce significant results. There is no literature supporting this claim, but it is a logical extension of partitioning mechanisms.

The basis for assuming behavior aids in partitioning the clients of the reef comes from Cote et al's (1998) cost-benefit model for client behavior. This model clarified previously unexplainable variation in posing behavior by client species by demonstrating variability in the rate at which posing benefits (makes cleaning more likely) accrue for different species under different conditions. Based on this, some species need not pose to be cleaned as they are innately attractive to cleaners, while less attractive cleaners must pose despite the costs (time lost foraging, exposure to predation, etc.) of doing so. This is supported by Lousy et al's (1995) finding that the Pomacentrid *Dascyllus albisella* raised naïve to cleaners posed immediately upon seeing a cleaner and suggests that this behavior may have evolved innate recognition of cleaners. If some species are more likely to present themselves for cleaning, at the fringes there would be ecological space for less conspicuous cleaners to stimulate the same tactile reward system in clients that are attractive but do not present themselves. It would follow that in the presence of other cleaners, an individual would perform their preferred solicitation behavior more readily; either to be more conspicuous or to attend to the clients they are most capable of acquiring. This mechanism would propose that each of these species-size class groups would have behaviorally distinct client groups they are most capable of attending to: *L. bicolor* Juveniles would be taken advantage of primarily by timid & unattractive individuals which would prefer (from their timidity) not to pose, but are required to in order to receive cleaning making cave stations their preferred posing locations. Adult *L. bicolor* would likely attend to timid & attractive clients as their slightly more robust body and inconspicuous coloration would allow them to cruise the reef & service clients that do not want to pose and are also not required to. Bold attractive clients would likely receive their cleanings from Adult *L. didmidiatus* as they are capable of presenting themselves for cleaning with relative security in the fact that they will receive the cleaner's attention. This is in contrast to Juvenile *L. dimidiatus* which is untrained as to preferred client groups and would likely clean attractive & unattractive clients without preference.

Here I have proposed a mechanism for how client fish could be partitioned among different developmental stages of different cleaning species. What is required is observational study determining if this mechanism results in differential cleaning rates of the different client species. Initial attempts at this type of study were plagued by the use of SCUBA, for though the cleaners are unaffected by the presence of a diver, the bubbles clearly perturb the clients less immune to predation and results in counts heavily influenced by the boldness of client species.

In conclusion, the high competition situation along the fringing reef walls in Moorea shows significantly different proportions of time allocated to different client solicitation behaviors. These differences in client solicitation behavior suggest this as a mechanism for the partitioning of behaviorally and nutritionally distinct groups of clients. The trend towards enhancement of a species-size group's preferred behavior in the presence of a competitive congeneric suggests that in competitive situations, an individual's preferred behavior increases the degree of partitioning, and may explain why this mechanism for partitioning has not been described in the more natural environments where cleaners have been studied in the past.

Acknowledgements

This study would not have been possible without Christine Huffard for field orientation, SCUBA assistance and video help. Thanks also to Nathan Garfield, Cheryl Logan, Mickey DeSalvo & Jim Hayward for SCUBA time. Additonal thanks to Carol Hickman for comments on an earlier draft of this paper. George Barlow & Alexandria Grutter were also of great help in providing preliminary natural hisory information.

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Intraspecific interactions and population structure of the coral reef fish *Thalassoma hardwicke* (Bennett 1828) in Cook's Bay, Moorea, French Polynesia

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ABSTRACT. Intraspecific interactions and population structures are not well known for coral reef fish, especially considering the variation in social systems among each species and differences between local populations. One such social system, is that of protogynous species, in which two types of individuals exist within a species – the initial phase, composed of females and some males, and the terminal phase, composed of males that were once females. An example of a protogynous hermaphroditic species is *Thalassoma hardwicke*, a member of the family Labridae, which is widely distributed throughout the Indo-Pacifc and found in the coral reefs of Moorea, French Polynesia. This study aims to determine if there is an underlying ratio of initial phase and terminal phase individuals that dictates the structure of the *T. hardwicke* population, and its behavior. Populations of *T.hardwicke* were studied by examining transects at fringing reefs, back reefs, and at a motu and by doing observational studies in Moorea, French Polynesia. At fringing reef and back reef sites initial phase individuals, or drabs, were more common than terminal phase individuals, or gaudies. At the motu site, however, the ratio was reversed. Certain behaviors also depended upon the life stage of the individual, with certain activities reserved for the different stages.

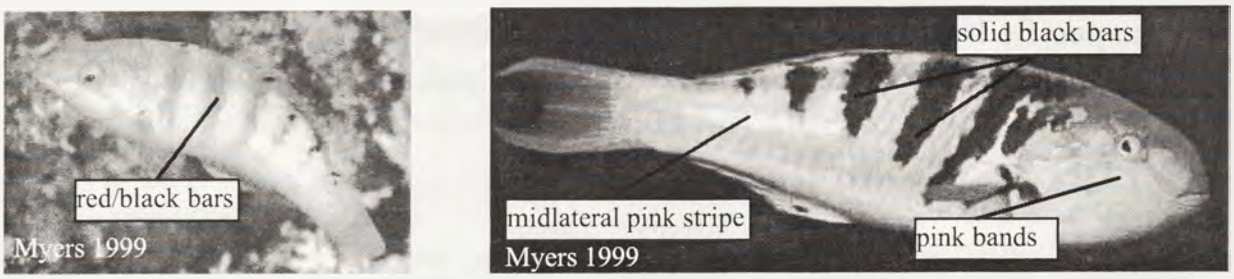
Introduction

In a coral reef system fish are a dominant feature. In the 720 km composing the Society Archipelago of French Polynesia, 633 fish species are found (Randall 1985). On the island of Moorea (17° 30 'S 149° 50' W) alone 217 species of fishes are found (Galzin and Pointer 1985). The social systems of these fish, including life histories and sexual patterns are equally diverse. Not only do life histories vary greatly between species of fish, but local populations have been shown to have variability as well (Warner 1991). One example of a varied life history exists in the Labrid family.

Wrasses, of the family Labridae, are known to be one of the most diverse reef fishes (Myers 1999). The family contains around 60 genera and 500 species, over half being found in the Indo-Pacific region (Allen 1994). Sex reversal for this family appears to be universal (Myers 1999). One life history strategy present in this family is seen in protogynous species, where the adult females change sex and become males. This population includes two types of individuals – initial phase and terminal phase (Shapiro 1991).

A common pattern of the sexual reversal process is that sex change is followed quickly by a change in color. In many wrasse species a predictable change in color patterns occurs as the individual ages (Warner and Robertson 1978). In species such as *Halichoere bivittatus*, *H. maculipinna*, *H. poeyi*, and *Thalassoma bifasciatum*, secondary males have been found within the initial phase population bearing transitional gonads (Warner and Robertson 1978). For these species sex change is completed before color change. Size is also a factor in determining initial and terminal phase individuals. In *H. bivittatus* and *T. bifasciatum* secondary males are restricted to the upper size class of the population (Warner and Robertson 1978). In some species, both males and females compose the initial phase population. The ratio of initial phase males to terminal phase males thus dictates the reproductive success of each stage, as is the case for *T. bifasciatum* (Warner and Hoffman 1980).

Thalassoma hardwicke (sixbar wrasse) (Bennett 1828) is a common fish found in a variety of habitats in the reefs of Moorea. *Thalassoma hardwicke* is a protogynous hermaphroditic species. The initial phase wrasses (drabs) contain all of the females of the population and few males, which are indistinguishable. They are light green with blackish and red bars located dorsally on the body, growing progressively shorter posteriorly (Figure 1). They appear to be drab in color and are smaller than terminal males. Terminal males



Initial phase – Drab Terminal phase – Gaudy

Figure 1. Initial and terminal phase *Thalassoma hardwicke* individuals. The initial phase (drab) is composed of all females and few males measuring around 1-10 cm. The body is a greenish color with red and black bars. The terminal phase (gaudy) is green dorsally and blue ventrally with solid black bars and a pink midlateral stripe. This phase is the larger size class.

(gaudies) are green dorsally and blue ventrally with solid black bars, having a gaudy appearance. A midlateral pink stripe is found on the body and is more evident posteriorly (Figure 1). Broad pink bands radiate from the eyes of individuals. These males are larger than the drabs (Randall 1990). The maximum standard length for this species is 17 cm, from the head to the crease where the tail is bent. The general color pattern remains the same although the actual color of the fish changes as it grows (Myers 1999).

There are 21 recognized species in the genus *Thalassoma* (Springer 1982). Wrasses of the genus *Thalassoma* are known to be active swimmers (Pitkin 2001). *Thalassoma hardwicke* is found widely distributed throughout the Indo-Pacific, excluding Hawaii (Carcasson 1977). Moorea is surrounded by a barrier reef, enclosing a series of shallow lagoons where sixbar wrasses are exclusively found (Shima 2000). A prominent feature of the *T. hardwicke* population is the ratio of drabs to gaudies and their various interactions. This ratio may be dependent on habitat in relation to anthropogenic activity. For example, in areas where tourism has driven the practice of fish feeding, fish communities might be altered. This could be devastating to local populations, as reef fish communities tend to have unstable structures. The composition of species at a particular site do not tend to rebuild themselves after addition or removal of fish (Sale 1977).

This study seeks to examine the underlying trends that structure the *T. hardwicke* population. Previous studies on *T. hardwicke* have focused on the juvenile stage (Shima 1999), and little is known about the behavior of *T. hardwicke* and the population structure as a whole. Another study of wrasses, including *T. hardwicke* focused on abundance of individuals at various habitats, which did not report any strong correlations (Boyd 1994). Combining these studies, the life history of *T. hardwicke* will become more known.

Questions

In an attempt to elucidate the population structure of *T. hardwicke*, this study poses three questions:

(1) What is the ratio of drabs and gaudies *in T. hardwicke* populations?
(2) How does this ratio, if in existence, shape intraspecifc interactions?
(3) Do anthropogenic activities, such as fish feeding, alter population structure?

Methods and Materials

Study Sites

The island of Moorea (17°30'S; 149°50'W) houses Cook's Bay on the northeastern side. I chose four study sites in this bay, two sites along the fringing reef and two in the back reef on either side of the reef pass (Figure 2). In front of the Gump Biological Station is a reef crest of the fringing reef (17°29.414'S; 149°49.133'W). The depth ranges from 1-2 meters. This site is referred to as the "Gump Station." Across the bay from the station is a reef crest ranging from 0.4-3 meters in depth, which drops off in a coral wall to the bottom of the bay. This site was located directly in front of an abandoned white hotel on the shore, and is referred to as "the White Hotel" (17°29.414'S; 149°49.566'W). Two other sites were chosen, both patch reefs by the barrier reef. "The West Back Reef" (17°28.660'S; 149°50.231'W) is 50 meters from the reef crest and is 1.5-2 meters in depth. It is west of the Cook's Bay pass. On the eastern side of the pass is the "East Back Reef"

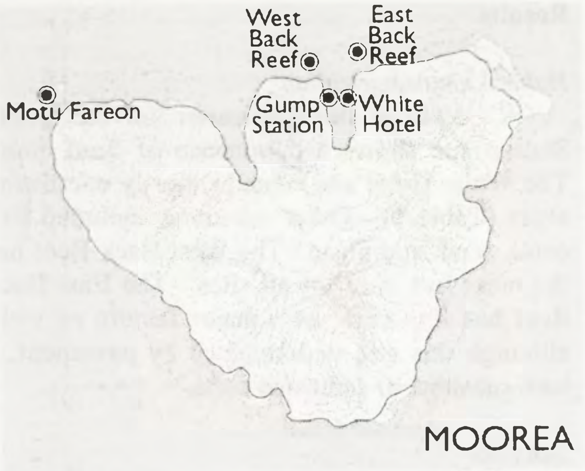


Figure 1. Map of study sites of *Thalassoma hardwicke* in Moorea, French Polynesia. The Gump Station and White Hotel sites occurred on fringing reefs. The East and West Back Reef Sites were located on either side of the reef pass, 50 meters from the reef crest of the barrier reef. These four sites are all contained in Cook's Bay. Motu Fareone is located just north of Club Med, and a lagoon is created due to its proximity to the neighboring motu, Motu Tiahura.

site (17°28.598'S; 149°49.252'W). This site is also 50 meters from the reef crest and is 1.5-2 meters deep.

Another study site, located on the western side of the island was near the shore of Motu Fareone (17°29.367'S; 149°54.833'). The study site was located in a lagoon between Motu Fareone and Motu Tiahura. The depth range was 1-2.5 meters. I chose this site because of its proximity to Club Med and other hotels known to feed fish in order to attract large groups of fish for tourists to see on snorkeling trips. A Club Med employee confirmed the presence of fish feeding.

Observations

I observed seventy-eight fish over a six week period in October and November, 2001, recording data using underwater paper while snorkeling. Fish observations involved following individual fish for a maximum of ten minutes, keeping a distance of about one meter from the individual. The size of each fish was estimated visually. Each fish was recorded as being drab or gaudy. Females and initial phase males were indistinguishable and seen as drab, and terminal phase males as gaudy. If there were any striking physical characteristics of the fish, such as an intermediate color state, they were noted. The habitat of the fish was recorded as well as the approximate area in which the fish traveled while under observation. This was calculated in square meters.

Behaviors of T. hardwicke

From these observations, some behaviors were scored and compared based on the size and color pattern of the fish. Two types of behaviors were scored; feeding, and conspecific interactions.

(1) Feeding behavior was scored based on the substrate bitten. These included algal turf, coral and encrusting algae, turbinaria, floating particles, and coral rubble. These data were later recorded as bites per minute for each substrate by dividing the number of bites a fish made on a particular substrate by the amount of time the individual was observed.

(2) Conspecifics were recorded if they were within 10 cm or less from the individual being studied. They were noted as either drabs or gaudies. Conspecific behaviors scored included chasing, retreating, following, and grouping. For these behaviors the size and color of each individual was noted. Chasing behaviors were recorded in terms of number of chases or instances being chased per minute. Per capita values for chasing and being chased were also recorded based on the amount of other six bar wrasses present. This number was taken as a total number of chases or times chased divided by the total number of both drabs and gaudies the individual encountered during observation. This gives a number of chases weighted by how many individuals were able to chase the fish being observed.

Transects

At each of the four sites in Cook's Bay I established three transects and recorded data on three different days for each transect. Transects were 15 meters long and were spaced 15 meters apart from each other. For the Gump Station and White Hotel sites, transects were located along the edge of the reef crest. The West Back Reef transects ran parallel to the reef crest and to each other. The transects at the East Back Reef ran parallel to the reef crest in a line running east to west. I characterized the habitat of each transect by examining 200 random points along the midline of the transect. This was accomplished by laying a chain with painted links along the bottom and recording the substrate under each painted link.

At each transect I took a visual census of all the sixbar wrasses. After the 15 meter transect tape was laid, I waited at least 15 minutes before beginning the census in order to let the fish adjust. The visual census was then completed by swimming at a constant speed along the length of one side of the transect, examining two meters out from the transect line for two minutes. Once the end of the transect was reached, the same process was repeated for the other side of the transect. Sixbar wrasse size was estimated and life phase, based on coloration (drab or gaudy), was noted.

Data Analysis

All observed fish were placed in one of three categories based on size and color. Drabs were classified as being 1-7 cm in size. Intermediates were all six bar wrasses, drab or gaudy, that were 8-10 cm. Gaudies were observed fish in the size range of 11-16 cm. Conspecific behaviors and range data was analyzed by one way analysis of variance (ANOVA), comparing activities of drabs, intermediates, and gaudies together and pairwise. ANOVAs were used to relate movement, feeding, and chasing data among the varying sizes. Data from transects were analyzed against each other and also grouped by fringing reef sites and back reef sites for comparison using a chi squared test.

Fed area

At the motu, the same transect method was performed, however, two transects were completed on two different days. The transects were laid parallel to the shore of Motu Fareon, 25 meters from the shore. Transects were 15 meters apart and ran north to south.

Transect data was compared to the Cook's Bay sites using a chi squared test. Preliminary observations of six bar wrasses were made as well.

Results

Habitat characterization

Habitat characterization for the Gump Station site shows a dominance of dead coral. The White Hotel site contains mostly encrusting algae (Table 1). Other substrates included live coral, sand, and algae. The West Back Reef has the most live coral of all sites. The East Back Reef has live coral as a major feature as well, although this site is dominated by pavement, a hard substrate of cemented coral.

Behavior

Movement values were combined for the White Hotel and Gump Station sites. The size of the area traversed by six bar wrasses during observations appears to increase with size of the individual and its life stage (Figure 3). Regression analysis reported a R square value= 0.63 and a P-value< 0.001. The ranges varied from 0.15 m^2 to 30 m^2. ANOVA tests compared drabs, intermediates, and gaudies, as well as all pairwise combinations. All P-values were statistically significant, except the P-value for the comparison of intermediates and gaudies (Table 2). Differences do exist among the size classes in terms of how large their range is, especially when comparing initial and terminal phase.

Feeding strategy was compared among the Gump Station and White Hotel sites. ANOVA tests examined the number of bites per minute of either algal turf substrate or live coral and coralline algae. At the Gump Station, combinations of drabs, intermediates, and gaudies, drabs and intermediates, and drabs and gaudies all reported P-values less than 0.05 from an ANOVA on algal turf substrate. The algal turf data at the White Hotel all reported P-values between 0.2187 and 0.7183. Algal turf feeding is highest among drabs at the station, and decreases with an increase in size (Figure 4).

Table 1. Average percent cover of substrate at four sites in Cook's Bay, Moorea, French Polynesia based on three 15 meter transects at each site. Percent cover of the West and East Back Reef are reported by Nathan Garfield.

substrate	Gump Station	White Hotel	West Back Reef	East Back Reef
Dead coral	74%	22.83%	31.5%	23.7%
Live coral	6%	13.5%	41.2%	29.5%
Sand	17.67%	0%	10.7%	2.5%
Encrusting algae	0%	43.83%		
Algae	0%	15.47%	5.3%	7.5%
Pavement			11.3%	36.8%

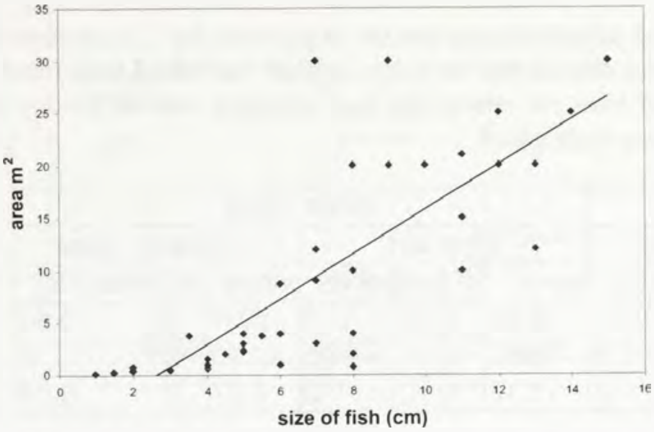


Figure 3. Range of area traversed by *T. hardwicke* individuals at the Gump Station and White Hotel sites during observations in Moorea French Polynesia. Regression analysis reports an R square value= 0.63 and a P-value < 0.001. As size increases, the area (m^2) traveled by the individual also increases.

Table 2. ANOVA data comparing range of area traversed by T. hardwicke individuals at the Gump Station and White Hotel sites during observations in Moorea, French Polynesia. Individuals were placed into one of three categories, D=drab, I=intermediate, G=gaudy, based on size and color.

size	*df*	*MS*	*F*	*P-value*
D,I,G	2	1066.312	21.11526	4.5E-07
D,I	1	800.5041	14.38021	0.000626
D,G	1	1881.176	49.50558	4.74E-08
I,G	1	138.5598	2.181786	0.156036

Regression analysis reports a R square value= 0.24 and a P-value= 0.003. No significant pattern seems appears for the White Hotel site. ANOVA tests comparing bites per minute of coral and encrusting algae at the Gump Station showed P-values from 0.7018-0.9314. At theWhite Hotel two P-values were less than 0.05, those being drabs, intermediates, and gaudies, and drabs and intermediates. The test comparing drabs and gaudies reported a P-value of 0.0760. The coral and encrusting algae feeding values for the Gump Station do not show a trend in feeding, however at the White Hotel, coral and encrusting algae feeding increases with decreasing size.

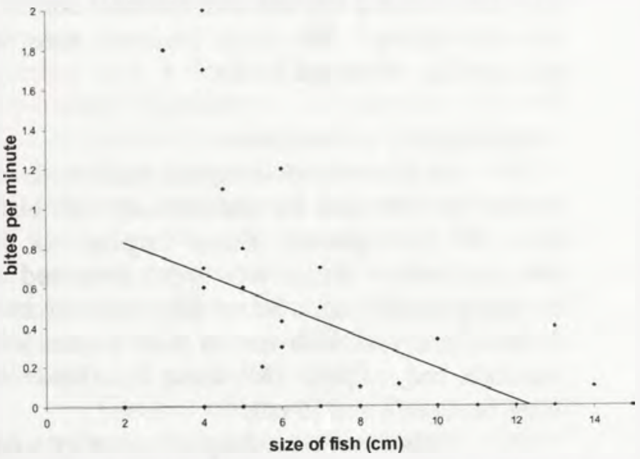


Figure 4. Algal turf feeding preference of T. hardwicke at Moorea, French Polynesia. Algal turf bites per minute observed at the Gump Station site. The amount of feeding is graphed versus the size of the individual observed. Regression of size versus bites per minute reports an R square value= 0.24 and a P-value= 0.003. As size increased, the bites per minute of algal turf substrate decreased.

Chi squared tests compared the amount of substrate available to the frequency of bites the substrate received (Table 3). The total number of bites per minute for each size class was divided by the total number of bites per minute given by the size class to each individual substrate. This number was then compared to the percent cover of the Gump Station and White Hotel habitat. Data was entered as percentages.This tested the expected number of bites (amount of available habitat) with the actual number of bites received by each substrate. For drabs at the Gump Station, the frequency of bites received for algal turf does not differ statistically from the amount of available dead coral on which the algal turf grows. At the White Hotel, the frequency of bites received for coral and encrusting algae does not differ statistically from the percent cover of that substrate. Gaudies, however, reported frequencies of bites that did not always correspond to the most available substrate at either site.

The number of chases received per minute from conspecifics does not have great variation among size classes. P-values for the ANOVAs ranged from 0.1946-0.3680. The number of chases given per minute by six bar wrasses being observed does have variation among size classes. ANOVAs reported P-values from 3.53E-06 – 0.0183. The gaudies had the highest average chases of 0.3775 chases per minute, whereas the intermediates had an average of only 0.092 chases per minute and drabs an average of 0.0219 chases per minute.

Table 3. Chi squared data comparing expected and actual bites per minute of substrate by *T. hardwicke* in Moorea, French Polynesia. Expected data used was that of percent cover of algal turf (dead coral) and a combination of coral and encrusting algae. Actual bites per minute for each substrate was the percent of total bites per minute. Statistically significant data are highlighted.

	Gump Station						White Hotel					
	algal turf			coral/enc. algae			algal turf			coral/enc. algae		
	value	df	prob.	value	df	probability	value	df	probablity	value	df	probability
drabs	0.76	1	0.38	2.53	1	0.11	9.65	1	0	0.37	1	0.54
intermed.	1.09	1	0.30	22.44	1	0	3.80	1	0.05	42.90	1	0
gaudies	11.04	1	0	10.92	1	0	11.17	1	0	0.77	1	0.38

For each chasing behavior, a per capita value was also calculated. ANOVA tests for both chasing behaviors reported values above .05. Per capita behavior does not vary due to life stage.

Transects

The transects performed at the Cook's Bay sites all show that communities of *T. hardwicke* contain more drab individuals than gaudy individuals. The Gump Station transects reported an overall combined total of 6 gaudies and 41 drabs, a ratio of 0.1463 gaudies per drab. The White Hotel reported a combined total of 19 gaudies and 133 drabs. This is a similar ratio to that of the station, 0.1428 gaudies per drabs (Figure 5). At the West Back Reef 4 gaudies and 133 drabs were recorded for the combined total, a ratio of 0.03 gaudies per drabs. The East Back Reef had a combined total of 2 drabs and 121 gaudies, a ratio of .0165 gaudies per drabs. This value is closest to that of the West Back Reef (Figure 5). Due to the similar ratios between the fringing reef sites and between the back reef sites, these data were grouped together for subsequent chi squared tests.

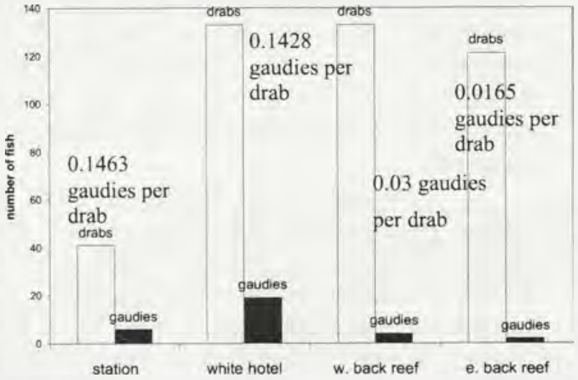


Figure 5. Number of drabs and gaudies present at four sites in Cook's Bay. Three transects were studied at four sites: Station, White Hotel, and West and East Back Reef. Numbers of drab and gaudy individuals were combined for each site.

The chi squared tests between the fringing reef sites and the back reef sites report a chi squared value of 18.824, df=1, and probability=0.00. When comparing all four sites to each other, the chi squared test reports a chisquared value of 19.000, df=3, and probability=0.00.The most common size of fish occurring at the Gump Station transects were 4 cm for drabs and 12 cm for gaudies. The most common fish size at the White Hotel was 7 cm drabs and 12 cm gaudies. At the West Back Reef 2 cm was the most common drab size and 14 cm was the most common gaudy size. At the East Back Reef 2 cm was also the most common size for drabs. The most common size for gaudies was 10 cm and 14 cm.

Natural history observations

In addition to the green body with red bars of the drabs and the whitish body with black bars of the gaudy, there appear to be intermediates. Some fish were observed as having purplish bars. Some fish were observed as having a yellowish tint to their bodies with blackish red stripes. All these fish observed were between 8 and 10 cm.

I observed a circling behavior by some gaudies. The individual would swim in a quick circle above a drab individual. This was observed in 6 gaudy observations. One observation included a gaudy swimming above another six bar wrasse while fluttering its tail.

Another behavior was witnessed 5 times. One gaudy would first join another gaudy. They would then speed up and swim parallel, cross paths as if to swim away,

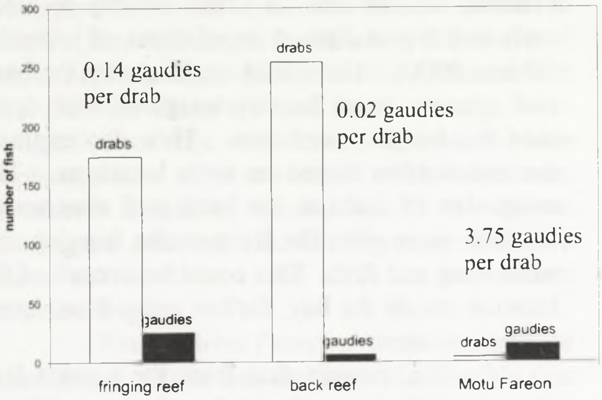


Figure 6. Numbers of drabs and gaudies in the fringing reef sites, back reef sites, and motu site. Numbers of drabs and gaudies are totals of individuals observed along a transect at two fringing reef sites and two back reef sites. The proportion of drabs to gaudies at the motu site is opposite that found at the fring reef and back reef sites.

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and then rejoin and swim parallel. This behavior lasted less than five seconds.

Fish fed area

Habitat characteristics. The habitat of the transects was comprised of over 75% sandy bottom. The rest of the transect was 14.75% dead coral and 8.5% live coral.

Transects. The total number of six bar wrasses recorded from the transects was 15 gaudies and 4 drabs (Figure 6). This ratio, dominated by gaudies is 3.75 gaudies per drab. The chi squared test comparing the numbers of drabs and gaudies between Motu Fareone and the fringing reef sites reported a chi squared value of 51.017, df=1.000, and probability=0.000. The chi squared test comparing the numbers of drabs and gaudies at Motu Fareone with the back reef sites reported a chi squared value of 149.417, df=1.000, and probability=0.000.

Gaudies which were 11 cm, 13 cm, and 15 cm were among the most common sizes of gaudies found at the Motu Fareone transects. The most common size for drabs at this site was 8 cm.

Observations. At Motu Fareone, the fish approached the observer closer than any six bar wrasse had at other sites. Two six bars were observed for ten minutes each, both gaudies. During the first observation 1 drab and 5 gaudies were present and 1 drab and 8 gaudies were present during the second observation. The only conspecific interactions noted were chases by the individual being observed. The gaudy chased two other gaudy individuals at different times.

Discussion

Behaviors

Movement. The trend of increasing range of area traveled with an increase in the size of individuals corresponds with the life stage of the fish. The P-values reported clearly show that the area of movement of drabs differs from that of intermediates and gaudies. The comparison of intermediates and gaudies, however, is less similar, indicating that in terms of movement sixbar wrasses at the intermediate stage behave in a manner more similar to gaudies than to drabs. This could be explained through the concept of bachelor males. Bachelor males are smaller or younger than males who are reproductively successful, much like an intermediate stage (Warner 1991). The smaller gaudies have to wait until they become larger before they may compete for space with the larger gaudies. During this stage the wrasses may move in larger areas than drabs, looking for a new range to occupy once they have become larger.

This trend is seen in another species of *Thalassoma*. *T. bifasciatum*, mentioned earlier, is also a protogynous hermaphrodite. Gaudy individuals of this species are more solitary and roam over a wider range than do drabs (Robertson and Choat 1974). *Thalassoma hardwicke* appears to behave in the same manner, the ranges of gaudies and intermediates are larger than that of drabs.

Feeding. At the Gump Station site, drabs were more likely to bite at algal turf substrate than intermediates and gaudies. As the size of an individual increased, the amount of bites per minute of algal turf decreased. Chi squared tests showed that drabs bit at the most available substrate, whereas gaudies and intermediates showed more preference for feeding substrate. Intermediates and gaudies move over larger ranges, and therefore have a greater selection of feeding substrates.

The same phenomena occurs at the White Hotel site as well. The P-values for coral substrate show a difference in feeding between drabs and intermediates, as well as between drabs and gaudies. This difference is eliminated when comparing intermediates and gaudies.

These data are reinforced with the chi squared results. Coral and encrusting algae are the dominant features of the habitat of the White Hotel, making encrusting algae the most available food source for drabs.

Intraspecific variation in diet is not well known for coral reef fishes (Jones et al 1991). However, ontogenetic differences in diet have been documented for other species of fish (Alheit and Scheibel 1982). *Thalassoma hardwicke* appears to have a shift in diet as it matures.

Conspecific interactions. The possibility of being chased in a given amount of time does not vary greatly by size class. The P-values, all of which were above 0.05, indicate that there is no preference for chasing a specific life stage. However, there does appear to be structure in terms of which life stage does the chasing. Gaudies are the most territorial of these three life stages. Perhaps intermediates behave more closely to drabs than to gaudies because they are not as reproductively successful as the gaudies, and must wait until they are bigger to show more dominance, reiniforcing the idea of bachelor males.

The instances of chases could be correlated with occurrences of spawning. *Thalassoma lunare*, another protogynous species, individuals exist in an unorganized overlapping manner, occasionally forming aggregations (Robertson and Choat 1974). Aggressive interactions among conspecifics are independent of spatial existence. Aggressive interactions occur when terminal phase males set up loosely defined spawning territories. When a subordinate male is detected, the gaudy makes an aggressive display (Robertson and Choat 1974).

Per capita data for chasing and for being chased did not prove to be significant. This could be because some individuals never encountered other six bar wrasses, so counts of zero were present, which could skew the data. Also, the per capita uses a combined total of drabs and gaudies, but gaudies are the primary chasers. A six bar wrasse appears to have a better chance of getting chased by a gaudy than by a drab, and therefore, the data should be somehow weighted for per capita chase data.

Transects

All four sites in Cook's Bay reveal a higher number of drabs than gaudies. The ratios pair the fringing reef sites together and the back reef sites together. The fringing reef sites are farther into the bay than the back reef sites. The back reefs are a site of larval settlement; in Moorea, six bar wrasses settle directly to patch reefs and create distinct populations of juveniles (Shima 2000). This could explain why the back reef sites are more heavily weighted with drabs than the fringing reef sites. This also explains the mean sizes found in both locations. The mean size of drabs at the back reef sites was 2 cm. The mean sizes for drabs on the fringing reef were 4 cm and 7cm. This could be a result of the location inside the bay, farther away from larval settlement sites.

The transect data from the Cook's Bay sites show that regardless of reef size and form, there is still an overall greater frequency of drabs over gaudies inside the lagoon.

Natural History Observations

Circling and chasing displays by gaudies appear to be similar to those of other *Thalassoma* species in regards to spawning behavior. For instance, when *T. bifasciatum* drabs approach gaudy territories, the gaudy swims above the drab in an aggressive and sexual display. These displays are very rapid and include vibrations of the body (Robertson and Choat 1974). When similar observations were made of *T. hardwicke* during the study, it was possible that the terminal males were involved in reproductive activities.

Future studies could also focus on the existence of the intermediate stage. By studying the coloration patterns and gonads of the phases, the process by which *T. hardwicke* changes sex may be explored.

Fish fed area

Motu Fareone was mainly composed of sandy bottom, differing in substrate from the non-fed sites. The ratio of gaudies to drabs ontradicted those recorded for the transects in Cook's Bay. A different social structure must be present. This could be the cause of many things. It is interesting to note that the most common size of drab found along the transects was that of 8 cm, a size classified as an intermediate in this study.

Varying habitats could be one explanation for this shift in community structure from Cook's Bay to Motu Fareone. The relationship between intraspecific variation and environmental variables of coral reef fish has not been studied extensively. The social environment from one intraspecific population to another can vary greatly (Warner 1991). Due to these uncertainties it is hard to determine the direct causation of the differences in population structure between sixbar wrasses found in Cook's Bay and those found along the shore of Motu Fareone. It is unclear as to whether or not the transect lines chosen for the study were indicative of the whole motu area. More replicates need to be conducted at Motu Fareone. Transects should be carried out all throughout the lagoon area and also leading away from the motu to see where the shift in population structure begins.

Since Motu Fareone is an area where tourism has driven fish feeding, it is possible that this anthropogenic activity is altering the community of *T. hardwicke*. The feeding could change the drabs' preference for eating from the most available substrate. Since juveniles have different requirements than adults, it is possible that a local population of juveniles may disappear due to disturbance, but the adult population will remain unchanged (Robertson 1998). Also, the feeding might affect the whole community, causing shifts toward certain species who are the best competitors. Intraspecific competition occurs among reef fishes. These effects are not on survivorship but on growth and maturation (Sale 1991). Most competition observed has been a result of limited food supply (Forrester 1990). A reliable food source at a regular schedule could cause bigger individuals to become more abundant.

Coral reef fishes may be vulnerable to anthropogenic activities because they are restricted to the reef environment and many have small home ranges with limited physical preferences (Russ 1991). More studies need to be conducted around Moorea in order to develop a larger profile of the *T. hardwicke* population which can then be compared to Motu Fareone. Once this has been completed, it will become more apparent as to what the causality of the population shift really is.

Conclusion

In Cook's Bay, a common population structure of *Thalassoma hardwicke* exists, with initial phase individuals being in more abundance than terminal phase indiviudals. At Motu Fareone, however, the population structure is the oppostite. The presence of two life stages within this species is a major driving force behind the structure of the population, and the interactions between conspecifics. By understanding the composition of local populations, it may become possible to monitor changes in the community composition which are possibly due to anthropogenic sources.

Acknowledgments

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Population size and structure of *Tridacna maxima* (Mollusca, bivalvia) on Moorea (French Polynesia) and its filtration and growth rates in different light intensities

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ABSTRACT. The balancing of heterotrophy (filter feeding) and phototrophy (translocation of photosynthates from zooxanthellae) was investigated in the giant clam *Tridacna maxima* via filtration rate and growth rate in 4 different light intensities (full light, ~63% light, ~37% light, and zero light) over a four-week period. It was hypothesized that growth rate would be proportional to the amount of light that the clam received, while filtration rate would be inversely proportional to the amount of light that the clam received. Mean growth rate decreased in the zero light regime, while the other three light intensities exhibited similar mean growth rates. The filtration rate remained relatively constant in the three highest light intensities, while it occurred at a much higher rate in the zero light environment. The increased filtration rate suggests that *T. maxima* has the ability to alter its filtration to compensate for changes in light levels, yet not to a sufficient level to maintain normal life processes, therefore signifying that *T. maxima* relies heavily on phototrophy as a means of sustenance.

Giant clams are highly prized for both their meat and their shells, and therefore, heavily hunted throughout most of their distribution. This has resulted in populations that are significantly altered in population size and structure. To ascertain if this is occurring on the island of Moorea (French Polynesia), the status of the *T. maxima* stock was investigated through an extensive survey of six sites covering six habitat types. *Tridacna maxima* showed distinct patterns of distribution, with the back reef and patch reef lagoon habitats having the highest clam densities. Roughly 5.2% of the population consisted of sexually mature clams (≥ 12 cm). The mean clam density, the mean shell size, and the size distribution suggest that the stock is unnatural in both population size and structure due to over hunting.

Introduction

Tridacnoideans ('giant clams') are the largest bivalves in the world, some species reaching upwards of 400 kg (Braley & Healey 1998). This group (Family Tridacnidae) contains 9 extant species, 2 in the genus *Hippopus* and 7 in the genus *Tridacna* (Klumpp & Griffiths 1994). Within the mantle of these bivalves resides the symbiotic alga *Symbiodinium microadriaticum* (also referred to as zooxanthellae in this paper), the same dinoflagellate genus found within reef building corals and sea anemones (Fisher et al. 1985; Fitt et al. 1986, Muller-Parker et al. 1996). The relationship between giant clams and these photosynthetic zooxanthellae is symbiotic (Yonge 1975). The zooxanthellae provide substantial quantities of organic matter, including glucose and other photosynthates, for the giant clam, and in return the giant clam provides a large protected and homeostatic environment for the zooxanthellae to inhabit (Cowen 1988; Ishikura et al. 1999; Sefton 1995; Yonge 1975).

Like other bivalves, giant clams also filter-feed to obtain protein and other needed nutrients (Yonge 1975). Although filter-feeding plays a significant role in meeting nutrient needs, all species in Tridacnidae, except for *T. gigas* and *H. hippopus*, lack the complete structure of the outer demibranch gills, thus lacking the full ability to filter-feed as their only source of nutrition (Braley & Healey 1998). Therefore, it can be inferred that giant clams rely heavily on the digestion of translocated photosynthates from their mantle inhabiting symbionts in meeting their nutritional requirements. Due to this dependence on zooxanthellae, giant clams only inhabit waters that are shallow and clear enough

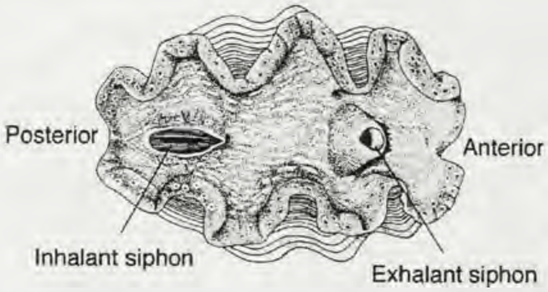

Figure 1. The shell, mantle, and major external features used in filtration (adapted from Braley & Healey 1998)

to allow sufficient sunlight absorption by their symbionts. However, giant clams occupy a wide range of coral-reef habitats, including waters as deep as 33 m (Klumpp & Lucas 1994). Hence, it would be expected that those giant clams living in deeper waters would rely more heavily on filtration due to the lower light intensity. Conversely, those giant clams that inhabit the upper meters of the water column would rely less on filtration due to the abundance of available light for zooxanthellae photosynthesis. However, none have investigated this balancing of filtration and use of translocated photosynthates as a food source in different light intensities. The present study looked at this balancing through filtration rate and growth rate in *Tridacna maxima* over a four-week period in different light intensities.

Although once found in the West Indies, northern Africa, and Europe, the Tridacnoideans are now confined to certain regions of the Indo-Pacific (Braley & Healey 1998; Juinio et al. 1989). Throughout much of this current distribution, giant clams are nutritionally and commercially important (Shau-Hwai et al. 1998; Green & Craig 1999). Their shells are extensively utilized in the shell trade industry and their meat, considered a delicacy by many, may wholesale for 40 dollars a pound (Juinio et al. 1989; Sefton 1995). But like many natural resources, they are easily exploited, due to their accessibility and life history characteristics, such as their sessile lifestyle and their slow development in reaching sexual maturity (Green & Craig 1999). This has resulted in threatened populations that have a disproportionately small percentage of sexually mature individuals, exacerbating the problem and diminishing many natural populations. This situation has aroused international interest in the conservation of these clams (Juinio et al. 1989). Since 1985, all giant clam species have appeared in Appendix Two of the Threatened Species List under CITES, the Convention on International Trade in Endangered Species of Wild Fauna and Flora in order to regulate the commercial trade of these species (Sefton 1995).

To forestall the further decimation of giant clam stocks, much research has looked at the status of those remaining populations in the Pacific. These include studies in the Philippines (Juinio et al. 1989), Malaysia (Shau-Hwai et al. 1998), Somoa (Green & Craig 1999), the Great Barrier Reef (McMichael 1975) and French Polynesia (Richard 1981; Planes et al. 1993). However, none have looked at the status of *T. maxima* (the only member of Tridacnidae still found in French Polynesia) on the island of Moorea (Planes et al. 1993).

In Moorea, a significant number of the residents hunt giant clams to sell to the many resorts on the island. Therefore, it could be expected that the population size and structure on the island of Moorea is disproportionately small in the number of sexually mature individuals, and therefore suffering from the effects of over hunting. The second part of this study looked at population size and structure in six different habitat types at six different sites to investigate the status of the remaining giant clam stock on the island.

Materials and Methods

Light study

In order to test filtration rate and growth rate in different light intensities, four tanks were constructed (12in X 20in X 10in) with light levels that ranged from normal light to zero light. Tank 1 was exposed to normal levels of light. In Tank 2, one layer of shade cloth blocked out ~37 % of the light, and therefore received ~63% of that received by tank 1. Tank 3, with two layers of shade cloth, received ~ 37 % of that received in the normal light intensity environment. In Tank 4, the zero light environment, all light was blocked out.

Twenty-four individuals of *Tridacna maxima* were collected from the Pao-Pao Bay/Pao-Pao Lagoon area. These clams inhabited the upper three meters of the water column, and were found imbedded in the reef or inhabiting the sandy lee of the reef. All collected *T. maximas* were either chipped out of coral rubble, using a chisel and underwater hammer, or dug out of the sand. Individuals attached to living coral were not collected in order to minimize disturbance to the reef community. The collected specimens were divided into three size ranges based on their shell length, with eight clams in each group (all clams were measured from posterior end to anterior end of shell, see Figure 2). The size ranges were as follows: 5 cm – 7.5 cm, 8.5 cm – 10.5 cm, and 11 cm – 13cm.

The eight clams from within the 8.5 cm – 10.5 cm range were marked and placed in individual tanks in order to determine an initial filtration rate. This was done by making a uniform solution of seawater and cocoa powder with a suspended particle density of .0005 g/ml. Due to the clam's failure to clear the water completely, a water sample from the initial

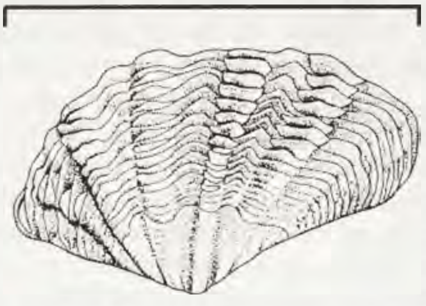

Figure 2. *Tridacna maxima* shell with line demonstrating how shell length was measured (adapted from Braley & Healey 1998)

filtration test was kept and referenced to in each proceeding filtration test. This initial sample was thereafter considered the blank. The time it took each clam to clear its tank of suspended particles to this concentration was measured. Although this did not allow an exact rate of filtration to be calculated, it allowed the calculation of a relative filtration rate between clams.

Following the initial filtration tests, two clams were randomly selected from each size range and placed in the four constructed tanks, thus creating four tanks with six clams in each. The statistics for the four tanks are listed in Table 1.

The clams were then weighed five times, to minimize the amount of water trapped inside their shells, and the smallest values were recorded. This was recorded as the initial weight. For the remaining four weeks the clams were weighed following the above-mentioned procedure. The growth rate per week of each clam was then found by dividing the total growth in grams over the four weeks by the number of weeks (4). This value was then analyzed using a one-way analysis of variance (ANCOVA) with length as the covariate to determine the significance of light intensity on growth rate.

Table 1. Minimum and maximum shell length, mean shell length, and standard error in each of the four tanks (cm)

	Min.	Max.	Mean	SE
Tank 1	6	13	9.667	1.014
Tank 2	5	12	9.333	1.174
Tank 3	5	13	9.583	1.129
Tank 4	5	12	9.417	1.076

The relative filtration rates of the two marked clams from each tank (those which were within the 8.5 cm – 10.5 cm range) were measured weekly for the following two weeks. In each test, the clam "cleared" its solution when the concentration matched that of the blank from the initial filtration test. The filtration times from each week were then analyzed using a one-way analysis of variance (ANOVA) to determine the significance of light intensity on filtration time.

Distribution study

This portion of the study documented the population size and structure of *Tridacna maxima* on the island of Moorea (Society Archipelago, French Polynesia) (17°30' S, 149° 50' W). Moorea, a high volcanic island, is encompassed by a barrier reef with twelve passes that connect the lagoon to the open ocean. On the northern side of the island, there are two large bays, Pao-Pao Bay and Opunohu Bay.

Six distinct habitat types were investigated on the island: the algal ridge, the back reef, the rubble flat, the patch reef lagoon, the near shore shallow lagoon, and the shallow waters of the bay. Three major habitat zones were not surveyed due to safety and logistical concerns: the fore reef, the deep sandy lagoon floor, and the deep bay floor. The algal ridge is dominated by coralline and other algae. This zone is exposed during low tides and experiences high wave exposure. The back reef consists of a large number of different coral and algal species with a rubble/sand floor. It experiences periods of mild to strong currents. The rubble flat experiences mild currents and is dominated by rubble with occasional coral heads interspersed. Sand is the dominant substratum of the patch reef lagoon, with isolated patches of *Porites* or *Montipora.* Normally the patch reef lagoon possesses very mild currents, with very low levels of turbidity. The near shore shallow lagoon is either dominated by *Porites rus,* in those areas with low turbidity levels, or in those near shore shallow lagoons that have higher turbidity, the zone is dominated by algae covered coral rubble. Very little wave action occurs here. The final habitat zone that was investigated was the shallow bay. The shallow bay waters are very turbid, and other than a few patch reefs at the mouths of the bays, algae covered coral rubble and rocks dominate the substratum. This area experiences mild wave action.

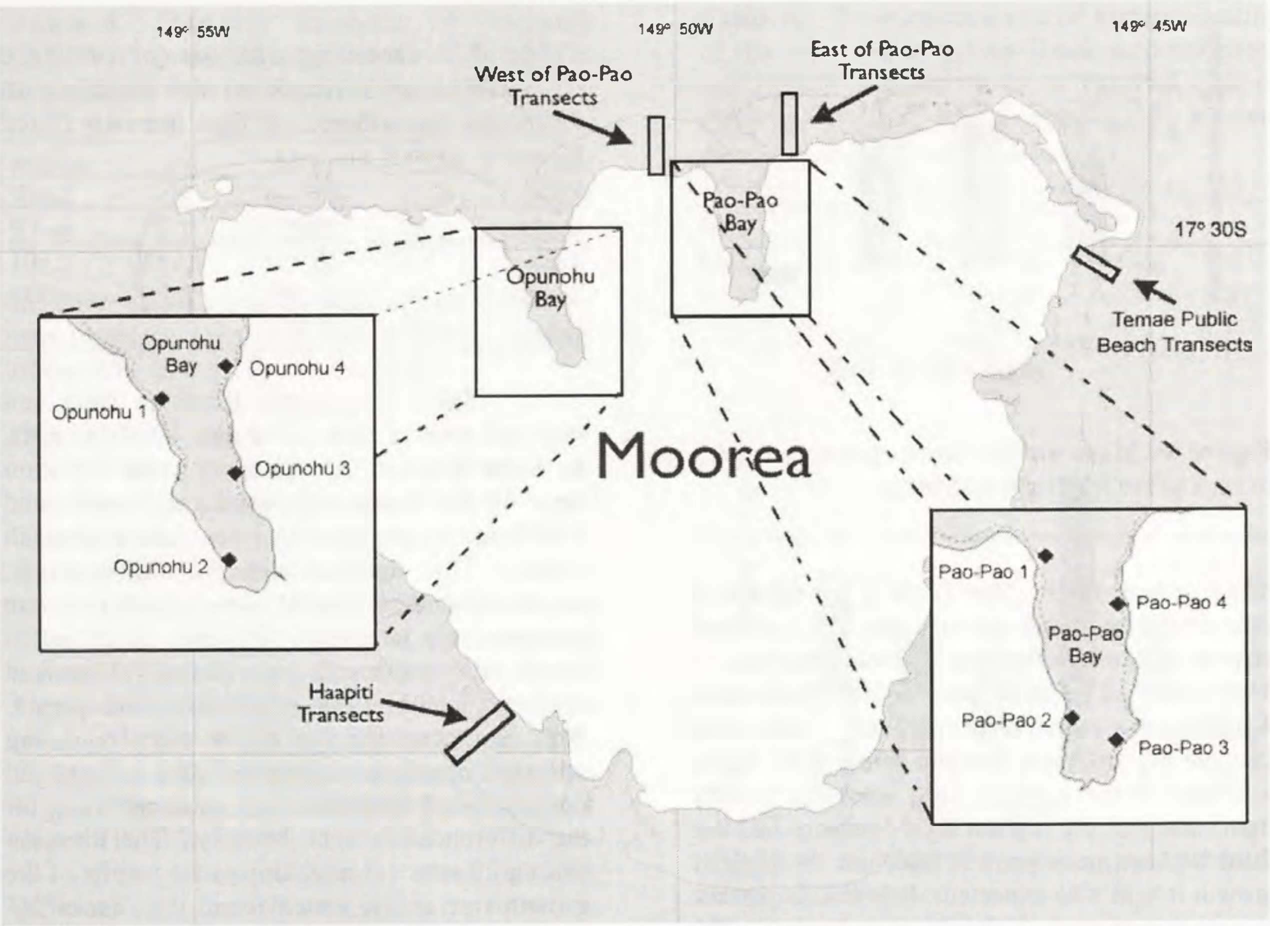

Figure 3. Map of Moorea, showing transects and enlargement of Pao-Pao Bay and Opunohu Bay

Six different sites were chosen to study these six habitat types. Four of these sites spanned from the algal ridge to the near shore shallow lagoons. They were: 300 m east of Pao-Pao Bay, 800 m west of Pao-Pao Bay, the Temae Public Beach, and 1.5 km southeast of Haapiti (referenced to as Haapiti site in this paper). (See Figure 3). In these four sites, all present habitat types were sampled. The two Pao-Pao sites and the Haapiti sites exhibited the following habitat types: algal ridge, back reef, rubble flat, patch reef lagoon, and near shore shallow lagoon. Temae Public Beach lacked a well-defined rubble flat and near shore shallow lagoon, therefore these areas were not sampled. In the other two sites, Opunohu Bay and Pao-Pao Bay, four locations were studied within each that fit the shallow bay habitat type (See Figure 1).

During the sixteen-day period, October 29 through November 13, 2001, field surveys were completed at the six sites. At all six sites, four transects (30 x 3 m) were examined in those habitat types present within the site. All transects ran parallel to the shoreline and in all habitat types, excluding the exposed algal ridge, were carried out in 1-3 m of water. All live *T. maxima*s were counted and measured (shell length).

Tridacna maxima densities were compared among habitats and sites using a two-way analysis of variance (ANOVA). Three separate ANOVAs were used because the Temae site, the Pao-Pao Bay site, and the Opunohu site all lacked distinct habitats that were seen in the other sites. The size distribution of *T. maxima* within sites and the size distribution of *T. maxima* within habitats were also compared using two separate one-way ANOVAs.

Results

Light Study

Figure 4 presents data on growth rate in each of the 4 different light intensities. The *Tridacna maximas* within the zero light intensity tank exhibited a mean growth rate of –0.317 grams per week, while the 100% light intensity to ~37% light intensity tanks exhibited similar

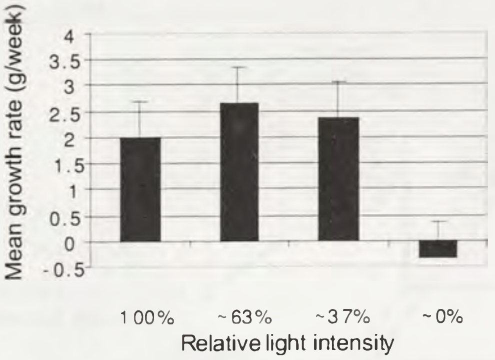

Figure 4. Mean growth rate in grams per week in each of the four light intensities

mean growth rates. See Table 2 for tabulated light intensity, mean growth rate, and standard error in each of the four tanks. Note that tanks 1-3 all exhibited positive growth rates, while tank 4 exhibited a negative growth rate. Also note that the highest mean growth rate (~63% light) was not in the highest light intensity (100% light) and that the highest light intensity had the third highest mean growth rate, not the highest growth rate as was expected. It is also important to note that in week 3, the clams in Tank 4 (~0% light) began to bleach, suggesting that the zooxanthellae in their mantles were either abandoning their habitats in search of higher light intensities, or the clams were consuming them at a higher rate then the zooxanthellae could reproduce.

As seen in Table 3, shell length is significant in the growth rate of *T. maxima*. The level of light intensity is also significantly correlated with the growth rate of *T. maxima*, thus demonstrating that growth rate depends on the shell length of the individual and the amount of light received by the individual. Figure 4 demonstrates this dependence on light intensity graphically.

Table 2. Percent of light intensity compared to that in full light, mean growth rate per week (GR), and standard error (SE) in each of the four light intensities

	% light	GR (g/week)	SE
Tank 1	100	2.012	0.218
Tank 2	~63	2.664	0.669
Tank 3	~37	2.382	0.558
Tank 4	~0	-0.317	0.366

Table 3. One-way analysis of variance (ANCOVA) with length as the covariate to determine the influence of light intensity (fixed factor) on growth rate

source	*df*	MS	F	P
Tank	3	10.994	12.582	<.001
Length	1	11.606	13.282	.002
Error	19	0.874		

Table 4 presents filtration time and standard error in each of the three filtration tests. In Tank 4 (zero light intensity), the filtration time of the clams decreased each week, and therefore, their filtration rates increased each week. This demonstrates the ability of *T. maxima* to increase its filtration rate in response to lower light intensities. However, the filtration times of the other clams (Tanks 1-3) stayed relatively constant during the same time period. This suggests that the clams were receiving adequate amounts of light in Tanks 1-3 and did not shift their filtration rates to compensate for the differences in light intensity. The filtration times of Tanks 1-3 also support the results of the growth rate study, which found that Tanks 1-3 had similar mean growth rates.

No statistically significant differences were found in the initial test of filtration time (prior to being placed in the fixed light intensities) ($p=0.643$) or after one week in the tanks ($p=0.164$). However, after two weeks in

Table 4. Mean filtration time (MFT) and standard error (SE) in the initial test (prior to being placed in the different light intensities), following one week in the fixed light intensities, and following two weeks in the fixed light intensities.

		MFT (min)	SE
Initial Test	Tank 1	202.5	7.5
	Tank 2	240.0	30.0
	Tank 3	232.5	37.5
	Tank 4	247.5	7.5
Week 1 Test	Tank 1	247.5	22.5
	Tank 2	232.5	7.5
	Tank 3	247.5	7.5
	Tank 4	195.9	15.0
Week 2 Test	Tank 1	247.5	7.5
	Tank 2	232.5	7.5
	Tank 3	262.5	7.5
	Tank 4	187.5	7.5

Table 5. One-way analysis of variance (ANOVA) to test the significance of tank (light intensity) on filtration rate after two weeks

source	*df*	MS	F	P
Tank	3	2100.000	18.667	0.008
Error	4	112.500		

the fixed light intensities, a significant difference was found between the tanks (p=0.008). Results of the ANOVA can be seen in Table 5.

Distribution Study

Field transects were conducted in six sites covering a combined area of 9360 m^2 on the island of Moorea (n=104 transects), and 291 *T. maximas* were recorded.

There were distinctive patterns of distribution and abundance of clams among habitat types and sites throughout the island. Figure 5 shows the numbers of *T. maxima* per transect in the six different habitat types. See Figure 6 for *T. maxima* per transect in each site. Figure 7 presents the number of *T. maxima* per transect in each site and each habitat.

In sixteen transects, covering four different sites, zero *T. maxima*s were found on the algal ridge, suggesting that the algal ridge is a difficult habitat type for giant clams to populate. Highest densities per transect were found in the patch reef lagoon habitat (7.75) and the back reef habitat (4.8125). Including the unusually large population found at the West of Pao-Pao site, clams were rare or absent in this habitat type (2.25). Throughout the island, the rubble flat habitat (1.4167) and the shallow bay habitat (1.4375) were also sparse in *T. maxima*.

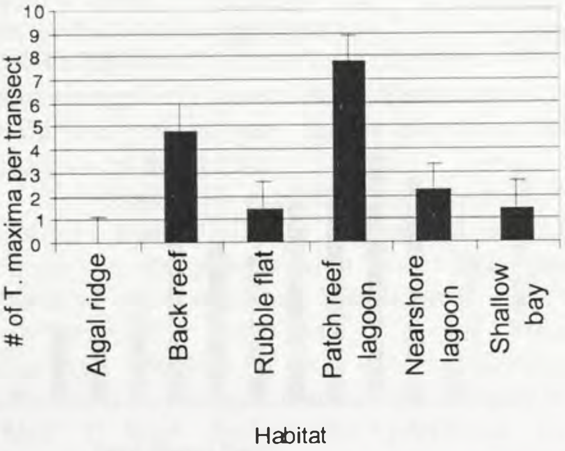

Figure 5. Number of *T. maxima* per transect in the six different habitat types

Table 6. Two-way analysis of variance testing of the influence of habitat (back reef and patch reef lagoon) and site (West of Pao-Pao, East of Pao-Pao, Haapiti, and Temae) on *T. maxima* distribution on the island of Moorea

source	*df*	MS	F	P
Site	3	82.198	7.836	0.001
Habitat	1	69.031	6.581	0.017
Site*Habitat	3	23.031	2.196	0.115

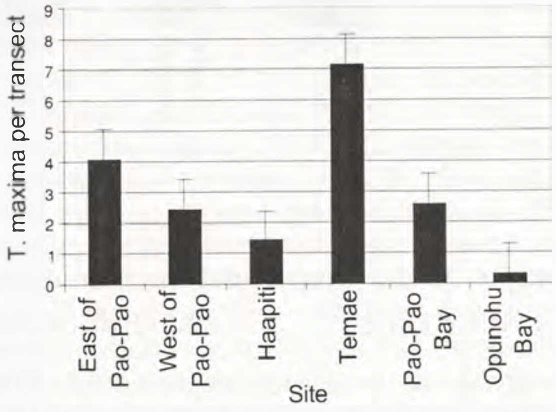

Figure 6. Number of *T. maxima* per transect in the six different sites

The Pao-Pao Bay and Opunohu Bay sites were not significantly different (p=0.072) in *T. maxima* density, most likely due to the high variation within the shallow bay habitat. The second ANOVA, which tested the difference between the back reef habitat and the patch reef lagoon habitat (in the East of Pao-Pao site, the West of Pao-Pao site, the Haapiti site, and the Temae site) on clam density found those results presented in Table 6. Both the site and habitat were found to be significantly different, while the site and habitat interaction was not. The significant difference between the habitats was expected, as shown clearly by the patterns of distribution in Figure 5. However, the significant difference between the sites was not predicted. Initially this was believed to be due to the high clam densities seen at Temae; thus resulting in high variation among transects between the sites. However, after testing the East of Pao-Pao site, the West of Pao-Pao site, and the Haapiti site for significant differences while considering the back reef, rubble flat, patch reef lagoon, and near shore lagoon habitats, a significant difference was still found between sites. This disproved the hypothesis that high *T. maxima* density at Temae had

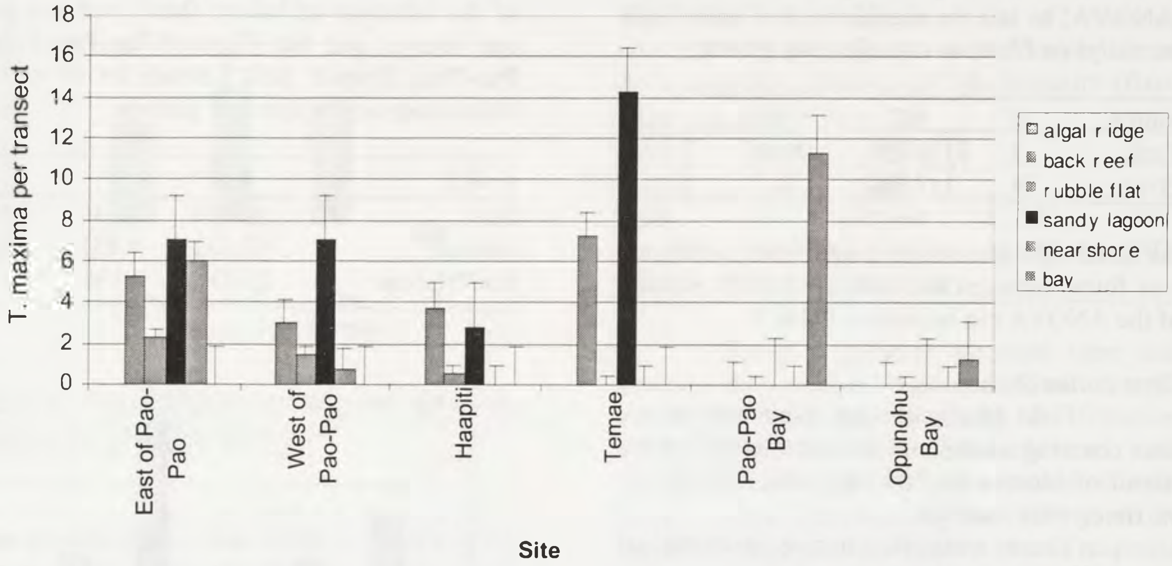

Figure 7. The number of *T. maxima* per transect in each site and habitat on the island of Moorea

skewed the results in the previous ANOVA. Consequently, some other factor must be playing a role in the distribution of *T. maxima*, not just habitat type. Also important to note from this third ANOVA is the significant difference between the habitats, once again demonstrating the distinct habitat usage by *T. maxima.* The site and habitat factor was significant, suggesting that when the factors of site and habitat are considered together, differential structuring within habitats at each site occurs.

Figure 8 presents data on size frequency of *T. maxima* on Moorea. The smallest recorded giant clam was 2 cm, while the largest was 15cm. The mean size on the island was 6.309 cm, with a standard error of 0.175. There was no significant difference between sites and sizes of clams (p=0.094). However, there was a significant difference with respect to habitat and size (see Table 8). Figure 9 shows mean shell length size in each of the habitats (excluding the algal ridge). The back reef habitat had the highest mean shell length of 7.15 cm, while the shallow bay habitat had the smallest mean shell length of 5.52 cm.

Discussion

Although it was found that *T. maxima* could increase its filtration rate when starved of light, it was unable to reach rates that would allow it to rely solely on filtration to maintain normal growth rate. All six *T. maximas* in the

Table 7. Two-way ANOVA testing the influence of site (West of Pao-Pao, East of Pao-Pao, and Haapiti) and habitat (back reef, rubble flat, patch reef lagoon, and near shore lagoon) on *T. maxima* distribution

source	*df*	MS	F	P
Site	2	46.312	11.43	<0.001
Habitat	3	41.410	10.228	<0.001
Site*Habitat	6	9.618	2.376	0.049

Table 8. One-way ANOVA to determine the influence of habitat on size.

source	*df*	MS	F	P
Habitat	4	26.080	2.991	0.019
Error	286	8.720		

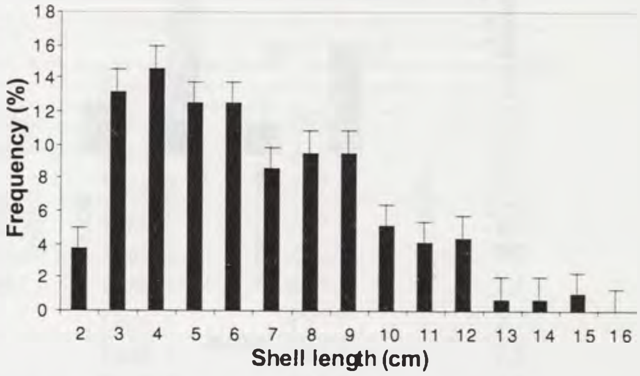

Figure 8. The size frequency of *Tridacna maxima* on the island of Moorea

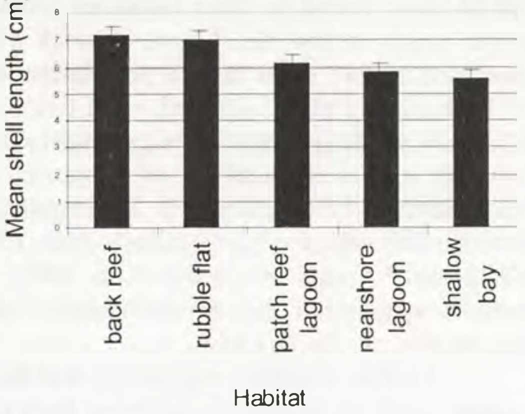

Figure 9. (above) Mean shell length (cm) in each of the habitats (excluding the algal ridge)

zero light intensity began to bleach after three weeks. Norton et al. (1992; 1995) found that bleaching is a direct result of stress on the giant clam. Both the decreased growth rate and bleaching demonstrate the importance of zooxanthellae to the normal growth and survival of *T. maxima*. These findings support previous studies that found that zooxanthellae might contribute approximately 50% of *T. maxima's* metabolic carbon requirements (Trench et al. 1981). However, recent studies suggest that this contribution may be even higher; Klumpp and Lucas (1994) investigated two other members of the Tridacnidae family and found that *T. derasa* receives 86 to 92% of the carbon required for growth and maintenance through phototrophy while *T. tevoroa* receives roughly 105% of that needed. No matter the contribution, it seems that *T. maxima* cannot maintain normal life processes (such as normal growth rate) without sufficient light. However, in the 4 weeks of study there was no loss of life in the zero light regime, therefore it remains to be seen if *T. maxima* can survive indefinitely in zero light conditions while maintaining lower growth rates. Future research could easily investigate this by extending the timeframe of the project.

Although *T. maxima* appears to require a certain amount of light to maintain normal growth rate, the growth rate in Tank 1 (full light intensity) was lower than both Tank 2 (~63% light intensity) and Tank 3 (~37% light intensity). While this was not significantly different, it may suggest that the light intensity in Tank 1 was above that preferred by zooxanthellae, and therefore above that preferred by giant clams. Although additional research would need to verify this hypothesis, it agrees with other findings on zooxanthellae. A similar study found that zooxanthellae in sea anemones had a reduced chloroplast volume when maintained in high levels of light (Tank 1 in this experiment) and the result was a decreased amount of photosynthates for the sea anemones (Muller-Parker et al. 1996). Ishikura et al. (1997) found that *T. crocea*, a close relative of *T. maxima*, exhibited the presence of significant amounts of mycosporine-like amino acids (MAAs), which absorb UV wavelengths of light. These MAAs protect zooxanthellae from UV irradiation and therefore allow *T. crocea* to inhabit shallow waters (Ishikura et al. 1997). It is unclear if MAAs are found within the mantles of *T. maxima*, however, the presence of them in *T. crocea* demonstrates that there is a upper limit to zooxanthellae irradiation and therefore there could be a preferable light intensity for *T. maxima*; less than that found at the surface, yet stronger than that found in waters in the deepest parts of the photic zone. Although additional research would need to confirm this hypothesis for the depth distribution of *T. maxima*, it agrees with field observations that found zero clams inhabiting waters less than 1 m.

Distinct habitat usage was found, with the back reef and patch reef lagoon habitat appearing to be the most suitable for *T. maxima*. Both the back reef and patch reef lagoon had the highest proportion of living coral, supporting the findings by Planes et al. (1993) that clam density is related to the amount of living coral cover. The Haapiti site, which had a high proportion of dead coral in its back reef and patch reef lagoon habitats, reinforces these findings in that it had a lower number of *T. maxima* compared to similar transects in the East of Pao-Pao site, the West of Pao-Pao site, and the Temae site (see Figure 7). The other habitats, excluding the algal ridge with its extremely high wave action and exposure during low tides, had very little living coral cover and very low clam density.

As stated earlier, there were significant differences in clam number between sites. This may have been due to human influences, including hunting and pollution, or it may have been due to natural differences between the sites, such as current, turbidity, and available habitat space. Although all of these factors may have played a role, it is most likely hunting of the highly prized clams that contributed to the differences between sites. Due to characteristics of the sites, such as current, lagoon width, and accessibility to the site, some sites would be much easier to hunt than others, thus resulting in higher levels of hunting at some sites, and consequently creating high variation between sites. Although the number of giant clam shells were not counted, the West of Pao-Pao site, the East of Pao-Pao site, and the Haapiti site all seemed to have much higher numbers of empty clam shells strewn about the habitat floors compared to that at Temae. The shells were often clumped together in a very nonrandom fashion suggesting that humans played a role in the death of these giant clams. These observations suggest that either a high level of hunting takes place at these sites, or, which seems to be very unlikely because of the placement of the shells, some sort of mass death event may have recently occurred. It seems that human predation must be the cause of the high density of empty shells, however, the amount of hunting at each site should be investigated.

The *T. maxima* stock on Moorea appears to be significantly threatened in clam number. In 104 transects covering 0.9360 ha on the island, 291 individuals were found. This is roughly 310.9 giant clams per ha. In a similar study by Richard (1981), that looked at the density of *T. maxima* on Takapoto atoll (French Polynesia), a mean density of 1400 giant clams per hectare was found. Although this atoll lacked some of the habitat types of Moorea, the mean density of Takapoto is far larger than that found in the patch reef lagoon (861 ha^{-1}), the most densely populated habitat type on the island. In another study in French Polynesia, *T. maxima*s were found at a remarkable density of 700,000 per ha. (Lucas 1994 taken from Green & Craig 1999). The survey procedure and location of this study is unknown, however, it demonstrates the potential of the species in comparison to that found on Moorea.

The most apparent sign that the stock of *T. maxima* on Moorea is unnatural in population size and structure can be seen in the size distribution of the species. Of the 291 individuals found, the average size was 6.3 cm. A similar size survey in the Philippines found mean shell length to be as large as 27.4 cm (Shau-Hwai et al. 1998). On Moorea the largest giant clam found was 15 cm, while other distribution studies have found *T. maxima* as large as 38 cm (Juinio et al. 1989). Only 15, or 5.2%, of the surveyed population was sexually mature ($\geq$ 12 cm) (Green & Craig 1999). In a nearly undisturbed refuge, Rose Atoll, Green and Craig (1999) found 34% of the *T. maxima* population to be sexually mature. The disparity between these mean numbers found on Moorea and of those found in other localities provides ample evidence that the Moorean stock of *T. maxima* is unlike other natural populations and has been significantly disturbed, most likely due to human predation on the larger individuals. Although further research should be carried out before laying the blame solely on humans, in both the Bora-Bora lagoon (Planes et al. 1993) and in the Philippines (Juinio et al. 1989) the reduced stocks of larger individuals were attributed to human predation.

Further evidence suggesting that the *T. maxima* stock on Moorea is suffering from over hunting can be seen in the size distribution with respect to habitat type. The smallest mean shell length was found in the shallow bay habitat, and closely followed by the near shore lagoon, while the largest mean shell length was found in the back reef habitat. These findings (Figure 9) show that mean shell length was proportional to the distance from the shoreline; the greater the distance of the habitat type from the shore, the larger the mean shell length. Planes et al. (1993) found that human predation targets the largest *T. maxima* individuals and the largest individuals in a given population can be found in the most difficult areas to hunt (the farthest from shore).

These findings all suggest that the *T. maxima* stock on Moorea is unnatural in population size and structure due to over hunting, yet, whether the stock has reached unhealthy levels remains to be seen. However, one thing is certainly clear, if the hunting of giant clams is not curtailed, then the population on Moorea could be seriously threatened. The current low percentage of sexually mature individuals could result in a bottleneck, and lead to the subsequent loss of genetic diversity and possible loss of the species on the island.

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Habitat and its Effect on Burrow Distribution of the Land Crab, *Cardisoma carnifex* (Herbst 1794)

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ABSTRACT. The association between habitat and burrow site choice of the tropical land crab, *Cardisoma carnifex*, was examined. Choice experiments, along with transects examining population density compared with ecological factors, were performed to elucidate the factors effecting the burrow distribution of *C. carnifex*. Captured crabs were put in enclosures designed to present two habitat choices in which to burrow. These choices mirrored the variety of habitats in which *C. carnifex* naturally resides. The results of these experiments were compared to burrow density data of natural crab populations. The results of the experiments showed that crabs preferentially burrowed in sand over dirt ($p = 0.002$), moist soil over dry soil ($p = 0.007$), and areas with artificial leaf canopy cover over areas with no cover ($p = 0.019$). Comparison between experimental data and natural burrow density data, which was strongly correlated with habitat location and canopy cover ($p = 0.0001$ in both cases), was inconclusive, but suggested the importance of canopy cover's effect on burrow density, which may outweigh the effects of sediment characteristics.

Introduction

There are many factors that contribute to the natural distribution of an organism. As summarized by Marshall (1997), animal distribution patterns are governed in part by: mechanisms for dispersal of young after naissance, probability of encountering suitable habitat, ability to recognize and utilize suitable habitat, and territoriality. Thus, elucidating the factors shaping a particular organism's distribution pattern involves an investigation of how that organism interacts with its environment.

Burrowing organisms are ideal for such distributional studies because they have patterns of distribution that are fixed in space (at least on the order of weeks). This allows for simple, accurate, and thorough data collection.

Land dwelling crabs, the most "terrestial" coming from the family Gecarcinidae, represent a vastly diverse group of organisms found in a myriad of habitats throughout the world that live in self constructed burrows for most of their life cycle (Hartnoll 1988). Land cabs become widely dispersed as young because females must return to the ocean water to distribute their spawn, where they develop before returning to the land as young crabs (for crabs in general see Adiyodi 1988, and for *C. carnifex* see Barnes 1974, Tarvyd 1999). Their spawning behavior, in which female crabs dip their gravid abdomens into the ocean, thus releasing thousands of crab offspring into the pelagic environment (Quinn et al. 1991), puts a selective pressure on juveniles when they return to the land to find suitable habitat. This means that crabs must actively choose appropriate habitats to survive. This creates a more interactive system between organism and environment than in cases where offspring are released over a short range and have all their necessary resources available in those areas, such as some species of spiders (Marshall 1997).

Once a habitat is chosen, a land crab's survival may be determined by a combination of factors including temperature, water availability, salinity, soil factors affecting burrow construction, proximity to the ocean, plant cover, food, and presence of predators (Wolcott 1988). Choosing a habitat where ecological factors are ideal for survival may be mediated by chemical or sensory cues to which crabs have evolved to respond. Crab burrow distributions give a clue into the factors that are necessary for their survival, and the ones that limit their success.

There have been studies on the factors affecting crab burrow distribution focusing both on *C. carnifex* and other burrowing land crab species. Jimenez et al. (1994) found high burrow densities associated with more structurally complex forests with greater total foliage and presence of mature fruits for the land crab *Gecarcinus planatus* (in the Gecarcinidae family). Mouton and Felder (1996) found greater fiddler crab burrow densities in vegetated areas than in non-vegetated areas (*Uca spinicarpa* and *Uca longisignalis*). In *C. carnifex*, Denhoy and Battersby (1992) found no correlation between food availability and burrow density from a food supplementation experiment, but did find higher burrow densities where the water table was shallower.

The literature on factors affecting burrow distribution is incomplete. The effect of food availability is unclear, and soil characteristics, which seem to be potentially influential ecological factors affecting burrow distribution, have been mostly ignored. Mouton and Felder (1996) found that one species of fiddler crab inhabited high clay content soil while another species found in close proximity utilized soil of low clay content. The possibility that land crabs may preferentially burrow in certain types of soil has not been addressed.

This study was conducted to test the hypothesis that both soil composition and canopy cover affect the burrow density of *C. carnifex*. A choice experiment was created to test if subjects preferentially burrowed in certain soil types and also if they would burrow in an area with artificial canopy cover over an area with no canopy. Choice data was then compared to distribution transects taken in the field along with ecological data at each transect. Experimental subjects were taken from three different habitats and distribution data was taken from these habitats plus one more. This was done to examine any differences in behavior these separate populations may display.

Such a study is important because *Cardisoma carnifex* has a major impact on the ecosystem as an herbivore and soil processor. These crabs feed on detritus, leaves, fruit, and seeds by clearing the ground of food items and bringing them back to their burrows (Lee 1988). They were found to remove 30-50% of leaf-fall in rain forests on Christmas Island and redistribute litter biomass to areas outside burrows, which significantly increased organic nutrients in the soil near burrows (O'Dowd and Lake 1989a,b). In addition, *C. carnifex* effects plant community composition by acting as a seed predator for some plants and a seed disperser for others (Lee 1988). Because these organisms play a significant role in ecosystem energy-transfer and plant-community composition, it seems relevant to elucidate certain aspects of their biology, in particular those associated with burrow habitat choice.

Materials and Methods

Natural History

Cardisoma. carnifex is a common decapod crustacean of the family Gecarcinidae found in near shore tropical environments ranging from upper intertidal zones to coconut groves, mudflats, and mangrove stands (Micheli et a.1991, Tarvyd 1999, Cheng, 2000). Found in great abundances from the Red Sea and East Coast of Africa to French Polynesia, the southern islands of Japan and the Great Barrier Reef (Foale 1999), these creatures live in underground burrows dug up to 2 m down into the substrate near the water table (Tarvyd 1999). The majority of their lives are spent in or near these terrestrial burrows. The species *carnifex* is one among seven that comprises the genus *Cardisoma* all of which are found in the tropics. They eat mainly leaves, flowers, and fruit of trees, but will also eat carrion if it is available and will resort to cannibalism if food is extremely scarce (Wolcott 1988).

Observations of undocumented natural history for *C. carnifex* were made informally during crab collection expeditions. An experiment was also conducted to see if individual crabs would occupy an abandoned burrow. When a crab was collected from a known burrow, that burrow was covered in plastic with a hole cut into it for accessibility to the burrow. The burrow was subsequently monitored informally and sediment buildup from a new burrow occupant was noted. If buildup was observed, the burrow was monitored for visual confirmation of new occupancy.

Study Site

This study was conducted on Moorea, French Polynesia (17° 30' W Lat., 149° 50' S Long), a small island in the Society Island archipelago, from October 7th to November 16th, 2001. The study sites included the shoreline along the Richard B. Gump South Pacific Biological Research Station property, the swamp at the base of the Oponohu Bay, hibiscus groves near Temae Bay, and the northern shoreline along the Fareone motu (see Figure 1).

The Gump site is a small grove of hibiscus trees and some coconut palms in close proximity of Cook's Bay. This area is characterized by a dense, semi-moist (approximately 15% soil moisture content), clay-like soil that is mostly bare except for coral rubble within the first 3 meters along the shore, and some grass patches near the road.

The Opunohu swamp is located at the base of the Opunohu bay and is south of the road near P.K 18. This site is dominated by hibiscus and has a soil texture similar to the Gump site, but is more moist (approximately 26% soil moisture content). It also has two brackish creeks that flow through the southern part of the swamp.

The Temae Bay site is located just off the perimeter road before it reaches the airport. This area has a dark, rich, moist soil that is possibly anaerobic (approximately 27% soil moisture). This area is dominated by hibiscus, but also has fig, mango, and coconut trees. This site is not in close proximity to the ocean, but skirts a brackish pond that is connected to the ocean.

No crabs were collected from the Fareone motu, but distribution data was collected there. This site is characterized by a sand and coral rubble sediment (approximately 9% soil moisture content) and a mixed canopy including iron-wood, coconut, and others.

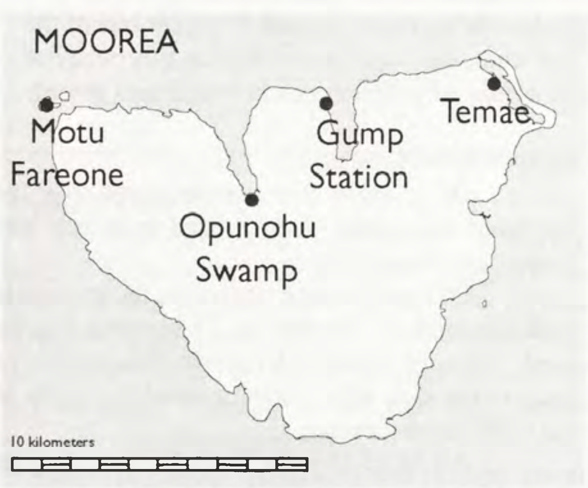

Figure 1. Map of study sites on Moorea French Polynesia (17° 30' W Lat., 149° 50' S Long.) where Cardisoma carnifex subjects were captured and burrow distribution data was collected.

Collection

Study subjects came from three habitat types to test for behavioral differences between the populations. They were captured in the swamp at the base of the Opunohu Bay (P.K. 18), the Temae swamp near the airport (P.K. 1), and the shoreline hibiscus grove on the southern portion of the Gump Station grounds (P.K.12).

Subjects were caught in each of the three study sites using a box trap method. This involved capturing crabs with an upside down box propped up with a stick. The traps triggered when the stick holding the box up was pulled away with a string. The box has food lures under it and when a crab walked under the trap to retrieve the bait, the observer (who waited in a tree to create as little disturbance as possible) pulled the trigger thus trapping the crab.

Experiment

To investigate the habitat preferences *C. carnifex*, various artificial habitat boxes were set up to mimic certain environmental variables the crabs encounter in nature. Each enclosure contained two possible "habitats" for the test subject to choose from. This was done to see if crabs would burrow preferentially in one habitat or the other, indicating a preference for that environmental condition.

All enclosures were fashioned from large buckets (.8 m diameter, .6 m height) filled up to .5 m with sediment and sealed with a mesh wire top to prevent subjects from escaping. There were two sets of all enclosure types to account for possible confounding factors in each enclosure. The replicate enclosures were arranged so that the variable habitats in each enclosure were opposite each other in order to eliminate any risk of the crabs burrowing habitually in the same spot and mistaking it for preference.

As many crab subjects as possible were cycled through all the enclosure types (and each replicate) in a randomized order. Test subjects were kept in buckets with water, food, and some sediment for at least 12 hours after its first trial was complete. This was done to prevent habituated responses when subjects were moved to new enclosures. Once inside an enclosure, test subjects were given 48 hours to build a burrow. A burrow was considered scoreable if it was deep enough for the crab to get its entire body under the surface of the sediment. Due to time and feasibility constraints, not all subjects completed all the trials and replicates. In addition, some trials were conducted at night to collect as much data as possible. Of the 46 choice experiment trials that were conducted, 13 were completed entirely at night. Their enclosures were checked before sunrise to see if crabs engage in burrowing activity at night.

Three enclosure types were used in the choice experiment, each testing a different ecological variable. One enclosure tested sediment-type preference (herein referred to as the sand/dirt enclosure). This enclosure was filled with one half sand and one half soil, both sediments collected from the Richard B. Gump Research Station grounds. Another enclosure tested for soil moisture preference by filling one half with the drier soil of the Gump Station shoreline, while the other half was filled with soil from the Temae bay swamp. The third enclosure type tested preference for plant canopy cover by artificially shading one half of the enclosure with hibiscus leaves and not shading the other half (shade enclosure). A wire mesh structure was built to raise the artificial canopy high above the sediment so crab subjects could not get to it. This enclosure was filled with the same dirt sediment from the Gump station as the sand/dirt enclosure. The angle of the sun was taken into account when setting up the shade enclosure replicates so that each half received the same angle of light throughout the day.

Field Data

Transect surveys were performed at the four study sites (Figure 1). Transects were distributed throughout the site in a purposeful manner to represent the diversity of microhabitats within each environment. This was to test whether the habitats were ecologically dissimilar and to see if these differences would affect burrow distribution.

Transects demarcated 5 x 5 m plots within which burrow density and habitat measurements were taken. Three types of data were collected: 1.) number of burrows per transect 2.) width of the largest burrow in each transect 3.) percent plant cover. With the burrow width measurement, if multiple burrows appeared to be the largest, all burrows were measured and the largest width was recorded. Plant cover was estimated by sight.

In addition, the soil in each habitat was briefly characterized to see if there was any affect on burrow distribution patterns and for descriptive purposes. Soil moisture was measured by taking wet and dry weights of soil samples. Soil was dried for ten minutes in a high powered drying oven. Soil type was characterized by the dominant visible composition of the soil.

Data Analysis

The results of the burrow habitat preference tests were analyzed for significance using a one-way Pearson's Chi Squares test. For the preference tests, each individual was counted once whether the subject completed one replicate or two. Density data was analyzed using an analysis of variance of the natural distribution data. Number of burrows per transect was used as the dependent variable with canopy cover and site location as independent variables, and burrow width was the dependent variable in a similar analysis. Data was analyzed using Systat.

Results

Natural History

In five out of ten burrows that had an occupant removed, a new *C. carnifex* individual moved in.

Larger crabs seemed to use intimidation and dominance to get food when other crabs were around, while smaller crabs tried to pull leaves and flowers into their burrows as quickly as possible before larger individuals could pull the food away.

C. carnifex individuals seemed to be attracted to the color yellow. This is based on the observation that crabs would try to grab a yellow string that was used to trigger one of the box traps, but they did not display this behavior on strings of different colors (white and green).

Experimental Results

Of 22 crabs that were captured over the course of this study, 16 were used in at least one burrow enclosure trial.

Of the 12 subjects used in the enclosure with the sand vs. dirt option, 12 burrowed in the sand. One of these individuals also burrowed once in the dirt, which was counted the same as the other burrow events.

All 11 of 11 individuals burrowed under the artificial canopy in the shade enclosure.

In the moist/dry enclosure, 10 of 11 individuals dug in the moist soil over the clay soil, and 1 individual chose dry soil. This was the same individual that chose dirt in the sand and dirt enclosure (all in Table 1).

A one-way Pearson's Chi Squared test revealed a significant difference among the burrowing preference of the crabs (Table 2).

Table 1. Results from the choice experiment showing the number of crabs that burrowed in each habitat type. Each enclosure is grouped as two.

Sand	Dirt	Shade	No-Shade	Dry	Moist
12	1	11	0	10	1

Table 2. Significance of choice experiment data from a one-way Pearson's Chi Squared test.

Enclosure	Chi^2 value	df	p
Sand/Dirt	9.380	1.000	0.002
Shade	5.500	1.000	0.019
Dry/Moist	7.364	1.000	0.007

Table 3. Results of ANOVA analysis on the dependent variables of burrow density and widest burrow width against the independent variables of percent canopy cover and site location.

Dependent Variable: # of burrows/transect

	DF	Mean Square	F -ratio	p
Site	3	16421.314	35.868	0.0001
Cover	1	66350.146	144.923	0.0001

Dependent Variable: Widest burrow width/transect

	DF	Mean Square	F -ratio	p
Site	3	32.259	4.620	0.017
Cover	1	982.421	129.725	0.0001

Transect Data

Transect data of factors effecting natural burrow density revealed a significant effect of site location (F = 35.868, p = 0.0001 ANOVA) and percent plant cover (F = 144.923, p = 0.0001 ANOVA) on the number of burrows per transect. Largest burrow size per transect was also significantly affected by site location (F = 4.260, p = 0.017 ANOVA) and percent cover (F = 129.725, p = 0.0001 ANOVA). These results were based on 8 transects at the Temae site, 8 at the Fareone motu, 5 at the Opunohu site, and 4 at the Gump site (Table 3).

Overall, transect data revealed that the swamp-like, highly vegetated sites (Temae and Opunohu sites) had the highest average burrow densities. Temae had an average of 114 burrows per transect, and Opunohu had an average of 55 burrow. The Gump site averaged 42 burrows per transect, and the sand beach of the motu had 7 burrows per transect.

Discussion

The results of the choice experiment seem to be in conflict with the field distribution data, and even among the different enclosure types. In choice experiments, *C. carnifex* test subjects burrowed preferentially in sand habitats over dirt habitats. This is inconsistent with the results of the moist/dry enclosure, where subjects preferentially burrowed in moist soil over dry soil. From this one would infer that crab subjects preferentially construct burrows in moist soils; however, the dirt in the sand/dirt enclosure had nearly twice the soil moisture content than the sand, yet the sand was clearly the sediment of choice for burrow construction. Further complicating this issue is the observation that transect data from the motu site, which consisted of a white sand beach, yielded the smallest burrow densities of the four study sites.

One possible explanation for this disparity is that in response to stress, crab subjects chose to burrow in sediments that presented the least resistance to burrow construction. A study by Jones and Boulding (1999) demonstrated that an intertidal snail preferentially selected complex microhabitats during times of increased temperatures because they provided more protection from desiccation. Similarly, under the stress of capture, handling, and foreign conditions, *C. carnifex* subjects may have responded by burrowing as quickly as possible as a defense mechanism. The most amenable substrates would, therefore, be the mostly likely choice for burrow construction in a stressful situation. Thus, the conflict between the choice experiment and the field data could be resolved if one accepts that the results of the choice experiment were dictated by stress responses and did not represent the subjects' actual habitat preferences.

An alternate explanation for the apparent disparity in data sets, is that canopy cover and vegetation availability play a more crucial role in burrow distribution than sediment characteristics. There are several lines of evidence that support this hypothesis. First, the field distribution data showed a significant correlation between canopy cover and burrow density. Second, test subjects in the choice experiment showed a significant preference for the artificial canopy. Third, crab burrows were observed in a myriad of sediments including some very extreme examples such as the root ball of coconut palms, sediment with gravel, and sediment topped by thick mats of grass. This suggests that *C. carnifex* may be a generalist in terms of burrow construction. If this is the case, it is possible that the availability of canopy cover outweighs any sediment preferences the crabs may have. The fourth line of evidence is that *C. carnifex* burrows were not observed where canopy cover was absent or further away than 5 to 10 m, but were found in almost any sediment type (not including rock or coral rubble).

More data is needed to discern the importance of canopy cover and sediment characteristics. The soil moisture data collected for this study was used only in a descriptive sense and was not robust enough to use for statistical analyses. It is apparent that soil moisture was correlated with canopy cover based on average soil moisture and canopy cover for each site, but it is unclear what effect these ecological variables had on burrow distribution. Until more work is done in this area, the hypothesis that canopy cover and soil characteristics, particularly soil moisture, both affect burrow distribution cannot be rejected.

Whether canopy cover is the dominant factor affecting burrow distribution is unclear, but this study demonstrated that canopy cover does play an important role in habitat choice for *C. carnifex*. What that role may be for terrestrial crabs has not been thoroughly investigated. Although its role as a food source and as desiccation protection have been suggested and primarily assumed (Wolcott 1988), no actual studies have been made on the role of canopy cover on the distribution of crab burrows. Data from this study suggests that the primary role of canopy cover for *C. carnifex* is as a food source.

There were six individuals that burrowed entirely at night in the shade enclosure, and all 6 burrowed under the artificial canopy. In these cases, no desiccation stress was possible, yet the crabs preferentially burrowed under the canopy. This suggests that the crab subjects could cue into the canopy and were attracted to it. This seems highly probable since the artificial canopy consisted of hibiscus leaves, the main food source of *C. carnifex* on the Island of Moorea. It is not surprising that *C. carnifex* individuals should have a strong attraction to their food source. *C. carnifex* lives in dense communities (4.5 burrows per square meter average for the Temae site) that are highly competitive. Also, given their ability construct a burrow in many sediment types in a few hours, it does not seem likely that desiccation is too great a concern for *C. carnifex*.

It is clear from this study that there are many interesting questions to investigate regarding the burrowing behavior of *Cardisoma carnifex*. Although it may not mimic natural behavior perfectly, there are many possible studies involving the artificial habitat enclosure technique. The interaction of canopy cover and sediment characteristics with habitat preference could be investigated using an enclosure that presents crabs with both variables at the same time in different combinations. An enclosure using various materials and plant species as artificial shade could reveal how sensitive the canopy sensing abilities of *C. carnifex* are. Along with this, the exact method crabs use to sense the canopy could be tested by covering the eyes of crab subjects to test if they still burrow preferentially under the canopy.

More natural distribution data is certainly necessary for elucidating the effects of ecological variables on burrow distribution. In addition to this, it would be interesting to document the demography of populations in different habitats and compare them to see if ecological variables have any effect on community composition and distribution. In this study, burrow width was significantly correlated with canopy cover suggesting that larger individuals may exploit more desirable habitat, or that size is a function of food availability. Older, larger individuals may be more concentrated in areas with greater resources, or there may be spatial arrangements according to sex. A study focusing on demography would involve capturing crabs in addition to taking distribution transects. This may present a problem since targeting specific crabs for capture is very difficult. A new method of capture may be necessary.

Finally, any investigation of the underground morphology of *C. carnifex* burrows would be fruitful since most of the work in this area has been on other crustaceans (see Mouton and Felder 1996, Steiglitz et al. 2000, Rowden and Jones 1995). Burrow morphology may be correlated with habitat and ecological variables as burrow depth varies with distance from the shore in the fiddler crab *Uca spinicarp* and *Uca longisignalis* (Mouton and Felder 1996).

This study is a first step to understanding the factors affecting the burrow distribution of *Cardisoma carnifex*. The implications for the importance of canopy cover may have further reaching applications into the realm of environmental management. Understanding the factors affecting an organism's distribution can aid in such issues (see Thomas et al. 2001, and Mezquita et al. 1999). If *C. carnifex* populations are tightly associated with hibiscus groves or other strand plant communities, care must be taken in managing those areas. Primary knowledge of how *C. carnifex* interacts with plant communities can act as a guide and warning flag for issues of development that are very timely in the South Pacific (Zann, 1999). Hopefully this and future studies can contribute to such a knowledge base.

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The Feeding Ecology of *Tetragnatha maxillosa* (Araneae: tetragnathidae*)* on Moorea, Society Islands, French Polynesia

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ABSTRACT. The feeding ecology of the long-jawed, orb-weaving spider *Tetragnatha maxillosa* was examined in a four part study addressing habitat distribution, web characteristics, prey and web site movement. Habitat distribution observations showed spiders built webs primarily on *Hibiscus* and on rocks over reaches of riffle in streams sampled at six sites. Web measurements and observations revealed mature spiders built larger webs slightly closer to the stream water's surface than those of immature spiders and that webs of larger size had more radii. A mark-recapture study showed infrequent movement over distances averaging less than 1 meter. Insect sampling was conducted to provide a general description of prey availability over water in the riparian feeding environment and showed that although insects collected from the webs and the environment were all families of Diptera, the insects found in the jaws of *T. maxillosa* were specifically of the family *Simuliidae* (blackflies). This suggests there may be a strong predator-prey relationship between *T. maxillosa* and this family of flies that are considered pests in French Polynesia.

Introduction

Tetragnatha is a genus of long-jawed, orb weaving spiders found in a diversity of environments throughout the continents with the exception of Antarctica (Aiken and Coyle 1999). Several radiations of *Tetragnatha* are associated with aquatic ecosystems throughout archipelagos in the Pacific Ocean including the Hawaiian Island, the Marquesan Islands, and the Societies where the island of Moorea is located. Research has been conducted on the Hawaiian Islands by Gillespie, but the ecology of species present in the Society Islands has not been studied extensively. Spiders of this genus have recently been described and documented on Moorea by Gillespie (1999; 2000). She identified six species of *Tetragnatha*, only three of which are found near aquatic ecosystems at low elevations on the island of Moorea. Of the three most commonly found at sea level, both *T. maxillosa* and *T. nitens* are suspected to be introduced species originating from India and Southeast Asia while *T. macilenta* is endemic to the island (Gillespie In Press).

Little is known about the feeding ecology of this genus in French Polynesia. As an orb-weaving spider, *T. maxillosa* must make important decisions concerning site location (Gillespie 1987), a factor that contributes to the spider's success in feeding, as insect capture is related to the habitat where the web is placed (Opell 1999). *T. maxillosa* is specifically associated with freshwater streams on the island of Moorea, although their habitat sometimes extends to the mouth of a stream where water may be more brackish. In 1987, Gillespie also showed that web site location for one species of *Tetragnatha* was indiscriminate and spiders would build on any available structural support within their habitat.

Hierarchical factors of conspecific competition in spider aggregation may be exhibited in their feeding patterns, particularly with respect to their relative site location. Spiders by nature are territorial (Nyffeler 1999; Foelix 1996), but may aggregate to share resources like drag lines since web silk is an energy consuming product that is largely ingested by spiders during web deconstruction (Gillespie 1987; Foelix 1996).

Feeding behavior is characterized by collection of prey from the webs, which is then often balled up and held in their jaws where external digestion commences (Foelix 1996). As for most orb weavers, only immature spiders and mature females build webs (Gillespie, pers. com; Foelix 1996).

Web construction is another significant factor in feeding success for orb weaving spiders. There are a number of variables that contribute to a webs success in capturing insects, which constitute the majority of most spiders' diets (Foelix 1996). Many studies have aimed to outline the determining factors of successful web construction. One such study suggests that increased capture area and stickiness of the silk components are important factors (Opell 1999). Web angle and orientation are also variables that affect prey interception and retention (Bishop and Connolly 1992; Choe et al. 1999; Eberhard 1989).

The goal of this study was to characterize the feeding ecology of *T. maxillosa* by addressing habitat associations, web characteristics, prey, and the movement of webs. The study asked what type of water flow the spiders were building over and what types of substrates they were building their webs on. It also aimed to observe any differences in web construction between immature and mature spiders, as well as correlations between the following web variables: Web angle, orientation, distance from shore, height above water, diameter, and number of radii. Prey was collected to ask what types of insects the spiders were feeding on. Frequency and distance between web site locations were also observed.

The broader significance of this study is to expand current knowledge of the ecological role of this species at a time when the biota of the island of Moorea are rapidly changing due to invasion by non native species such as Miconia and Sharpshooters (Mishner, pers. com). Spiders may have the potential to be a biological control agent for such pests as *Simuliidae*, a family of Diptera that is considered a pest by the French Polynesian government because of their affect on the tourist industry (Seychant, pers. com.).

Methods and Materials

Study Sites

The study was conducted on the island of Moorea, French Polynesia (17°30'S, 149°50'W) between October 15 and November 15, 2001. There were four parts to this study, performed at six stream sites. Stream 1 in PaoPao at the base of Cooks Bay served as the site of the mark-recapture (Figure 1). Site 2 was in Afareaitu, a small bay on the east side of the island. It was observed that Site 2 had less allochthonous input than any of the other five sites and less vegetative overhang, and was therefore only sampled for habitat transecting. Four of the stream sites are located in Opunohu valley, an agricultural valley that feeds into Opunohu Bay (Figure 2). Figure 3 presents a summary of the sampling performed at each location.

Mark-Recapture

A mark-recapture study was performed to observe how frequently the spiders were moving their webs and if so how far relative to their original web location over a two week period. The mark-recapture also outlines patterns in spider aggregation, and how closely they were constructing their webs to each other.

On October 15 and 16, 2001, *T. maxillosa* were marked at Site 1 using an enamel based paint. Only mature females were marked because mature male spiders do not build webs and die soon after mating. Immature spiders were too delicate to be handled. Spiders over 7 mm in length were considered mature because females of this size were observed mating. A schematic marking guide was used to correspond the location and color of the marked individual with its reference number. Sex and maturity, height above water, and length of body were all recorded before each spider was marked with a small dot of colored enamel on the metatarsus, tibia or femur of pair 1, 2, 3, or 4 of the left or right side of the body as illustrated in Figure 4. In this fashion, twenty-four spiders were marked with each color of enamel. Spiders were sought for recapture every evening between October 17 and October 26, 2001 for a total of nine days. When an individual was identified, web measurements were recorded and spider location was marked with a small piece of colored tape labeled with the spiders reference number and the date.

On October 29, 2001, measurements from two points were made to each location marked. The points were later used to construct a map of the spider's movement over time, with reference numbers and dates. This was used to calculate how often the spiders moved and how far. The map also depicts aggregations of spiders within the plot. The mean distance and frequency of individual's movements were calculated. Aggregations of spiders were counted and recorded.

Web Measurements

Measurements of web variables were recorded to examine patterns in web construction and location. The following web measurements were made for mature individuals: angle and diameter of web, orientation, number of radii, height above water, distance from shore and the size of spiders measured as length of body from abdomen to head, excluding the jaws in overall body length.

Only angle, diameter, orientation and height above water were observed for immature spider's webs. It was hypothesized that there would be significant differences in the data sets for immature versus mature spider's web characteristics.

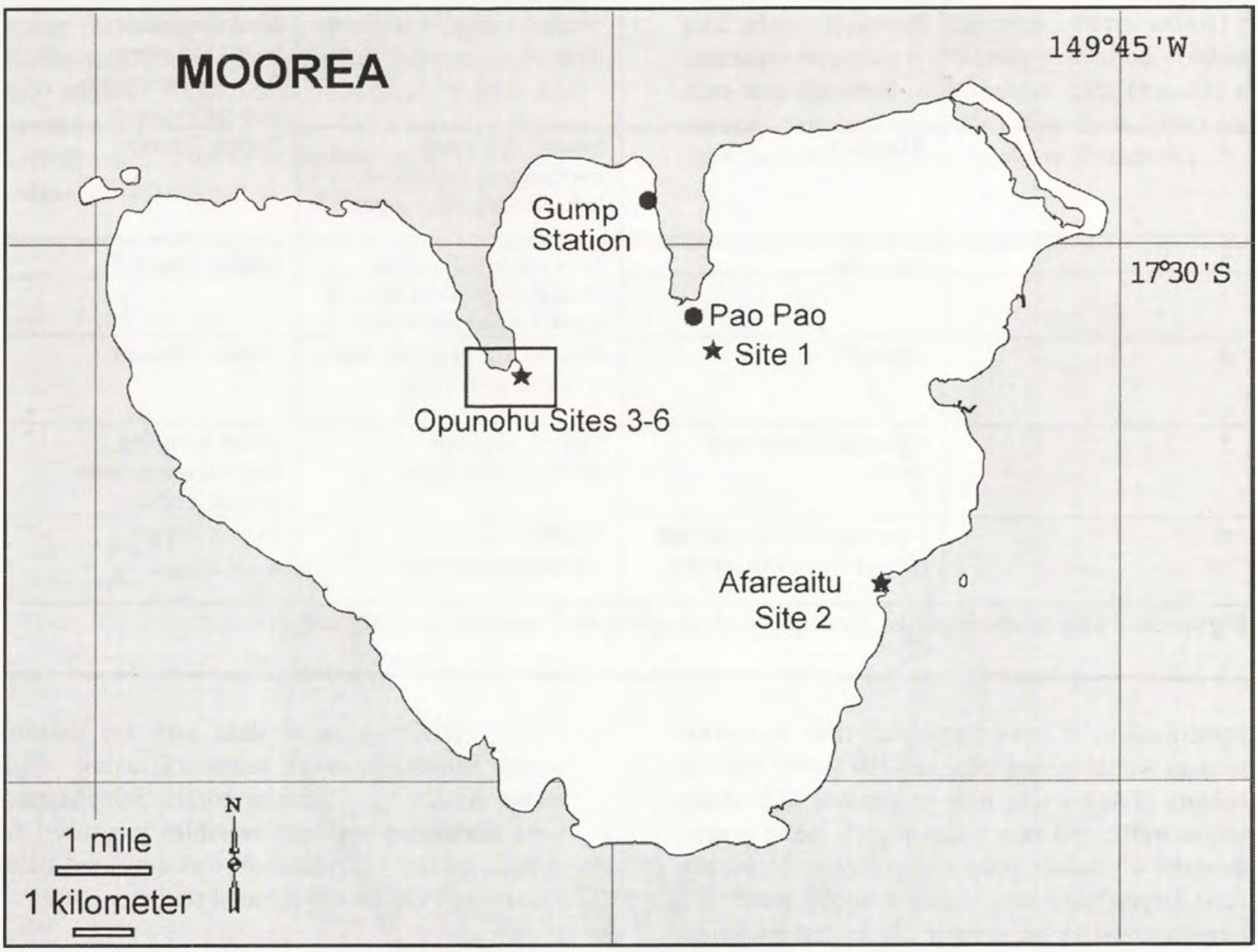

Figure 1: Map of Moorea indicating locations of sampling sites. Box indicates more detailed map (below).

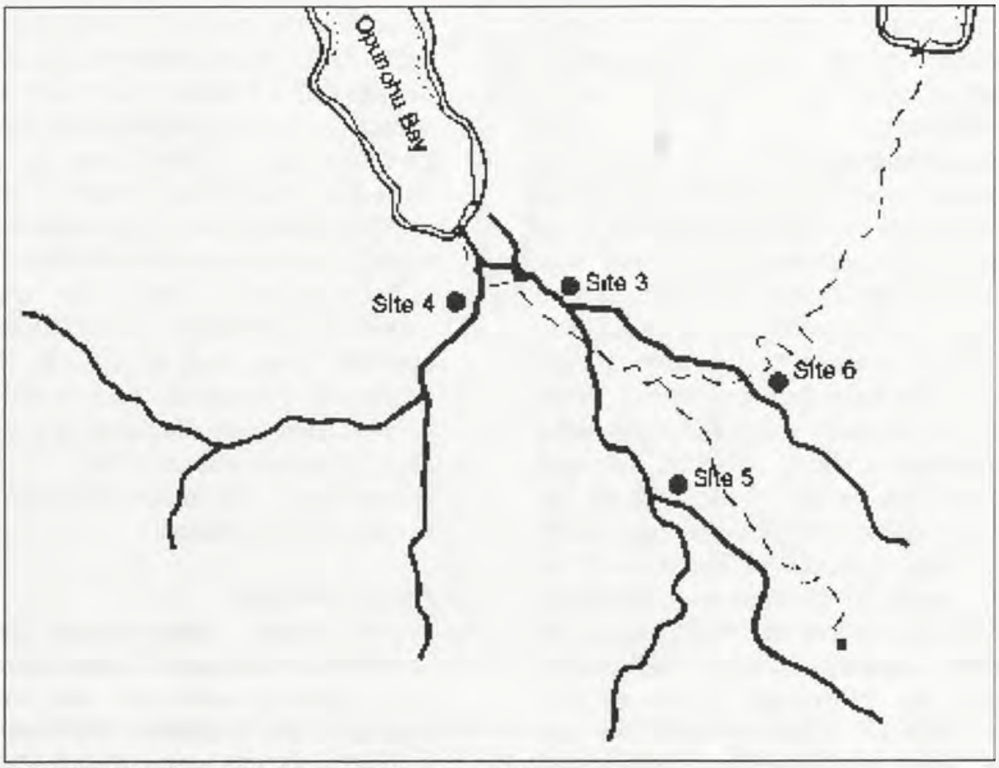

Figure 2: Opuhohu Valley sampling sites 3-6.

Stream #	Location	Description	Research Performed
1	PaoPao	Shaded, high allochthonous input	Mark-Recapture Insect Sampling Web Measurements Habitat Transect
2	Afareaitu	Shaded, not much overhanging vegetation, low allochthonous input	Habitat Transect
3	Opunohu	Near construction site, shaded, high allochthonous input, low water clarity	Habitat Transect
4	Opunohu	Shaded, lots of algae, high allochthonous input	Habitat Transect
5	Opunohu (3 coconuts Trail)	Shaded, medium allochthonous input	Insect Sampling Web Measurements Habitat Transect
6	Opunohu (by Agricultural School Nono Plantation)	Shaded, lots of allochthonous input	Habitat Transect

Figure 3: Table of descriptions, locations and sampling at *T. maxillosa* study test sites.

Specifically, it was expected that immature spiders would be building smaller webs, because spiders of larger size may be capable of building larger webs, and may consequently be in greater demand of higher prey capture rates to sustain their larger body size. Height above water was hypothesized to be greater for immature spider because of suspected conspecific competition for web locations, and a suspicion that web sites closer to the water's surface may be more optimal for interception of prey. The diameter and number of radii were suspected to be positively correlated.

All web measurements were made using a standard metric ruler or measuring tape, except for the angle of webs, which was measured using a clinometer. The orientation of the web was recorded as the direction the web angle opened towards. Data was recorded for the orientation of the web as a score of 1 for webs facing downstream, 2 for webs facing upstream, 3 for webs facing the southeast shore and 4 for webs facing the northwest shore. Size of web was measured from the center of the web to the outermost silk spun. Number of radii were counted. Distance from the surface level of the water to the center of the web was measured. Directional orientation was recorded as the angle of the web opening towards upstream, downstream, the Northeast shore or the Southwest shore. General data on air temperature, wind, and weather were recorded before measurements were made.

Differences in data sets for matures versus immatures were compared using single factor ANOVAs. Pearson correlation matrixes were computed between variables measured for mature spiders. Orientation was analyzed using a two way Pearson chi-squared statistic.

Insect Sampling

Insect near streams were sampled to determine the prey of *T. maxillosa*, using four techniques. Sticky traps were first employed at Sites 1 and 3 however they were ineffective in collecting insects. Malaise traps were ultimately used to sample at Sites 1 and 3. Insects were collected from individual's webs during recapture and after web measurements were made. Insects were also collected from the jaws of *T. maxillosa*. These balls were analyzed under a microscope to determine what the contents were, and to classify the types of organisms consumed. Insects collected from malaise traps were identified to provide a rough idea of what was available in the riparian environment. All insects collected were placed in vials of 70% ethanol.

Habitat Sampling

Stream habitat transects were employed to observe what type of water flow the spiders were building webs over and what types of vegetation and substrates they were building on. Transects of 150 meter length were walked at stream sites 1-6 to characterize the habitat preferences of *T. maxillosa.* Vegetation type and water flow type were recorded. The type of water flow was recorded as riffle, run or pool. Riffle was characterized as a shallow reach of the stream with some turbulent water and partially exposed substrate. Run was defined as a swift reach with little surface agitation and no major obstructions to flow, and pools were defined as reaches with deep water that is deep and slow moving. Samples were taken of unknown vegetation and later identified. Habitat data was recorded as frequency data (number of spiders per vegetative or water flow type) and analyzed using Pearson chi squared statistics.

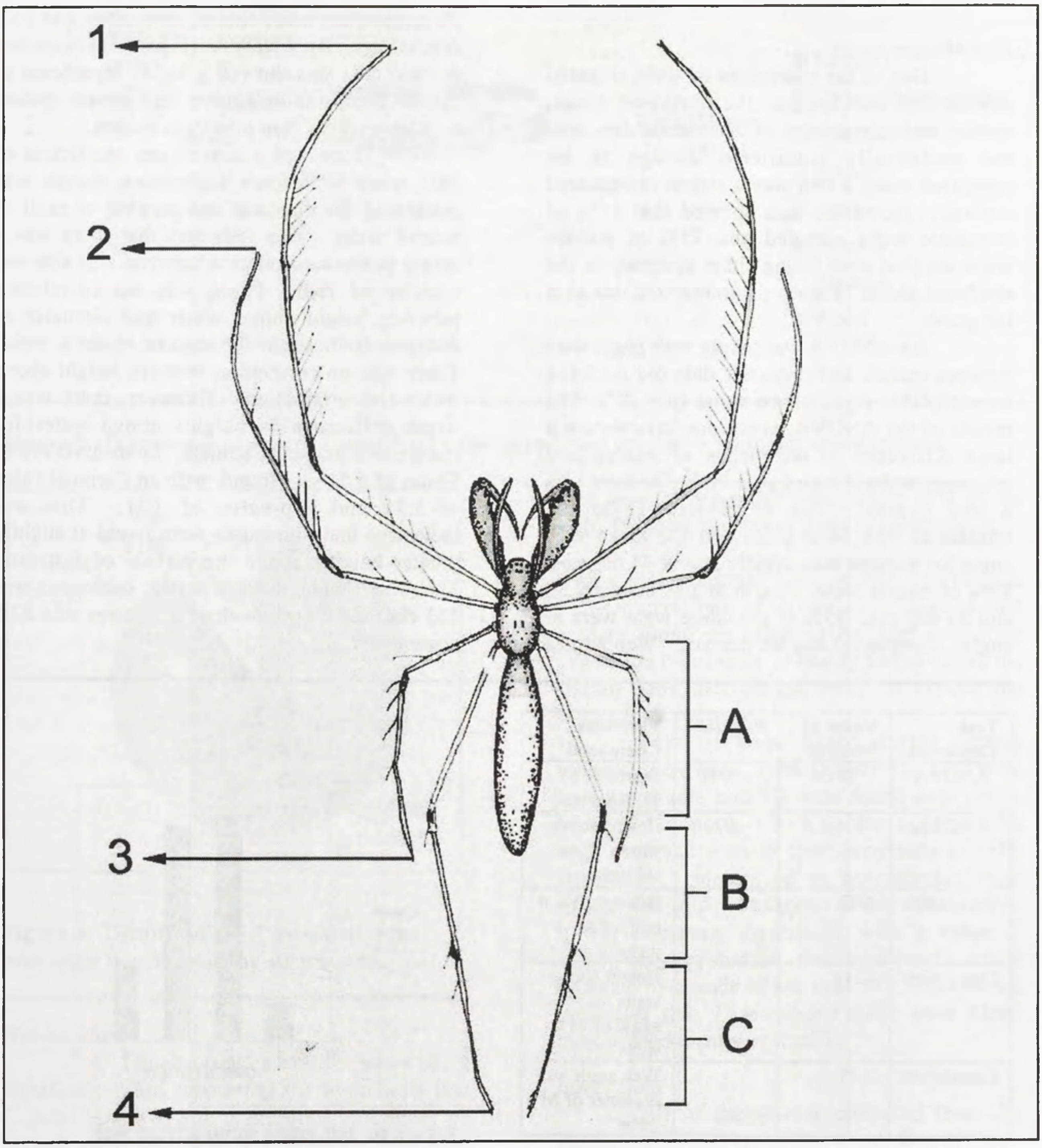

Figure 4: Schematic diagram of marking locations on *T. Maxillosa*. *On left side* (1) Left 1 (2) Left 2 (3) Left 3 (4) Left 4. *On right 4 leg* (A) Femur (B) Tibia (C) Metatarsus. Redrawn and adapted from Koch, 1872.

Results

Mark-Recapture

Individual spiders recovered moved a mean distance of .58 meters. Spiders moved web site location an average of every three days. Five major aggregation sites along the plot were visible. The longest web site tenure was seven days; the shortest was 1 day.

Web Measurements

Due to the sparseness of webs oriented downstream and towards the northwest shore, mature and immature web orientation data was not statistically significant enough to be compared using a two way Pearson chi-squared statistic. However, data showed that 81% of immature webs sampled and 75% of mature webs sampled were facing either upstream or the southeast shore. Figure 6 summarizes this in a bar graph.

An ANOVA comparing web angle data between mature and immature data did not have an acceptable significance value (p = .07). The results of the ANOVA would not have shown a large difference in the means of mature and immature webs if found significant, as there was a low f-ratio. The mean web angle for immatures was 54 degrees and the mean web angle for matures was slightly less at 44 degrees. 95% of mature webs were at angles between 38 and 50 degrees. 95% of immature webs were at angles between 40 and 68 degrees. Web angles showed two major correlations with other variables. Using only mature data for statistical analyses, web angle and height above water showed a slight negative correlation with a coefficient of -.214. Web angle was positively correlated with web diameter, with a coefficient of .245. There was no correlation between web angle and orientation.

The mean diameter of mature webs was 26 centimeters while that of immatures was 16.1 centimeters. An ANOVA (Figure 5) comparing the two data sets showed a highly significant p-value. Data was indicative that mature spiders built larger webs than immature spiders.

There was a correlation coefficient of .602 when a Pearson correlation matrix was calculated for diameter and number of radii of mature webs. This indicates that there was a strong positive correlation between web size and number of radii. There was no correlation between height above water and diameter or distance from shore for mature spider's webs. There was no correlation between height above water and orientation. However, there was a slight difference in heights above water for mature and immature spiders. In an ANOVA an f-ratio of 5.51 was found, with an f critical value of 3.95 and a p-value of .021. This was indicative that immatures were found at slightly greater heights above the surface of a stream. The mean height above water for immatures was 113 centimeters while that of matures was 87.5 centimeters.

Test Employed	Value of Statistic	P-Value	Variables Compared
ANOVA	F=64	< .001	Diameter M vs. IM webs
ANOVA	F = 5.5	.02	Height above water M vs. IM webs
Correlation	.602	-	Diameter vs. # radii of M webs
Correlation	-.214	-	Height above water vs. angle of M webs
Correlation	.245	-	Web angle vs. diameter of M webs

Figure 5: Table of significant web measurement results for *T. maxillosa*. (M) Matures (IM) Immature.

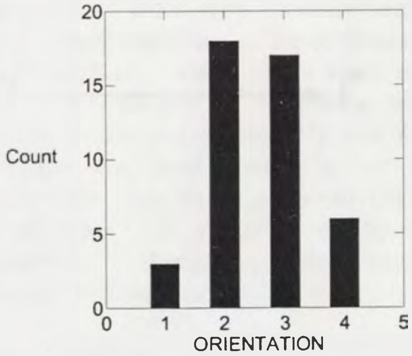

Figure 6: Bar graph summarizing web orientation for *T. maxillosa* at study sites. (1) Downstream (2) Upstream (3) Southeast shore (4) Northwest shore.

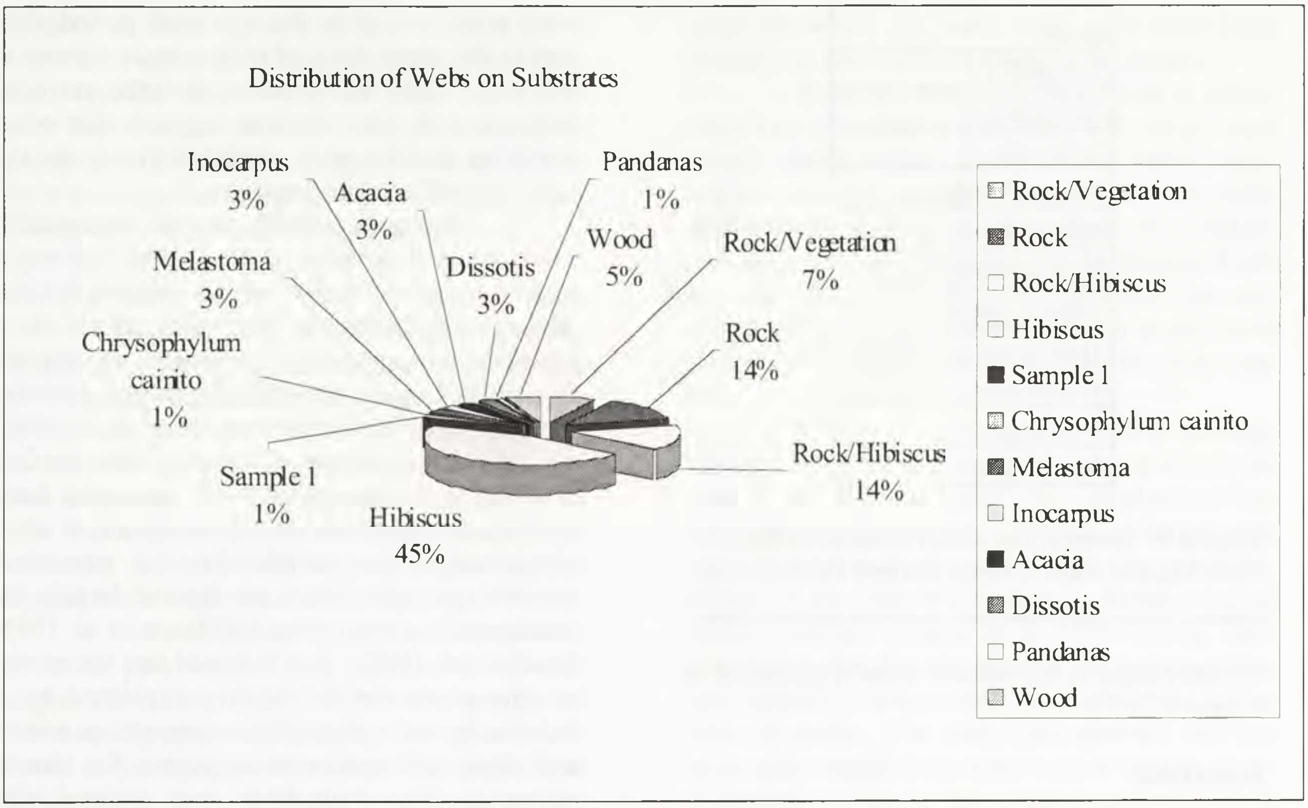

Figure 7: Distribution of mature *T. Maxillosa* webs on rock and vegetative substrates above streams.

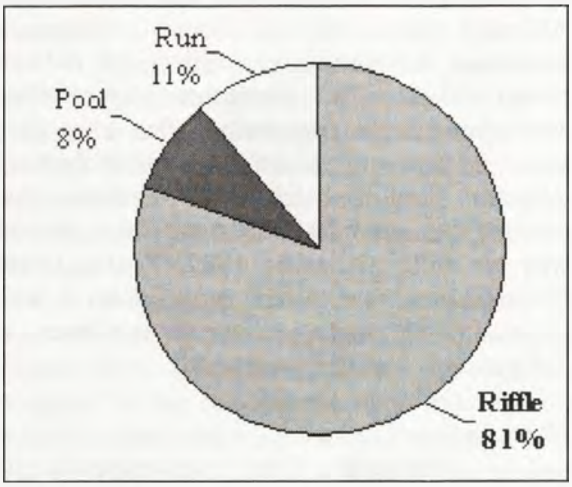

Figure 8: Distribution of *T. maxillosa* webs over water flow types along six transects.

Habitat Data

A chi-squared statistic showed a significant value supporting the hypothesis that *T. maxillosa* web site distribution is greater on certain substrates with a probability less than .001 that this result would be found by chance. 45% of spiders sampled were found in *Hibiscus* vegetation and 14% were found solely on rock. 32% were found on rock or rock in combination with another vegetation type, while 75% of the webs found on vegetation types in combination with a rock substrate were on *Hibiscus* (Figure 7). Observations of stream sites showed that spiders found on other native and nonnative vegetation types were primarily found where the stream bank had eroded away to expose the vegetation's roots.

81% of webs found during stream transects were above riffle (Figure 8). 11% were found over run, and 8% were found over pools. Most of the pools where spider aggregations were observed were in close proximity to riffle (less than 3 meters up or downstream from riffle). Chi squared analyses of this data showed it was extremely significant, with a value of 110.33 and a probability that these results would be found by chance of less than .001. Results are indicative that *T. maxillosa* webs were found most predominantly over riffle.

Insect Data

All of the insects collected from the jaws of *T. maxillosa* were *Simuliidae* (blackflies). 82% of insects captured from spider's webs were of the superfamily *Culicoidea* (midges), while the remaining insects captured from webs were identified as non-midge Diptera. A summary of the types of

Insect
Culicoidea 1
Culicoidea 2
Culicoidea 3
Diptera family 1
Diptera family 2
Diptera family 3
Diptera family 4
Hymenoptera 1 (small)
Hymenoptera 2 (large)
Orthoptera
Lepidoptera

Figure 9: Summary of insect families collected from Malaise traps at Opunohu and PaoPao sites.

insects caught in the malaise traps is presented in Figure 9.

Discussion

Web Site Tenure

The results of this study indicate that *T. maxillosa* individuals at the mark-recapture site moved webs infrequently and over short distances. The spider's frequency of movement suggests that individuals at the mark-recapture site were successfully feeding as web site tenure for most spiders is positively correlated with prey capture (Caraco & Gillespie, 1986; Smallwood 1993; Williams et al 1995). However, a 1986 study by Gillespie and Caraco showed that for a North American counterpart, *T. elongata,* web site tenure was in fact conversely related to the quantity of prey captured. *T. elongata* in poor habitats moved webs less than those in prey rich habitats, and when spiders were moved between sites it was shown that they maintained the same behavior.

The results of the Gillespie and Caraco model present some doubt in assuming that site tenure is always positively correlated to prey capture for spiders of this genus, and can be simply taken into account by stating that a sites viability for feeding success cannot be entirely gauged by the length of time a spider stays at a location. The Gillespie and Caraco model also measured site location on a smaller temporal scale than this study.

Despite the doubt presented from previous work in the field, *T. maxillosa* at the mark-recapture site must be in some respect successful because many individuals captured were seen throughout the two week period, data that is also supportive of prey capture success at the site. The short mean distance traveled between web site locations suggests that other variables besides prey availability may be the cause of web site movement.

One such variable may be intraspecific competition from other spiders. The five major aggregations of webs within the study site showed individuals building webs within close proximity to each other. This data also suggests that spiders were successfully feeding, because their solitary and territorial nature (Foelix 1996) would otherwise prevent them from locating webs in such tight quarters. Past studies have shown that population density for spiders is often correlated to prey availability but numerical assemblages of spiders are limited largely by intraspecific competition (Williams et al 1995; Smallwood 1993). It is believed that the results of this study can be largely explained by a balance between the spiders competitive nature and their willingness to aggregate for shared resources like drag lines and optimal site locations. For such short distances, movement between web site locations can also be explained by the physical properties of web construction. Although spiders may not choose to relocate to new sites, as mentioned earlier, the delicate nature of their webs requires them to deconstruct and rebuild webs frequently, often on a daily basis. Spiders will often allow some of their silk into the wind to catch on a substrate, thus creating the initial drag line from which the web will be built (Gillespie 1987; Foelix 1996). Environmental factors such as variations in wind patterns could easily account for movement of webs at such a small spatial scale.

Insect Data

Although the predominance of *Simuliidae* consumed by *T. maxillosa* is not indicative that they are feeding exclusively on blackflies, it does suggest that blackflies constitute a large proportion of the spider's diet. The study also suggests that *T. maxillosa* individuals are feeding almost entirely on Diptera, which is supported by previous studies (Lesar & Unzicker 1978) on the diet and prey preferences of *Tetragnatha*

Specifically, in 1999 a Nyffeler study sampled five groups of orb-weavers and showed 40% of *Tetragnatha* species diets consisted of Diptera, with the lowest breadth of diet, indicating more specialized feeding behavior. Spiders may be generalists as predators but relative to other genera, *Tetragnatha* may be more of a specialist feeding group. As many web-weaving spiders rely on lesser variety in prey captured but more abundance within prey groups (Nyffeler 1999), it appears that there may be a strong predator-prey relationship between *Tetragnatha maxillosa* and *Simuliidae*.

Web Variables

Web angle data had several interesting correlations, decreasing slightly with height above water while increasing with the diameter of the web within the data set. While these two correlations may not be entirely accounted for, web angle data can be considered significant in that webs were neither more horizontally or vertically oriented with respect to gravity, but instead had a mean value of 44 degrees.

Webs of vertical angle have been shown to retain prey longer than those of horizontal angles (Eberhard 1989). But many groups of orb-weaving spiders including *Tetragnatha* are found to consistently have mean web angles that differ largely from vertical orientation to gravity (90 degrees). Habitat limitations or increased prey capture are the two most probable explanations for this behavior based on prior studies.

Increased prey interception appears to be the more reasonable explanation for *T. maxillosa* mean web angles at the sites of this study, because of the types of prey found to be consumed. As previously mentioned, predation of *Simuliidae* (blackflies) was supported most by the data.

Simuliidae larva have an interesting way of making the transition from water to flight. Most *Simuliidae* become projectiles, wrapped in air bubbles as they shoot to the water's surface using buoyancy (Crosskey 1990). The flies can often make this transition to their adult stage without the intermediate use of legs, beginning immediately with flight (Crosskey 1990). The interception of larva as they hatch from the water's surface may be accountable for the construction of webs both facing upstream and with web angles close to 45 degrees. A web of directional orientation towards upstream would be optimal for intercepting prey as it exits in a downstream orientation using the waters velocity, the stream's downstream current, and the buoyancy of an air bubble as driving forces. This does not account for the number of webs that were oriented towards the southeast shore, which may be explained by environmental variables such as habitat structure. The *Hibiscus* trees on which the webs were built were both notably on the southeast shore of the stream.

Data showed that there was a slight negative correlation between web angle and height above water. Does a more horizontally angled web catch more prey for individuals building at heights farther from the waters surface? Studies comparing the directional flight patterns of recently matured *Simuliidae* as well as trends in web angles for spiders at different stages of maturity would be beneficial to test this.

If prey is exiting the water's surface, then the slightly greater height above water for immature spiders can be explained by conspecific competition. Spiders of larger size may be getting more optimal web site locations, closer to the stream's surface. Ambrose et al found similar results in a 1995 study, and concluded that for one species of *Tetragnatha*, intraspecific competition was observed on a vertical scale. The study also showed that the most prey caught over water was at heights of 1-2 meters, similar to the mean height of near 1 meter above the water's surface for *T. maxillosa*.

Mature versus immature data signified that web size was greater for mature spiders. Spiders of larger size and metabolism may be capable of building larger webs, but may also need to build larger webs to sustain their larger body size. A study on a non-riparian species of *Tetragnatha* speculated that web size was less related to body size than it was to the instar and degree of sexual maturity of the spider (LeSar & Unzicker 1978). However, environmental factors such as substrate availability may still be variables in web size since mature spiders were found at heights closer to the waters surface, where substrates may be found farther apart. Webs of greater height above the water encounter more foliage. It appears more likely that immature spiders are building smaller webs both as a result of their smaller body size and because it is easier and more practical to build smaller webs in locations of denser foliage, but that the prior factor holds more influence because the heights above water were only slightly greater for immature spiders.

Resource allocation can also account for the number of radii increasing with the size of the web's diameter. Most orb weavers carefully construct their webs and it is logical that the number of radii should increase with the size of the web, but it may also be a result of the smaller spiders inability to allocate as many metabolic resources to web building that limits the size of their webs. As well as it is less necessary to build larger webs in order to capture enough prey to sustain their smaller body size. This serves as an explanation why there was no direct correlation between web size and height above water, because web size is related to spider size and there was not an extreme range in the heights of immature and mature spiders above the stream. Further studies quantifying prey capture at different heights above water may prove helpful in connecting the variables.

Habitat Data

While this study's data clearly suggests that spiders are web building on mainly hibiscus and rock vegetation, there are numerous possible reasons why. One reason suggested by the data is that prey availability is higher over riffle, and that prey or prey larva are associated with this water flow type. This hypothesis is supported by the fact that all the insects found in the jaws of *T. maxillosa* were *Simuliidae*, while those in the webs were primarily other Diptera. *Simuliidae* have a strong association with lotic environments, particularly with riffle because of both oxygen content and filter feeding (Crosskey 1990). It cannot be assumed that blackflies are the cause of spider web location over riffle but data infers that both are organisms associated with riffle, which may be why they are feeding on *Simuliidae*. *T. maxillosa* may also be found above riffle for the more practical reason that, by definition there is more debris and rock protruding from the water in the riffle sections of the stream, thus there are more substrates for web building. A similar explanation can be employed to explain why the spiders are found mainly on *Hibiscus*. There could be an association between their prey and *Hibiscus* trees, but a more practical reason would be that *Hibiscus* is one of the most frequent types of vegetation overhanging the stream. Although spiders built on a number of other vegetative types in lower frequency, all but webs built on *Hibiscus* were observed to be mainly on the roots of the plant where the stream bank had eroded away. The overhanging branches offered by *Hibiscus* permitted the spiders to web-build as close as possible to the waters surface. This also accounts for the large percentage of rocks built on.

Conclusions

Spider of the species *Tetragnatha maxillosa* were found to be building webs over riffle, on rock and *Hibiscus* substrates. Their prey appeared to be predominantly of the fly family *Simuliidae*, which has an association with riffle and lotic water environments. Web variables, especially those of web angle and orientation also suggest that there may be a strong predator-prey relationship between *T. maxillosa* and *Simuliidae*. Web site tenure and distance between web site locations supported this in indicating that the spiders were successfully feeding and consequently remained in the same feeding locations.

Studies quantifying insect prey capture rates may provide more insight into the feeding ecology of *Tetragnatha maxillosa* and may prove to illustrate conspecific competition between spiders of different size and maturity within the species.

Further studies comparing the distributional patterns of *Tetragnatha maxillosa* with those of *Simuliidae* may also elucidate whether there is a strong predator-prey relationship, as suggested by the results of this study. Many species of blackflies are associated with a particular order of stream (Crosskey 1990) and distributional patterns of blackflies may therefore be predicted by species. First determining whether any of the species of Simuliidae on the island of Moorea are associated with particular stream orders, and then sampling for both T. maxillosa and Simuliidae could test a correlation. Examination of the relationship of similar species of *Tetragnatha* to prey may prove insightful as means for biological pest control agents in French Polynesia. Blackflies are a considerable nuisance to the tourist industry, especially on islands of the Marquesas where *Nonos* (blackflies) are extremely prone to feeding on humans, and have prevented the tourist industry from developing on one side of the North Island (Crosskey 1990; Seychan pers. com.). Studies on the comparable native species, *Tetragnatha macilenta*, found often in the same habitat as *Tetragnatha maxillosa* could aid in understanding the biota of the island before further invasion by non-native species.

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Reproduction and Distribution of *Hibiscus tiliaceus* from the Coast to the Mountains on Moorea, French Polynesia

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ABSTRACT. *Hibiscus tiliaceus* (Malvaceae) is a common tree throughout the Pacific islands. Its origin of arrival to the islands as either a native plant or an introduced species is debated. This study primarily focuses on reproductive and seed biology to suggest how *H. tiliaceus* has been able to disperse by hydrochory and exploit numerous habitats across a wide range of altitude on the Pacific islands, more specifically the island of Moorea. On Moorea, studies were conducted up to 400 meters. Bagging experiments were performed to suggest the modes of pollination for this tree, which was determined to be a generalist through these experiments and visual observations. *H. tileaceus* receives pollen from a variety of sources. A black bee and two species of Nitidulidae beetles were found to transport pollen. Along with the insects found, it is suggested that wind is capable of disrupting pollen on to the pistil of the same flower. Statistically significant seed set and buoyancy data suggest that there is an ecotypic variation among elevational populations of *H. tiliaceus*. More specifically, there was a significant difference in the coastal sites compared to those between 100-400m elevation. Higher elevation sites showed no significant difference when compared to one another. Overall, coastal trees were found to have fruits with a higher seed set and the seeds were more buoyant than those collected in the higher elevation sites. There are a number of possibilities that may explain the variation observed. These may include genetic differences, pollinator presence/absence, timing of pollination, pollen competition, and pollen tube growth rates. Buoyancy experiments showed that seeds from all elevational sites are able to float in salt water. Despite the variations observed, and the possibly increased hydrochory ability of coastal trees, it is suggested that *H. tiliaceus* varieties observed from the coast to the mountains on Moorea may be able to disperse by water and establish on new coastal shores.

Introduction

Hibiscus tiliaceus (Malvaceae), commonly known as beach hibiscus (Anonymous 1998), is the only native hibiscus plant on the island of Moorea. It is a tree that grows in a variety of habitats, ranging from the coastline to the high mountain ranges. *H. tiliaceus* is a small to medium-sized tree, up to 15m high, common to abundant in the littoral forest and mangrove forest margins of atolls and high islands. It is one of the most common secondary forest trees and is frequently found in disturbed forest ranging up into the mountains at over 800 meters of altitude in French Polynesia (Whistler 1980; Petard 1986). Dispersion along a large elevation and ecological range is unusual for a single species. It is therefore suspected that ecophenotypic variation is present within this species, possibly due to genetic differences.

The reproductive biology of *H. tiliaceus* is, for the most part, unknown. Understanding the reproductive biology of this species may shed light on how it has dispersed between and within tropical islands. In addition, important plant and insect associations that may be found could suggest conditions necessary for ideal development of this tree. It is suspected that *H. tiliaceus* is highly adaptable to a variety of habitats and lives in a broad association with a number of plant and insect species.

H. tiliaceus is a very useful plant to the people of French Polynesia. The medicinal and functional uses of *H. tiliaceus* are numerous. The soft wood is used in construction, canoe parts, and for many other purposes. The fiber from the bark is used to make cordage, mats, and strainers (Whistler 1980). Medicinal preparations include all parts of the plant. The leaves are used as wraps around fractured bones and sprained muscles; the fluid from the bark is used to promote menstruation, treat skin diseases, eye infections, and stomachaches; the flowers are used to make a salve; and an infusion of the leaves is used to aid in childbirth (Anonymous 1998). The importance of this plant to the people of Polynesia is evident in its usages.

Insight into the reproductive biology and varietal distribution of *H. tiliaceus* would be of value in understanding how this plant has adapted and radiated across the Pacific islands. There are many interesting questions regarding the reproduction and distribution of *H. tiliaceus*. Since so much is unknown, it is an ideal indigenous plant to study. Following is a series of questions of *H. tiliacues* that were addressed in this project:

Inflorescence biology

1. How long do the flowers last?
2. What is the growth rate of the buds and fruits?
3. What is the arrangement of the inflorescence?

Reproductive biology

4. What is the mode of pollination?
5. Does this mode or pollinator change along an elevational gradient?
6. Is this species self-compatible?

Seed biology

7. What is the frequency of seed set at different elevations?
8. What is the range of soil and water types in which seeds of this species can germinate?
9. Are seeds from different elevations equally buoyant?

Natural history surveyed

10. When do flowers change color?
11. Is the change in flower color a consequence of fertilization?
12. How many varieties of H. tiliaceus are on Moorea, and what ecosystems do they inhabit?

The flowers of *H. tiliaceus* are believed to have a short duration. Flower color is suspected to change after the flower is pollinated, and would be independent of elevation. The short life of the flower, along with the suspected quick development of the buds and fruits, would make it an ideal study species with which to examine different reproductive qualities.

Since *H. tiliaceus* is a very successful plant throughout the Pacific islands, from the coast to the mountain ranges, it was suspected that it is a generalist in its mode of pollination, and probably self-compatible. Due to trends on other islands of insects living in gradients along the elevational changes, the more native species living at the higher altitudes (Gilespie pers. com), it was suspected that there would be a difference in the types of insects pollinating *H. tiliaceus* along an elevational gradient. If these pollinators are equally successful in pollinating the flowers, it is believed that seed set would be uniform across elevational changes. The seeds were suspected to be buoyant in sea water and able to germinate under saline conditions. Hydrochory, or water dispersion, would be the primary mode of dispersal for H. tiliaceus due to the size of the seeds.

It is suspected that *H. tiliaceus* contains ecotypic differences within the diverse elevation and environment types it inhabits. This project aims to study some of the dynamics of the distribution and reproductive biology of *H. tiliaceus* along an elevational gradient on the island of Moorea.

Materials and Methods

Three sites were chosen for the study of *H. tiliaceus* (Figure 1). Approximately 15 trees in all sites were used. Sites 1a and 1b are located at sea level. The first of these sites, located adjacent to the UC Berkeley Gump Station, was the primary location for all experiments. Site 1b at the Opunohu swamp was a location for seed, soil, and water collection. Site 2 was spread across an elevational gradient of 100-180 m along a road, due to the difficulty of acquiring flowers and fruits in the high canopy. The road to the Belvedere was used, which contained clearings at the two maraes-Tahitian ceremonial sites, pk (pointe kilometrique) 4, and the Belvedere. These clearings enabled low-lying branches to be easily accessed. The high elevation site, Site 3, was located along the Three-coconut ridge at an elevation of 406 m. Studies conducted at or using specimens from sites 1a, 2, and 3 include bagging experiments, seed set counts, buoyancy experiments, and germination studies. The high-elevation site was the least well studied due to its remote location and frequent poor weather conditions. Two suspected varieties were used for the experiments in this study because they were found at all elevational sites. The names of these varieties, from one local source, differ from those described by Brown (1935). These are "purau ro'a" and "purau tahatai" (Appendix 1). There was no distinction made between these varieties for the experiments performed.

Inflorescence biology

Preliminary observations at the coast determined that the flower of *H. tiliaceus* lasts for one day, blooming around 6:00am and falling off the tree between 2:00pm and 9:00pm. Occasionally, a peach flower from the previous

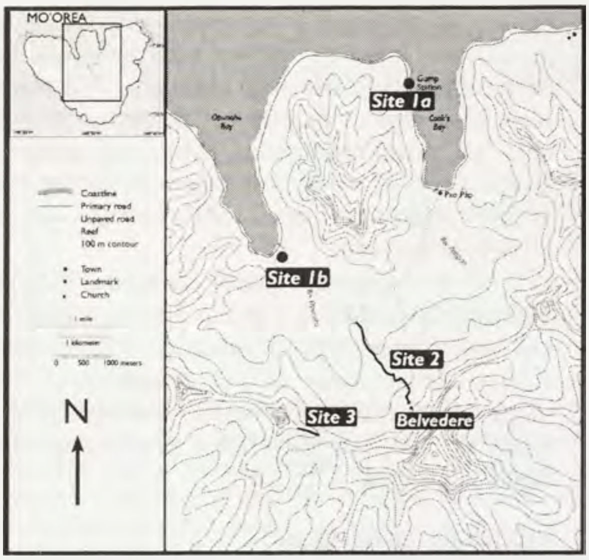

Figure 1. Map of site locations on Moorea. Site 1a: UC Berkeley Gump Research Station, sea level; Site 1b: Opunohu Swamp, sea level; Site 2: Road to Belvedere, 100-180 m; Site 3: 3 Coconut Ridge, 406 m.

day was seen still attached until 11:00am the following day. This attribute made it an ideal specimen for pollination and reproduction studies.

The growth and arrangement of the inflorescence of *H. tiliaceus* was observed at Sites 1a, 2, and 3. The growth of the buds and fruits was diagramed over 24 days.

Reproductive biology

Preliminary removal of stamens on flowers at the Sites 1a and 3 showed that pollen is being brought in from other flowers. This initiated further pollination experiments. Most experiments were conducted at the station site due to blooming time limitations. Many flowers appeared to be initially pollinated before 9:00am.

In order to study the mode of pollination along an elevational gradient, visual observations were conducted along with a series of bagging experiments at sites 1a, 2, and 3. Numerous trials were run between 10/15-11/12/2001 under weather conditions varying in sun, rain, and wind. A total of ten hours was spent at each site watching flowers for potential pollinators. Either one or ten minute intervals were used in observing individual flowers. During this time, and while performing bagging experiments, visiting insect specimens were collected and examined for pollen presence.

Bagging experiments were conducted to look for modes of pollination of *H. tiliaceus*. Flower bagging experiments conducted included removing the stamens and style using a size ten scalpel blade, self-pollination using toothpicks to distribute pollen, wind-force pollination, and using different mesh sizes to eliminate certain sized pollinators. Materials used included square bags with a width of approximately 15cm, more than adequate to house the open blooms without disturbing their normal form. Two types of mesh were used. A finely woven, white nylon material was used to insure that no insects could enter the flowers. This bag type will be referred to as the closed bag, which was needed for self-pollination, destylizing and wind-force pollination experiments. The second material was a gray 2mm mesh, which was large enough to allow small beetles and ants frequently seen on flowers to pass freely and prevent larger potential pollinators from entering. The bags were sewn closed on three of four sides. The fourth side was pinned, then sealed with tape for marking and complete closure.

Bags were placed on flowers between 6-7:00am, usually before the buds had fully opened. Bags with the fallen flowers were collected between 6-9:00pm once the flowers had detached. Although the pollen is visible to the naked eye, the flowers were examined with a microscope in order to get a more accurate pollen count. As the movement in the bags could dislodge pollen, an initial observation of presence or absence of pollen was usually noted just after the bags were removed from the trees.

Chi-square tests were performed to look at the relationship of pollination and bag type, using a p-value ≤ 0.05 showing statistical significance for the bagging experiments. An Excel graph was plotted to show the total percentages of the outcomes of the bagging experiments in Sites 1a, 2 and 3.

During the coarse of the bagging experiments, all fruits were tagged with a day and their corresponding identification number. On the last day of data collection, the fruits from Sites 1a and 2 were collected, measured, and the number of seeds set along with the total number of seeds possible in each fruit were counted if the fruits were old enough and there was a definite difference between the filled and not filled seeds. Fruits from Site 3 were left out of this analysis due to their inaccessibility and small sample number. A site map recorded the location of the experiments for each day. Fruits that were not relocated were assumed dead because when the fruits die, they quickly detach from the tree and the flagging markers were lost.

Seed biology

To look at the potential variation of seed set across an altitutinal range, mature fruits were collected at all four sites. *H. tiliaceus* has dehiscent fruits, meaning that the fruits ripen and open, dispersing seeds, while still connected to the tree. Filled, or fertilized, seeds were counted along with the total possible number of seeds in each fruit. Filled seeds are around 5mm in length and are easy to distinguish from ones that have not been fertilized, which are usually 1-2mm long. The arcsine of the proportion of seeds set to the total number of seeds present was used to normalize the distribution of data. This was analyzed using the program Systat to perform a 1-way analysis of variance to suggest what the pollination rate may be at different elevations.

Two experiment types were used to look at how seeds from sites 1b, 2 and 3 would compare in germination trials using different soil and water types. This was done to test whether significant genetic differentiation in germination ability has taken place along an elevational gradient. These experiments ran for 21 days. Experiment A used seeds from each site, which were each planted in soil from the Three-coconut site, the mid-elevation site, and compost as a control. These flats were watered with fresh water daily. Experiment B consisted of seeds from each site which were planted in Opunohu swamp soil and compost as a control. They were watered daily with estuary water collected near the mouth of the stream entering Opunohu Bay at pk 18. This water was predominately fresh. It's salinity measured 8.5ppt. Five replicates of each combination had been planted for statistical analyses.

Flats used for planting were 20 x 15 x 4 cm in dimension. Approximately 2.5 cm of soil was uniformly distributed in the flats. Fifteen seeds were planted in each flat. A total of 75 flats were planted. Flats were arranged randomly using cards in order to decrease environmental factors on germination rates. They were placed underneath a hibiscus tree at sea level on the Gump Station. A shade cloth was used to cover the flats, which reduced the light by approximately 33 percent. Since 32 seeds germinated out of 1125 planted and some seedlings were eaten by crabs, observations were recorded, but no analyses were performed.

The buoyancy of seeds from sites 1b, 2 and 3 was tested to look at the potential of *H. tiliaceus* seeds from all elevations to disperse across oceans. Mature seeds were collected and placed in cups containing approximately 1.5 cups of sea water, which was changed at least every other day. These trials were run for 14 days from 10/19-11/03/2001. The disturbance of the experiment caused it to be terminated prematurely. The number of seeds sunk was observed daily. Turbulence caused by water changing was minimal, but enough to break the water tension and ensure that the entire surface of the seed was moistened. This was done to look at the potential for genetic differentiation in seed buoyancy along an elevational gradient.

Natural history surveyed

The flowers of H. tiliaceus change color gradually throughout the day from a lemon yellow, to a range of peach-red colors. There is a wide variation in the color change of flowers, some specific to individual trees. Other patterns appear randomly among flowers on the same tree. The majority of blooming buds were already observed around 6:00am to often have thick, red-peach margins on their petals.

To study color change as a result of fertilization, flowers were treated in three ways. They were bagged with the closed mesh bags, destylized, and removed from the tree in early morning prior to pollination. All of these experiments revealed that color change from yellow to peach-red occurs without pollination. Generally, the color change in these flowers was of the same intensity as pollinated flowers from the same tree. Although it was initially suggested that color change may be linked to fertilization, it could still be hypothesized that the color change throughout the day may be some sort of a marker associated with pollination. The variety of color patterns could be further studied to look at patterns in pollination and fertilization.

There are five varieties of H. tiliaceus on Moorea, according to one source (Appendix I). The two ornamental varieties were seen only on the coast, usually associated with home gardens. The other three varieties, "purau ro'a", "purau tahatai", and one with a three pointed-leaf, were seen in a broader distribution. "Purau ro'a" and "purau tahatai" were observed over an elevational gradient from the coast up to 406m.

Results

Inflorescence biology

The growth rate of *H. tiliaceus* was determined to be rapid from observations. Inflorescences that had initially been composed only of buds were completely composed of fruits, and fruits had

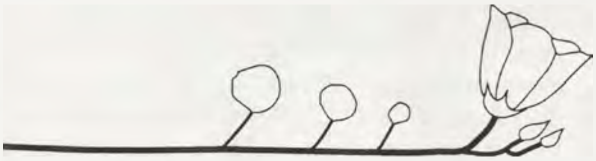

Figure 2. Inflorescence diagram for *H. tiliaceus*. Youngest buds are found on the most distal ends of the branch. The flower would be located near the end of the branch, behind the buds. Progressively older fruits are located towards the trunk of the tree.

Mesh type		***Pollen present***	***Pollen absent***	***p-values***
Closed	OBS	20	8	
Closed	EXP	14	14	0.023
2 mm	OBS	8	4	
2mm	EXP	6	6	0.25

Table 1. Chi-square analysis for bagging experiments from Site 1a, n=40. Statistically significant results seen for closed bag experiments, p=0.023 with $p \leq 0.05$ being significant. This suggests that wind forces may be responsible for pollination. Bagging experiments using 2 mm mesh did not show statistical significance, but pollen presence suggests small insects may be involved in pollination, along with wind forces. The insignificance suggests that larger insects may be responsible for pollination.

been observed to mature and drop their seeds. Fruits were seen to mature in a few weeks time once they reached their approximate maximum size of 2.1 cm long and 1.9 cm wide. This rapid growth rate made *H. tiliaceus* an ideal species to observe in the short duration of this project to gather information about its reproductive development.

The arrangement of the inflorescence is such that the youngest buds develop at the end of the branch. Moving inward toward the trunk of the tree, progressively older fruits are encountered. This is seen in Figure 2.

Reproductive biology

Bagging experiments suggested that *H. tiliaceus* is a generalist in its modes of pollination. This was further supported by visual observations and insect visitor inspection. Table 1 shows, with a p-value of 0.023, that a statistically significant amount of pollen is being deposited on pistils by the end of the day for the closed bag experiments conducted at the coast. This suggests that wind forces may act to displace pollen, which can then fall onto the pistils that lie below. The flowers tend to hang from the trees at an angle of 10° or more, which places the pistils on the end of the style below the stamens that are fused to the shaft of the style. When the pistil is fully opened the stigmas of *H. tiliaceus* do not curl and come into contact with the anthers, as is seen in other hibiscus varieties. Although the chi-square test performed on the 2mm mesh bags for Site 1a did not show that there was a statistically significant amount of pollen being deposited on the pistils at the end of the day, the high presence of pollen suggests that small insects, along with wind, may be responsible for some pollination. This lack of significance for the 2mm bags also suggests that a larger insect may be a primary pollinator.

The sample sizes for Sites 2 and 3 are small, which makes it difficult to infer the size of pollinators based soley on the bagging experiments. However, the presence of pollen was seen in most bagging experiments (Figure 3). This suggests that other pollinators, as well as wind, are potentially present at the higher elevations. In Site 3 there is no presence of pollen from flowers covered with the 2mm bags, but there was the observation of small Nitidulidae beetles that were later found to passively transport pollen within the flowers. The bagging experiment data must be taken into consideration along with the visitors found on the flowers. Numerous visiting insects were collected on *H. tiliaceus*. One species of black bee (Appendix I), which is being identified, was seen pollinating coastal trees at Site 1a. Slides of pollen recovered from the pollen ball carried by the bee and from its pollen-covered abdomen, showed that it was identical to pollen from *H. tiliaceus*. Two species of beetles from the Nitidulidae family (Appendix I) were observed visiting the flowers at Sites 1a and 3. The brown species of these two beetles was also observed at Site 2. They were passively seen to carry pollen all over their bodies while crawling along the style and depositing it on the pistil. Although ants were seen to visit the flowers, attracted to the extra-floral nectaries on the undersides of the leaves and petioles and the nectaries at the base of the style (Peracca 2000), they were never observed transporting pollen. Due to the frequency with which ants are seen within the flowers, it is suspected that they may also passively pollinate. H. tiliaceus was found in the

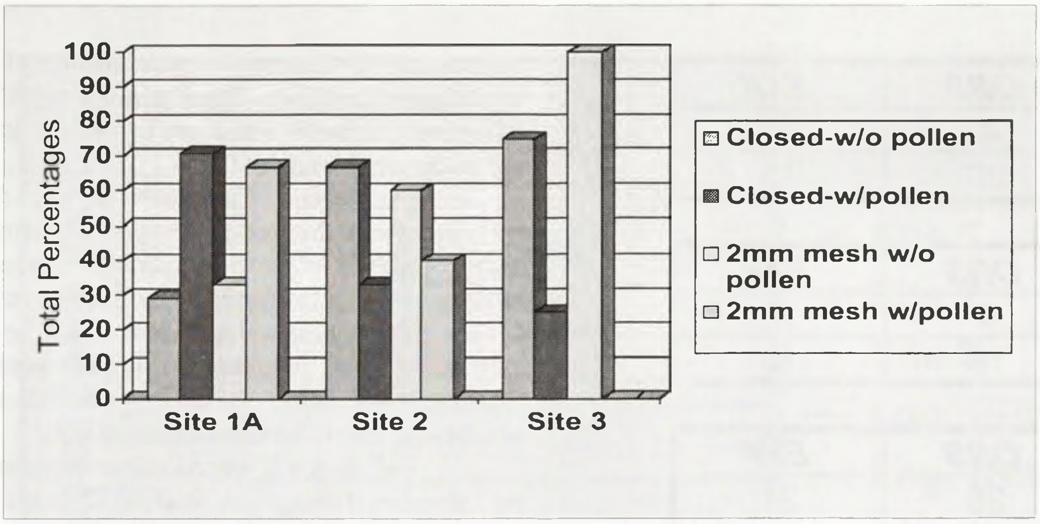

Figure 3. Graph of the total percentages of the results from bagging experiments conducted at Sites 1a, 2, and 3. Site 1a has the largest sample size (n=40), and was analyzed in Table 1. Sites 2 and 3 had samples sizes that were too small for statistical analyses. This graph shows that pollen was present in most cases, except in Site 3, where no pollen was found in the 2 mm mesh bags.

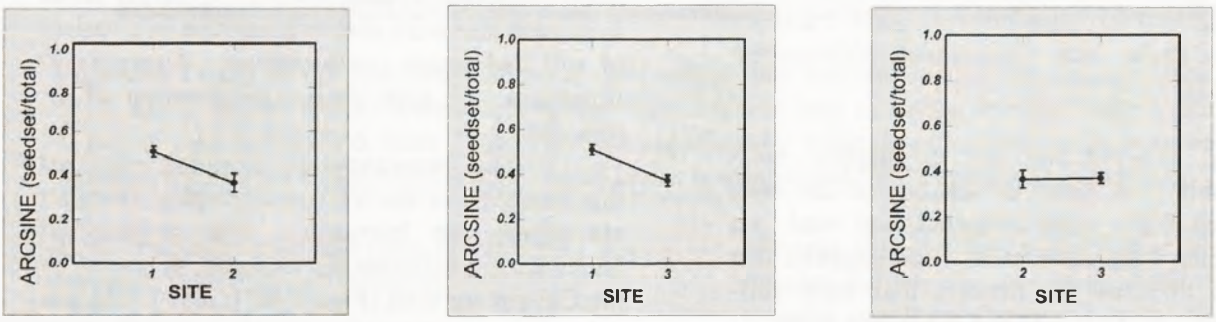

Figure 4. One-way analysis of variants on seed set for general fruits collected from Sites 1 (1a, 1b), 2, and 3. Mode of pollination unknown. Site 1 is compared individually with 2 and 3. Site 1 is found to be significantly different from both Sites 2 and 3 with p values of 0.005 and 0.000, respectively. This shows that seed set for Site 1 is significantly higher than Sites 2 and 3. Sites 2 and 3 are not significantly different from one another (p=0.65). $P \leq 0.05$ is statistically significant.

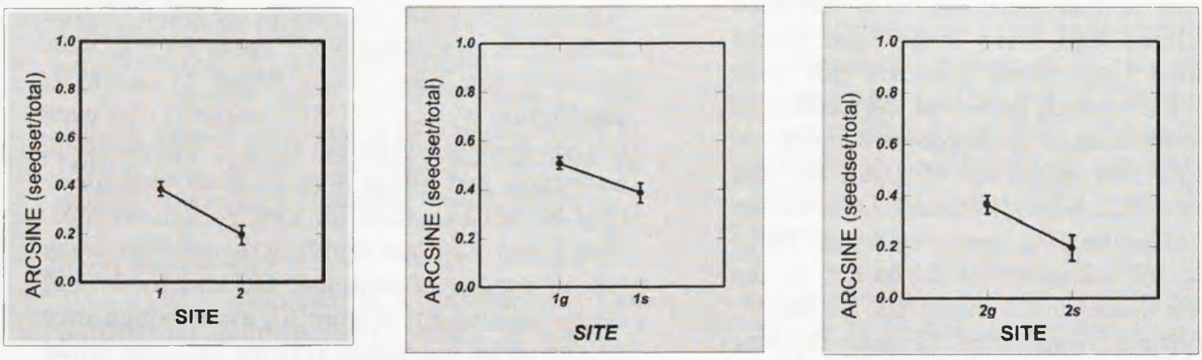

Figure 5. One-way analysis of variance of self-pollinated fruits and comparison of self-pollinated fruits to general fruits from Sites 1a and 2. Mode of pollination unknown for general fruits. Site 1a has a statistically significant higher seed set than Site 2 (p=0.011). In comparing self-pollinated fruits to general fruits, seed set was found to be significantly higher in the general fruits of both Sites 1 (1a, 1b) and 2 (p=0.011).

Sites	OBS	EXP
1	4	15
2	26	15

Sites	OBS	EXP
1	4	10
3	16	10

Sites	OBS	EXP
2	26	21
3	16	21

Figure 6. Chi-squared analysis of seed bouyancy experiments for seeds from Sites 1b, 2, and 3. Seeds sunk were measured. Site 1b shows a statistically significant higher bouyancy than both Sites 2 and 3 ($p=5.9 \times 10^{-9}$ and p=0.007 respectively). Sites 2 and 3 do not show any significant difference (p=0.123).

bagging experiments to be highly self-compatible. A total of 23 out of 29 self-pollinated fruits were collected, and seed set frequencies were calculated. This suggests that close to 80% of the flowers that were self-pollinated were fertilized and developed fruits.

Seed biology

In order to look at the fertilization frequencies of flowers at different elevations, numerous mature fruits were collected and filled seeds were counted along with the total number of seeds, filled and not filled, that were contained in each fruit. Filled seeds are those that have been fertilized and were larger and easily distinguished from those that had not been fertilized. The general fruits that were collected had been pollinated in an unknown manner. A large sample size attempted to adjust for this natural pollination, which assumed that a variety of modes of pollination were occurring. These data show the variation of seeds set at the different elevational sites. Seed set for Site 1, including fruits from Sites 1a and 1b, was significantly higher having p-values of 0.005 and 0.000, in comparison to Sites 2 and 3 (Figure 4). Although significantly lower at Sites 2 and 3, there is still a relatively high frequency of seed set at high elevations. There is not a significant difference between seeds set in Sites 2 and 3, shown by the p-value of 0.65.

The seeds filled within the fruits collected from the self-pollination experiments were compared to those frequencies of seed set in general fruits collected from respective sites (Figure 5). In both comparisons it can be seen that there is a significantly higher seed set frequency in fruits from the coastal sites, whether they were self-pollinated or not.

The data for germination experiments are shown in Table 2. A total of 32 seeds were germinated out of 1125 planted, and more were sprouting when the experiment was terminated. It takes anywhere from five days to at least 21 days for seeds to germinate. The main result of interest in these trails is that seeds from the Three-coconut site are able to germinate in soil from the Opunohu swamp while being watered with the slightly saline water. Overall seeds from each site were able to germinate in a variety of soil and water combinations. Appendix II illustrates the germination progression of H. tiliaceus.

The buoyancy experiments revealed that seeds from the varieties sampled from all elevations are buoyant. The degrees of buoyancy are different for each site, as shown by the Chi-square tests (Figure 6). It was found that there is a statistically significant difference between Site 1 (here 1b), and Sites 2 and 3. These results suggest that seeds from Site 1b are more buoyant than seeds from Sites 2 and 3. Again Sites 2 and 3 did not show any significant difference between one another. This suggests that there may be some difference in the buoyancy abilities of *H. tiliaceus* seeds from different elevations. As seen in the graph (Figure 7) the rates of sinkage of the seeds from all sites follow a log plot, which began to reach an equilibrium by day 17. This suggests that even though there are different rates of buoyancy, at least some percentage of seeds from each site would be able to float for long periods of time. Sites 2 and 3 are not significantly different in the overall number of seeds that had sunk by the end of the experiment (Figure 6), even though they appear so in this graph (Figure 7). The initial rate of bouyancy loss was the steepest for Site 2. Studies of a longer duration, and including a larger sample size, would be able to ascertain

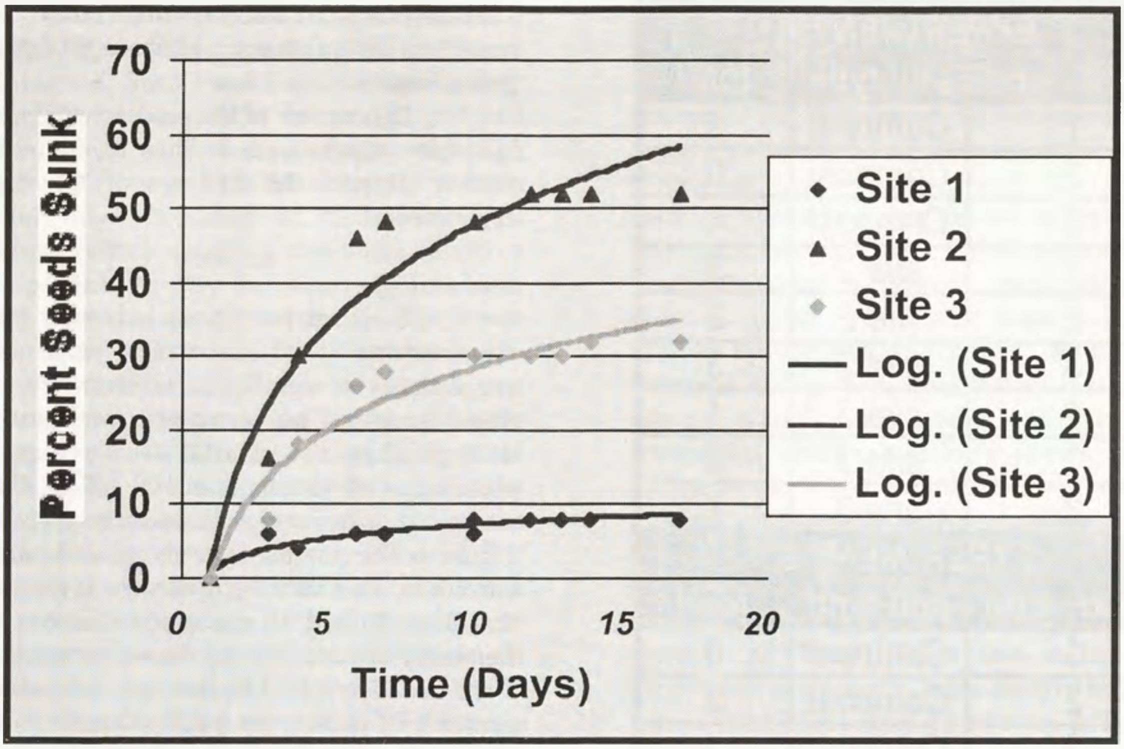

Figure 7. Graph of the percentage of seeds sunk versus time buoyancy experiment using seeds from site 1b, 2, and 3. Site 1b has the lowest sinking rate. Site 2 is seen here as having a higher sinking rate than Site 3, but as seen in Figure 6, Sites 2 and 3 are not significanlty different in the total number of seeds sunk. Sinking rate follows a logarithmic curve. Equilibrium is approached by the end of the experiment.

more precisely what the rates of buoyancy are for seeds from different elevations.

Discussion

Inflorescence biology

The rapid development of the fruits and buds (Table 1) suggest that this tree is fast growing in general. This, along with this tree's ability to self-propagate, have aided its exploitation of a variety of habitats. It also made this species an ideal one to study for different aspects of reproductive development.

Further study could be done on the growth rates of this tree to look at its ability to radiate into new or disturbed environments in comparison to the surrounding vegetation.

Reproductive biology

H. tiliaceus is a generalist in its pollination requirements, which includes a variety of insects and suggested wind forces. The size and open structure of the flower permits a wide variety of organisms to be able to enter the flower. A primary pollinator at the coast is suggested to be a black bee. The black bee is morphologically compatible with the *Hibiscus tilicaeus* flower. Since the flower has a large opening the bee can maneuver inside the flower, usually touching the pistil with the ventral pollen-covered portion of its abdomen. Wind was found to be a statistically significant force, most likely responsible for some self-pollination (Table 1) because the pollen grains are too large to be transported by wind alone. It is suspected that there are other insects that pollinate the flowers. Even though the results of the chi-square analysis on the 2mm bagging experiments at Site 1a were not found to be significant (Table 1), visual observations discovered small insects transporting pollen and pollinating flowers. Other pollinators at Site 1a include the small Nitidulidae beetles, which passively transport pollen on their bodies while crawling within the flowers.

Evidence from the observations and bagging experiments conducted at Sites 2 and 3 (Figure 3), weakly suggest that flowers are being pollinated by small insects and wind forces that disrupt the pollen within an individual flower. The observation of the two Nitidulidae beetles inside flowers at Site 3, and of one of the species at Site 2, shows that they are present and may be responsible for some pollination. Further

EXPERIMENT A

seed type	soil type	total
1	Compost	2
2	Compost	0
3	Compost	4
1	site 2	3
2	site 2	2
3	site 2	3
1	site 3	1
2	site 3	3
3	site 3	7

EXPERIMENT B

seed type	soil type	total
1	Compost	0
2	Compost	2
3	Compost	1
1	site 1b	0
2	site 1b	2
3	site 1b	3

Table 3. Germination experiments for seeds collected from Sites 1b, 2, and 3. Experiment A combined seeds from these three sites with soil from Sites 2 and 3. Compost was used as a control. Fresh water was used for this experiment. Experiment B combined seeds from the three sites with soil from Opunohu Swamp and compost as a control. Estuary water of 8.5 ppt was used in this experiment. Overall, seeds from the three sites germinated in a wide variety of soil and water types. Note especially the germination of seeds from Site 3 in Opunohu Swamp soil watered with saline estuary water.
bagging experiments at these two sites may show some statistical significance.

bagging experiments at these two sites may show some statistical significance.

Data suggest that there is some diversification of the insects that pollinate *H. tiliaceus* along an elevational gradient. The black bee, believed introduced, was seen only at the coast. This does not hold true for the Nitidulidae beetles, one species of which was found in Sites 1a, 2 and 3. Overall *H. tiliaceus* is a generalist in its pollination requirements, which would allow it to reproduce sexually and asexually when arriving on a new island, regardless of the presence or absence of potential pollinators.

Discussion of the seed set analyses and buoyancy experiments at Sites 1a, 2 and 3 will further support the findings of the bagging experiments.

Seed biology

Seed set frequencies recorded from all sites, along with buoyancy experiments, suggested with significant results in most cases that there is an ecotypic variation that distinguishes the coastal trees from those observed at elevations from 100-406 m. Data for seed set at different elevations can be seen in Figure 4. The number of seeds set at the different elevations does vary significantly. It can be seen that Sites 1a and 1b at the coast have a higher frequency of seeds setting than is present at Sites 2 and 3. This could be due to a decrease in the presence of pollinators for *H. tiliaceus* at higher elevations. The black bee observed pollinating *H. tiliaceus* at the coast was not observed at higher elevation sites. It is therefore proposed that this black bee is a primary pollinator for *H. tiliaceus* along the coast. Although there are other insects, including the two Nitidulidae species, that are involved in pollination, it is suggested that these insects are not as successful in pollination. Both beetles were found at the coast site and up at the Three-coconut site. The brown species was also found at Site 2. It is suggested that both species exist along the elevational gradient studied and may offer a small, but potential mode for pollen transportation between flowers. They probably are involved in primarily self-pollination due to the short distances they travel. *Hibiscus moscheutos* is pollinated by a specialist anthophorid bee, *Ptilothrix bombiformis* and by the bumble bee, *Bombus pensylvanicus*. These two pollinators have short flight distances between flowers, which results in a predominately homogeneous mixture of pollen leading to increased inbreeding. Physically isolated populations may as a result become genetically isolated. This indicates that pollen transport within *H. tiliaceus* populations may be limited by the size of the pollinators. Since pollen dispersal may be low in *H. tiliaceus* as seen in *H. moscheutos*, hydrochory may be an important means for gene flow between populations (Kudoh et al. 1997). This will be discussed in relation to seed buoyancy.

Seed set data seen in Figures 4 and 5 show that Site 1 differs significantly from Sites 2 and 3, but that Sites 2 and 3 are not significantly different from one another in both general and self-pollinated fruit comparisons. Although significantly lower at Sites 2 and 3, there is still a relatively high frequency of seed set at high elevations, which suggests that some mode of active pollination may be occurring here even though it was not directly observed. The lower seed set at the higher elevations in comparison to seed set from Sites 1a and 1b also suggests that either there may be fewer pollinators at higher elevations or that there may be some other variable, such as a genetic variation, that has resulted in an overall lower seed set of the ovules. Although it was initially believed that the black bee may be present at Site 2 and 3, due to the presence of a honey bee, yellow wasp, and a smaller black wasp found at all sites, this is not supported by the analyses of the data. It is believed that the two species of Nitidulidae beetles are present at least along the elevational gradient examined, and may be responsible for some pollination.

Self-pollination studies showed *H. tiliaceus* to be highly self-compatible, but the frequency of seeds set was not found to be as high as those fruits that were randomly pollinated. This random pollination, although unknown, is suspected to be a combination of self-pollination and cross-pollination. Comparing the expected seed set and the observed between Sites 1a and 2 shows that there is a significant statistical relationship. Site 1 (1a and 1b) had a higher seed set frequency in both cases.

Low self-pollination rates could possibly be explained by the timing of numerous applications of pollen on a flower's stigmas during a limited time interval. Competition among pollen grains in *H. moscheutos* occurs within the pistil, where pollen grains vie for unfertilized ovules. It is possible in *H. tiliaceus* that a variation in pollen-tube growth rates and in the ability of unfertilized ovules to be located that decreased numbers of seeds set may occur. Even when a load of 100-250 pollen grains applied on the pistils of *H. tiliaceus* in the self-pollination experiments was assumed to ensure full seed set, as seen in the experiments on *H. moscheutos*, this may not have resulted in the full seed set potential of the self-pollinated fruits in Sites 1a and 2 (Spira 1996).

Since the seed set frequencies found at Site 2 comparing the general fruits with those that had been self-pollinated (Figure 5) showed a significantly lower rate for the self-pollinated fruits, it is suggested that a larger, active pollinator may be present. If the flowers at Site 2 were primarily being self-pollinated by wind forces or small insects, then the seed set comparisons of the general and self-pollinated fruits may not have been significantly different. Competition of a flower's own pollen with outcross pollen can result in faster pollen tube growth by the outcross pollen, which would decrease selfing rates when pollen loads of this mixture is applied at the same time. This, along with other studies that have shown outcross pollen to be more successful in producing a higher seed set in self-compatible species, support the idea that a large insect may be cross-pollinating flowers at the higher elevations. But pollen tube competition does not appear to be a general mechanism that can enhance the proportion of progeny from outcrossing in *H. moscheutos*. The data on relative pollen tube growth rates that support this conclusion suggest that the pollen tube competition between self and outcross pollen is highly variable among individuals. Some plants may be more prone to selfing than others when they are exposed to both self and outcross pollen. One explanation may be that genetic and/or environmental factors are responsible for the differences in pollen competitive ability. Another explanation suggests that some plants have more vigorous pollen, which is highly competitive whether it is involved in selfing or outcrossing (Spira et al. 1993). Previous research conducted by Snow and Spira (1991a, b) and Spira et al. (1992) demonstrated that pollen-tube competition is present in *H. moscheutos*, and that the success of fertilization is influenced by pollen tube growth rates that differ for mixed pollen (self and outcross) applied to the stigmas at the ends of the pistil. These studies offer possible suggestions for the variation seen in fruits that had been pollinated by an unknown mode or self-pollinated.

Buoyancy experiments also suggest an elevational difference between the coastal trees and trees at higher elevations. It was initially suspected that seeds from Site 3 would be the least buoyant. It is seen in Figure 7 that Site 2 had a higher rate of sinkage of seeds than Site 3, but Figure 6 shows that the final number of seeds sunk is not significantly different between Sites 2 and 3. The important data is shown in the significant difference between Site 1 and Sites 2 and 3 as seen in Figures 6 and 7. These data suggest that there is some ecotypic variation in trees at the coast in comparison to their phenotypic counterparts in higher elevations. This variation may be due to some genetic differences that have occurred as a result of living in different habitats and elevations or have been selected for and allowed them to exploit other environments.

H. tiliaceus is one of many pantropical coastal plants that produces buoyant disseminules. In one study a group of H. tiliaceus seeds and fruits were floated in sea water. Although some of the seeds began to sink, a number of disseminules remained floating for two or three months (Nakanishi). Even during the two week period that the seeds for this study were observed, a large proportion of at least 50% remained afloat. The morphological characteristics that allow these seeds to be buoyant include air spaces present between the folds of the cotyledons, the hypodermis is two-layered, where as it is usually one layer (Kumar 1990). According to other sources, "the seeds are buoyant and resistant to salt water hence the species is widely distributed throughout the tropics along coasts worldwide" (Anonymous 1998). Seeds from all sites were found to be buoyant and in sea water, so it can be suggested that this species can easily disperse throughout the tropics.

Germination studies conducted for this project showed that high-elevation trees produce seeds that are capable of germinating in saline, coastal conditions. Studies have shown that *H. tiliaceus* has a high tolerance for saline conditions, even when transplanted from higher elevations to moderately saline environments. However, the trade off for this adaptation is in the photosynthetic ability of the plant. Although variation in photosynthesis and growth rates are seen in upland *H. tiliaceus* when exposed to saline conditions, there are compensatory mechanisms in the coastal trees that result in no net difference in growth rates when trees grown in their normal environments are compared (Santiago 2000). A significant number of H. tiliaceus seeds germinated in a study of seeds that had been subjected to artificial sea water (Nakanishi 1988). The data concerning the germination of high-elevation seeds in swamp soil while being watered with saline water support the adaptability of H. tiliaceus varieties to saline conditions. The seeds were found to tolerate water that was saline to at least 8.5ppt. Although much lower than the salinity of 35ppt of Cook's Bay, next to Site 1a, this supports the possibility for migration of this species, even of mountain varieties, across the ocean. Future buoyancy studies over longer periods of time, followed by viability tests through germination would be another way to measure the seed salinity tolerances of *H. tiliaceus*.

Future genetic analyses along elevational gradient could be incorporated with the reproductive and seed biology studies suggested to form a more complete picture of the distribution of *H. tiliaceus* varieties on Moorea.

Conclusion

This study of *H. tiliaceus* suggests that there are ecotypic differences in this species along an elevational gradient on Moorea. This conclusion is supported by significant evidence found in seed set and buoyancy experiments. Along with the differences in these experiments, it was shown through germination studies that the trees at varying elevations are capable of germinating in a variety of soil types and able to withstand slightly saline estuary water. This suggests that although there may be some potential genetic variations in these ecotypes, that it is not rigid. The species as a whole has remained plastic, which would allow trees from any elevation, given enough of a chance, to be able to disperse seeds through ocean currents to distant shores where they would be able to germinate and establish themselves. This study demonstrates how island plants that disperse by water have been able to radiate to different islands. H. tiliaceus is an abundant tree and highly exploits new and disturbed land as observed during this study and discovered in discussion with locals. It is not apparently in danger of declining populations, but this study can help us to appreciate and understand the dynamics of water-dispersion, exploitation and adaptation of a species to a wide range of habitats on Moorea and across the Pacific islands.

Acknowledgements

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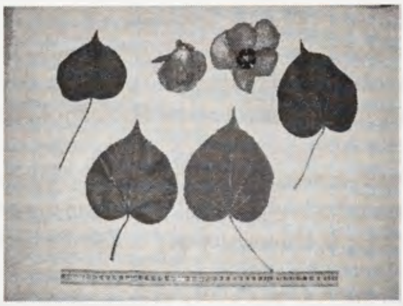
APPENDIX I

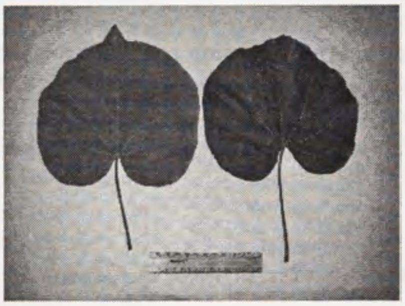
According to Petard, Brown distinguishes three botanical varieties of *H. tiliaceus*: *H. tiliaceus tiliaceus, H. tiliaceus henryanus,* and *H. tilicaceus sterilis.* Within the variety *H. tiliaceus tiliaceus,* the Tahitians recognize four sub-varieties: Purau Ahue, Purau Hiva, Purau Opi'Opi, and Purau Maohi. (Purau is the Tahitian word for *H. tiliaceus).* (Petard 1986) Five varieties of H. tiliaceus were described to be on Moorea, according to one local source.

Deposited in the Herbarium at UC Berkeley:

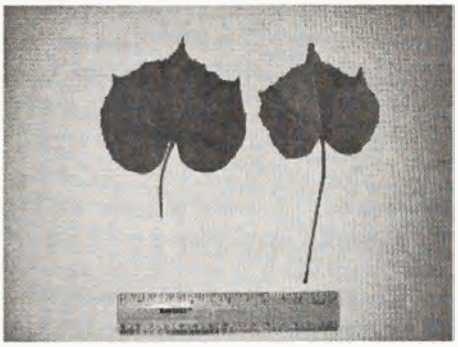
1. MMKF #1: "Purau ro'a" is known as the mountain hibiscus. It has a small leaf, with rounded margins. The tree is primarily used for its wood, which has a hard, black heartwood. According to Brown's (1935) descriptions of leaf, wood, and habitat, this variety is believed to be *Hibiscus tiliaceus sterilis*



2. MMKF #2: "Purau tahatai" is a water-loving tree, usually found growing along the shores of the ocean and streams. It has a large, floppy leaf with rounded margins. The wood is pale brown, and its lightness allows it to be used in making the outrigger portion of a canoe or anything that needs to be buoyant. This variety has many medicinal uses and the bark is used for making the traditional grass skirts. Based on Brown's (1935) descriptions of leaf, wood, and habitat, this variety is believed to be either *H. tiliaceus typicus* or *H. tiliaceus Henryanus*.



3. MMKF #3: A third variety has leaves with three pointed edges, which grows in association with MMKF #1 and MMKF #2. The leaves are thick and rough, like sand-paper. These first three varieties commonly grow together. According to Brown (1935) Nadeaud distinguishes this variety as a variety of *H. tiliaceus sterilis* known as *Paritium trilobatum*. *Paritium* is the older name for the hibiscus genus. Known as "fau rau maire" in Tahitian.



4. MMKF #4: Decorative variety, no Tahitian name given, not matched with any scientific description. Observed along the coastal strand or in gardens in Pao Pao and Opunohu valleys. Uniformly green leaves on same tree as multi-colored leaves of white, red and green splotches. Suggested variety of *H. tiliaceus tiliaceus*.

5. MMKF #5: Decorative variety, no Tahitian name given, not matched with any scientific description. Observed along the coastal strand or in gardens in Pao Pao and Opunohu valleys. Narrower, elongate, uniformly colored leaves that are darker green and red. Suggested variety of *H. tiliaceus tiliaceus*.

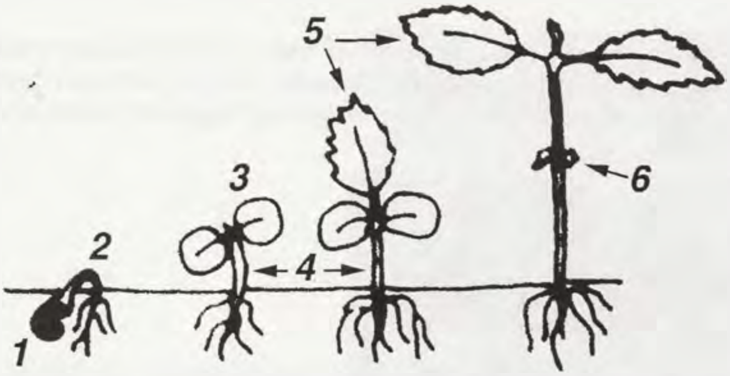
Deposited in the Entomology collection at UC Berkeley:

6. MMKF #6: Black bee observed pollinating a *H. tiliaceus* tree at Site 1a, collected on 10/28/200, next to coast road just south of the UC Berkeley Gump Research Station.

7. MMKF #7: Nitidulidae beetle (brown species) collected while inside *H. tiliaceus* flower at Site 1a on 10/19/2001 m.

8. MMKF #8: Nitidulidae beetle (black species) collected while inside *H. tiliaceus* flower at Site 1a on 10/19/2001.

APPENDIX II



1. seed coat
2. hypocotyl
3. cotyledons
4. hypocotyl
5. foliage leaves
6. withered cotyledons

Illustration of the germination process of H. tiliaceus as observed from numerous seedlings during germination experiments. Seed germination in Hibiscus tiliaceus is epigeous. The cotyledons are carried above ground by the elongating hypocotyl.

Factors affecting the distribution of an ethnobotanical resource, *Thespesia populnea* (Malvaceae) on Moorea, French Polynesia

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ABSTRACT. Populations of *Thespesia populnea*, a culturally and economically important coastal strand tree on Moorea, French Polynesia, appear to be declining. Because of its numerous traditional medical and functional uses, it is important to examine what factors may be contributing to its declining population. To evaluate the level of the decline, this study maps the distribution of the tree in Cook's Bay, a developed area and the less impacted Opunohu Bay. It also tests several variables which may be affecting *Thespesia. populnea's* distribution including: salt water buoyancy, optimal germination conditions, herbivory by burrowing land crabs, and anthropogenic shoreline modification. A questionnaire on the ethnobotanical uses and natural history of *Thespesia populnea* was distributed to local Tahitians. This study shows that distribution of trees in the two bays was not equal and could not be predicted easily. *Thespesia populnea* had relatively high dispersal potential, was able to germinate in multiple soil and light treatments, and was not threatened by herbivory from land crabs. Shoreline modification, resulting in land and ecosystem fragmentation and human harvesting was found to most affect *Thespesia. populnea's* distribution. Further study of both, human consumption of *Thespesia. populnea*, and shoreline modification on Moorea are recommended.

Introduction

Throughout Pacific Island archipelagos, floral species have been vital to island peoples over the course of history. Floral species have been colonizing the island archipelagos since their volcanic formation in the Pacific Ocean. Additionally, over the course of human occupation of these islands, the plant assemblages have been altered significantly with the introduction of new species and changes in the landscape. In Polynesia, species are described in records of traditional plant uses (Whistler 1992).

Since European contact with Pacific Island cultures, plant use has changed significantly. Though much traditional plant use has changed since European contact, some knowledge persists within Polynesian cultures. Thus, it is critical to preserve this knowledge in written form. Furthermore, only a small percentage of Polynesian plants have been analyzed scientifically, while others have yet to be described in Western scientific literature. Thus, work on such species is pertinent, particularly in cases of declining plant populations.

The coastal tree *Thespesia populnea*, of the Malvaceae family (Mueller-Dombois 1998), known as *miro* or *amae* in Tahitian, may be among those ethnobotanical plants that appear to have a declining population (Welsh 1998). Little is known about its biology.

The family Malvaceae is large and widely distributed throughout the world (Brown 1931) except for arctic regions (Hillebrand 1888.) The native distribution of *Thespesia populnea* ranges from East Africa to Eastern Polynesia. Welsh (1998) and Petard (1986) consider it a possible Polynesian introduction, however, Whistler (1992) cites it as indigenous. Considered a component of mangrove communities, Thespesia populnea is widely distributed in the Society Islands (Welsh, 1998). It is usually found along the coastal strand and littoral forest (Merrill 1945), with smaller populations in the high elevations of the high islands (Matarau, pers. com.) According to Fosberg (1996), the tree is found on the islands of Tahiti, Tetiaroa (seedlings only), Moorea, Huahine, Raiatea, Tahaa, Bora Bora, Maupiti, and Tupai.

Historically, Tahitians considered *Thespesia populnea* sacred, and as such was planted around *maraes*, temple sites (Petard 1986). The wood was used to make *toares*, ceremonial drums, *umetes*, bowls used for grinding medicine or serving, *tikis*, idols, and canoes for priests (Chabouis 1971), and their paddles, harpoon handles and the cross pieces in pirogues (Petard 1986.)

Traditional uses of plant species vary over their geographic distribution and over time. However, *Thespesia populnea* is an important wood source thoughout its distribution due to its decay resistance, durability, hardness, and aesthetic value (Whistler 1992). The tree is also valued for its tannin, dye, oil, and gum (Neal 1965).

In addition to *T. populnea*'s traditional uses, it has also been found to have bioactivity in several of its structures (Nagappa 2001; Kavimani et al. 1999; Inbaraj et al. 1999; Hewage et al. 1998; Shirwaikar et al. 1995). This, increases the importance of assessing the population and studying its biology.

Increasing development is widely known to have affects on the surrounding ecosystem. However, islands are special case due to their increased sensitivity as a result of isolation (Whittaker 1998.)

The study was conducted during October and November 2001 on the island of Moorea in the Society Island Archipelago of French Polynesia at the University of California at the Richard B. Gump Biological Research Station.

The goal of this research was to increase baseline information about the distribution, germination biology, and human uses of *Thespesia populnea* in French Polynesia. The primary objectives were to determine 1) how *Thespesia populnea* was distributed in Cook's Bay and Opunohu Bay, and 2) suggest possible factors that are structuring the distribution. Secondarily, four variables of distribution were tested. These were (a) optimal conditions for *Thespesia populnea* germination, (b) salt-water buoyancy, (c) herbivory by burrowing land crabs, and (d) human use. A more thorough understanding of *Thespesia populnea* in French Polynesia, and the factors affecting its distribution on Moorea would improve our understanding of the population status and may help establish conservation priorities for it where needed.

Methods

Study Sites

The study sites were in Cook's Bay and Opunohu Bay on the high island of Moorea (S°17 30', W149° 50'), in the Society Archipelago in French Polynesia. Both bays are located on the north side of the island and are situated on the north-south axis (Figure 1).

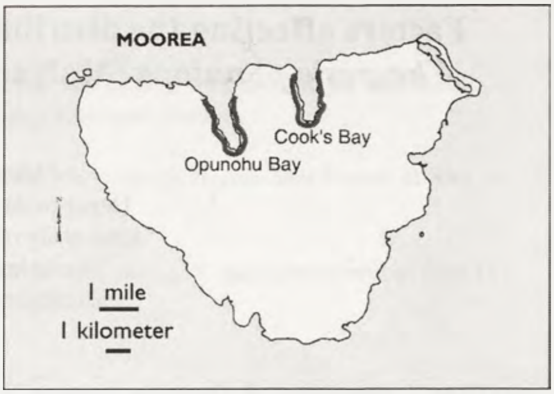

Figure 1. Map of Moorea, French Polynesia, showing *Thespesia populnea* distribution study sites, Opunohu Bay (approximately 6.5 km) and Cook's Bay (approximately 5 km.)

Distribution in Opunohu Bay and Cook's Bay

The distribution of *T. populnea* trees was mapped along the coast of Opunohu Bay and Cook's Bay (Figure 1) to establish population size and structure in conjunction with shoreline status. Mapping was conducted using two methods: walking the perimeter road and kayaking the perimeter. The Cook's Bay site was mapped form PK 7 to PK 12 (5 kilometers) and the Opunohu Bay site was mapped from PK 15 to PK 21 (6 kilometers). Morphological data on the tree circumference was measured at 40 cm from the base using a standard metric tape measure and tree height was measured using a Suunto optical reading clinometer (model PM-5/S PC). Data on the soil type, associated flora within a 3-meter circumference of the tree base was collected, as well as the adjacent shoreline environment that each individual tree was found in. The soil and shoreline types were classified into three types and four types, respectively (Table 1.)

Shoreline Mapping

Shoreline mapping was conducted to assess the ratio of shoreline categories in conjunction with the number of *T. populnea* individuals in each category. Mapping of the shoreline was conducted by kayak using a Garmin eTrex Global Positioning System (GPS). The kayak was rowed along the perimeter of each bay to mark waypoints on the GPS as changes in the shoreline were observed. Each shoreline classification found in Table 1. was further divided into a "managed" or "unmanaged" category.

Table 1. A classification of soil types and shoreline types found in the *Thespesia populnea* distribution study conducted on the perimeter of Opunohu Bay and Cook's Bay, Moorea, French Polynesia.

Soil Types	Shoreline types
Sandy soil	Sandy Beach
Basalt/coral soil	Rocky Beach
Forest soil	Broken wall
	Intact wall

Germination Tests

Germination tests were conducted to study optimal seed recruitment conditions for *T. populnea* in its coastal habitat. The germination tests were conducted in three different soil types and in three different light conditions, each with a corresponding control. The soils tested for optimal growing conditions were sand (type 1), basalt/coral rubble soil (type 2), and a forest clay (type 3), which were collected from sites where *T. populnea* shoots were found growing. Nine replicates of each soil type were exposed to one of the three light conditions for 33 days. Each replicate consisted of a 22x12 cm plastic tray filled with soil and planted with eight *T. populnea* seeds collected from two adjacent trees at PK 16 in Opunohu Bay.

The light conditions tested were full sun, partial sun, and full shade. Light exposure was measured using an Extech Instruments light meter (model 401025), with Lux setting on "50000" and Foot-candle (Fc) set on "fast." Full sun replicates were exposed to full daylight from sunrise to sunset. The partial sun replicates were covered with one layer of green shade cloth over one layer of white shade cloth to give a light meter reading of 740 luxes, approximately half the reading of the full sun light reading of 1459 luxes measured with no cloud cover. The full sun and partial sun light replicates were watered two times daily, and the full shade replicates were watered once per day to prevent seed rot, except when precipitation eliminated the need for watering. The germination tests ran for 33 days.

Salt Water Buoyancy Test

Salt water buoyancy tests of *T. populnea* seeds and seed pod disseminules were conducted to study dispersal potential in an aqueous medium. *T. populnea* seeds, broken seed pods, and intact seed pods were floated in a 35x40x50 cm Plexiglass circulated aquarium filled with salt water for 18 days (403 hours). The tank was divided in three equal chambers by verticaly fixing two pieces of plastic netting to the aquarium sides. Chamber 1 held 100 seeds, chamber 2 held 15 broken seed pods, including seeds, and chamber 3 held 15 intact seed pods. The aquarium was monitored daily to note changes in buoyancy.

Land Crab Herbivory Test

Herbivory on *T. populnea* sprouts by coastal burrowing land crabs (*Cardisoma carnifex*) was studied to assess herbivory as a factor influencing distribution. Burrowing land crabs were placed in 45x40x70 cm buckets filled with two cups of soil and three *T. populnea* shoots for seven days. The crabs were given 1 cup of water daily and were not provided with an alternate food source. The buckets were placed in a shaded environment with natural air circulation.

Ethnobotanical Questionnaire

The questionnaire on the uses of *T. populnea* was distributed to Tahitian people living on the island of Moorea to study its management by humans. An interpreter translated the questions into French and Tahitian. The questionnaire was posed to nine households on the island (Figure 2.) Interviews were conducted at the homes of local Tahitians with the eldest member(s) of the household or those who volunteered themselves

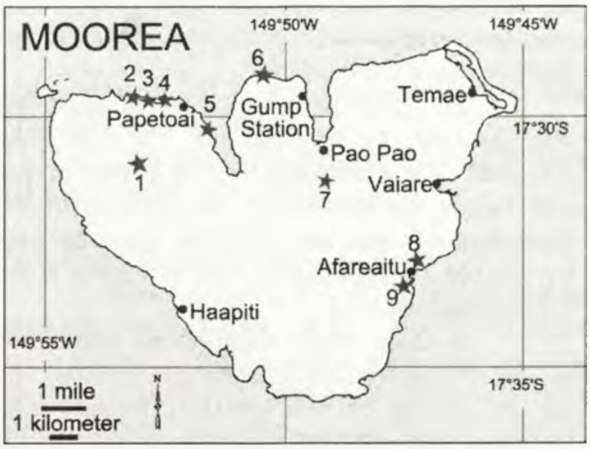

Figure 2. Map of Moorea, French Polynesia, showing the locations of collaborators' homes (stars = locations) where interviews were conducted. See Appendix B for the collaborators' names, home location, and date of interview.

as having the most knowledge about *T. populnea.* See Appendix A for questionnaire format. The collaborators' names, home location, and the date they were interviewed can be found in Appendix B.

Statistical Analyses

Single-factor ANOVA tests generated in the program Systat were used to test for relationship between population structure and abiotic soil type and shoreline type factors. Chi-squared test generated in the program Microsoft Excel was used to test for independence of each shoreline type with the bay. Log linear model multi-way contingency tests generated in the program Systat were used to test the interaction of shoreline type and bay in explaining the abundance of *T. populnea*.

Results

Distribution in Opunohu Bay and Cook's Bay

The distribution of *T. populnea* trees was mapped along the coast of Opunohu Bay and Cook's Bay to establish population size and structure in conjunction with shoreline status.

A comparison of the population size of *T. populnea* in Cook's Bay and Opunohu Bay showed four times as many *T. populnea* individuals in Opunohu Bay. However, the Cook's Bay site measured 5006 meters (~5 km), while the Opunohu Bay site measured 6483 meters (~6.5km). Accounting for the difference in distance of the two bays, without considering the correlation with shoreline type ratios, there may be closer to three times as many trees per distance along the shoreline.

The population structure of each bay was analyzed by single-factor ANOVA tests. The tests were performed on tree circumference and height measurements in relation to the associated soil type and shoreline types for each bay (Table 2). Significance was measured at the 0.05 confidence level.

In Cook's Bay, significance was found for the soil type-circumference test ($p=0.04$) and the soil type-height test ($p=0.04$). Results from Opunohu Bay showed significance only in the soil type-height test ($p<0.001$).

Human Use. Evidence of human use was found during the distribution study in the form of cuttings made to the bark or branches of *T. populnea.* In Cook's Bay, 13.5% of the trees showed branch cuttings and 2.2% had bark cuttings. In Opunohu Bay, these percentages were higher at 31.4% and 2.5%, respectively.

Shoreline Mapping

Geographic Information Systems (GIS) software was used to map the shoreline into segments of distinct types in Opunohu and Cook's Bays (Figure 4). Chi-squared tests were performed for both bays on the frequency of trees within each shoreline type and were significant (Cook's Bay, $p<<0.01$ and Opunohu Bay, $p<<0.01$). A graphic model of the tree frequency per 100 meters vs. shoreline type shows more total trees per shoreline type in Opunohu Bay than in Cook's Bay (Figure 3). In addition, a log linear model multi-way contingency test of shoreline type and bay was performed to explain the abundance of trees ($X^2 = 1181$, $df = 15$, $p<0.0001$). Where there was no shoreline type, these cells were specified as "structural zeros" in the model. With this interaction term in the model, the model explained significantly more variation in the data than without this term ($X^2 = 13.02$, $df = 5$, $p<0.0232$.)

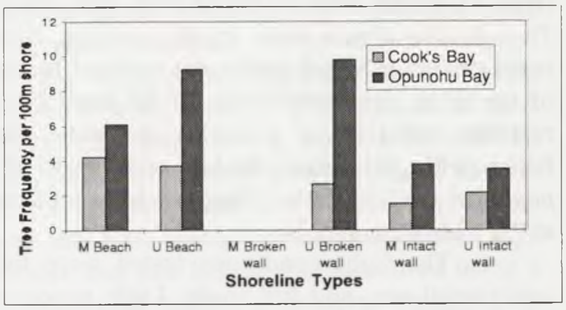

Figure 3. Histogram comparing the frequency of *Thespesia populnea* per 100 meters of shoreline with the shoreline types in Cook's Bay and Opunohu Bay, Moorea, French Polynesia. Shoreline classifications: M-managed shoreline, U-unmanaged shoreline.

Table 2. A comparison of P-values for multiple variables measured in Cook's Bay and Opunohu Bay performed in a single-factor Anova tests. Legend: s - soil type, sh - shoreline type, c - tree circumference (cm), h – height (m), r – ratio of soil types/ shoreline types.

P-vaules	s-c	s-h	sh-c	sh-h	r-c	r-h
Cook's Bay	.04	.04	.30	.17	.33	.32
Opunohu Bay	.32	.00	.37	.06	.64	.64

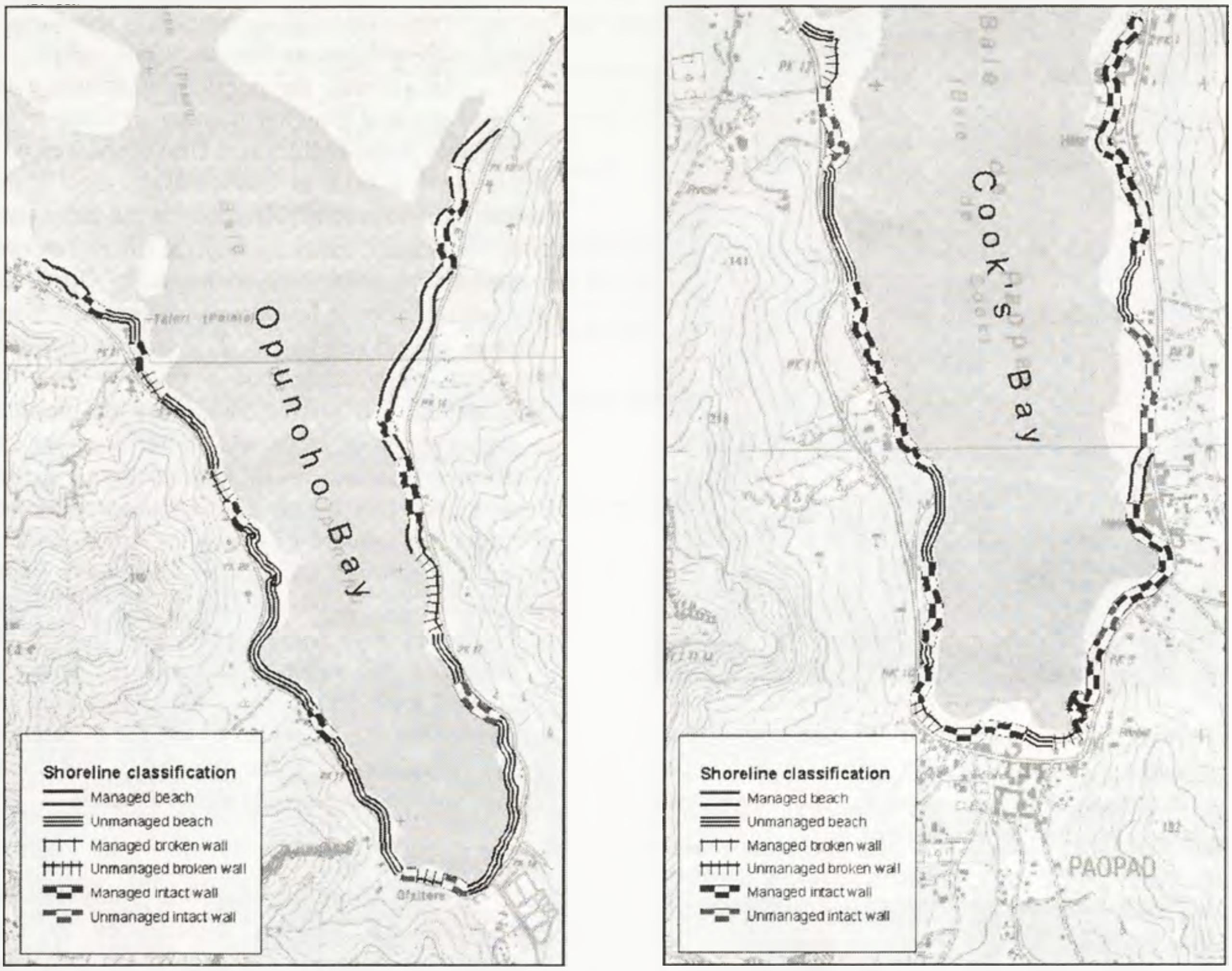

Figure 4. Maps of Opunohu Bay and Cook's Bay, Moorea, French Polynesia. The shoreline is classified into categories based on the type of shorelines and management status. Base map from E. Edlund, University of Montana GIS Dept. Used with permission.

Germination Tests

Results of the germination tests showed seed germination in all soil types over the 33 day test period.

Partial light replicates showed seed sprouts germinating at 15 days in the forest soil (type 3). In total, 12 seeds sprouted and had an average growth rate of 0.53 cm/day with an average height of 5.58 cm by the end of the test. At 28 days, seeds began to germinate in the basalt/coral soil (type 2) (Figure 5.) By day 33, there were 3 sprouts in 3 different replicates of basalt/coral soil. At 31 days, one seed sprouted in sandy soil (type1) and had a final height of 1.5 cm by the end of the test period.

Full shade replicates showed 2 sprouts in 2 replicates of basalt / coral soil on days 26 and 27. Their average growth rate was 1.18 cm per day.

Full sun replicates showed no sprouts for the duration of the test.

Salt Water Buoyancy Test

The results of the salt water buoyancy tests showed that after 18 days 96 of 100 seeds were floating. Broken seeds pods showed 46% floating, and 60% of intact seed pods were still floating over the duration of the test period (Figure 6). A Chi-squared test on the number of seeds, broken pods, and intact seed pods still floating over the duration of the test period yielded a p-value of 0.006 which shows a significant difference at the 0.01 significance level.

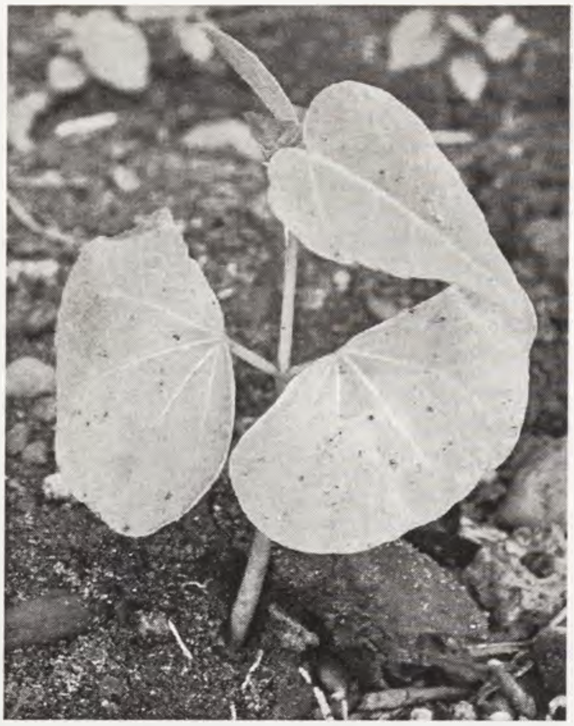

Figure 5. Young shoot of *Thespesia populnea*, growing in basalt/coral soil, approximately eight centimeters in height, showing the cotyledons (below) and first leaves (above).

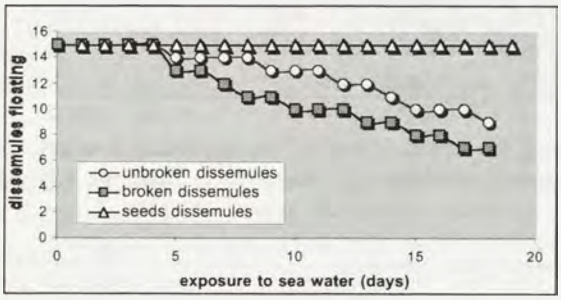

Figure 6. Salt water bouyancy test of *Thespesia populnea* seed pod dissemules. Seeds, broken pods, and unbroken pod dissemules showing differing floatation potential

Land Crab Herbivory Test

The test for herbivory by burrowing land crabs (*Cardisoma carnifex*) showed that *T. populnea* shoots were not eaten by the 5 crabs over the 7 day test period. All the land crabs died shortly after the 7 day test period.

Ethnobotanical Questionnaire

Results of the questionnaire are summarized in terms of (I.) percent "yes," answers from collaborators, and (II.) percent concurrence with the questions asked (Table 3.)

Discussion

Distribution in Opunohu Bay and Cook's Bay

In general, the population structure in the bays showed a higher number of large trees Differences in the smaller size classes showed no Opunohu Bay than in Cook's Bay. significant results. This structural difference in the large size category is most likely a result of the difference in development in the two bays. In Opunohu Bay, where there is less development, and more Tahitian owned residences, *T. populnea* is more abundant, suggesting that a relative lack of development and private ownership are needed to preserve older trees. A higher number of vacation residences, hotels and markets exist in Cook's Bay (Harbaugh 2000), which may be affecting the amount of available space. Older, larger *T. populnea* individuals may have been removed to accommodate encroaching development. As a consequence, new recruits of *T. populnea* and other strand species may be competing with development for space; thus, in the future, we should expect to see the difference in the populations in the two bays and their relative size classes become more disparate.

Shoreline Mapping

The shoreline mapping study shows that there are striking differences in the frequency of trees per 100 meters in each bay (Figure 3). The number of *T. populnea* in Opunohu Bay is consistently higher in every shoreline category except "managed broken wall," in which there were no trees observed in this category in either bay. As discussed above, this is likely due to the relative amounts of development in each bay; however, in this case, , development will be strictly defined by shoreline types. Development can be viewed on a continuum in which "beach" categories are the least developed, "broken wall" is more developed, and "intact wall" is the most developed type of shoreline. Further definition can be made in each of these categories by dividing them into "managed" and "unmanaged" categories, where "managed" shoreline would be found in front of residences or other development, and "unmanaged" shoreline may be on public property or deserted plots. Therefore, "unmanaged beach" would be considered the least developed type of shoreline and "managed intact wall" would be the most developed.

Table 3. Adaption of the questionnarie distributed on Moorea, French Polynesia. The answers are presented in percentages in terms of (I.) "yes" or "no" responses, and (II.) percent agreement of answer.

I. Question	**Percent "yes" answers**
1) Is *Thespesia populnea* growing near here?	1) 66%
2) Was it planted there? 2a) Or did sprout alone?	2) 33% 2a) 66%
3) Do you ever harvest *Thespesia populnea*?	3) 100%
4) Are there different varieties of *Thespesia populnea*?	4) 22%
II. Question followed by the most common answer	**Percent concurrence**
5) What is the average life span of *Thespesia populnea*? Greater than 100 years.	5) 88%
6) How much has the number of trees changed since you were a kid? The number has decreased by 95%.	6) 66%

Based on the delineations above, the most notable difference between the two bays is in the "unmanaged beach" category, in which the population of Opunohu Bay is over four times that of Cook's Bay. Another notable difference is between "beach" shoreline, both "managed" and "unmanaged", and "broken wall" and "intact wall" shoreline types, where in both bays there are more trees in both "beach" categories than in other, more developed shoreline types. In addition, the "intact wall" category has the lowest number of trees per shoreline type. This result suggests sea-dispersed seeds or that seed pods have a higher seedling recruitment in "beach" shoreline segments, and a much lower recruitment in " intact wall" shoreline segments. This may be explained by the fact that in geographic localities where the tidal flux is small, such as Moorea, sea–dispersed seeds may need large wave action to land seeds in suitable germination conditions. However, in areas of shoreline that have been modified by sea barricades like the walls observed along the two bays, only the largest wave action would be sufficient to land seeds in potentially suitable germination conditions.

Sea walls can be viewed as a type of landscape fragmentation which occurs when a naturally joined ecosystem such as that of the shore becomes split into ocean and the beach sections (Sanders et al. 1991.) This type of modification alters the dynamics of the ecosystem and is essentially a disruption of existing ecological connections (Jochen 2000.)

The shoreline mapping study shows strong evidence to support that the distribution of *T. populnea* is affected by shoreline type and by management regime.

The GIS map of shoreline types in Cook's Bay and Opunohu Bay will help guide further study of coastal *T. populnea* populations.

Germination Tests

The results of the treatments in three different soil types and in three different light conditions indicated that the relative combined optimal growing conditions for *T. populnea* are in forest soil with a partial light source. This may be due to the combined factors of the soil's water retention capacity and the constant moisture level held in the partial shade environment. The combination of these factors also may have provided a suitable temperature and pH for germination.

Although some germination occurred in basalt/coral soil and in sandy soil in the partial sun treatment, it occurred much later in the test period and yielded much lower seedling counts. However, if this test had been run for a longer period, the seedling count in all the soil treatments, and perhaps all light treatments may have been higher.

Seedling germination in the full light treatment did not occur over the course of the experiment. A possible explanation is that the soils in the full sun treatment dried out too rapidly, thus, there was not enough moisture available to induce germination. Despite the absence of seedling germination in the full sun treatment, observations in the field showed that germination was possible at this light intensity.

Salt Water Buoyancy Test

Salt water buoyancy tests indicate that seeds, broken seed pods, and intact seed pod disseminules are not equally buoyant. However, all had 46% or greater buoyancy at the end of the 18 day period, suggesting that *T. populnea* may be adapted for sea-dispersal. Support for this idea could be tested by salt water immersion tests of seeds to study viability. However, the fact that *T. populnea* is primarily a mangrove tree, which are know for their adaptation to saline or brackish environments, suggests that the tree has a high salt tolerance.

Although this study did not test viability of seeds after salt water immersion, a study conducted by Nakanishi (1988) showed that germination increased on average after exposure to salt water for one to three months.

Land Crab Herbivory Test

The test for herbivory by burrowing land crabs (*Cardisoma carnifex)* was found to be inconclusive because the crabs died. If the cause of death was starvation, this suggests that herbivory is not a factor affecting the distribution of *T. populnea* in the two bays. However, if their deaths were due to other stress then the tests should be conducted again, perhaps in a less synthetic environment to reduce stress variables.

Ethnobotanical Assessment

The ethnobotanical information collected was from a relatively small sample size; however, there was high agreement among collaborators from different locations across the island.

In studies on humans, it is often difficult to eliminate bias, thus I have noted below where particular bias may be influencing the data.

Question one (Table 3) may have some degree of biased due to the fact that some collaborators were chosen for interviews based solely on whether *T. populnea* could be seen growing near the home. However, the other interviews were arranged by the local translators, which were chosen for collaborators' knowledge on traditional medicine or traditional Polynesian culture. This may pose bias in question three, since those people known to have knowledge in traditional medicine or cultural would presumably know more about important plants such as *T. populnea* than an average Tahitian would.

Question two addressed the conservation issue. A majority of the collaborators had not planted the tree(s) found growing adjacent to their property. Most trees were reported to have self-established. This does not necessarily suggest that people are not planting trees.

Question three examined the issues of harvesting and demand. All collaborators reported to have cut part of the tree at some time in their life for use. This suggests that the tree is still an important ethnobotanical resource to Tahitians.

In question four, a majority of collaborators indicated that *T. populnea* had no varieties. Two collaborators suggested that thee were morphological differences between populations in the mountains and that of the coast, but explained that either one had the same uses. However, a preference was noted by collaborators who were carpenters, for the wood from mountain trees, due to its higher functional and aesthetic quality as harder darker wood. The difference between the two qualities of wood was ascribed to the soil each grew in.

Question five showed that a majority of the collaborators thought the tree lived more than 100 years. There have been no studies on Polynesian populations that indicate the average life span of *T. populnea,* thus a comparison of ethnoecological knowledge to the literature is not available.

Question six addressed collaborators' perspectives on the degree of population change, which was measured as a function of their lifetime. Because most collaborators were elders in the community, the answers reflect a change over an average of the last 65 years. A majority of collaborators reported that the number of trees has declined by 95% in their lifetime. While this number was an estimate, it suggests a rapid decline in the number of trees over the last decade. Collaborators were asked for an explanation for the cause of the decline. The most common reasons were: irresponsible harvesting for selling, and cyclone activity.

In general, the results of the questionnaire suggest that *T. populnea* is a relatively important ethnobotanical resource and that Tahitians are well aware of its declining numbers. However, it is not clear if people have made the connection between the declining population and the need to plant the tree to alleviate this problem. The challenge is to find appropriate, long-term solutions to conservation of *T. populnea* which meet the needs of the community (Cunningham 2001).

Future Work

If the trend toward development continues, the GIS mapping data on the shoreline types in Opunohu Bay and Cook's Bay will be useful in monitoring the population of *T. populnea* in relation to shoreline development. This data could also be used in future studies, which examine the effects of shoreline development on the distribution of all coastal strand species. However, the shoreline of Moorea needs to be mapped in its entirety to carry out such studies on a larger scale.

A long-term study on human use of *T. populnea* would allow a more thorough assessment of the consumption rate and could potentially result in recommendations for appropriate conservation strategies.

Molecular analyses of Polynesian populations would assess the genetic variation between and within island populations, which could potentially help establish conservation priorities.

Conclusion

Because *T. populnea* shows considerable salt water buoyancy, which allows for both short and long distance dispersal, it can easily distribute itself. In addition, *T. populnea* is readily able to germinate in all soil types found along the shore. However, the study suggests that its ability to establish itself on shore may be affected by the type of shoreline it encounters and how this shoreline is managed. Use by humans is a factor affecting its distribution; however, further study would be needed to assess the affects. Harvesting the wood for its aesthetic quality for the tourist industry may have a larger affect than harvesting for personal medicinal use.

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Appendix A

The questionnaire format, which was translated into French and Tahitian for the interviews. The names of all collaborators, the location of their home, and date interviewed in Appendix B.

Informant's name: **date:** **PK maker:**

Is miro (*Thespesia populnea*) growing near here?

If yes, how old is the tree(s)?

If yes, was it planted there or did it sprout alone?

Do you ever harvest miro (*Thespesia populnea*)?

How is miro (*Thespesia populnea*)?useful?

What is the average life span of miro (*Thespesia populnea*)?

Have you noticed the number of miro trees change
in the last 5 years, 10 years, 20 years?

If yes, is it declining or increasing?

Are there different varieties of miro (*Thespesia populnea*)?

If yes, what characteristics distinguish each as a variety?

Other comments:

Appendix B. A list of the collaborators who were interviewed with the ethnobotanical questionnaire. Collaborator numbers correspond to numbers in Figure 2.

Collaborator	Name(s)	Location	Date Interviewed
1	Mita Garmain	PK 22.5	11/12/01
2	Pangnifou-Afou & Pang-fat Terii	PK 22.4	11/8/01
3	J. Marie Pirakaueke	PK 21.5	11/12/01
4	Papa Matarau	PK 21.5	11/7/01
5	Marae Terii & Poheroa Tore	PK 19	11/6/01
6	Tinihau Itaia	PK 14	11/6/01
7	Louise Faehau	PK 14	11/6/01
8	Mimi Terai	PK 9	10/29/01
9	Lucie Tapu	PK 10	10/29/01

Morphological Phylogenetic Analysis of *Tetragnatha* and *Leucauge* Spiders (Araneae: Tetragnathidae) of French Polynesia

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ABSTRACT. Tetragnathid spiders are generally homogeneous in morphology and ecology except in the Hawaiian Islands where they are diverse. The Society Islands share some similarities with the Hawaiian islands but also the Society Islands have some key physical differences compared to the Hawaiian Islands. Do the biological patterns share these similarities or differences? Based on the physical differences between the Hawaiian Islands and the Society Islands, I hypothesize that the patterns of diverse morphology and ecology of Tetragnathidae found in the Hawaiian Islands will not appear as aptly in the Society Islands. This study first looks at the morphological phylogeny of the Tetragnathidae spider fauna of French Polynesia to better understand the morphological diversity and relationships. Secondly, three secondary variables, geography , habitat and elevation, are mapped on that phylogeny. The results of the morphological phylogeny are presented and discussed here. The secondary variables mapped on the phylogeny suggest some similarities and some difference between the Tetragnathids of the Society Islands and the Hawaiian Islands. With further study, these similarities and differences between the biological systems of the Hawaiian Archipelago and the Society Islands Archipelago may offer insight to the understanding of evolutionary patterns and processes.

Introduction

Evolutionary patterns and processes exemplify the complexity of life. The difficulty in elucidating evolutionary patterns and processes can be lessened in unique environments (Chiba 1996). Hot-spot oceanic archipelagos present an example of this unique environment because of three specific characteristics (Simon 1987; Gillespie, Croom et al. 1997): 1. They offer a linear chronology. 2. They are isolated. 3. They display heterogeneous habitats. Because of these characteristics, a hot-spot oceanic archipelago presents an ideal 'natural laboratory' for the study of evolutionary patterns and processes.

The Hawaiian Islands archipelago has been biologically studied intensely for many years (Simon 1987). The Society Islands represent a lesser-studied archipelago (Berland 1934; Gillespie In Press). There are many similarities and dissimilarities between the Hawaiian Islands and the Society Islands. This creates reason to further study the biodiversity in the Society Islands.

The Society Islands share the characteristics of linear chronology, isolation, and heterogeneous habitat with the Hawaiian Islands (Gillespie In Press). Also, between the archipelagos, some fauna and flora correlation exist (Mueller-Dombois and Fosberg 1998). However, there are key dissimilarities between the Society Islands and Hawaiian Islands:

1. Age Range: The Hawaiian Islands formed between 0.38 mya -5.1 mya and the Society Islands formed between 1.0 mya - 3.3 mya.
2. Size: The largest island in the Hawaiian Islands is ~170 km in diameter and the largest island in the Society Islands is ~40 km in diameter.
3. Extent of Isolation: The Hawaiian Islands are 1600 km from the next island and 4000 km from a major land mass, where the Society Islands are only 400 km from the next nearest island but 6000 km from a major land mass.
4. Altitude Range: The Hawaiian Islands range in altitude from 0 meters to 4205 meters and the Society Islands range from 0 meters to 2300 meters.

Because of these physical differences there is reason to presume there are also biological differences. A notable study on the difference in the diversity of crab spiders between French Polynesian Islands and the Hawaiian Islands supports that there are different biological patterns between the Society Islands and the Hawaiian Islands (Garb and Gillespie 2000). Further comparative studies between the biology of the Society Islands and the Hawaiian Islands will augment greatly to the understanding of evolutionary patterns and processes.

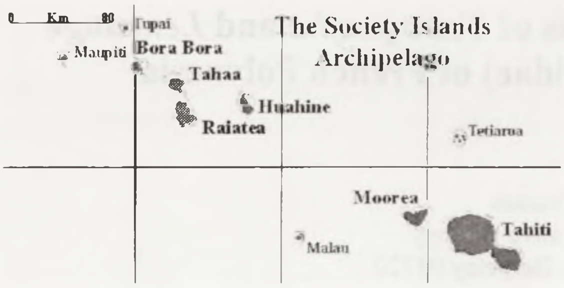

Figure 1. Map of the Society Islands.

The Society Islands, located south of the Tuamotos and north of the Austral Islands in Polynesia, represents a unique archipelago of six islands. In chronological and geographic order: Tahiti, the furthest southeast, is the youngest at 1.0myrs. Moorea is next order followed by Raiatea. The oldest two islands are Huahine and Bora Bora, the furthest northwest, which is 3.3myrs (Figure 1). The terrestrial habitats of all the islands differ due to their geological history. Tahiti, being the youngest is mountainous and has the most heterogeneous environments. Bora Bora, the oldest is much like an atoll and has a much more homogeneous environment than the younger islands.

This study focuses on the biodiversity of the Tetragnathid spiders of the Society Islands. Tetragnathid spiders are abundant worldwide (Levi 1981) and are considered homogeneous in morphology and ecology (Kaston 1948; Dabrowska Prot, Luczak et al. 1968; Gillespie 1986; Gillespie 1991) except for in the Hawaiian Islands where they are diverse in morphology and ecology (Simon 1987; Gillespie 1991; Gillespie, Croom et al. 1997). In the Society Islands do Tetragnathid spiders follow the homogeneous pattern found worldwide or the diverse pattern seen in the Hawaiian Islands? This paper will address this question by asking:

1. What are the morphological phylogenetic relationships of Tetragnathid Spiders, the genera *Leucauge* and *Tetragnatha*, in the Society Islands?
2. Are there environmental (habitat occupied and elevation) and/or geographic variables (geographic position on the Islands) that correlate with the morphological phylogenetic relationships?

Based on the described physical differences between the Hawaiian Islands and the Society Islands, I hypothesize that the patterns of diverse morphology and ecology of Tetragnathidae found in the Hawaiian Islands will not appear as readily in the Society Islands, even given the similarities of being archipelagos.

Materials and Methods

Biology and Biogeography of the Society Island Tetragnathids

The spiders of the Society Islands are a particularly understudied group of organisms (Gillespie In Press). Recent expeditions to the Society Islands by R. Gillespie and colleagues lead to the first comprehensive knowledge of the spider fauna.

The literature states that there are eight species of Tetragnathidae on the Society Islands of Tahiti, Moorea, Raiatea, and Bora Bora (Berland 1934; Marples 1957; Chrysanthus 1963; Gillespie In Press). Gillespie's in press recently revised the descriptions of the genus *Tetragnatha* on the Society Islands. There are six described species of *Tetragnatha* on the Society Islands. The six species are described as follows *T. rava* (endemic), *T. moua* (endemic), *T. tuamoaa* (endemic), *T. nitens* (introduced found in Puerto Rico (Gillespie, Croom et al. 1997)), *T.maxillosa* (introduced found in Burma, Malaya, India, and New Guinea), *T.macilenta* (indigenous found in Somoa, Solomon Islands, Cook Islands, Norfolk Islands, Australia, New Guinea, Tonga, Admiralty Islands, New Britain). Although not recently revised, there are two species of *Leucauge* on the Society Islands described in the literature (Berland 1942; Marples 1957; Chrysanthus 1963). These species are *L. granulata* (introduced found in Somoa (Marples 1957), Australia, New Zealand, New Guinea (Chrysanthus 1963)) and *L. tuberculata* (introduced found in the Austral Islands (Berland 1942), Somoa, Fiji, Tonga). The Hawaiian Islands have over fifty morphospecies of Tetragnathidae described.

Collection and Examination

The specimens used for this study were collected at the following places, GPS was taken where available:

1. **Tahiti**; A) Mt. Aorai S17°36.042' W149°29.621' elevation of 1870 meters, and B) Mt. Marau elevation of 1300 meters, C) Farehape elevation of 200 meters, D) Mt. Urufa, Papenoo elevation of 600 meters
2. **Moorea**; A) Three Coconuts Pass S17°32.828' W149°50.521' elevation of 390 meters, B) Belvedere elevation of 270 meters, C) River behind Paupau S17°30.990' W149°49.104' elevation of 30 meters , Two

Rivers behind Opunohu Bay River D) S17°32.072' W149°50.104' elevation 24 meters, E) S17°32.238' W149°50.122' elevation of 49 meters, Mangroves lining Cook's Bay and Mt. Mauaputa S17°31.582' W149°48.195' elevation of 300 meters and 840 meters

3. Raiatea; A) Uturoa, Temehani Pl. 800 meters
4. Bora Bora; A) Mt. Hue/ Pahia 600 meters

Several specimens of each species of the genera *Leucauge* and *Tetragnatha* were collected from the above named areas. Included in the analysis were several specimens already collected by R. Gillespie and colleagues from Tahiti, Moorea, Raiatea and Bora Bora. All specimens were collected both at night and day to avoid composition misinterpretation (Green 1999). All specimens were stored in 75% ethanol for subsequent character analysis. Both alien and native species were included in the collection.

All specimens were examined using a basic dissecting microscope and a microscalpel to identify anatomy for character analysis. Every spider used in this study is cataloged in Appendix 1.

Morphological Characters

All morphological characters used in this study appeared to follow the basic cladistic code (Mishler 2000): 1) the characters were homologous 2) the characters represented discrete states 3) all characters were independent from one another 4) and all characters had genetic basis.

The characters for this phylogenetic analysis were defined using three methods. First, several characters defined in the literature were examined and if they followed the code outlined above were used (Berland 1942; Marples 1957; Chrysanthus 1963; Okuma 1987; Gillespie In Press). The following are the characters used in the literature to describe and diagnose Tetragnathid spiders:

- *Leucauge* – Posterior median eyes canoe tapetum present (Scharff and Coddington 1997); Double fringe of femoral hairs on posterior leg (Kaston 1948); Color/ Pattern (Berland 1942; Marples 1957; Chrysanthus 1963); Size (Marples 1957); Silvery Marks (Marples 1957); Eye differences (Marples 1957); Teeth differences (Marples 1957); Abdomen length (Marples 1957; Chrysanthus 1963); Leg length (Marples 1957); Epigynum shape (Chrysanthus 1963).

- *Tetragnatha* – Male genitalia (Hormiga, Eberhard et al. 1995; Gillespie, Croom et al. 1997; Scharff and Coddington 1997); Epigynum absent (Scharff and Coddington 1997); Spur on base of fang in males (Kaston 1948); Teeth differences (Marples 1957; Okuma 1987; Gillespie 1991); Length of chelicerae (Okuma 1987); Length of legs (Okuma 1987); Macrosetae (spination) on legs 1 and 3 because they represent divergence in function (Gillespie 1991); Color / Pattern (Berland 1942; Marples 1957; Okuma 1987; Gillespie 1991); Eye spacing and size (Berland 1942; Marples 1957; Okuma 1987; Gillespie 1991); Fang structure (Okuma 1987; Gillespie 1991)

Secondly, morphologically discreet characters came from observation of specimens. These observed characters, not found in the literature, were mainly pigmentation patterns. Not many were used.

Finally, some characters were found taking continuous measurements. Measurements were taken of the anterior most leg, the abdomen, the head, the jaw, the eyes, and the fang. After the operational taxonomic units (OTUs) were defined using the characters from the literature and observation, the measurements were grouped based on the OTU diagnosis and run through an ANOVA in congruence with a multiple range test, Tukey Test, to show significant difference. If a significant difference in OTUs was found, character states were formed accordingly. All statistics were done on the program SYSTAT 4.0.

The three methods just described were used to diagnose character states for the morphological phylogenetic analysis.

Phylogenetic Analysis

The matrix created by the morphological characters diagnosed was analyzed using the programs MacClade 4.0 and PAUP 4.1.1. The data matrices were constructed in MacClade and then tested heuristically in PAUP for the most parsimonious phylogenetic tree. A strict tree consensus was then created to display the phylogeny used in the results. The outgroup is designated as the genus *Nephila* based on previous morphological phylogenies (Hormiga, Eberhard et al. 1995; Scharff and Coddington 1997).

Secondary mapping of habitat, elevation and geographic variables followed the production of the morphological phylogeny.

Results

Morphological Characters

Many characters were considered for this study and some were omitted due to variability, coding impossibilities or they did not follow the criteria for a good character state. One character that was omitted due to lack of information was the male palp morphology (further consideration is given to this fact in the discussion). The following characters were created using the three methods outlined before and put into a matrix shown in Appendix 3:

Character 1: Number of promarginal teeth of chelicerae: 0, three teeth; 1, six teeth; 2, seven teeth; 3, eight teeth; 4, nine teeth

Character 2: Number of retromarginal teeth of Chelicerae: 0, four teeth; 1, five teeth; 2, seven teeth; 3, nine teeth; 4, ten teeth; 5, greater than ten teeth

Character 3: Macrosetae on first leg's femur: 0, absent; 1, present

Character 4: Macrosetae on first leg's patella: 0, absent; 1, present

Character 5 : Macrosetae on first leg's Tibia: 0, absent; 1, present

Character 6: Macrosetae on first leg's metatarsus: 0, absent; 1, present

Character 7: Macrosetae on first leg's tarsus: 0, absent; 1, present

Character 8: Macrosetae on third leg's femur: 0, absent; 1, present

Character 9: Macrosetae on third leg's patella: 0, absent; 1, present

Character 10: Macrosetae on third leg's tibia: 0, absent; 1, present

Character 11: Macrosetae on third leg's metatarsus: 0, absent; 1, present

Character 12: Macrosetae on third leg's tarsus: 0, absent; 1, present

Character 13: Outer eye's size comparison: 0, equal in size; 1, posterior outside eye is larger than the anterior outer eye; 2, anterior outside eye is larger than the posterior outer eye

Character 14: Do the outer eyes touch: 0, no; 1, yes

Character 15: Pattern on head (Figure 2) : 0, no pattern; 1, strait line across half of head starting posterior; 2, radiating pattern of lines from center of head; 3, pattern >< on head; 4, Y-shaped pattern; 5, complete line across head; 6, V-shaped pattern

Character 16: Presence of canoe-shaped tapetum: 0, absent; 1, present

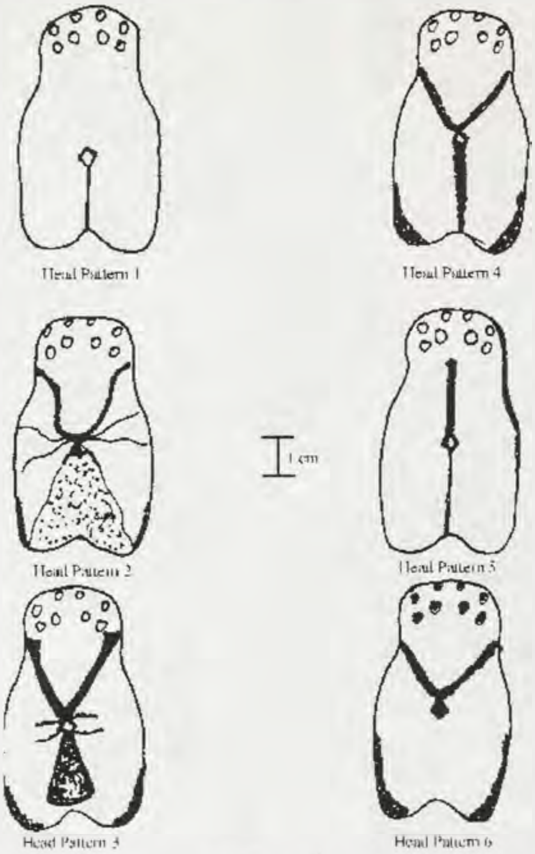

Figure 2. Head patterns used for Character 15 in morphological phylogenetic analysis

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Character 17: Distance of between inside posterior eyes compared to the distance of the inside posterior eyes to the outer posterior eye: 0, anterior eye further apart than posterior; 1, anterior eye equidistant apart as posterior; 2, posterior eyes further apart that anterior

Character 18: Pigment line on ventral side of abdomen: 0, absent; 1, present

Character 19: Two white pigment lines on ventral side of abdomen: 0, absent; 1, present

Character 20: Presence of epigynum: 0, no epigynum and less than one millimeter distance from book lungs; 1, no epigynum and one millimeter from book lungs; 3, epigynum present

Character 21: Distance between first and second teeth on promarginal side of chelicera: 0, absent; 1, present

Character 22: Distance between first and second teeth on retromarginal side of chelicera: 0, absent; 1, present

Character 23: Four brown pigmented spots on back: 0, absent; 1, present
Character 24: Iridescent pigmentation on dorsal side of abdomen: 0, present; 1, absent
Character 25: Light pigmented microsetae on side of abdomen: 0, present; 1, absent
Character 26: White pigmented dots on ventral side of abdomen: 0, present; 1, absent
Character 27: White pigmented dots on dorsal side of abdomen: 0, present; 1, absent
Character 28: Double row of hairs on upper femora of posterior most leg: 0, absent; 1, present
Character 29: Enlarged first tooth on promarginal side of chelicera: 0, present; 1, absent
Character 30: Enlarged first tooth on retromarginal side of chelicera: 0, present; 1, absent
Character 31: Spur on fang: 0, absent; 1, present
Character 32: Spur on male chelicera: 0, absent; 1, present
Character 33: Length of first leg (Figure 3) : 0, group 1 from ANOVA; 1, group 2 from ANOVA
Character 34: Jaw length (Figure 4): 0, group 1 from ANOVA; 1, group 2 from ANOVA
Character 35: Abdomen length (Figure 5): 0, group 1 from ANOVA; 1, group 2 from ANOVA

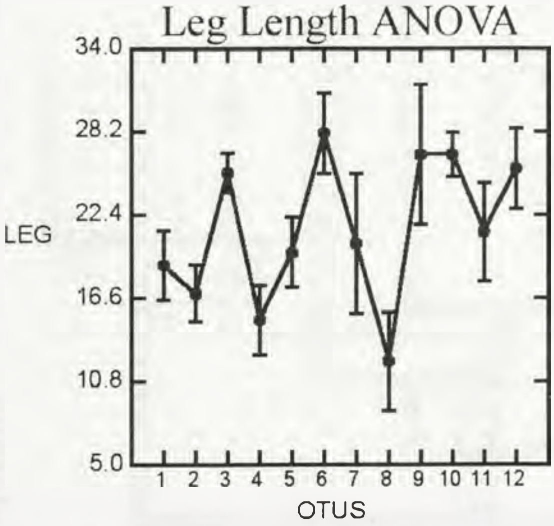

Figure 3. ANOVA of Length of Anterior Most Leg. Tested with Tukey Test for significance. Two groups were established. Group 1 with character state = 0 consisted of OTU1, OTU2, OTU4, OTU5, OTU8, and OTU11. Group 2 with character state = 1 consisted of OTU3, and OTU6. OTU9, OTU10, OTU12, and OTU7 fit in neither group and was scored as ?.

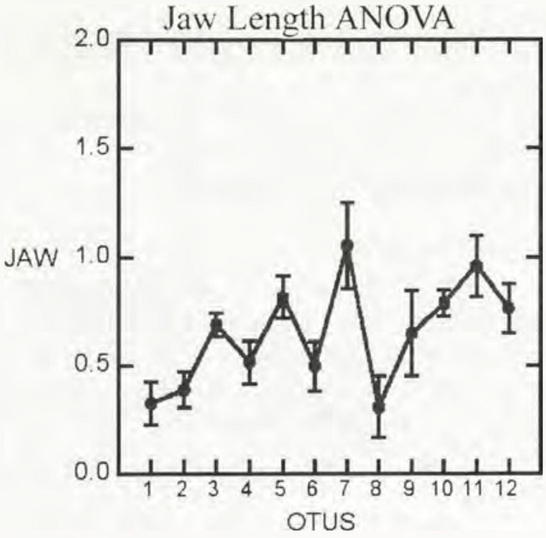

Figure 4. ANOVA of Length of Jaw. Tested with Tukey Test for significance. Three groups were established. Group 1 with character state = 0 consisted of OTU7, and OTU11. Group 2 with character state = 1 consisted of OTU3, OTU5, OTU10, and OTU12. Group 3 with character state = 2 consisted of OTU1, OTU2, and OTU8. OTU4, OTU6, and OTU9 fit in neither group and was scored as ?.

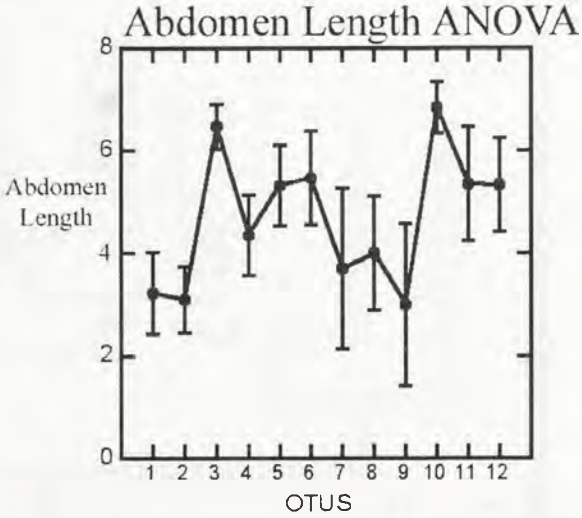

Figure 5. ANOVA of Length of Abdomen. Tested with Tukey Test for significance. Three groups were established. Group 1 defined as character state = 0 consisted of OTU1, OTU2, OTU4, OTU7 and OTU9. Group 2 defined as character state = 1 consisted of OTU3 and OTU10. Group 3 defined as character state = 2 consisted of OTU5, OTU6, OTU11 and OTU12. OTU8 fit in neither group and was scored as ?.

Secondary Variable Character 36:
Macroenvironment: 0, Ocean; 1, Stream; 2, Forest
Secondary Variable Character 37: Elevation: 0, Elevation lower than 300 meters; 1, Elevation higher than 300 meters

Phylogenetic Analysis

With the 35 characters described above thirteen operational taxonomic units (OTUs) were designated. Pictures of the OTUs are shown in Appendix 2. Three *Leucauge* and ten *Tetragnatha.* The three *Leucauge* OTUs are (OTU1, OTU2, OTU13). The other OTUs are *Tetragnatha.*

Using PAUP and MacClade, all 35 characters were used in the final phylogenetic analysis. All the characters were unordered and given equal weights. Seventeen cells had missing data. The matrix used is shown in Appendix 3.

Three most parsimonious trees were yielded. The three tress differ only in the connection of OTU1, OTU2 and OTU13 which probably results from only having one specimen of OTU13. The strict consensus tree obtained with the characters described above is shown in Figure 6.

Secondary Characterss

The results of mapping on the secondary characters, geography, habitat and elevation, are shown in Figures 7, 8, and 9 respectively.

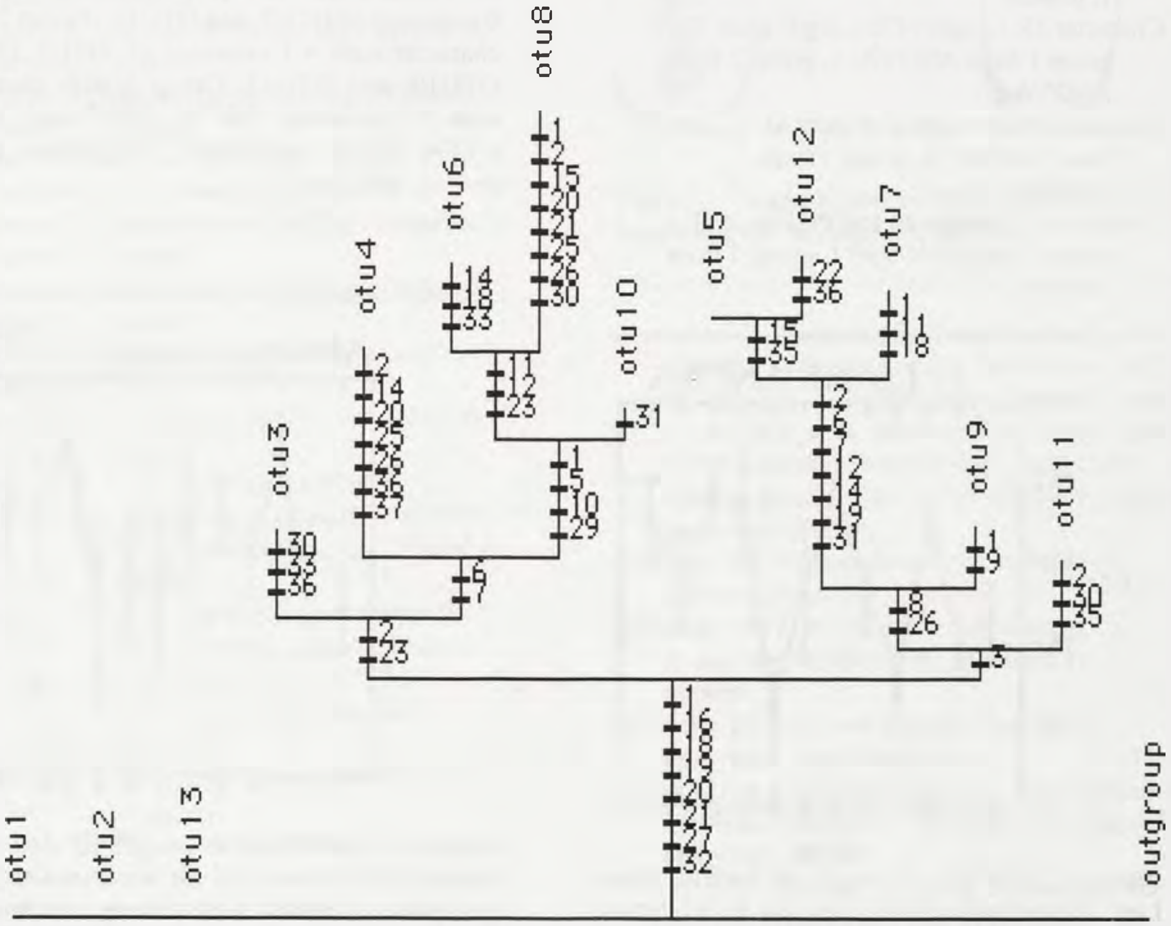

Figure 6. Strict consensus tree of the Society Islands Tetragnathid spiders morphological phylogeny. This phylogeny was produced using the morphological characters described in the programs PAUP 4.1.1 and MacClade 4.0.

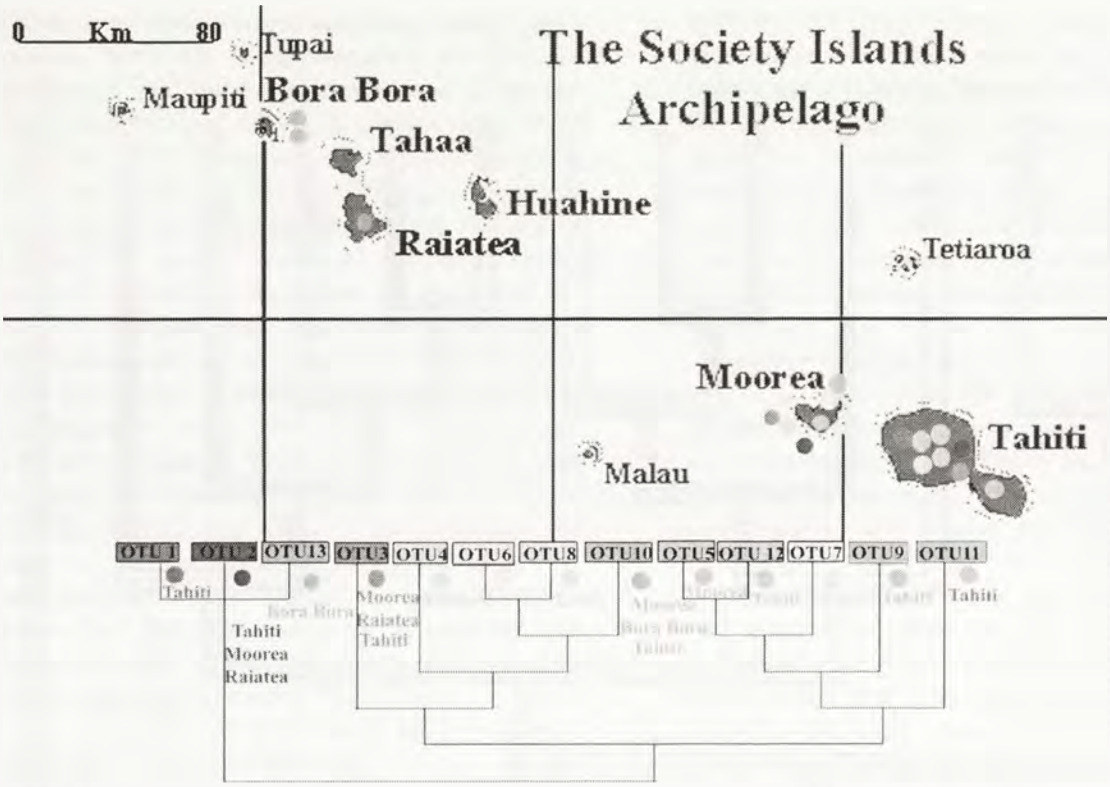

Figure 7. Geographic placement on the Society Islands of the OTUs defined. OTU1 is only in Tahiti. OTU2 is found in Tahiti, Moorea and Raiatea. OTU3 is found in Tahiti, Moorea and Raiatea. OTU4 is found in only Moorea. OTU5 is found in only Moorea. OTU6 is found in only Moorea. OTU7 is found in only Moorea. OTU8 is found in only Tahiti. OTU9 is found in only Tahiti. OTU10 is found in Tahiti, Moorea and Bora Bora. OUT 11 is found in only Tahiti. OTU12 is found in only Tahiti. OTU13 is found in only Bora Bora.

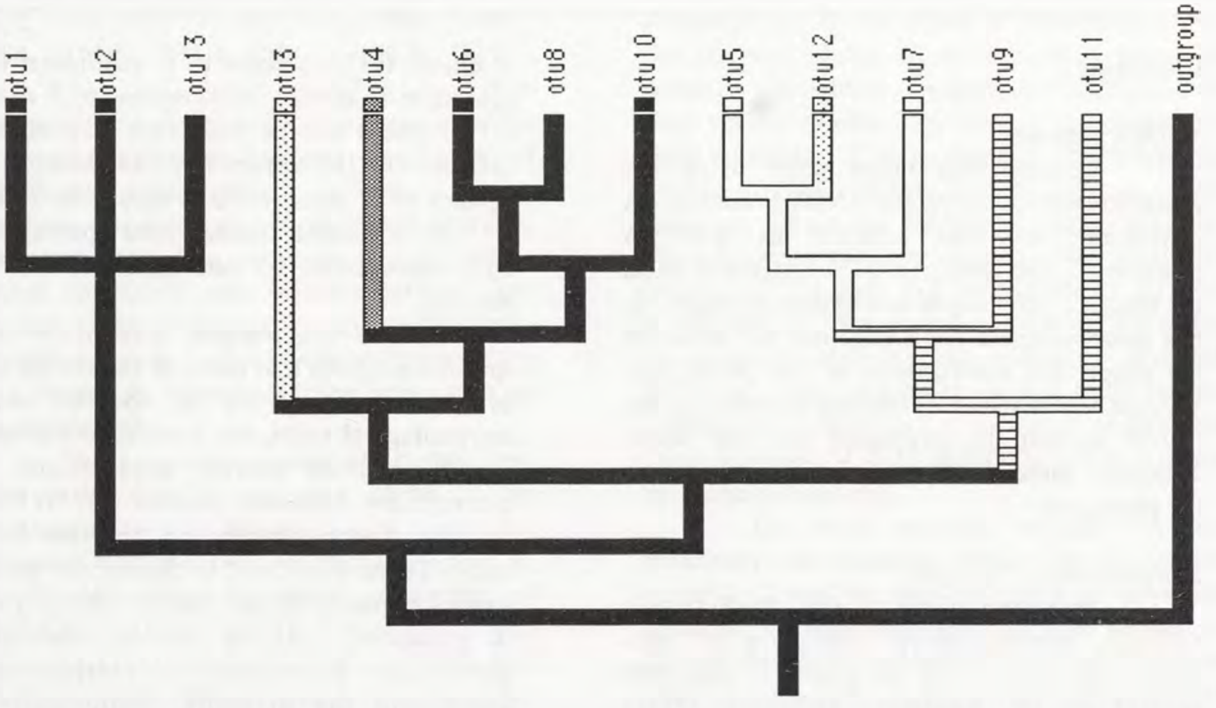

Figure 8. Environmental variable mapped on the morphological phylogeny. The dark shading is stream and forest dwelling . The dark gray shading is forest dwelling only. The dotted shading is stream dwelling only and the white shading is ocean dwelling. The horizontal lined shading is unknown.

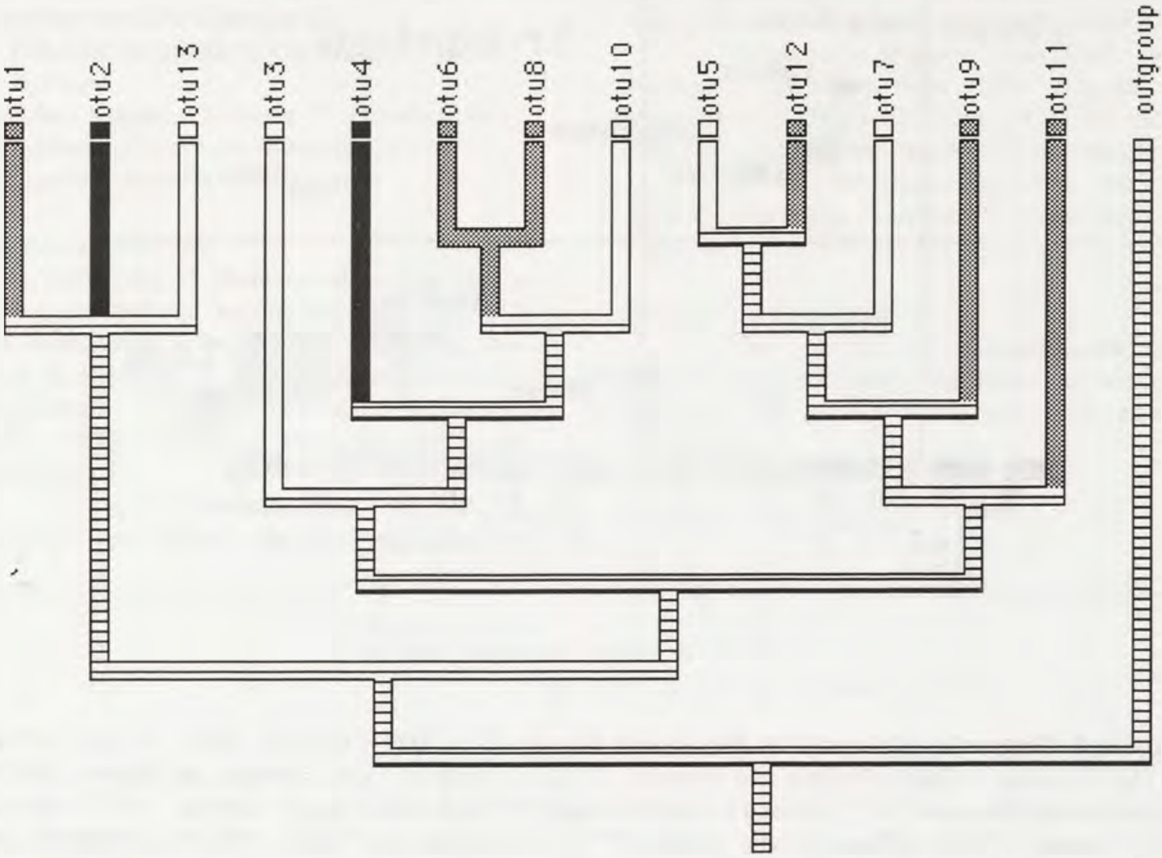

Figure 9. Elevation variable mapped on the morphological phylogeny. The black shading is high elevation. The dark gray shading is high and low elevation. The white shading is low elevation. The horizontal lined shading is unknown.

Discussion

ANOVA Statistics

Caution was taken when using the characters developed by the ANOVA method. A phylogeny was run without the ANOVA characters. The three ANOVA characters used, leg length , jaw length and abdomen length, in this morphological phylogeny did not influence the shape and connections of the phylogeny. Without the ANOVA characters described in the results section the phylogeny was the same. Therefore, these characters only added support to the phylogeny.

Morphological Phylogeny

Independent from the phylogenetic analysis, representative specimens of each diagnosed OTU were assigned to described species by Dr. Rosemary Gillespie. OTU1 equates to *L. tuberculata.* OTU2 equates to *L. granulata*. OTU3 equates to *T. maxillosa*. OTU4 equates to *T. tuamoaa*. OTU5 equates to *T. nitens*. OTU6 equates to *T. macilenta*. OTU7 equates to *T. nitens*. OTU8 equates to *T. rava*. OTU9 could not be identified as a described species. OTU10 equates to *T. macilenta*. OTU11 equates to *T. maua*. OTU12 equates to *T. nitens*. OTU13 is another unidentified species but is only represented by one specimen that is a juvenile.

This independent assessment of the specimens shows that many of the OTUs, I have as separate based on the specific outlined morphological traits, are considered one species based on their current descriptions. This discrepancy between number of OTUs and describe species causes no problem for this study. There is no need to discuss the debate of species concepts (Pinna 2000). This phylogeny is produced only to discuss biologically significant differences in morphology to understand the diversity, ecologically and morphologically, in the Society Island Tetragnathid spiders. Because the OTUs of this study show strongly supported branches by the presence of many synapomorphics, there is good reason to keep them separate to discuss ecological and geographic positions. I do not define my outlined OTUs as species rather they are workable groupings of morphologically separate entities. When referring to species separation I use the describe groups defined in Gillespie in press because of the conservative methods with which the species are described

Another important aspect to discuss is the lack of the use of male palp morphology in this phylogeny. Most spider morphological phylogenies use the palp morphology extensively (Scharff 1997) but as a result of lack of male specimens for OTU1, OTU2, OTU5, OTU9, OTU12, and OTU13 the palps are not used. Th palp is a good character and should be used in future morphological phylogenies of this group but for this study the morphological characters used sufficiently collect the diversity of Tetragnathids to discuss conclusions.

Caution is taken with all conclusions made from the morphological variables due to possible morphological convergence not uncommon in Tetragnathids (Gillespie, Croom et al. 1997). A morphological phylogeny of Tetragnathids may not show monophyletic groupings. Even with caution there are meaningful conclusions on a big-scale produce from this phylogeny.

For the phylogeny seen in Figure 5, there is a strong separation of *Leucauge* (OTU1, OTU2, and OTU13) and *Tetragnatha* (OTU3, OTU4, OTU5, OTU6, OTU7, OTU8, OTU9, OT10, OTU11, and OTU12). The synapomorphies supporting the separation of the *Tetragnatha* clade from *Leucauge* are number of teeth on promarginal side of chelicera is greater than three, presence of canoe-shaped tapetum, dark pigmented line on ventral side, white pigment on ventral side, distance between first and second tooth on promarginal and retro marginal side, a lack of two rows of hairs on the upper femora on the posterior leg, and a spur on the male chelicera.

There is also a separation into two clades within the *Tetragnatha* clade. The separation shown in Figure 5 is between the clade consisting of OTU3, OTU4, OTU6, OTU8, and OTU10 versus the clade consisting of OTU5, OTU7, OTU9, OTU11, and OTU12. The clade with OTU3, OTU4, OTU6, OTU8, and OTU10 is supported by the synapomorphies number of retromarginal teeth and iridescent pigmentation on dorsal side of abdomen. The synapomorphy supporting the clade of OTU5, OTU7, OTU9, OTU11, and OTU12 is macrosetae on anterior most leg's femora. The separation within the Tetragnatha clade separates the described species of *T. nitens and T. moua* from the described species *T. maxillosa, T. macilenta, T. tuamoaa,* and *T. rava.* The separation of *T. nitens* from *T. maxillosa and T. macilenta* is also seen in a phylogeny produced by Gillespie personal communication 2001.

Secondary Variables

First, the secondary variable of geographic position mapped onto the phylogeny is shown in Figure 6. There are two different patterns seen here. Geographically one *Tetragnatha* clade shows lineage progression from west to east (oldest to youngest) while the other from east to west (youngest to oldest). The one conclusion that can be made by these patterns is that within a morphologically similar group there is a wide geographic distribution. This is similarly seen in many Hawaiian Tetragnatha (Gillespie, Croom et al. 1997).

The second variable mapped on the phylogeny was the habitat. The habitat variables chosen, stream, forest and ocean, are liberal groupings specifically to observe a pattern if one exists. But, as a result of them being broad groupings not much can be concluded other than the pattern itself. The habitat variable mapped on the phylogeny in Figure 7 shows separation of morphology in habitats between clades. Although, within the clade of Tetragnathids on the Society Islands there is a wide range of diversity in habitat occupation, Tetragnathids with similar morphology appear to occupy the similar habitats. This is shown by the following groups of OTUs within similar habitats. The Leucauge all occupy forest and stream habitats. Then between the Tetragnatha, the group of OTU6, OUT8 and OTU10 (defined by the morphological characters number of teeth on promargin of chelicera, macrosetae on first and third leg's tibia, and enlarged first tooth on the promargin of chelicera) also occupy stream and forest habitats. OTU5 and OTU7 both occupy the ocean side habitat.

The third variable mapped on the phylogeny was elevation. Again, like the habitat variable, I kept the defined groups of elevation nebulous, defining only low and high elevation. Despite the all-encompassing elevation variables there appears to be no pattern of morphological traits associated with a specific elevation. This supports the idea that morphological variation in *Tetragnatha* varies with environment (Simon

1900; Gillespie 1991; Gillespie, Croom et al. 1997).

Suggested for further study, a molecular phylogeny would allow further conclusion about the patterns seen in this morphological phylogenetic analysis. Also a more in depth analysis of the specific microhabitats would give more insight to the correlation between morphology and habitat use.

In conclusion, this study, although preliminary, supports the pattern of morphologically low diversity of Tetragnathids in the Society Islands when compared to the Hawaiian Islands. Yet, like the Hawaiian Islands the morphological diversity that does exists among the Tetragnathid spiders in the Society Islands is spread among different environments and geographically. With this preliminary study of the Tetragnathids on the Society Islands there are many interesting patterns that with further study may show conclusive patterns comparable to the Hawaiian Islands biologically.

Acknowledgments

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Appendix 1: Materials examined.

All specimen used in this study are listed here in the appendix. Specimens are listed alphabetically by genus and species. Localities are given with GPS if available. Specimens if not specified are deposited in the University of California Museum of Entomology.

- *Leucauge*
  Leucauge granulata: French Polynesia: Moorea, Tahiti and Raiatea, Specimens number A11, A20, A12, A40, A6: **From the California Academy of Sciences**: New Guinea: Morobe: District, Wau, Big Wau Creek, Specimen 28-VII-1972 Coll. T.W.Davies: New Zealand: N Island: Auckland: Waitakere Ranges nr. Bethello Bch, Specimen 30.X.1995 coll. J.Bouth: Solomon Islands; Guandal Canal Specimen II-IX-45 coll. H.M. Malkin
  Leucauge tuberculata: French Polynesia: Tahiti: Mt Aorai, Specimens number A45, A46, R6.1-R6.10
  Leucauge sp.: French Polynesia: Bora Bora: Mt Hue, Pahia, Specimens number R11
- *Nephila*
  Nephila sp.: Costa Rica; Heredia Prov.; Puerto Viejo 7-VIII-1965 at 85 meters coll. S.J. Arnold
- *Tetragnatha*

Tetragnatha macilenta: French Polynesia: Moorea & Tahiti & Bora Bora, Specimens number A21.1-A21.2, A24, A13, A14, A38, R4.1-R4.3, R2.1-R2.2

Tetragnatha maua: French Polynesia: Tahiti, Specimens number R10.1-R10.5

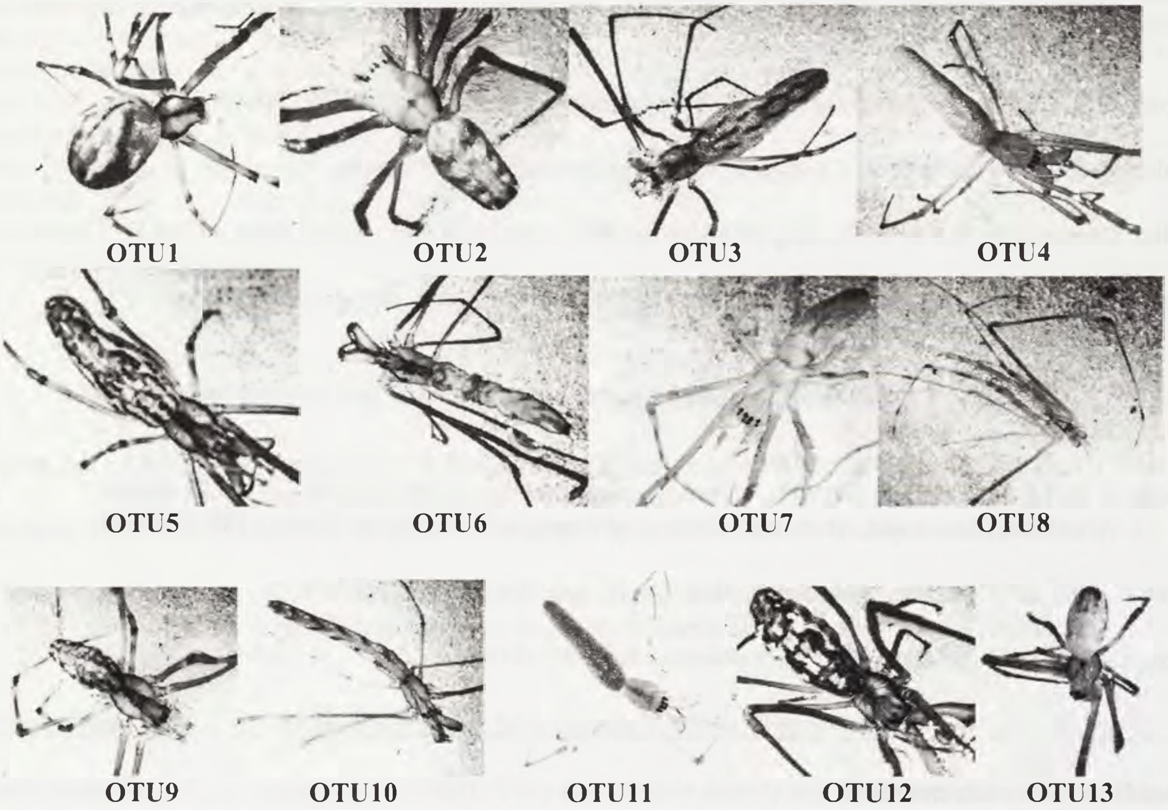
Tetragnatha maxillosa: French Polynesia: Moorea & Raiatea, Specimens number A35.1-A35.4, A36.1-A36.6, A5, A18

Tetragnatha nitens: French Polynesia: Moorea & Tahiti, Specimens numberA32.1-A32.3, A34, A33.1-A33.2, R3, R1.1-R1.3, R5, A32

Tetragnatha rava: French Polynesia: Tahiti, Specimens number R8.1-R8.5

Tetragnatha tuamoaa: French Polynesia: Moorea, Specimens number A2.1-A2.2, A10

Appendix 2: Pictures of each OTU.



OTU1 OTU2 OTU3 OTU4

OTU5 OTU6 OTU7 OTU8

OTU9 OTU10 OTU11 OTU12 OTU13

Appendix 3: Data Matrix

	Ch 1	Ch 2	Ch 3	Ch 4	Ch 5	Ch 6	Ch 7	Ch 8	Ch 9	Ch 10	Ch 11	Ch 12	Ch 13	Ch 14
otu1	0	1	0	0	1	1	1	0	0	1	1	1	0	1
otu2	0	1	1	0	1	1	1	0	0	1	1	1	0	1
otu3	3&4	4	0&1	0	1	1	1	0	0	1	1	1	1	0&1
otu4	3	3	0	0	1	0	0	0	0	1	1	1	1	0
otu5	3	5	1	0	1	0	0	1	0	1	1	0	1	0
otu6	2	4	0	0	0	0	0	0	0	0	0	0	1	0
otu7	2	5	1	0	1	0	0	1	0	1	0	0	1	0
otu8	1	5	0	0	0	0	0	0	0	0	0	0	1	1
otu9	1	1	1	0	1	1	1	1	1	1	1	1	1	1

otu10	2	4	0	0	0	0	0	0	0	0	1	1	1	1
otu11	3	3	1	0	1	1	1	0	0	1	1	1	1	1
otu12	3&4	5	1	0	1	0	0	1	0	1	1	0	1	0
otu13	0	1	0	0	1	1	1	0	0	1	1	1	0	1
outgroup	0	0	0	0	0	1	1	0	0	0	1	1	2	0

	Ch 15	Ch 16	Ch 17	Ch 18	Ch 19	Ch 20	Ch 21	Ch 22	Ch 23	Ch 24	Ch 25	Ch 26	Ch 27	Ch 28
otu1	5	1	1	0	1	1	0	0	0	1	0	0	0	1
otu2	1	1	1	0	1	1	0	0	0	1	1	0	0	1
otu3	2	0	2	1	0	0	1	0	1	0	1	0	1	0
otu4	4	0	2	1	0	2	1	0	1	0	0	1	1	0
otu5	3	0	2	1	1	0	1	0	0	0	1	1	1	0
otu6	4	0	2	0	0	0	1	0	0	0	1	0	1	0
otu7	0	0	2	0	1	0	1	0	0	0	1	1	1	0
otu8	6	0	2	1	0	2	0	0	0	0	0	1	1	0
otu9	4&6	0	2	1	0	0	1	0	0	0	1	1	1	0
otu10	4	0	2	1	0	0	1	0	1	0	1	0	1	0
otu11	0	0	2	1	0	0	1	0	0	0	1	0	1	0
otu12	3	0	2	1	1	0	1	1	0	0	1	1	1	0
otu13	6	1	1	0	1	1	0	0	0	1	1	0	0	1
outgroup	0	1	0	0	1	1	0	0	0	0	0	0	0	0

	Ch 29	Ch 30	Ch 31	Ch 32	Ch 33	Ch 34	Ch 35	Ch 36	Ch 37
otu1	0	0	0	0	0	2	0	3	1
otu2	0	0	0	0	0	2	0	3	2
otu3	0	1	0	1	1	1	1	1	0
otu4	0	0	0	1	0	?	0	2	2
otu5	0	0	1	1	0	1	2	0	0
otu6	1	0	0	1	1	?	2	3	1
otu7	0	0	1	1	?	0	0	0	0
otu8	1	1	0	1	0	2	?	?	1
otu9	0	0	0	1	?	?	0	?	1
otu10	1	0	1	1	?	1	1	3	0
otu11	0	1	0	1	0	0	2	?	1
otu12	0	0	1	1	?	1	2	1	1
otu13	0	0	0	0	0	2	0	?	0
outgroup	0	0	0	0	?	?	?	?	?

A Floristic Study of the Gingers (Family: Zingiberaceae) in Moorea, French Polynesia

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ABSTRACT. A floristic study of gingers in Moorea was done through documenting and describing all species found and conducting informational interviews with residents of Moorea. Twelve species, including six species previously undocumented in Moorea, were documented and described along with their uses. A key based on morphological characteristics was created for the species found in Moorea. In addition, two experiments were conducted to understand the ecology of gingers in gardens. First an allelopathic experiment between two varieties of *A. purpurata* was administered. Second a germination experiment on the seed of *E. elatior* (red variety) was conducted. Plant variety, but not soil type, was found to be significantly related to differences in plant viability. The pink variety of *A. purpurata* appeared to be more viable then the red variety. In the germination experiment on the seeds of *E. elatior*, one seed germinated out of approximately 300 seeds. More research is needed to understand if allelopathy is occurring between the two varieties of *A. purpurata* and if *E. elatior* has the potential to escape from gardens through viable seeds.

Introduction

Zingiberaceae, the ginger family, with roughly 44 genera and 700-1000 species, is distributed throughout the tropics (Smith 1987, Wagner et al. 1990). Although the morphology of gingers has been well studied, knowledge of basic morphological characters is still inadequate in many groups within the family (Larsen et al. 1999). Both the structure of the ginger's inflorescence and its fragile short-lived flowers (lasting less than 24 hrs) are difficult to preserve accurately in the herbarium; many old herbarium ginger specimens are lacking in floral characteristics. Since the inflorescences and flowers are key in identifying species, taxonomic problems in gingers are numerous. Ginger taxonomy has been found to be best constructed with photographs and measurements of living specimens (Larsen et al. 1999).

Ginger family characteristics

The family consists of perennial herbs with oil cells, aromatic rhizomes, and distichous pinnate leaves. Its shoot, like many monocots, is a pseudostem formed by sheathing leaf bases. The inflorescence can either terminate in a leafy shoot (considered "terminal") or be on a leafless shoot separate from the leafy shoot (considered "radical"). Spirally arranged bracts are often the showy part of the inflorescence and subtend a single flower or a cluster of flowers. Ginger flowers are perfect (having both stamens and carpels) but highly modified; out of a flower's five stamens, only one is fertile. Two of the stamens are connately fused, forming a petaloid labellum or lip. The other two may form petaloid staminodes or be absent. Both the calyx and the corolla are three-lobed. The filamentous style is often found inside the groove formed by two anther thecae. Placentation can be axile, parietal, or free-central. (Holttum 1950, Smith 1987, Wagner et al. 1990).

The family Costaceae is included as a subfamily in Zingiberaceae in some literature, but since it lacks the oil cells and distichous leaf arrangement that other members of Zingiberaceae have, it has been lately considered separate from the ginger family (Larsen et al. 1999).

Documented gingers in Moorea

The familiar commerce ginger, *Zingiber officinale,* known in Tahitian as "re'a tinito" (translated as Chinese ginger), is only one of the many gingers found and used in Moorea (Welsh 1998). Despite the presence of Zingiberaceae on Moorea, little is known about them, because the flora of French Polynesia are relatively unknown compared to the flora of Hawaii, New Zealand, Australia, and New Caledonia and other Pacific islands (Murdock 1999). Only two references (Fosberg and Stoddart 1996) and (Welsh 1998) list gingers in Moorea. Varieties found in Moorea according to Fosberg and Stoddart (1996) include *Alpinia purpurata*, *Alpinia zerumbet*, *Curcuma longa*, and *Etlingera* *cevuga* (referred to as *Amomum cevuga).* Welsh (1998) did not find *A. purpurata* and *A. zerumbet*, but added *Zingiber officinale and Zingiber zerumbet* to this list. *E. cevuga* and *Z. zerumbet* are indigenous according to Welsh (1998). Welsh described both naturalized and cultivated species, since he listed a specimen collected in Papeari Botanical Garden in Tahiti and indicated that some of his listed species were based on observations in the market.

Because gingers are relatively unknown and there is conflicting information on their presence in Moorea, a complete collection of gingers in Moorea needs to be compiled. Inadequate identification prevents ecological, economic, and many other types of research on tropical ecosystems (Fosberg 1989). This research presents a floristic study of gingers in Moorea by:

1. Documenting and describing all ginger varieties found in Moorea;
2. Conducting informational interviews with residents of Moorea to establish when and why these various gingers are cultivated;
3. Conducting preliminary tests between varieties of the same species; and
4. Comparing the morphology of these gingers to see whether generalizations can be made for all species, whether they can be distinguished from each other without inflorescence morphology and which morphological characters can be considered for phylogenetic analysis

Materials and Methods

Species documentation and description

Species collected in this study were identified based on descriptions and photographs using Welsh (1998), Wagner et al. (1990), Konczak (unknown date), Nirav (1992) and Saquet (1996). Identifications were aided by comparing collected plants to specimens in the Museum of the Islands, discussions with local Tahitians, French and Chinese, and e-mail correspondence with Chelsea Specht, a researcher at New York Botanical Gardens. Collections made for this research were deposited in the University and Jepson Herbaria, UC Berkeley. Digital pictures were taken of all twelve species (15 varieties) presented in this study.

Study sites

All species presented in this study were found on roadsides, along paths, in gardens, or in plantations, with the exception of the indigenous gingers *Zingiber zerumbet* and *Etlingera cevuga*, which were found behind Marae Tetiiroa. Measurement sites were mostly in private gardens, where different varieties of ginger could be found in one single location. All sites were either near Opunohu Bay, Cook's Bay, or the inland road between these two bays. Sites were reached by walking or with the aid of vehicles.

Site 1: PK 21 (Brotherson's family property)
Site 2: PK 18 (Manu's plantation)
Site 3: PK 17.5 (Kellum's garden)
Site 4: PK 11.5 (Gump Station and nature path)
Site 5: PK 9 (Wong's family property)
Site 6: Paopao Valley
Site 7: Intersection of Belvederre road and road bordering Agriculture School
Site 8: behind Marae Tetiiroa

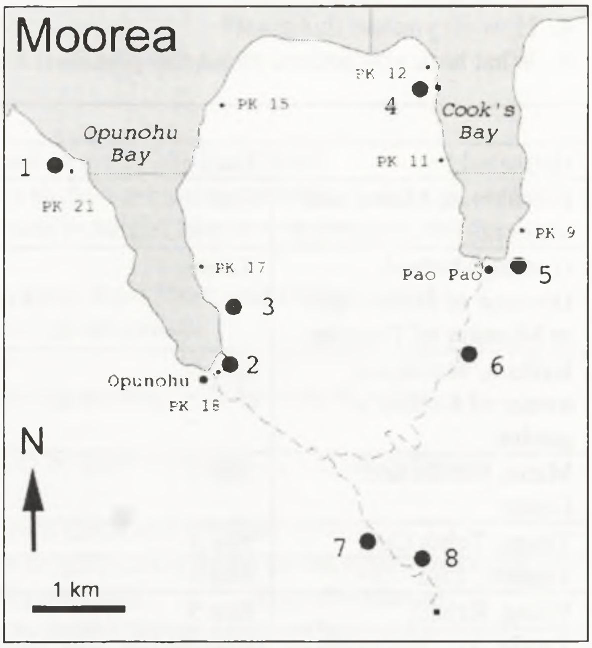

Figure 1. Sites where gingers were collected

Morphological characters obtained for collected specimens

Measurements were taken with metric tape or ruler. Flower and bract observations and dissections were aided by using a dissecting microscope and razor blades. Seventy-one characters were described (see Appendix 1). For each species sample size was five plants, with the exceptions of *A. purpurata* (five samples from each variety), *E. cevuga* (four samples), and *H. gardnerium* (two samples since only two samples existed). Since some gingers are seasonal in presence of shoots or inflorescences, so some characters were missing in these gingers. Colors and shapes of morphological characters were described for each specimen.

Ethnobotany

Interviews with eight households (listed in Table 1) were conducted in order to understand the differentiation, arrival times, Tahitian names and uses of gingers found in Moorea. Photographs of different gingers were brought to most interviews for easier identification. Discussions were conducted with the help of French, Tahitian and Hakka Chinese translators.

Questions asked were:

1. What varieties of ginger are in your garden? How do you distinguish them?
2. When have you noticed this ginger on this island? Have you noticed it in the wild?
3. What is the Tahitian name of this plant?
4. How do you use this plant?
5. What have you noticed about this plant?

Household	Location
Brotherson, Manea and Valentine	Site 1
Guerin, Michael, Director of Ecotourism in Ministry of Tourism	Tahiti
Kellum, Marimari, owner of Kellum's garden	Site 3
Manu, Gerald and Loana	Site 2
Tehau, Tchoi Cam	Site 5
Teuhiro, Lise	Site 2
Wong, Kristin	Site 5

Table 1. Interviewed Households and their locations; site locations in Moorea are mapped in Figure 1.

Ecological experiments

Alpinia purpurata shoot experiment. An experiment was conducted to test whether *A. purpurata* (pink variety) has an allelopatric effect on *A. purpurata* (red variety) in gardens. The null hypothesis is that both plants will have the same viability across different soil conditions. The hypothesis is that the red ginger plots planted in soil underneath the pink ginger will grow less than those with other conditions and that the pink ginger is not affected by soil differences.

Anecdotal evidence from ethnobotany interviews suggested that the pink variety tends to exclude the red variety from gardens (Kellum, Table 1, Wong, Table 1). This was tested by assigning thirty-six plots with different soil conditions: red, pink, or terrain. Red soil was defined as soil dug from underneath the red ginger, pink soil was defined as soil underneath the pink gingers and terrain soil was control. All soil was obtained at the Gump Station.

Plots were 0.5 m^2 each. New shoots (18 red ginger shoots, 18 pink ginger shoots) were taken from four different *A. purpurata* inflorescences in the Gump Station and randomly assigned different soil conditions. Six replicates were done for each condition:

1. Red ginger with red soil;
2. Red ginger with pink soil;
3. Red ginger with terrain soil;
4. Pink ginger with red soil;
5. Pink ginger with pink soil; and
6. Pink ginger with terrain soil

A cylindrical volume of approximately .00067 m^3 of terrain soil was replaced with pink ginger soil, red ginger soil, or repotted in terrain soil for each plot. The experiment was conducted from October 17 to November 9. Plants were noted as either "green" or "dried" on November 9. Green was defined as having at least one green leaf. If a plant is green, it is considered to be viable. Microsoft Excel was used for chi square analysis of data.

Etlingera elatior seed experiment. A germination study was undertaken to determine the seed viability of *E. elatior*, since it appeared that all gingers asexually reproduce through rhizomes and not through seeds. *A. purpurata* (kimi variety), *A. zerumbet*, and *E. elatior* (red torch ginger variety) had been observed to also have fruits, but only the fruit of *E. elatior* was eaten. The pink variety of *E. elatior* lacked fruits. The null hypothesis was that the seeds would not germinate, since *E. elatior* asexually reproduces. Four fruits (3 fresh fruits, 1 dried fruit) taken from the same specimen, each containing approximately 50-100 seeds with an average seed size of 3-5 mm, were placed between 4 layers of tissue paper in 4 closed petri dishes-- one petri dish for each fruit.

The experiment began on October 15 and terminated on November 9. Initial watering was 20 mL water on the first day, 10 mL water on the second day, and 5 mL of water every five days after. Petri dishes were placed on a window ledge in the dry lab of the Gump Station and shaded with a mesh cloth since torch gingers generally prefer semi-shady environments (Nirav 1992).

Results

Species descriptions including ethnobotanical information

For each species, morphological descriptions on shoot, leaf, inflorescence, bract, flower features, rhizome are given below. Photographs on all species were taken in Moorea and are included in Appendix 2. A *Unique features* section is included for each species to highlight distinguishing characteristics. All morphological data were based on primary observations unless literature is specifically cited for characteristics not present at the time of collection. The Tahitian name and use of each plant are based on interview data unless otherwise cited.

The six species previously documented in Moorea are designated with an asterisk next to their descriptions below. They were found at multiple sites, with the exception of the rare *H. gardnerianum*, which was only found in one location. They were all documented in the neighboring island of Tahiti in some form. Five were described in Welsh (1999) and Fosberg and Stoddart (1996), two were shown as photographs in Saquet (1996), and all were found as voucher specimens in the Museum of the Islands in Tahiti.

Table 2. Key to the Zingiberaceae of Moorea based on morphological data

1. Inflorescences on leafless shoot, separate from leafy shoot (radical)
    2. Rhizome interior orange .... *C. longa*
    2'. Rhizome interior white or pale yellow
        3. Leaf curls toward upper (adaxial) side, toward shoot axis (adaxial curl)
            4. Plant large ca 5 m in height; leaves slightly pungent, undulate .... *E. elatior*
            4'. Plant medium to large ca. 97-218 cm in height, leaves very pungent, entire .... *E. cevuga*
        3'. Leaf curls toward lower (abaxial) side, away from shoot axis (abaxial curl)
            5. Rhizome interior white .... *Z. spectabile*
            5'. Rhizome interior pale yellow
                6. Shoot maroon, beginning development by early October .... *Z. zerumbet*
                6'. Shoot green, dying off by early October .... *Z. officinale*

1'. Inflorescences on a leafy shoot (terminal)
    7. Leaf curls toward lower (abaxial) side, away from shoot axis (abaxial curl)
        8. Shoot green, ca. 99-248 cm in height; leaves green on the upper (adaxial) side and maroon on the lower (abaxial) side....... *H. gardnerianum*
        8'. Shoot green, ca. 103-207 cm in height; leaves green on both sides
            9. Flower yellow .... *H. flavescens*
            9'. Flower white....... *H. coronarium*
    7'. Leaf curls toward upper (adaxial) side, toward shoot axis (adaxial curl)
        10. Rachis partially or completely obscured by bract....... *A. purpurata*
        10'. Rachis visible
            11. Leaves uniformly green, glossy; bract closed, open during flowering, white with pink tips; labellum of flower yellow with red veination. .... *A. zerumbet*
            11'. Leaves variegated green and white; bract open, pink with green streaks; labellum of flower white....... *A. vittata*

Alpinia purpurata (Vieill.) K. Schum.
[*Guillainia purpurata* Vieill., *Languas purpurata (*Vieill)]
Common name: Red ginger, pink ginger
Tahitian name: Opuhi tahiti (red ginger variety), opuhi (pink ginger variety), kimi, double opuhi
Shoot Green for all varieties; "opuhi tahiti" variety 75-112 cm tall, 1-2 cm wide, internode between 9-14 cm; "opuhi" variety 90-158 cm tall, 1.5-2.5 cm wide, internode between 8-20 cm; "kimi" variety 200-264 cm tall, 3-4 cm wide, internode between 25-44 cm; "double opuhi" variety 207-293 cm tall, 2.2-4 cm wide, internode between 19-38 cm
Leaf Green, adaxial curl toward shoot axis, margin slight yellow, lanceolate, petiolate for all varieties; "opuhi tahiti" variety 12.5-37.5 cm long, 4.5-11 cm wide, 21-26 veins; "opuhi" variety 24-47 cm long, 6.5-12.5 cm wide, 15-24 veins; "kimi" variety 41-73.5 cm long, 11-19 cm wide, 15-19 veins; "double opuhi" variety 46-59 cm long, 12-16 cm wide, 12-14 veins
Inflorescence Terminal, rachis partially obscured by bracts for all varieties; "opuhi tahiti" variety 17.5-27 cm long, 5-7.5 cm wide; "opuhi" variety 20-39 cm long, 3-7 cm wide; "kimi" variety 18-31 cm long, 8-11 cm wide; "double opuhi" variety 27-64 cm long, 15-32 cm wide
Bract "Opuhi tahiti" variety 4.2-4.6 cm long, 2.3-3 cm wide; "opuhi" variety 4-5 cm long, 2.5-3.5 cm wide; "kimi" variety 4.2-5.3 cm long, 3-5 cm wide; "double opuhi" variety 5-6 cm long, 4-4.5 cm wide
Flower features Calyx pink and white, 2 cm long, .6 cm wide; corolla three unequally-lobed, white 3 cm long, .5 cm wide; three-lobed labellum; anther 1.8 cm long, 1.2 mm wide, adnate on petaloid, placentation axile
Rhizome Tan outside, white inside
Uses Inflorescence often sold as cut flowers or placed in bouquets
Introduction: European; "opuhi tahiti" variety came from Soloman islands around 1930s, the "opuhi" and "kimi" variety came within the last ten years (Guerin, Table 1).
Unique Features

The "opuhi tahiti" and "opuhi" varieties are the most common ornamental gingers found in gardens and bordering paths. Young shoots from inflorescences can be removed and planted; these shoots develop into smaller plants with rhizomes. However, plants that developed from the original rhizome will grow taller than those from the inflorescence. (Tehau, Table 1).

"Opuhi tahiti", "opuhi", "kimi" and "double opuhi" are the four varieties found in Moorea.

--See Appendix 2, figure 2-5

**Alpinia vittata* W. Bull
Common name: n/a
Tahitian name: Opuhi
Shoot Green, 165-360 cm tall, 1.5-2 cm wide, internode between 8-15 cm.
Leaf Green with white bands, 31.5-42 cm long, 5-9 cm wide, adaxial curl toward shoot, 19-26 veins, lanceolate or elliptic-lanceolate with sinuate margin, petiolate
Inflorescence Terminal, 14 cm long, 5 cm wide, rachis glabrous and clearly visible between bracts
Bract Pink with green marks, 1.5 long, 5.5 wide
Flower features Calyx pink and white, 2cm long, .6 cm wide; corolla three-lobed, white 3 cm long, .5 cm wide; labellum three-lobed; anther 1.8 cm long, 1.2 mm wide, adnate on petaloid, placentation axile
Rhizome Tan outside, white inside
Uses Foliage plant in gardens for its variegated leaves
Unique Features

It is the only ginger in Moorea that has variegated green and white leaves. It was misclassified as *A. nutans* in Saquet (1996).

--See Appendix 2, Figure 6

Alpinia zerumbet (L.) Sm.
[*A. speciosa* K. Schum, *Languas speciosa* (K. Schum) Small]
Common name: Shell ginger
Tahitian name: n/a
Shoot Green, 214-240 cm tall, 2-4 cm wide, internode between 11-30 cm
Leaf Green, glossy, 35-62 cm long, 11.5-14 cm wide, adaxial curl toward shoot axis, 11-14 veins, lancelate and slightly ciliated with brown hair on margins, petiolate
Inflorescence Terminal, 33-44 cm long, 8-13 cm wide, rachis pubescent and seen
Bract White with pink tip at top, 3-3.5 cm long, .75-1.3 cm wide
Flower features Calyx white with pink tip, 2.4-2.5 cm long, 2 cm wide; corolla three unequally-lobed, white with pink tip 3.5-4.5 cm long, 1-2 cm wide; one-lobed chordate shaped labellum, yellow with red venation, somewhat ruffled, 4-6 cm long, 3-4.5 cm wide; anther 1.5-2 cm long, 0.2-0.4 cm wide, stamen, 3.8-4.5 cm long, style 4.8-5 cm long; ovary villous, placentation axile
Rhizome Tan outside, white inside

Uses cultivated ornamentally in gardens
Introduction European
Unique Features
Shell ginger flowers are the showy part of the inflorescence, unlike most gingers in Moorea, which have showy bracts and small flowers.

One orange fruit is found attached to the rachis of the shell ginger. A bumblebee (genus *Bombus*), was observed visiting the flowers of the shell ginger.
-- See Appendix 2, Figure 7

Curcuma longa L.
Common name: Tumeric
Tahitian name: Re'a or re'a Tahiti
Deciduous - Only the rhizome was found in the spring, so the floral description has been adapted from Welsh, 1998 and Wagner et al., 1990.
Shoot Green, 150 cm tall
Leaf Green, 30-50 cm long, 8-18 cm wide, lanceolate, oblong or elliptic, glaborous
Inflorescence Radical, 10-20 cm long, 5-8 cm wide, rachis not clearly visible.
Bract White and green to pink, 5-6 cm long, width n/a
Flower features calyx, 0.9-1.3 cm long; corolla pink to yellow or green 2.7-3.9 cm long; labellum white with a yellow medium band, 1.5-1.9 cm long, 1.5-1.9 cm wide; stamen ca 0.6 cm long; staminode present; ovary three-celled
Rhizome Orange-red
Uses Tahitians use the rhizome as a medicine, dye, or flavoring. The rhizome can be ground up, mixed with coconut milk, and applied to skin as a tanning lotion.
Introduction Polynesian
Unique Features
C. longa has a uniquely colored rhizome.
-- See Appendix 2, Figure 8

Etlingera cevuga (Seem.) R.M. Sm.
[*Ammoum cevuga* Seem., *Geanthus cevuga* (Seem.) Loesener]
Common name: n/a
Tahitian name: Opuhi
Shoot Leafy shoot green, 97-218 cm tall, 1.6-2 cm wide; leafless shoot (Welsh 1998) 6-15 cm long
Leaf Green, 26-41 cm long, 4-13 cm wide, adaxial curl toward shoot axis, 16-21 veins, oblong-lanceolate, petiolate
Inflorescence not seen. Below description has been adapted from Welsh 1998, Wagner et al. 1990, and from a specimen from the Museum of the Islands in Tahiti.
Inflorescence Radical, rachis not seen
Bract Purplish-red, pubescent, ciliated along margins, closely imbricate; sterile ones ovate, 2.7-4 cm long, 1.8-2.4 cm wide, fertile bracts oblong-oblong lanceolate, 2.7-3.5 cm long, 1.2-2 cm wide
Flower features Calyx red, 2-2.5 cm long, pubescent; corolla red 3.5-3.8 cm long, sparsely pubescent, width n/a; labellum peach colored with yellowish medium band, somewhat ruffled, 1.2-1.8 cm long, 1.1-1.5 cm wide; anther 0.5 cm long; ovary villous
Rhizome Tan outside, white inside for old plants; red outside, white inside for young plants
Uses The pungent leaves were used as mattress padding in ancient times (Petard 1986).
Introduction Native or Polynesian
Unique Features
"Opuhi" is a name applied to many different gingers, but *E. cevuga* is the original "opuhi" (Kellum, Table 1).
-- See Appendix 2, Figure 9

**Etlingera elatior* (Jack) R.M. Sm
[*A. elatior* Jack, *A. magifica* Roscoe, *Nicolaia elatior* (Jack) Horaninow, *Phaeomeria magnifica* (Roscoe) K. Schum.]
Common name: Torch Ginger
Tahitian name: Rose de Porcelaine
Shoot Green, leafy shoots above 456 cm tall, 4-5 cm wide, internode between 29-35 cm; leafless shoots green for pink variety, red for red variety, 135-200 cm tall, 1-3 cm wide
Leaf Green, red hue on only the red variety, 62-88.5 cm long, 12.5-18.5 cm wide, adaxial curl toward shoot axis, 17-21 veins, oblong, undulate and petiolate
Inflorescence Radical, 12-18 cm long, 9-17 cm wide, rachis obscured by imbricate bracts
Bract Red or pink with white outline, 9.5-10.5 cm long, 5-9 cm wide
Flower features Calyx three-toothed, semi-transparent with pink hue and red stripes, 3.5 cm long, 1.5 cm wide; corolla tubular, semi-transparent with pink hue and red stripes, three equal lobes, 3.5 cm long, .45 cm wide; one-lobed labellum, red with either white (pink variety) or yellow (red variety) margin, 4.1 cm long, 1.8 cm wide; anther 0.9 cm long, 0.2 cm wide, stamen, 3.3 cm long, style 3.2 cm long; ovary villous, axile placentation
Rhizome Brown outside, yellow inside for old plants, red outside, white inside for young plants
Uses Inflorescence often sold as cut flowers or placed in bouquets.
Introduction European

Unique features

The largest Moorean ginger plant, it has hooks on the tips of its sterile bracts. It has previously been documented in Tahiti but not in Moorea, even though it has been present in Moorea since the 1960's (Kellum, Table 1; Tehau, Table 1; Teuhiro, Table 1).

Two varieties, red and pink, are seen in Moorea. They vary in stem, leaf and inflorescence color. Only the red variety is seen fruiting (Kellum, Table 1). The honeybee (*Apis mellifera*) has been observed to visit the flowers.

-- See Appendix 2, Figure 11

**Hedychium coronarium* Koenig in Retz
Common name: White ginger
Tahitian name: n/a
Shoot Green, 103-171 cm tall, 1-2 cm wide, internode between 11-15 cm
Leaf Green, 32-36 cm long, 4-6 cm wide, abaxial curl away from shoot axis, 13-21 veins, oblong-lanceolate or lanceolate, sessile
Inflorescence not seen. Below description has been adapted from Welsh 1998, Wagner et al. 1990.
Inflorescence Terminal, 20-30 cm long, 8 cm wide, rachis obscured by bract
Bract Green, oblong, imbricate, 3.5-5.5 cm long, 2-3 cm wide
Flower features Calyx white, 2.5-3.5 cm long; corolla white; floral tube ca 5 cm long; labellum yellow or yellow green at center, 4.5-5 cm long, 5 cm wide; stamenodes prominent, as long as labellum length, 3.5-5.5 cm wide; anther orange
Rhizome Tan outside, white inside for old plants, red outside, white inside for young plants
Uses Flowers were placed in bras to function as perfumes before French perfumes became widespread (Brotherson, Table 1).
Introduction European
Unique features The leaves are a slightly lighter green than *Hedychium flavescens*; otherwise it is hard to differentiate this species from *H. flavescens* without the flower. Both *H. flavescens* and *H. coronarium* are documented in Tahiti but not in Moorea (Fosberg and Stoddart 1996 and Welsh 1998).
-- See Appendix 2, Figure 13

**Hedychium flavescens* Roscoe, Monandr.
Common name: Yellow ginger
Tahitian name: n/a
Shoot Green, 172-207 cm tall, 2-3 cm wide, internode between 12-17 cm
Leaf Green, 43-59 cm long, 9-11.5 cm wide, abaxial curl away from shoot axis, 8-15 veins, oblong to lanceolate, sessile
Inflorescence Terminal, 14-20.5 cm long, 3-7 cm wide, rachis obscured by imbricate bracts
Bract Green, 4 cm long, 2.5 cm wide
Flower features Calyx three-toothed, semi-transparent light green, 4.2-4.5 cm long, 1 cm wide; corolla yellow; floral tube 8.5 cm long, 0.3-0.4 cm wide 4.0-4.2 cm long, 0.5 cm wide; labellum two-lobed, yellow, 4.6-4.7 cm long, 4 cm wide; staminode prominent, 4.5-4.7 cm long, 1.7-2 cm wide; anther 1.6-1.7 cm long, 0.1 cm wide; stamen, 5.6-5.7 cm long, 0.2 cm wide; style 5.5-5.9 cm long; placentation axile
Rhizome Tan outside, yellow inside for old plants, red outside, white inside for young plants
Uses Same as *H. coronarium*
Introduction European
-- See Appendix 2, Figure 14

**Hedychium gardnerianum* Roscoe, Monandr.
[*H. pallidum* Regel]
Common name: Kahili ginger
Tahitian name:
Shoot Green, 90-248 cm tall, 2-2.4 cm wide, internode between 8-13 cm
Leaf Green upperside, maroon underside, 41-42 cm long, 6.5-7 cm wide, abaxial curl away from shoot axis, 14-20 veins, linear to elliptic, sessile
Inflorescence not seen. Below a description has been adapted from Wagner et al. 1990.
Inflorescence Terminal, 16-45 cm long, primary bracts widely space on rachis, rachis glabrous
Bract Color n/a, ovate-elliptic, enfolding cincinni
Flower features Calyx cylinderical, 3-lobed; corolla greenish-yellow; floral tube ca 5-5.5 cm long; labellum orange at center, 2.5-3 cm long; stamenodes prominent, yellow, 3 cm long; anther orange, ovary glabrous
Rhizome Tan outside, white inside
Uses n/a
Introduction European
Unique features

The kahili ginger is a rare species in Moorea. Fosberg and Stoddart (1996) documented it in Tahiti.

-- See Appendix 2, Figure 15

Zingiber officinale Roscoe, Trans. Linn.
Common name: Ginger, common ginger
Tahitian name: Re'a tinito (Chinese ginger)
Deciduous – Qualitative observations on leaf and shoot were taken before the shoot died around October 12. Only the rhizome was found

afterwards, so the below floral description has been mostly adapted from Welsh 1998.

Shoot Green, 75-150 cm tall, width n/a

Leaf Green, 15 cm long, 1.5 cm wide, abaxial curl away from shoot axis, lanceolate

Inflorescence Radical, 4-5 cm long, 2 cm wide, rachis not seen

Bract Green with a submarginal band, 2.5 cm long

Flower features Calyx 1 cm long; corolla yellowish 2.5 cm long; labellum dark purple with cream blotches and base; anther, cream with dark purple appendages; staminode present, 6 cm long, 1-1.2 cm wide

Rhizome Tan outside, pale yellow inside

Uses The rhizome is used as food and medicine. In Tahitian medicine, the rhizome is ground up and mixed in coconut oil for rheumatoid arthritis (Manu, Table 1)

Introduction Chinese, around 1850 (Kellum, Table 1)

Unique features

This ginger is the common ginger found in grocery markets in the United States and in Tahiti.

--See Appendix 2, Figure 16

**Zingiber spectabile* Griffith

Common name: Black gingerwort

Tahitian name: n/a

Shoot Leafy shoot green, 92-121 cm tall, 2-2.8 cm wide; leafless shoot green, 30 cm long, 2 cm wide

Leaf Green, 37-41.5 cm long, 7-9 cm wide, abaxial curl away from shoot axis, 14-17 veins, lanceolate or linear, sessile or petiolate

Inflorescence Radical 10 cm long, 5.5 cm wide, rachis hidden within imbricate bracts

Bract Yellowish-green, closely imbricate, 4 cm long, 4 cm wide

Flower not seen. Below description has been adapted from Hottum 1950.

Flower features Calyx pink or cream, 2.7-3 cm long, globrous; corolla pale yellow ca 5.7 cm long, 0.6 cm wide; labellum dull dark purple with many small pale yellow spots, 2.5 cm long, 1.4 cm wide; anther 1.5 cm long; ovary pubescent; staminodes present, 1 cm wide

Rhizome Tan outside, white inside

Uses Inflorescence placed in bouquets

Introduction n/a, most likely European

Unique features

Based on observations *Z. spectabile* appears to be sessile in sunny environments and petiolate in shady environments.

--See Appendix 2, Figure 17

Zingiber zerumbet (L.) J. E. Smith
[*Amomum zerumbet* L.]

Common name: Shampoo ginger or wild ginger

Tahitian name: Re'a moeruru

Shoot Leafy shoot maroon, 26-47.5 cm tall, 0.75-1 cm wide, internode between 26-47.5 cm

Leaf Green, 14-30.5 cm long, 5.5-7 cm wide, abaxial curl away from shoot axis, 18-28 veins, lanceolate, sessile

Inflorescence not seen. Below a description has been adapted from Welsh 1998, Wagner et al. 1990.

Inflorescence Radical, rachis hidden by imbricate bracts

Bract Green when young, red when old, closely imbricate, ovate, 1.5-4 cm long, 1.25-4 cm wide

Flower features Calyx 1.3-1.7 cm long; corolla whitish to dark yellow 3.5-5.5 cm long; labellum orange to yellow, 1.2-2 cm long, 1.5-2 cm wide; anther 0.8-1 cm long

Rhizome Tan outside, pale yellow inside

Uses Juice from inflorescence used as shampoo

Introduction Native or Polynesian

Unique features

Its maroon shoot makes this ginger distinctive from other gingers, which have green shoots

-- See Appendix 2, Figure 18

Ecological experiments

A. purpurata shoot experiment. Figure 19 illustrates the number of plants in each variety and the plant viability status (dried or green) on November 9. Two red gingers remained green from October 17 to November 9, whereas thirteen pink gingers remained green. Figure 20 illustrates the number of plants in each type of soil and the plant viability status. Based on chi square statistics viability differences between varieties were statistically significant at the 0.01 significance level. No significant correlation at the 0.01 significance level was found between soil type and plant viability. Table 2 lists the chi square analysis of Figures 19 and 20.

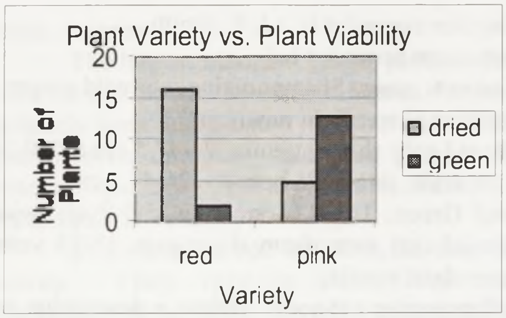

Figure 19. Number of plants in each variety that are either viable (green) or dead (dried).

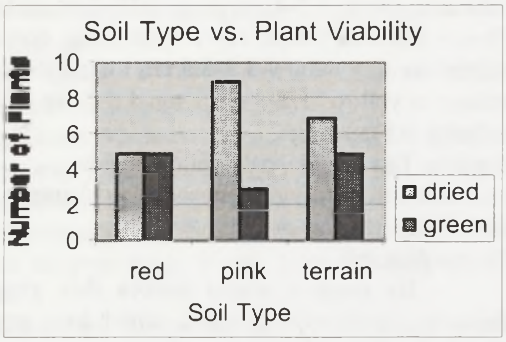

Figure 20. Number of plants in each soil type that are either viable (green) or dead (dried).

	Plant variety vs. Plant viability	Soil type vs. Plant viability
Chi-squared	13.8	1.758
Degrees of Freedom	1	2
Probability	0.000	0.415

Table 2. Chi-squared analysis on plant variety and soil type vs. plant viability

E. elatior germination experiment. Only one seed from a *E. elatior* fruit in a petri dish of 71 seeds germinated. Seeds from other fresh fruits on the same inflorescence and those from the dried fruit did not germinate. Approximately 300 seeds were tested in this experiment.

Discussion

How to identify gingers - morphological comparisons

Larsen et. al. (1999) stated that the "structure of inflorescence and flowers is of prime importance in classification and identification of the *Zingiberaceae*." Keys for gingers are generally based on inflorescence and floral morphology (Ridley 1924, Stone 1970, Wagner 1990). Since most gingers have flowers that are short-lived or rare, identification based on floral morphology can be difficult (Larsen et al. 1999). In addition, the consistency of the flower form is the character that separates *Zingiberaceae* from all other plants; thus basing a key on floral characters is unsatisfactory (Holttum, 1950). Holttum was the first to base his key almost entirely on inflorescence and bract characters, instead of on primarily floral characters. The easiest way to identify gingers is by their inflorescences, which are quite distinct between each species. The exception to this rule is *H. flavescens* and *H. coronarium,* which appear to be disinguishable only by flower coloration (Welsh 1998).

Most gingers are seasonal in their inflorescences; so even a key only based on inflorescence and bract characters may not help in identification. Table 2, "Key to the Zingiberaceae of Moorea," is mostly based on non-floral characteristics. One important distinguishing characteristic is the way a leaf curls; this has not previously been discussed or considered in literature as an identifying characteristic in gingers. All ginger leaves, especially when removed from the rest of the plant, prefer to curl in one direction, whether toward the shoot axis (having an adaxial curl) or away from the shoot axis (having an abaxial curl). Generally, leaves that have abaxial curls are less stiff and have a thinner, more velvety feel than those that have adaxial curls. Leaf pubescence exists in all leaves with abaxial curls, but not vice versa. These characteristics are potentially useful for differentiating between ginger species, because both leaf curl and pubescence are discrete characteristics consistent for all gingers within the same species. They appear to be genetically, rather than environmentally, based.

Another important leaf characteristic is whether the leaf is petiolate or sessile. It appears to be a distinct identifying characteristic for all Moorean gingers except for *Z. spectabile,* which, based on observations, appears to have a petiole only when the species is found in the shade.

Ginger flowers have a general form overall: three-toothed calyx, three-lobed corolla, one labellum, one fertile stamen with the carpel inserted between the two thecae of the stamen (Wagner et al. 1990). Despite the general consistency of form, flowers vary in size, shape, presence of staminodes (infertile stamen), anther attachment, arrangement on the bract, and coloration. Floral morphology (size and shape of floral structures) is important to examine in determining whether a plant belongs to a ginger variety or a new species; inflorescence (bract) morphology, color, and development can vary within species but flowers appear to be consistent between varieties. Some gingers (such as the *Hedychium* genus) have two prominent stamenoides that look like petals; while others observed in the study (*Alpinia* and *Etlingera* genuses) lack or have reduced stamenoides. Another distinct floral character for morphological comparisons is whether the anther is attached to a petaloid structure facing the labellum, or if it is on a filament.

Rhizome color, a key morphological characteristic, varies in some species depending on the age of the plant; whether it varies within that particular species is noted in the "Results" section under "rhizome." Definitive coloration (rhizome interior orange, yellow, or white) was used in the key only if it appeared consistent for that species.

Shoot height, an age-dependent morphological character, can help distinguish between large ginger plants (such as *E. elatior*, which can be taller than 5 m) and small ones (such as *Z. zerumbet*, whcih ranges from 26 cm to 47.5 cm), but varies too much with the age of the plant and environmental factors to be a central identifying character.

Phylogeny

Selections for phylogenetic characters are based on whether they are discrete states, independent, homologous, and genetically determined. Leaf curl can potentially be a characteristic for phylogenetic analysis since it fits the above criteria. Presence or absence of a petiole may not be a characteristic to consider for phylogenetic analysis because it varies for one species. Any characteristics that vary by age, such as size of plant structure, should not be considered for phylogeny but can work well as comparative morphological characters.

Generally, floral characters are useful characteristics for phylogenetic analysis. The presence or absence of a staminode appears to be a discrete, homologous, and genetically determined character. Another distinct floral character for phylogenetic analysis is whether the anther is actually attached to a petaloid structure facing the labellum, or if it is on a filament. Not enough characteristics were available to create a phylogeny of gingers in Moorea because six of the twelve species found on Moorea were not flowering during the time of this study.

Ethnobotany

Residents of Moorea were essential in finding and identifying gingers. Because six out of twelve ginger lacked inflorescences at the time of study, there was difficulty in identification of the species. Also, two of the twelve gingers lacked shoots, so it was impossible to find them without help from the people in Moorea. They knew the location of and information about Zingiberaceae because gingers are highly used in Moorea.

Ginger uses in Moorea generally fall into two categories: ornamental uses or functional uses. Gingers have been relatively recently used as ornamentals or as landscape plants, as compared to more ancient uses in traditional medicine and spices (Larsen et al. 1999). The showy inflorescences of the ornamental gingers, with brightly colored bracts and floral parts, are often used as cut flowers in decorations or placed in bouquets. According to Guerin (Table 1), people prefer newer varieties of ginger. Thus, it is not surprising that the recently introduced pink and "kimi" varieties of *A. purpurata*, the most popular ornamental ginger grown, are already found all over Moorean gardens and in bouquets.

Different parts of gingers have a wide variety of uses functionally. The rhizomes of gingers *Z. officinale* (common ginger) and *C. longa* (tumeric) are used in Tahitian medicine or as a food spice. *C. longa* rhizomes can also be ground and applied topically with coconut oil as a skin-darkening lotion (Manu, Table 1). The pungent leaves of *E. cevuga* are used as mattress pads or rooftops of temporary shelter (Petard 1986). Juice from the inflorescence of the shampoo ginger, *Z. zerumbet*, is used as shampoo (Petard 1986, Whistler 1992, Manu, Table 1). Flowers from *H. flavescens* and *H. coronarium* are placed in bras as perfumes before French perfumes became common in Moorea (Brotherson , Table 1).

Alpinia purpurata shoot experiment

Based on statistical analysis using chi-squared analysis, the pink ginger variety appears to be more viable than the red ginger variety of *A. purpurata*. Regardless of soil differences, the pink ginger appeared to survive better than the red ginger through the duration of this study. That may explain why pink gingers appear to exclude red gingers. It is not possible to determine whether the pink ginger actually has allelopathic effects on the growth of red gingers, because soil difference had no significant effect on plant viability. More tests with a larger sample size and a longer time period need to be conducted to strengthen the hypothesis that allelopathy or exclusion is occurring.

Etlingera elatior seed experiment

The presence of a germinating seed indicates that torch ginger seeds are likely to be viable based on this experiment. However, only one out of approximately three hundred seeds germinated, suggesting that germination conditions could have been improved to give a higher yield. Since torch ginger fruits are eaten based on observations, a more realistic germination experiment, such as placing mechanical stress on the seeds to simulate animal digestion, may give a higher yield. The most likely pollinator is the *Apis mellifera* found on the red torch ginger but not on the pink; perhaps that explains why only the red variety is fruiting. The red torch ginger thus has the potential to reproduce sexually and migrate far away from the source plant, but the results were not strong enough to confidently discount chance.

Conclusion

Gingers are a morphologically varied and highly useful family. The lack of a complete documentation of gingers in Moorea necessitated this study of ginger as groundwork for future studies of ginger in Moorea. Twelve species and fifteen varieties of gingers in Moorea were described in detail; six of these were previously undocumented. All were different morphologically and used either ornamentally or functionally on Moorea. A key was created with primarily non-floral characteristics.

An allelopathy experiment was conducted between varieties of the common ginger *A. purpurata* to understand varietal differences. The results of this experiment suggest that the pink variety is more viable than the red variety in the short-run. Future studies on differences between the two varieties of *Alpinia purpurata* can determine why one variety appears to exclude another variety. A seed germination experiment on the red variety of *E. elatior* was done to determine whether *E. elatior* can sexually reproduce in Moorea. Since only one seed germinated out of three hundred seeds, the results suggest that the seeds are viable. More research is needed to strengthen the idea that *E. elatior* can reproduce through seeds. Understanding varietal differences can help in knowing what varieties to cultivate and which varieties are becoming endangered.

Without first identifying or classifying a species, in-depth research on that species would be difficult to continue. A complete documentation of gingers in Moorea will allow easier ginger identification there. From thorough morphological descriptions, a phylogenetic study based on morphological characters can follow in understanding the evolutionary relationship between these species in Moorea. A key containing non-floral characteristics, such as that in Table 2, along with thorough morphological descriptions, will help in identifying gingers when they lack flowers. Since this key is primarily based on non-floral characteristics, it may be useful for the creation of similar keys in other parts of the world. Since inflorescences are not always present, such keys will be very useful in reliably identifying gingers worldwide. Future studies on gingers are necessary to create a key completely based on non-floral characteristics. Ethnobotanical information on gingers can lead to more ginger research conducted in Moorea to benefit not only the people of Polynesia through supplementing their knowledge but also help in advancing biological research on gingers. Continued collaboration with people in Moorea is needed to benefit gingers research. Moorea provides an ideal place to study gingers because gingers have been scientifically unexplored in Moorea and yet have been heavily utilized in Polynesian culture.

Acknowledgements

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Appendix 1. Morphological Characters Measured

Characters measured:

1.	Stem length
2.	Internode length (between first and second leaf from ground)
3.	Stem diameter (measured at 20 cm height from ground)
4.	leaf shape (2nd leaf from top): 1- linear; 2- lanceolate; 3- oblong; 4- elliptic; describe
5.	leaf length (2nd leaf from top)
6.	leaf width @ midpoint (2nd leaf from top)
7.	venation characteristics: 1- ridged; 2- colored; 3- both; 4- none; 5- other
8.	number in venation across mid-leaf (measured 1 cm from mid-vein)
9.	leaf margins: 1- entire with membrane; 2- ciliate; 3- undulate 4- entire without membranes
10.	leaf arrangement: 1- distichous; 2- spiral
11.	leaf curl: 1- inward; 2- outward
12.	apex: 1- acuminate; 2- apiculate; 3- attenuate; 4- other
13.	base: 1- acute; 2-attenuate; 3- other
14.	leaf coloration: 1- unified; 2- different on parts
15.	leaf attachment: 1- sessile; 2- petiolate, more than .5 cm
16.	leaf texture: 1-smooth; 2- velvety (pubescent back); 3- ridged, 4- other
17.	petiole attachment: 1- liguate
18.	stamen length
19.	stamen number observed: 1- one; 2- two; 3- other
20.	stamen attachment: 1- adnate to petaloid; 2- not adnate to petaloid
21.	dithecal anther: 1- yes; 2- no
22.	style between anther: 1- yes; 2- no
23.	anther shape
24.	anther length
25.	anther width
26.	stigma shape
27.	stigma apex pubescent: 1- yes; 2- no
28.	stigma color different from style color: 1- yes; 2- no
29.	style length
30.	calyx color: 1- same as subtending bract; 2- different
31.	calyx length
32.	calyx width
33.	corolla shape: 1- irregular/zygomorphic; 2- tubular; 3- bilabiate
34.	petal length
35.	petal width @ widest part
36.	secondary petal width
37.	petal shape
38.	petal color: 1- same as sepal color; 2- different
39.	labellum shape: 1- one-lobed; 2- two or three-lobed
40.	labellum length
41.	labellum width
42.	labellum number
43.	labellum color: 1- same as petal color; 2- different
44.	transverse placentation types: 1- axile; 2- parietal; 3- two chambered
45.	longitudinal placentation types: 1- basal; 2- free central
46.	inflorescence type: 1- raceme; 2- thyrse; 3- head; 4- cyme; 5- other

47.	inflorescence existence: 1- absent; 2- present
48.	inflorescence color change: 1- yes; 2- no
49.	inflorescence shape
50.	inflorescence length
51.	inflorescence width
52.	shoots/secondary inflorescence found on inflorescence: 1- yes; 2- no
53.	if found, shoots start/end at
54.	flowers found on inflorescence: 1- yes; 2- no
55.	if found, flowers start/end at (on inflorescence)
56.	inflorescence attachment: 1- terminal; 2- radical
57.	inflorescence insect associations: 1- ants; 2- aphids; 3- bumble bee; 4- honey bee; 5- scale, 6- other
58.	flower insect associations: 1- ants; 2- aphids; 3- bumble bee; 4- honey bee; 5- other
59.	number of flower subtended by bract: 1- one; 2- two
60.	bracts: 1- open; 2- closed (considered closed if any bract found on inflorescence is closed)
61.	bract length
62.	bract width
63.	rachis seen: 1- throughout; 2- half-way; 3- none
64.	bract arrangement: 1- spiral
65.	bract color change: 1- yes; 2- no
66.	bract color: 1- red; 2- pink; 3- orange red; 4- light green; 5- magenta; 6- fuschia; 7- white with pink; 8- other
67.	rhizome seen: 1- yes; 2- no
68.	rhizome diameter
69.	rhizome color: 1- white to slight pale yellow; 2- bright yellow; 3- orange-red
70.	longest root length
71.	rhizome orientation to leaf: 1- parallel; 2- transverse; 3- both

Appendix 2. Pictures of gingers taken in Moorea

Figure 2. "Opuhi tahiti" variety (red variety) of *A. purpurata*

Figure 3. "Opuhi" variety (pink variety) of *A. purpurata*

Figure 4. "Kimi" variety of *A. purpurata*

Figure 5. "Double opuhi" variety of *A. purpurata*

Figure 6. *A. vittata*

Figure 7. *A. zerumbet*

Figure 8. Rhizome of *C. longa*

Figure 9. Shoot of *E. cevuga*

Figure 10. Red variety of *E. elatior*

Figure 11. Inflorescence of the red variety *E. elatior*

Figure 12. Pink variety of *E. elatior*

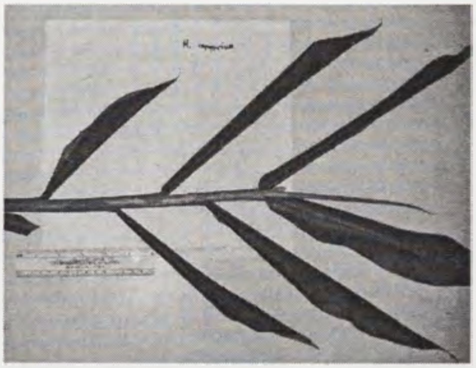

Figure 13. Shoot of *H. coronarium*

Figure 14. *H. flavescens*

Figure 15. Rhizome of *H. gardnerianum*

Figure 16. Rhizome of *Z. officinale*

Figure 17. Shoot and inflorescence of *Z. spectabile*

Figure 18. *Z. zerumbet*

Appendix 3. Specimens are deposited in the University and Jepson Herbaria, UC Berkeley

Voucher	Date	Species	Location	Observations	Pressed Parts
MC # 1	11-Oct	*Alpinia purpurata*	Loana's plantation, Opunohu, PK 18	Old alpinia with elongated bract, red bracts, green leaves, white flower	inflorescence, leaf, psuedostem and flower
MC # 2	11-Oct	*Alpinia purpurata*	Loana's plantation, Opunohu, PK 18	on inflorescence- Pink bracts on bottom, white bracts on top, green leaves	inflorescence and leaf
MC # 3	19-Oct	*Alpinia zerumbet*	Marimari Kellum Garden, Opunohu, PK17.5	Pink tip on white bracts, green leaves, brown rachi, fuzzy ovaries	inflorescence and leaf
MC # 4	19-Oct	*Alpinia zerumbet*	Marimari Kellum Garden, Opunohu, PK17.5	Pink tip on top of white bract, yellow labellum with red veins (MC #4-7 pressed on one newspaper)	flower
MC # 5	19-Oct	*Alpinia purpurata*	Gump Station, PK 11	White flower, red bract (MC #4-7 pressed on one newspaper)	bract and flower
MC # 6	19-Oct	*Alpinia purpurata*	Gump Station, PK 11	red and white bracts (MC #4-7 pressed on one newspaper)	white bract
MC # 7	11-Oct	*Alpinia purpurata*	Loana's plantation, Opunohu, PK 18	red bracts (MC #4-7 pressed on one newspaper)	secondary inflorescence
MC # 8	20-Oct	*Curcuma longa*	Nardi land, on inland road from Paopao to Opunohu, in Paopao valley	yellow, orange rhizomes	rhizome and root
MC # 9	12-Oct	*Hedychium flavescens*	Valentine's property, Papetoai, PK 21.5	yellow flower, green bract, orange stamen	inflorescence
MC # 10	22-Oct	*Calathea crotalifera*	Agriculture School—Loana obtained for me	yellow with green tint on bottom, not in ginger family	inflorescence
MC # 11	22-Oct	*Hedychium coronarium*	Loana's plantation, Opunohu, PK 18	light green leaves	leaves
MC # 12	14-Oct	*Alpinia purpurata*	border of house on PK 19	new shoot off an old inflorescence, green leaves, red bracts, green stem, light brown rhizome	inflorescence, leaf, psudostem and
MC # 13	23-Oct	*Etlinger elatior*	Marimari Kellum Garden, Opunohu, PK17.5	green leaf w/ maroon edges (4 specimens pressed to show front and back view	leaves
MC # 14	22-Oct	*Zingiber spectabile*	Loana's plantation, Opunohu, PK 18	fan like arrangement on leaves, green leaves and psudostem, yellow rhizome-no bract currently but if there was bracts, it would be orange yellow according to Loana	psudostem, leaves, rhizome slice
MC # 15	22-Oct	*Zingiber zerumbet*	Loana's plantation, Opunohu, PK 18	maroon/brown psudostem, green leaves, yellow rhizome	leaf, psudostem, rhizome and root

MC # 16	24-Oct	*Alpinia vittata*	neighbor next to Loana's plantation, Opunohu, PK 18	green leaf w/ white strips	leaf, inflorescence and flower
MC # 17	15-Oct	*Alpinia purpurata*	next to stream by pig farm, near Belvadere	pink inflorescence, green leaf	psudostem, inflorescence and leaf
MC # 18	29-Oct	*Alpinia purpurata*	Loana's plantation, Opunohu, PK 18	green leaf	leaf
MC # 19	30-Oct	*Hedychium gardnerianum*	Marimari Kellum Garden, Opunohu, PK17.5	green leaf with abaxial side maroon	psudostem and leaf
MC # 20	6-Nov	*Etlingera cevuga*	behind Marae Tetiiroa, near Belvedere	green leaf	leaf

An ecological survey of the epiphytic orchid species on Moorea, French Polynesia, with investigation into the factors influencing distribution and of the leafless epiphytic orchid, *Taeniophyllum fasciola*

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ABSTRACT. In order to identify the factors that may influence the differential distribution of the epiphytic orchids on Moorea, French Polynesia, a survey of the epiphytic species of the family Orchidaceae was made from sea level to selected high points on the island (up to 830 meters). Attributes such as elevation, moisture availability, sunlight exposure, substrate, and host species were examined and correlated with the species of epiphytic orchid found at that site to determine if there is a relationship between distribution and the ecological requirements of each species. *Taeniophyllum fasciola*, a leafless epiphytic orchid with photosynthetic roots, was examined separately to correlate distributional factors with certain reproductive indicators including fruit-to-flower ratios and reproductive success in a community. These data contribute to determining if the reproductive capacity of a population is limited by a trade-off between resource allocation for present reproduction and future growth and reproduction.

Introduction

It is intriguing to research an organism that is so widely recognized and studied. The human-interest significance of orchids is remarkable due to their great economic and aesthetic value, yet orchids are of extreme scientific interest as well. Orchidaceae is the most species-rich family of flowering plants in the world. An incredible number of genera have diversified to live in a variety of locations and habitats worldwide. Epiphytic representatives of this family thrive in many landscapes, but especially in the tropics where temperature and desiccation stress are less of a concern for plants living away from the soil due to the equable humid climate. While epiphytic species comprise not quite ten percent of higher plants worldwide (Kress 1986), in tropical rain forests epiphytes can make up nearly fifty percent of the flora (Kelly 1994). Although successful in this ecosystem, the epiphytic orchids in these environments still must compete for resources such as substrate, ecological position on the host tree (or phorophyte, as Nieder (2000) uses to indicate the nonparasitic relationship between epiphytes and the trees they use for mechanical support), and water availability with the associated epiphytes of the host phorophyte. A distinctive limitation many epiphytic orchids also have is an obligate association with mycorrhizal fungi. Most orchids possess a delicate specificity to being associated with fungal mycorrhizal symbionts for germination and occasionally nutrient acquisition into their adult stage. It is interesting to note whether these tropical epiphytic orchids on Moorea also have symbiotic fungi, due to the relative remoteness of the Society island group from a continental land mass. It is possible these orchids have evolved to survive without specific mycorrhizal associates because the chance of fungal spores and their orchid hosts both immigrating to such a remote island is unlikely.

Epiphytic orchids, in particular, being especially sensitive to moisture and stable substrate concerns, may be important indicators of habitat health. Due to their specialized habitat requirements, they can be effective indicators of the integrity of ecosystems. (Williams-Linera *et al.* 1995) It is therefore interesting to investigate the factors that influence this distribution of the several orchid species found on an island along an altitudinal gradient and between host trees, and what allows them to exist as epiphytic individuals with such restrictions. These factors might include available moisture (both from the moss associates, and the substrate they trap, the substrate of the bark of the phorophyte, and in terms of distance from terrestrial water source), sunlight, altitude, and specificity of potentially obligate epiphytic associates on the host (such as mosses, lichens, and fungi). Epiphytes are dependent on their hosts only for structural support, yet environmental factors surrounding and influencing this host, including type of bark, type of forest, available light, and proximity to water, all may affect distribution of the orchid epiphytes, regardless of whether the host is directly providing water or nutrients to the epiphyte.

Seven genera (twelve species) of epiphytic orchids have been confirmed to exist on the island of Moorea (Fosburg et al. 1996; Welsh 1998), part of the Society Island archipelago of French Polynesia in the South Pacific. This tropical volcanic high island rises to 1207 meters in elevation, and is thus an ideal study system for a survey of epiphytic orchids. The climate, altitudinal variation, and tropical humidity should support a diversity of species, and the results of this study will present a preliminary list of the epiphytic orchid species found on Moorea. Due to the sensitivity of orchids to environmental circumstances, this information will in turn provide an indication of the biodiversity and health of the island environment.

This study also examines the epiphytic orchid *Taeniophyllum fasciola*, a leafless species obtaining its nutrients from its photosynthetic roots grasping the host branches. Due to this adaptation, it is limited in its capacity for growth and potentially reproductive energy allocation. Each orchid fruit capsule can contain thousands of seeds, yet many times orchids suffer from other limitations constraining population growth. (Tremblay 1997) The study will contribute to determining if the reproductive capacity of a population is limited by the trade-off between resource allocation for present reproduction and future growth and reproduction, as suggested by Melendez-Ackerman (2000). Reproductive success clearly affects population growth rate although many ecological factors may result in substantial bottlenecks for growth rate.

Materials and Methods

A field study of epiphytic orchids was conducted on Moorea (17°30'S, 149°50'W), a volcanic high island of French Polynesia. Preliminary observations began on 17 September 2001.

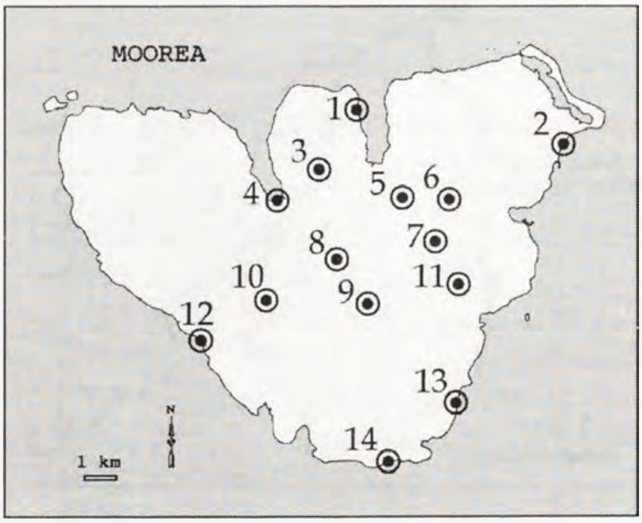

Figure 1. Study sites for between-species survey of epiphytic orchids on Moorea, French Polynesia, 17 September 2001 through 15 November 2001.

Data acquisition for the study ended on 15 November 2001. This study consisted of two different, but not entirely separate investigations into ecological factors influencing and limiting the distribution of epiphytic orchid species on Moorea. The first was a between-species comparison of all epiphytic orchid species found in a two-week survey of the island, and in doing this a second study was extrapolated to examine ecological factors that affect within-species distribution and reproductive capacity of a population of the leafless epiphytic orchid, *Taeniophyllum fasciola*.

Between-species comparison

Sites were located around the island at varying elevations to survey the composition of epiphytic individuals of the Orchidaceae family and assess the factors influencing their distribution (Figure 1, Table 1). These sites were found in a two-week survey around the island: along the coast road, up in the valleys, streams, trails, ridges, passes, mountain tops.

Table 1. Location of study sites on map (Figure 1) for between-species survey of epiphytic orchids on Moorea, French Polynesia, 17 September 2001 through 15 November 2001.

Map Site	Location	Elevation
1	UC Berkeley Gump South Pacific Biological Research Station	sea level
2	Temae	sea level
3	summit Mt. Rotui	898m
4	base of Opunohu Bay, mouth of Opunohu River	sea level
5	Pao Pao Valley, stream tributary	20m
6	Vaire-Paopao track, pass on ridge between Mt. Mouaputa and Mt. Tearai	320m
7	summit Mt. Mouaputa	830m
8	Opunohu River Valley	100m
9	Belvedere	266m
10	3 Coconuts Trail, pass on ridge between Mt. Mouapu and Mt. Mouaroa	450m
11	Putoa waterfall, Afareaitu Valley	120m
12	Haapiti	sea level
13	PK 12, S of Afareaitu	sea level
14	PK 15, southernmost point on Moorea	sea level

As many varying locations, elevations, and habitats as possible were surveyed during this period, to record all the epiphytic orchid species found and attempt to compile an accurate list of all species present on the island.

Data taken on each individual found included: species identity, location, elevation, estimated percent canopy cover, distance from water source (stream), distance above ground, information on the host (species, circumference of branch, inclination, position on the branch), and estimated cover and composition of associated epiphytes (mainly mosses and fungi). Also recorded was the estimated height of the orchid and whether the individual was fruiting or flowering. This data was compiled into a pilot list of the epiphytic orchid species found on Moorea, and supplemented with a key of the ecological features of each species. Voucher specimens were deposited in the University of California Herbarium, December 2001. (Appendix A)

Within-species comparison

One population ("Site 1") of the leafless epiphytic orchid, *Taeniophyllum fasciola*, was located at sea level at exactly PK18 at the base of Opunohu Bay approximately 30 meters inland from the water of the bay. This population was over 500 individuals large. One other small population of approximately 50 individuals ("Site 2") of *T. fasciola* was found at 100 meters elevation in Opunohu Valley along one of the stream tributaries of the Opunohu River, behind the agricultural school of Moorea, 200 meters the pig farm on the Col des 3 Cocotiers (3-Coconuts Trail). (Figure 2)

Both populations were compared along several ecological factors. These included elevation, distance from water source (stream), distance above ground, light intensity measured with a light meter (measured in *lux* units, 1 lumen/m^2), information on the host (species, circumference of branch, inclination, position on the branch), and estimated cover and composition of associated epiphytes (mainly mosses, lichen and fungi). Also recorded was information on each individual orchid including the estimated height of the plant, whether the individual was fruiting or flowering and counting plants per tree to estimate population density at the two sites. Each individual was measured counting the number of root extensions, and measuring the five longest roots and height of the plant with calipers. The lengths of the five longest roots were averaged to obtain a measure of plant size. ANOVA was run on each of the ecological variables to determine if the populations were significantly different.

The population at Site 1 was sampled through establishing 5m^2 quadrats in the *Hibiscus* sp. swamp parallel to the shore of the Opunohu stream. The entire swamp was 25 meters in width along the stream, and over 100 meters in length away from the stream.

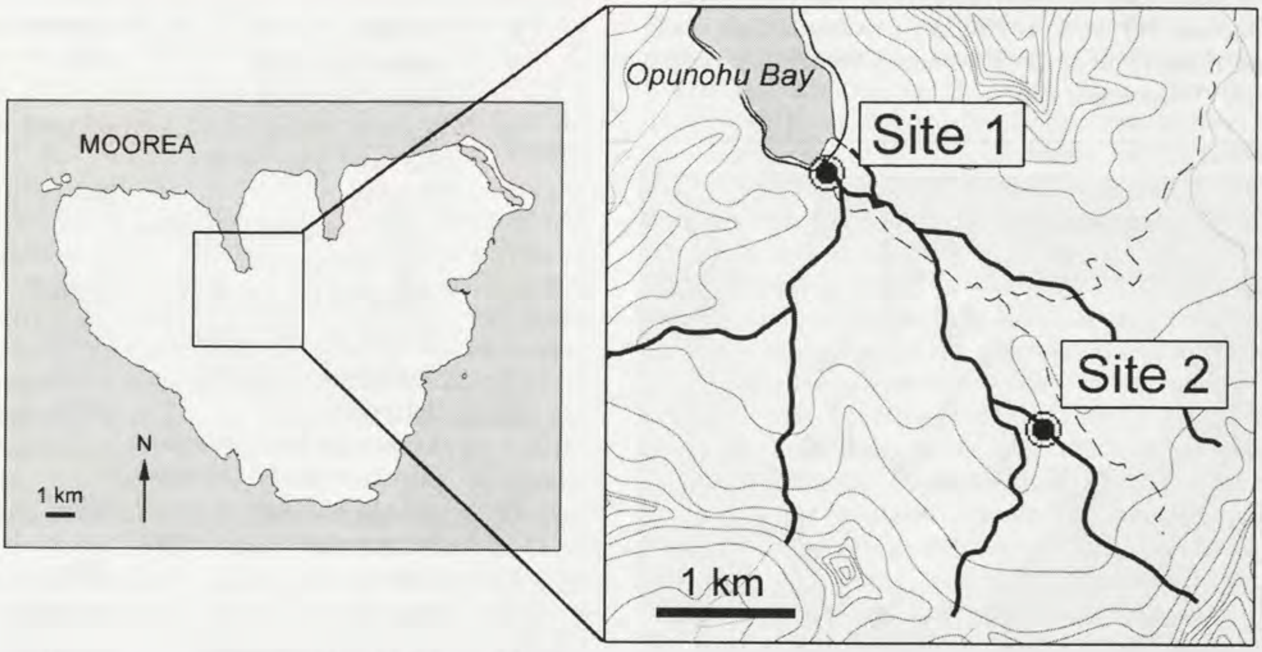

Figure 2. Study sites of *Taeniophyllum fasciola* at sea level (Site 1) and 100m elevation (Site 2), on the Opunohu River Valley system (darkened lines are the branches of the Opunohu River, dotted lines are roads) on Moorea, French Polynesia, 17 September 2001 through 15 November 2001. (100m contour lines)

Quadrats were established only within 25 meters of the stream due to there being no *T. fasciola* individuals present beyond that point in the swamp. Every *T. fasciola* individual present in each quadrat was counted and measured. All trees in each quadrat were measured and identified, as well.

The within-site comparison of *T. fasciola* indicated whether there was variation within a population due to host size, associated epiphyte cover, distance from water source, and light availability, and conclusions were made concerning the reproductive status of a population. The same data taken for the between-site comparison of this orchid was used for this within-site study of reproductive capacity and within-population variation. (These included elevation, distance from water source, distance above ground, light intensity, information on the host (species, circumference of branch, inclination, position on the branch), estimated cover and composition of associated epiphytes, size of the plant, and fruiting or flowering.) Linear regressions were run on the data to determine if there were any correlations between plant size, reproductive ability, and the environmental variables measured.

An examination of the potential mycorrhizal symbionts associated with the photosynthetic roots of *T. fasciola* was completed. This information was obtained by making cross-sections of the roots of selected specimens taken from the sea level site at the base of Opunohu Bay at PK18. Ten individuals were selected at varying distances from the stream. Half were young, non-reproductively-active individuals with >90% of roots less than 5cm in length. The individuals comprising the other half of specimens selected had an average root length greater than 5cm, or were reproductively active, (either fruiting or flowering). Twenty cross-sections were mounted on seven standard microscope slides, set with gel and sealed with nail polish. The vouchers were examined by Lewis Feldman, John Taylor, and Tom Bruns, of the Department of Plant Microbial Biology at the University of California, Berkeley, to determine if there were symbiotic mycorrhizal fungus associated with *T. fasciola*.

Results

Between-species comparison

Results compiled from the intensive two-week survey of the island were combined with information obtained on all epiphytic orchid species observed during the entire nine-week study period. Observations and ecological measurements were recorded for all epiphytic orchid species found, and specimen collection took place during this time. Almost all individuals (99.7%) were found within 100 meters of a water source (stream), with most (80%) being found within 10 meters of a stream. Individuals were rarely sampled higher than 2 meters above the ground due to sampling constraints and information from observations indicating that epiphytic orchids were rarely located higher than 2 meters above the ground. Nearly all (98.8%) of individuals sampled were on *Hibiscus* sp. host tree, with *Metrociteros* sp. and stumps of the fern *Angiopterus* sp. being the other species' found to host epiphytic orchids. Although a variety of mixed habitats were, including stands of trees that were primarily *Inocarpus* sp., and stands of the invasive *Miconia calvescens*, both near and away from streams, neither of these were found to be suitable hosts for epiphytic orchids, and any individuals found within these stands were isolated to the *Hibiscus* sp. or Angiopterus sp. hosts found within. Few individuals were found fruiting, and even fewer flowering.

Table 2. Orchid species found in island-wide survey of Moorea, 17 September 2001 - 15 November 2001.

species	# individuals	elevation	avg. distance from stream	avg. moss cover
Bulbophyllum longiflorum	8	110-500m	15.75m	40%
Bulbophyllum tahitense	1	610m	300m	30%
Dendrobium biflorum	22	110-480m	14.27m	75%
Dendrobium crispatum	4	3-110m	5m	96.3%
Dendrobium involuntum	1	195m	60m	30%
Eria rostiflora	1	204m	2m	70%
Oberonia equitans	8	0-460m	17.62m	26.9%
Phreatia myosurus	2	110-270m	54m	55%
Spathoglottis pacifica	66	100-445m	6.11m	91%
Taeniophyllum fasciola	547	0-110m	20m	60%

Ten species of epiphytic orchids (seven genera) were found on Moorea during the nine-week survey of the island. (Table 2) Individuals were found from sea level to some of the highest ridges on the island. The elevation range of *Dendrobium biflorum* was 110-480 meters, while the elevation range of *Dendrobium crispatum* was 3-110 meters.

Linear regressions run on plant size of each species against the environmental variables recorded yielded several trends. For the eight individuals of *Bulbophyllum longiflorum* found, there was a significant positive correlation between plant height and elevation (R^2=0.634, std. error 2.059), slope (R^2=0.455, std. error 2.246), and distance from ground (R^2=0.22, std. error 2.686). For the twenty-two individuals of *Dendrobium biflorum*, there was a significant positive correlation between height of plant and circumference of host tree (R^2=0.149, std. error 7.533). For the four individuals of *Dendrobium crispatum*, there was a significant positive correlation between plant height and distance from ground (R^2=0.465, std. error 1.044), circumference of host tree (R^2=0.613, std. error 0.057), and a significant negative correlation between plant height and moss cover (R^2=0.451, std. error 6.805). For the eight individuals of *Oberonia equitans* found, there were significant negative correlations between plant size and distance from a water source (R^2=0.765, std. error 9.861), and inclination of host tree (R^2=0.667, std. error 1.667), and a significant positive correlation between plant size and circumference of host tree (R^2=0.79, std. error 0.034). For the sixty-six individuals of *Spathoglottis pacifica* found, no significant correlations were found. Statistical analysis was not possible for *Bulbophyllum tahitense*, *Dendrobium involuntum*, *Eria rostiflora*, and *Phreatia myosurus* due to the number of individuals found being too low.

Within-species comparison

A four-week study of *Taeniophyllum fasciola* conducted at two sites on Moorea yielded over 500 individuals of this orchid species. All (100%) were found within 25m of a water source, and all individuals found were on *Hibiscus* sp. host trees. Regression data analysis was run to determine if there was a correlation between size of the plant and reproductive ability, and whether environmental factors such as distance from a water source, associated epiphyte cover, light availability, and size of host tree are correlated with plant size, or significantly affect the distribution of *T. fasciola*. An ANOVA was run on these two populations to determine if the environmental variables vary between populations with variance in size of plant, and analysis indicated that there was no significant difference between the two populations along these environmental variables. Linear regressions also indicated that there were no significant correlations between any environmental factors and plant size.

The population at 100m elevation only had 50 individuals, while the population at sea level had nearly 500 individuals. While the individuals at sea level were found within 25m of the stream, all individuals at 100m elevation were found within one meter of the stream. The light intensity at each site varied (average 3870 lux at the sea level site, and 618 lux at the 100m elevation site). *T. fasciola* individuals were never found near other epiphytes that were greater than 2cm in height. The average inclination of branch that *T. fasciola* was found on was 22° (std. dev: 24°).

For the within-site study of *Taeniophyllum fasciola*, there were significant positive correlations between plant size and flowering (R^2=0.211, std. error 0.570), and fruiting (R^2=0.437, std. error 0.687). 37% of orchids found at Site 1 were reproductively active (fruiting or flowering), while 30% of individuals at Site 2 were reproductively active. A 4:7 ratio of flowers to fruits was found for this population.

Although there was nothing in the root cross-sections that immediately looked like classic orchid mycorrhizae, there was evidence of fungal mycorrhizal hyphae coils found in the root cross sections taken from the younger specimens (average root length <5cm, not reproductively active). There was also evidence of ectomycorrhizal symbionts on the up-facing side of the root (as opposed to the side in contact with the phorophyte) found within 1cm of the root tip on several of the younger specimens. Several cross-sections also contained unidentified fungal spores and structures that were not mycorrhizal. Blue-green algae was found in the root cells of most of the cross-sections: in clusters as well as in layers closer to the surface, and the heterocyst was visible.

The orchid velamen layer was clearly visible in the root sections. There were slits on the outside layer of cells touching the phorophyte, as well as small connections (like tunnels) between each of these cells of the outer layer. The epidermis was morphologically different on either side of the root. Spoke-like transfer cells were also present near the central vascular system of the root, as well as filamentous structure cells. Idioblast cells were present containing raphids, small packets of calcium oxylate crystals.

Discussion

In examining the ecological factors that may influence a population of plants, it is nearly impossible to take into account all of the variables that may be affecting variation between populations. It may be especially difficult to define influential variables for tropical species, in particular, due to their being able to take advantage of a relatively equable year-round climate, constant humidity, and periods of intense rainfall that all contribute to temperature and moisture availability being consistent across the board for all populations and species on an island. This being, the data gathered for this study attempted to define ecological factors that do influence and limit the distribution of epiphytic orchids on Moorea, French Polynesia beyond factors such as rainfall, humidity, and temperature.

In the survey of the island of all epiphytic orchid species, it is curious that one common terrestrial species, *Spathoglottis pacifica*, was also found epiphytically. This species has not previously been recorded as an epiphytic species (Fosburg et al. 1996; Welsh 1998), although a possible explanation may lie in the degree of epiphytism that the individuals were found. That is, the *S. pacifica* individuals recorded in this study were found epiphytically (up to 4m above the ground), but mostly on heavily decaying branches and stumps. This most likely provided ample substrate for *S. pacifica* seeds to germinate, thus mimicking terrestrial soil, as opposed to a true phorophyte surface, as epiphytes must deal with.

The hypothesis that epiphytic orchids are located within a certain radius of a water source was not disproved (99.7% of all epiphytic orchids surveyed on the island were found within 100 meters of a stream, and 80% were found within 10 meters of a stream). This could be an indication that distance from a water source is an influential factor in the moisture availability for the orchid. These results could also be skewed to reflect the sampling pattern. Although many different areas of the island were sampled, trails to get to ridges and higher points on the island usually followed stream valleys, so areas within a certain distance of streams might be over-represented in the data. This might also be reflected in the species diversity found for the epiphytic orchids of Moorea. Ten species were found, while the literature (Fosburg et al. 1996; Welsh 1998) suggests that there are 17 species of epiphytic orchids on Moorea. It is possible that several species have disappeared from the island since 1998, but it is more likely that sampling technique over the nine weeks was focused in these areas, and was not sufficient enough to establish a complete accurate list of all epiphytic orchid species present on Moorea.

In both the between-species survey of all epiphytic orchids, and the within-species study of *Taeniophyllum fasciola*, nearly all (98.8%) of individuals found were on *Hibiscus* sp. host trees. Santos (2000) found that epiphytes do indeed show patterns of host preference, and Beckner (1979) suggests that orchids may be found on a particular host because they have highly specialized needs. The composition of the bark of the host tree certainly has an effect on what epiphytes are able to colonize there, yet even in mixed stands of trees where *Hibiscus* sp. was not the dominant phorophyte, epiphytic orchids seemed to be preferentially growing on *Hibiscus* sp. trees. This could be because the bark was ideal for germination of the tiny seeds of orchids – rough enough to act as a seed trap, catch the seeds and capture a bit of substrate to germinate in, but smooth enough to not gather enough substrate for larger epiphytes to grow that might out-compete the orchids or shade them too much. This preference pattern for host tree might also be attributed to complementary environmental factors for both the orchid and the host tree (light, humidity, epiphyte association).

The two species of *Dendrobium* found on Moorea were distributed differently along an elevational gradient. *Dendrobium biflorum* was found from 110m-480m elevation, while *Dendrobium crispatum* was found from 3m-110m elevation. Fosburg *et al.* (1996) suggests that the elevational gradient of these two species is reversed (*D. biflorum* from 240-500m, *D. crispatum* from 1000-1200m). This information would indicate that *D. crispatum* is only found on the highest mountain on Moorea, but individuals were found during this study as low as 3m in elevation.

Although statistical analysis was completed on all species surveyed on Moorea, it is likely that none but *Taeniophyllum fasciola* and *Spathoglottis pacifica* were sampled in great enough numbers to yield significant statistical conclusions. Neither of these species showed significant correlations (using linear regression analysis) with the environmental variables measured. Nevertheless, with small sample size, several correlations were identified for other species including *Bulbophyllum longiflorum*, *Dendrobium biflorum*, *Dendrobium crispatum*, and *Oberonia equitans*. Plant size of *B. longiflorum* was positively correlated with elevation, (R^2=0.634, std. error 2.059), slope (R^2=0.455, std. error 2.246), and distance from ground (R^2=0.22, std. error 2.686). Plant size of *Dendrobium biflorum* was positively correlated with circumference of host tree (R^2=0.149, std. error 7.533). Plant size of *Dendrobium crispatum* was positively correlated with distance from ground (R^2=0.465, std. error 1.044), circumference of host tree (R^2=0.613, std. error 0.057), and negatively correlated with percent moss cover (R^2=0.451, std. error 6.805). These data for *Dendrobium* make sense because a larger plant would need a larger host to support it. Also, germinating seeds and younger plants might need more moss cover to capture more substrate to grow in. As the plant grows larger, it out-competes the moss and thus moss cover is less in association with larger (older) plants. Data for *Oberonia equitans* showed negative correlations between plant size and distance from a water source (R^2=0.765, std. error 9.861), and inclination of host tree (R^2=0.667, std. error 1.667), and a positive correlation between plant size and circumference of host tree (R^2=0.79, std. error 0.034). These results are consistent with the hypothesis of larger plant size closer to a water source, as well as larger plants needing to be on larger host trees. The fact that there were no significant correlations between ecological factors and plant size for *Spathoglottis pacifica*, although the sample size was 66, may be due to the fact that this orchid was found in great numbers as a fully terrestrial plant, and to run statistical tests on only the ones deemed epiphytic may be misleading, and misrepresentative for the entire population.

Only two populations of the leafless epiphytic orchid, *Taeniophyllum fasciola*, were found on the entire island. This might be expected, however, as Hietz *et al.* (1995) suggests, due to the sharing of the same mycorrhizal partner that might not be found elsewhere. That study found that several orchid species showed an extremely high incidence of intra-specific association (or clumping). For the within-species comparison of *T. fasciola*, the sample size (487 orchids at Site 1 at sea level, and 50 orchids at Site 2 at 100m elevation) was sufficiently large to obtain statistically significant results, although linear regressions did not indicate any significant correlations, and the ANOVA analysis comparing the two populations did not suggest and significant differences between the them along the ecological parameters measured for this study. The reasons for these statistically insignificant results may be many. The variables chosen to compare the two populations were most likely not appropriate to address the larger study question of why these orchids are epiphytic in these locations, and not others. Although it has been found (Benzing 1983; Johansson 1974) that light and moisture availability are the major environmental factors limiting epiphyte distribution between hosts, perhaps distance from a stream was not an appropriate variable to test for moisture availability. It is also possible that specific characteristics measured of the host tree do not affect plant size or reproductive ability.

These populations were compared along several measurements, and compared in terms of reproductive capacity. This between-site comparison was examined to find out if there are significant differences in the populations in terms of size, host preference, and reproductive capacity, due to variables between the sites such as elevation, light availability, and moisture availability.

Individuals at the 100m-elevation site were found within 1 meter of the stream. This might be due to the fact that this site was located in a small valley, while the sea level site was a swamp, with all points in the *Hibiscus* sp. grove being on an equal level slope. Light intensity was much greater at the sea level site (average 3870 lux at Site 1, and 618 lux at Site 2). *T. fasciola* density and population size was also much greater there. This correlation is supported by de Rezende Cardoso, (1997) who found that epiphytic orchids were found in large numbers where light and air movement were more favorable. The sea level site was also near the marine influence of the bay, which might provide more airflow to the site. Air movement might aid in seed transfer, as orchid seeds are very tiny and light, and are easily transported by wind. This would lead to a greater population density at this site. Low average host branch inclination (average 22°) was characteristic for *T. fasciola*. This might be due to ease of germination and growth on a more horizontal substrate plane.

Due to it being leafless, this orchid perhaps needs to grow where there are minimal associated epiphytes, especially tall ones that might shade this leafless orchid that needs access to sunlight for its photosynthetic roots, thus because it is only found where associated epiphytes do not exceed 2cm in height, this hypothesis is confirmed.

Correlations between plant size and reproductive ability would be expected for almost any plant, and the data obtained in this study confirms that hypothesis. There were significant positive correlations between plant size and flowering (R^2=0.211, std. error 0.570), and fruiting (R^2=0.437, std. error 0.687). Approximately one-third of each *T. fasciola* population was reproductively active (fruiting or flowering). Ackerman *et al.* (1990) found there to be a significant negative correlation between reproductive ability and plant growth in the following years for orchids. This would indicate that the plant is allocating more energetic resources to reproduction, and thus has less to allocate to growth. Because this study only spanned nine weeks, this hypothesis could not be confirmed, but results suggest that both populations are reproductively healthy. The stronger correlation between plant size and fruiting rather than flowering is perhaps that a larger plant has more resources to put into nectar production to offer pollinators, and they are subsequently attracted to these individuals more to pollinate. However, pollinators were never observed for this orchid in the field, and it is unknown whether this orchid might in fact be nectarless.

The 4:7 ratio of flowers to fruits (capsules) found for this population of *T. fasciola* does not reflect the results of previous work done, or of trends seen in the Orchidaceae family (Bartareau 1995; Montalvo *et al.* 1987) that suggest higher flower-to-capsule ratios for resource-limited orchids. Natural selection should set an optimal flower-to-fruit ratio for plants that need to allocate for present reproduction as well as future growth and reproduction. Yet this orchid does not seem to be limited by costs of capsule production and maturation. Ackerman *et al.* (1996) suggests that the answer lies in an orchid's pollination ecology and not in other ecological factors such as energy allocation. Murren *et al.* (1996) suggests, however, that differences in resource availability (influenced by habitat variability) affect plant size and thus, reproductive ability. There is not enough data or information on *T. fasciola* from this study to be able to speculate how this orchid maintains reproductive capacity. More detailed work should be done focused on the reproduction of this species to determine if this unusual flower-to-capsule ratio is consistent, and what this implies about the reproductive ecology of this orchid.

Fungal infection in orchids usually represents true symbiosis. (Boullard 1997) In the root cross-sections of *T. fasciola* there was evidence of fungal mycorrhizal hyphae coils from the younger specimens (average root length <5cm, not reproductively active). This information provides the verification that mycorrhizal fungi do indeed associate with this orchid at a young stage and probably assist the orchid in germination and nutrient acquisition. The orchid then digests and consumes the fungus once reaching a certain stage, thus there are only traces of these hyphal coils left in only the youngest individuals. Thus this adult orchid relys solely on its photosynthetic roots for all energy acquisition.

There was also evidence of ectomycorrhizal symbionts on the up-facing side of the root (as opposed to the side in contact with the phorophyte) found within 1cm of the root tip on several of the younger specimens. This may be due to these root meristems needing additional support in obtaining nutrients, and these fungi aid in this process. Blue-green algae was found in the root cells of most of the cross-sections: in clusters as well as in layers closer to the surface, and the heterocyst was visible. These prokaryotes aid in nitrogen-fixation, and it is curious that this organism was found within the roots of this orchid. Future studies might address in more detail the role of this prokaryote in the life process of *T. fasciola*, or orchids in general.

The orchid velamen layer was clearly visible in the root sections. There were slits on the outside layer of cells touching the phorophyte, as well as small connections (like tunnels) between each of these cells of the outer layer. This allows for the efficient capture and retention of water when it becomes available during the intermittent tropical rain showers. The connections between the epidermis cells allow the water to spread quickly within the root, and be retained for absorption into the vascular system. This morphological adaptation to the cells only on the phorophyte surface of the root has this clearly defined purpose to efficiently capture moisture as it becomes available. Spoke-like transfer cells were also present near the central vascular system of the root, as well as filamentous structure cells. This morphology of the root allows maximum efficiency, as this orchid must balance the need for structural support with the requirement to transfer moisture to the central vascular system. Idioblast cells were present containing raphids, small packets of calcium oxylate crystals. These crystals are present as protection against predation (insects, most likely). Countless chloroplasts were present throughout the root, as well, especially in the larger, older specimens, as Zotz (1997) found for the epiphytic orchid, *Dimerandra emarginata* (photosynthetic capacity increases with plant size). Because this orchid is leafless, it relys on its green roots for photosynthesis. Clearly this orchid has many special adaptations to living epiphytically, as well as in a tropical environment.

Conclusion

Through an island-wide survey of the epiphytic orchids, ten species (7 genera) were found on Moorea. Most were on *Hibiscus* sp. host phorophytes, and most were within 100 meters of a water source (stream), not disproving the hypothesis that epiphytic orchids need to be near a water source (moisture availability).

Two populations of *Taeniophyllum fasciola* were closely studied and compared to assess the factors that limit and permit its distribution in the two areas in which it is found, further examine factors determining the dispersal of this species and to determine whether reproductive capability is correlated with the size of the individual. *T. fasciola*, an epiphytic member of the family Orchidaceae, was examined to attempt to correlate distributional factors with certain reproductive indicators including fruit-to-flower ratios and reproductive success in a community. This data contributed in attempting to provide answers to the question if reproductive capacity of a population is limited by the trade-off between resource allocation for present reproduction and future growth and reproduction, as suggested by Melendez-Ackerman (2000). No significant environmental factors were found to be correlated with plant size, although reproductive capability was correlated positively with larger plant size.

The epiphytic orchid *Taeniophyllum fasciola* is leafless, relying on its photosynthetic roots and mycorrhizal symbionts at a young age to obtain nutrients. Epiphyte and orchid diversity may be very good indicators of the degree of disturbance suffered by a habitat in the humid tropics. (Turner et al. 1994)

Due to it being leafless, this orchid perhaps needs to grow where there are minimal associated epiphytes, especially tall ones that might shade this leafless orchid that needs access to sunlight for its photosynthetic roots.

It is likely that many biological and physical factors determine the distribution of epiphytic orchids in a tropical forest. Mycorrhizal association may be an especially unique factor for orchids that other tropical epiphytes do not need to deal with thus affecting the limited distribution of epiphytic orchids on Moorea, French Polynesia, though this factor was only investigated in the leafless epiphytic orchid, *Taeniophyllum fasciola*.

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APPENDIX A

Voucher specimens deposited in University of California Herbarium, December 2001.

VOUCHER	Species	Date	Location	Host	Habitat
COCHRANE 1	*Taeniophyllum fasciola*	24-Sep-01	sea level, PK18 Opunohu Bay, Moorea	*Hibiscus* sp.	*Hibiscus* swamp
COCHRANE 2	*Dendrobium biflorum*	24-Sep-01	266m, just above Belvedere, Moorea	*Hibiscus* sp.	mixed forest
COCHRANE 3*	unknown **#1** (*Phreatia* sp.?)	24-Sep-01	270m, just above Belvedere, Moorea	*Hibiscus* sp.	mixed forest
COCHRANE 4	*Dendrobium biflorum*	27-Sep-01	204m, Fare Hape, Tahiti	*Hibiscus* sp.	riparian
COCHRANE 5	unknown **#2** (*Eria rostiflora?)*	27-Sep-01	204m, Fare Hape, Tahiti	*Hibiscus* sp.	riparian
COCHRANE 6	*Bulbophyllum tahitense*	27-Sep-01	610m, PK20 Papenoo Valley Rd, Col Urufaau, Tahiti	*Metrociteros*	dry ridge, lone tree
COCHRANE 7*	*Bulbophyllum longiflorum*	13-Oct-01	75m, Opunohu Valley, Col des 3 Cocotiers, Moorea	*Hibiscus* sp.	mixed forest, riparian
COCHRANE 8*	*Oberonia equitans*	13-Oct-01	75m, Opunohu Valley, Col des 3 Cocotiers, Moorea	*Hibiscus* sp.	mixed forest, riparian
COCHRANE 9*	*Dendrobium crispatum*	13-Oct-01	75m, Opunohu Valley, Col des 3 Cocotiers, Moorea	*Hibiscus* sp.	mixed forest, riparian
COCHRANE 10*	unknown **#5**	22-Oct-01	archery platform marae, Opunohu Valley, Moorea	*Hibiscus* sp.	*Inocarpus* stand
COCHRANE 11	*Taeniophyllum fasciola*	31-Oct-01	sea level, PK18 Opunohu Bay, Moorea	*Hibiscus* sp.	*Hibiscus* swamp
COCHRANE 12	*Taeniophyllum fasciola*	31-Oct-01	sea level, PK18 Opunohu Bay, Moorea	*Hibiscus* sp.	*Hibiscus* swamp

*ANNOTATIONS:

COCHRANE 3: leaves ~7cm, 8cm total height
COCHRANE 7: 1.5m up, 10m W of stream
COCHRANE 8: 3 individuals,1cm apart on runner; 0.5m up; roots <1mm wide, 6.5cm long; branch 0.47m circ; 40% canopy
COCHRANE 9: 4m up, 60% canopy
COCHRANE 10: 1m up; branch 0.63m circ, 90°

Ethnobotany of Type II Diabetes in French Polynesia: Plant use, preparation, and morphology

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ABSTRACT. This study investigates the use of traditional medicine for the treatment for type II diabetes in French Polynesia. First, plants used for treatment and their preparations were investigated. Secondly, the distribution of two major plants used for treatment, *Morinda citrifolia* and *Casuarina equisetifolia,* was studied. Thirdly, the morphology and ethnotaxonomy of the *Morinda citrifolia* tree was studied and possible varieties were considered. Interviews were conducted with healers on the island of Moorea, French Polynesia, to obtain ethnobotanical data. Distribution data was obtained using transects along randomly selected sections around the circumference of the island. Morphological characteristics of sixty two *M. citrifolia* trees were studied to determine the existence of possible varieties. Ethnobotanical interviews confirmed that a variety of plants were used by Polynesian healers for the treatment of diabetes, including *Morinda citrifolia, Casuarina equisetifolia, Persea Americana,* and *Cymbopogon nardus.* The distribution study showed that both *M. citrifolia* and *C. equisetifolia* occur in abundance around the island and are easily accessible to the Polynesians. The ethnotaxonimcal study produced significant p values suggesting that there are multiple varieties of *M. citrifolia* on the island, including a smaller Tuamotu variety as suggested by one informant.

Introduction

Ethnobotany is defined as the study of the relationship between plants and human cultures. This relationship has existed for thousands of years, as humans have been using plants since ancient times. Ancient man most likely discovered by trial-and-error that certain plants, when applied topically or ingested, produced beneficial effects. This use is documented through biblical time with the use of aloe vera by the Israelites (Whistler 1992). Ethnobotany is not limited to the use of plants for food, clothing, and shelter, but also includes their use for religious ceremonies, ornamentation, and health care (Schultes 1992).

As modern civilization arose, medicinal focus turned to synthetic drugs such as aspirin. These drugs could be developed in a laboratory, packaged, and marketed with ease. Now in the twenty-first century, modern scientists and doctors are beginning to realize the full benefit of medicinal plants (Reid 1993). Society is now realizing that research and preservation of this knowledge is essential to the health of the world.

While the cost of manufacturing and distributing synthetic drugs in Western countries is rarely a debilitating problem, it can be a huge issue in developing countries. These countries do not have the funds to develop or distribute new synthesized drugs and therefore rely on the traditional medicine that they have used for centuries. This medicine, unlike the synthetic Western counterpart, is both easily accessible and usually free (Whistler 1992).

Polynesia, which consists of the triangle in the Pacific with Hawaii at the Northern angle, New Zealand at the Southwestern angle, and Easter Island at the Southeastern angle, was settled by the Polynesians around 1300 B.C. (Whistler 1991) Plant species found in Polynesia can be divided into two categories; 'native', or species arriving unassisted by human transport, and 'introduced', or plants brought to the islands by either Polynesians or Europeans. The majority of useful plants are introduced and were carried with the Polynesians as they settled the islands (Lepovsky et al. 1996). The Polynesians brought with them a full pharmacopeia consisting of seventy-two species, some which included *Curcuma longa, Morinda citrifolia, Piper methysticum, Casuarina equisetifolia,* and *Pandanus spurius* (Whistler 1991). However, medicinal plants brought by Europeans have since been incorporated into the Polynesian pharmocopeia as well.

Much has changed since the ancient times of Polynesian exploration and settlement, and now the islands face many modern problems. French Polynesia is an overseas department of France, and thus receives support in the form of money and resources (Finney 1992). The predominant health care found on the island is one of French infrastructure, with French doctors and Western medical practices. However, the Polynesians value their culture and history greatly, and retain much of their own system of health care as well. This system relies on knowledge brought with them as they settled the islands and is based on plants easily grown in their living environment (Whistler 1992).

In modern Polynesia, diabetes is a growing problem of the islanders. Over the last decade there has been a shift from traditional subsistence living to a more sedentary life style (Nguyen Ngoc et al. 1998). Current figures show that 39 percent of French Polynesians are obese, determined by having a Body Mass Index of over 30. In the period of 1996 to 1999, the obesity of children ten years old increased 50% (Nguyen Ngoc et al. 1998). These rising levels of obesity have possibly contributed to the development of insulin resistance and type II diabetes in Tahiti (Harding et al. 2001).

Type II diabetes, or diabetes mellitus, is a condition in which the body develops a decreased ability to effectively use insulin, a hormone secreted by the pancreas. Insulin is responsible for the body's conversion of sugar to energy. Type II diabetes can be controlled by increasing physical activity and monitoring blood glucose levels. It is important to treat diabetes, as it can lead to blindness, leg and foot amputations, pregnancy complications, and kidney failure (Harding et al. 2001).

Early literature review suggested that a number of plant species are used to treat diabetes mellitus, including *Morinda citrifolia*, *Casuarina equisetifolia*, and *Persea americana*, along with many others (Whistler, 1992). Also investigated was the concept of ethnotaxonomy, or the local people's recognition of different varieties of plants. This research raised a number of questions which this study investigates. What is the total pharmacopeia used for diabetes and how are the treatments prepared? Are these plants easily accessible to the population? And finally, is there evidence of possible varieities in one of these plants, *M. citrifolia?*

Materials and Methods

Ethnobotany

Ethnobotanical data was collected during interviews conducted from 23 October to 6 November 2001. Interviews with three healers on the island of Moorea (17°30' S, 149°50' W) were conducted with the aid of a Tahitian translator. Two of the healers, Lucie Tapu and Mimi Terai, lived in the town of Afareaitu on the Southeastern side of the Moorea. The other healer, Mama Lucie, lived near the village of Haapiti, on the Southwestern side of the island.

The goal of the meetings with the healers was to obtain knowledge on traditional Polynesian medical practices. No formal questionnaire was used, so as not to limit the scope of the interviews. Instead, a simple question and answer dialogue through a Tahitian translator was employed. Illustrated field guides were also used to facilitate the healer's identification of plants for discussion. All questions were asked with the purpose to obtain information regarding traditional Polynesian medicine and culture, specific plants used in the treatment of diabetes, and methods used to prepare them. The healers were also asked about relative frequency of diabetes on the islands and their views towards the disease.

In addition to a question and answer session, Lucie Tapu and Mimi Terai also demonstrated the preparation of treatments during the course of the interview. Plants were collected, the treatment was prepared, and general information such as proper dosage was provided. Each interview also included a tour of the healer's garden, where important and relevant medicinal plants were described and identified.

Distribution

In order to assess the abundance of plants used for traditional treatments of diabetes on Moorea, transects were conducted around the circumference of the island. The coastal road that circumnavigates Moorea is marked by *Pointe Kilomètre*, or PK markers. The entire length of the road is sixty kilometers. Twelve random one-kilometer sections of road were determined using a random number table from zero to sixty. PKs were chosen, and transects were conducted proceeding in a counter-clockwise fashion (Figure 1).

The kilometer transects were performed by walking the road on both mountain and ocean sides, looking for the presence of *Morinda citrifolia* and *Casuarina equisetifolia*. These two trees were chosen because of their frequency in traditional preparations and their ease of visual identification from the road. Transect width was determined by the visual limit from the road – never more than 50 meters on the ocean side due

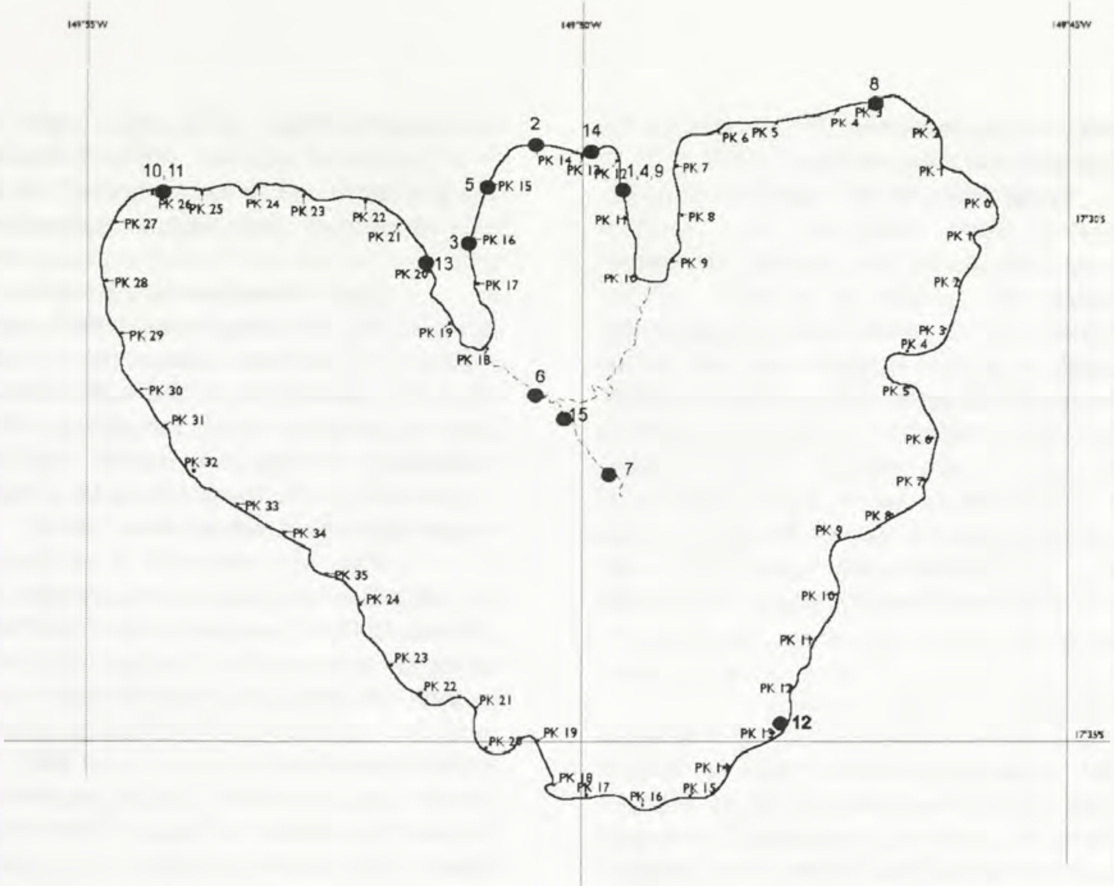

Figure 1. A map of Moorea showing the 14 sites used for *M. citrifolia* data collection.

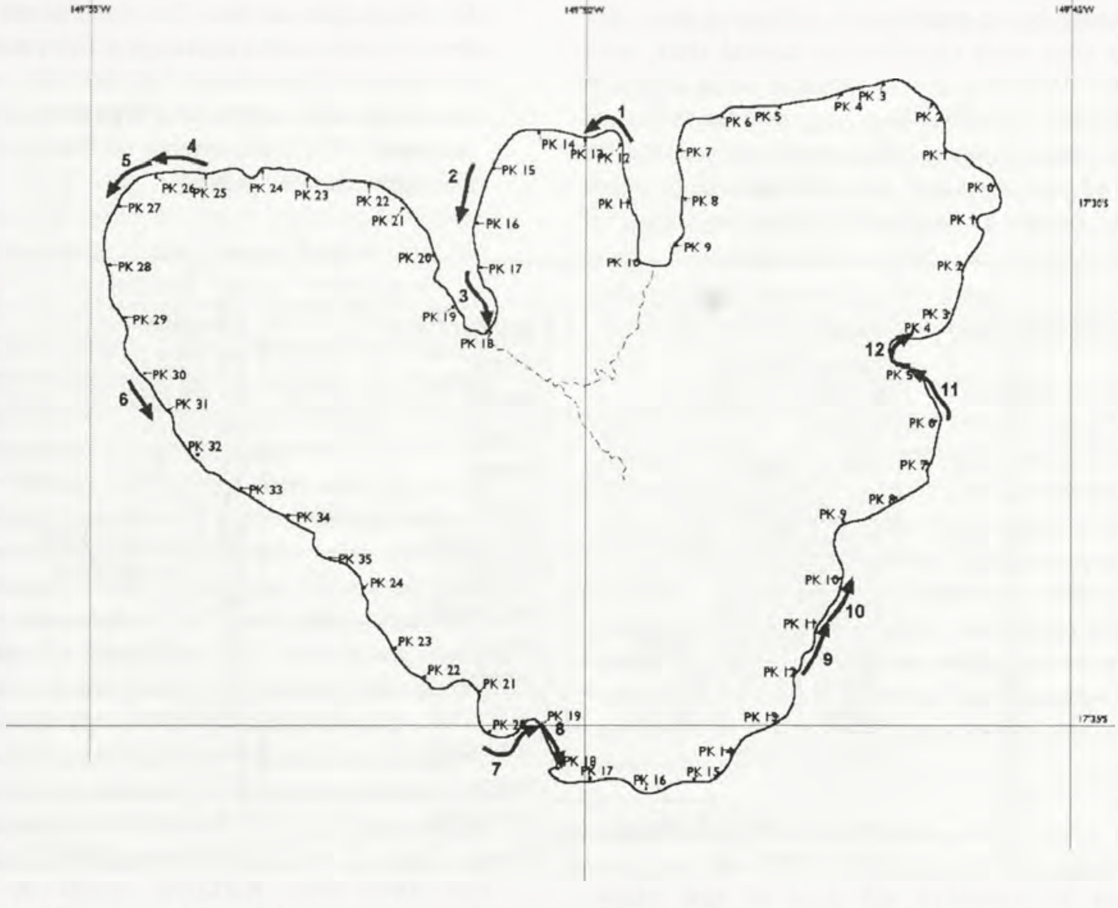

Figure 2. A map of Moorea showing the twelve distribution transects conducted.

to water, and no more than 200 meters on the mountain side due to the extent of vision.

Populations of *M. citrifolia* and *C. equisetifolia* were observed and counted. Observed individuals were placed into one of two categories; public or private. Plants considered to be on public land were those that were easily accessible from the road side and no permission would be needed to pick the fruit or leaves. This included *C. equisetifolia* used as a border plant on the outside of the property. Plants considered to be on private land were those clearly inside a marked boundary of the property, where permission would have to be obtained from the property owner to pick the fruit or leaves.

Morphology

A morphological study was conducted in order to test for the existence of varieties in *Morinda citrifolia* on the island of Moorea. Sixty-two *M. citrifolia* trees distributed over fifteen sites around the island were studied. Each site was placed into one of three categories; cultivated, natural, or "sauvage". Six sites were classified as cultivated sites, which were currently being maintained and cared for. Six other sites were classified as natural sites, with no maintenance and that appeared to be naturally occurring. Finally, three sites were determined to be sauvage sites, which were cultivated sites that were planted and once maintained, but now have become overgrown due to lack of maintenance (Table 1, Figure 2). Up to six trees were studied at each site. On each tree, leaf and fruit dimensions were recorded, as well as environmental observations and soil testing results.

Leaf measurements were taken to look for possible difference in leaf morphology. The lowest twenty-five leaves were measured on each tree, and the length and width of the leaf at the widest point were measured. Branches stemming off the main trunk less than ten centimeters above the ground were not included in part of the twenty-five lowest leaves.

The *M. citrifolia* fruit progresses through five stages as it approaches ripeness (Petard 1986). Stage one is when the fruit is the most green colored and the least ripe. Stage five is a totally ripe, white fruit. Between stages one and five the *M. citrifolia* fruit goes through a color change, with a stage three fruit being a shade of green yellow. Fruit measurements were conducted, and for the sake of consistency all fruits judged to be a stage one or a stage five were discounted and not measured. This is because at many of the sites, stage one fruits are barely developed and stage five fruits are picked for distribution or use. The dimensions of up to five fruits on each tree, ranging from a stage two to stage four were measured. Their circumference, length and ripeness stage were recorded. The total number of fruits on the tree was approximated as well.

Site Number	Name	Classification	Location
1	Gump Property	Natural	PK 11.8 (-)
2	East of Sheraton	Natural	PK 14 (-)
3	PK 16	Savage	PK 16 (+)
4	Nature Trail	Natural	PK 11.8 (-)
5	Tiarje's Plantation	Savage	PK 15 (-)
6	Agricultural School	Cultivated	PK 1.6, inner road
7	Belvadere High	Natural	PK 4, inner road
8	Boulin's Farm	Cultivated	PK 3 (-)
9	Gump Property High	Natural	PK 11.8 (-)
10	Royal Tahiti Low	Cultivated	PK 26 (-)
11	Royal Tahiti High	Cultivated	PK 26 (-)
12	Nelli's Farm	Cultivated	PK 13 (+)
13	PK 20	Savage	PK 20 (-)
14	Louie Boulaville's	Cultivated	PK 13 (-)
15	Belvadere Low	Natural	PK 2, inner road

Table 1. An index of site numbers, their classification, and location on the island. A negative sign indicates that the PK is counted counter-clockwise around the island, while a positive sign indicates that it is counted clockwise around the island.

Tree height was also measured, and circumference of the trunk at breast level was recorded. The live crown ration of the tree was approximated, giving the ratio, by height only, of the active-leaf producing branches on the tree to total tree height. Population spacing was determined by measuring the distance from the study tree to another *M. citrifolia* tree in the four cardinal directions. The total population number of *M. citrifolia* trees was counted in small populations. In larger populations it was either estimated or obtained from the land owner.

Shade was measured through ocular estimation or a fish-eye lens. Slope was approximated as well using a compass, and elevation and coordinates were determined using a GPS when possible. On coastal sites, the position was determined using the PK markers along the coastal road of the island.

Soil samples were taken according to procedures described by Sarrantonio et al. (1996). Rooting depth was determined by measuring the depth at which coarse roots (>5mm) appeared in a soil core. Soil moisture content was determined by massing a soil sample, microwaving the sample on a high setting for five minutes, and massing again. The difference in mass was determined to be moisture released, and a moisture percentage was found by dividing this value by the original mass of the sample.

One site, Site 14, was a small *M. citrifolia* plantation of about one-hundred trees on the property of Mr. Louis Boulaville. Mr. Boulaville recognized three varieties of *M. citrifolia*, distinguished by their fruit. They consisted of a small variety, originating from the Tuamotus; a medium sized variety, originating from the Society islands, and a large variety, originating from the Marquesas.

A suite of ANOVA tests was run on the data collected to determine if statistically defined varieties were found in the study. Two variables were tested in the ANOVAs. The total fruit volume, calculated by multiplying the fruit length by the circumference, was tested, along with the total leaf area, calculated by multiplying the leaf width by the length and dividing by two.

Site 14 was tested against different cultivated populations. One test compared it to the populations at Sites 6 and 12, while the other compared it with populations at Sites 10 and 11.

A third ANOVA test was run comparing the supposed variety at Site 14 with the trees at Sites 6 and 12 and also with the trees at Sites 10 and 11. Finally, a test was run comparing hypothesized varieties at Sites 8, 9, and 14 with Sites 5, 6, and 12. This produced results from a total of 8 ANOVA tests.

Results

Ethnobotanical

There were many different plants found to be used by healers around the island for the treatment of diabetes. The most common plants utilized were *Morinda citrifolia, Persea americana*, and *Casuarina equisetifolia*. All of the healers interviewed either used these plants or were familiar with the technique and recipe that involved these plants. *Cymbopogon nardus*, *Psidium guajava*, *Cocos nucifera*, and *Curcuma longa* were also used by some of the healers.

A summary of these plants, including their method of introduction to Polynesia, can be found in Table 2 (Cox and Banack 1991). A number of preparations were recorded and can be found in Appendix A. General information regarding the Polynesian system of healers and their views on diabetes was also documented and recorded. These views included their perception of the severity of the problem on the island and ideas about traditional and Western medicine.

Distribution

Distribution data is expressed in Table 3, where population numbers are given that show both public and private *Morinda citrifolia* and *Casuarina equisetifolia* trees. Around the total circumference of the island, the average coastal *M. citrifolia* population per kilometer was 19.1, while the average *C. equisetifolia* population was 52.6.

Out of 229 total *M. citrifolia* trees, a total of 72, or 31.4%, were private trees. The remaining 157, or 68.6%, were public trees accessible to everyone. A similar distribution was found for the *C. equisetifolia* trees, with 191 trees out of a total of 631 being private, or 30.3%. The remaining 440, or 69.7%, were public *C. equisetifolia* trees. It should also be noted that in many cases even though trees were recorded as being private, permission could easily be obtained by talking to the land owner (pers. obs.).

Morphology / Ethnotaxonomy

The main objective of the morphology study was to look for evidence of varietal differences in *M. citrifolia*. A series of interviews on the island provided conflicting results, with most informants reporting that there were no

Scientific Name	English Name	Tahitian Name	Introduction
Casuarina equisetifolia	Ironwood	Aito	Polynesian
Cocos nucifera	Coconut	Haari	Native
Curcuma longa	Tumeric	Rea Tahiti	Polynesian
Cymbopogon nardus	Citronella	Ti	European
Morinda citrifolia	Noni	Nono	Polynesian / Native
Persea Americana	Avocado	Avota	European
Psidium guajava	Guava	Tuava	European

Table 2. A list of plants used in the treatment of diabetes in French Polynesia, listed by scientific name. Also included are their English and Tahitian names, as well as their perceived method of introduction (Cox and Banack 1991, Mabberley 1997). Polynesian indicates a Polynesian introduction; European indicates a European introduction; native indicates a species native to the islands.

recognized varieties of *M. citrifolia*. However, one informant, Louis Boulaville, reported in an interview on 7 November 2001 that there were proposed varieties of *M. citrifolia*, including one type from the Tuamotus and another type from the Marquesas.

Data used for statistical analysis were the average fruit volume and leaf area of each tree (Table 4). Statistical analysis of M Boulaville's plantation (Site 14) and others is expressed below and in Table 5. An ANOVA test comparing the fruit volume and leaf area of Site 14 with two other cultivated sites, Sites 6 and 12, yielded a p value of .015 for fruit volume and .038 for leaf area. An ANOVA test comparing Site 14 with cultivated Sites 10 and 11 yielded a p value of .003 for fruit volume and a p value of .016 for leaf area.

A similar ANOVA was run comparing Site 14, Sites 6 and 12, and Sites 10 and 11. This test yielded a p value of .002 for fruit volume and a p value of .042 for leaf area. Finally, an ANOVA test was run comparing sites 8, 9, and 14 with Sites 5, 6, and 12. A p value of .004 was obtained for fruit volume and a p value of .002 was obtained for leaf area.

Discussion

Ethnobotany

Preliminary research suggested that at least three plants were used to treat diabetes mellitus in French Polynesia; *Morinda citrifolia*, *Casuarina equisetifolia*, and *Persea Americana*. However, four other plants were found to be used by healers on the island of Moorea as well. These include *Cocus nucifera*, *Psidium guajava*, *Curcuma longa*, and *Cymbopogon nardus*. All of the plants used by the healers have tested positive for bioactivity in other research.

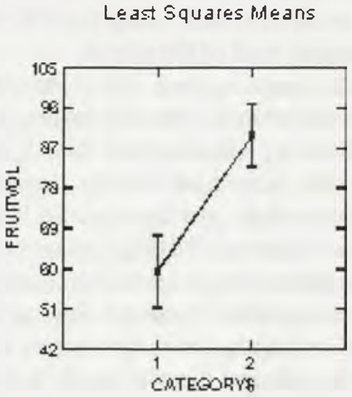

Figure 3. ANOVA results for fruit volume, expressed in cm^3 at Sites 14 (category 1) with Sites 6 and 12 (category 2). P value equals .015.

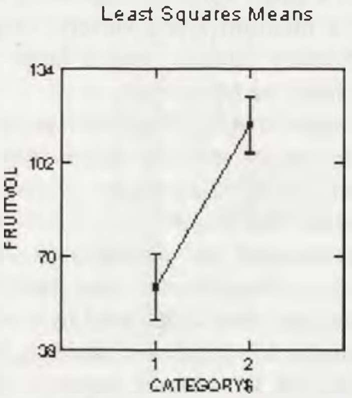

Figure 4. ANOVA results for fruit volume, expressed in cm^3 at Sites 14 (category 1) with Sites 10 and 11 (category 2). P value equals .003.

Index	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
PK	**12 -**	**15 -**	**17 -**	**25 -**	**26 -**	**30 -**	**20 +**	**19 +**	**12 +**	**11 +**	**6 +**	**5 +**
Nono	5	47	5	19	14	14	15	81	15	14	0	0
Aito	41	125	0	102	4	2	6	36	110	100	52	53

Table 3. Data from 12 one-kilometer transects conducted around the island. Numbers indicate total population, both public and private, of *M. citrifolia* (nono) and *C. equisetifolia* (aito) found within proximity of the road. Transects were conducted starting at PK marker indicated and consisted of the one kilometer stretch of road going counter-clockwise around the island. A negative sign indicates that the PK is counted counter-clockwise around the island, while a positive sign indicates that it is counted clockwise around the island.

Site	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Fruit Vol	92	59	50	0	61	75	73	74	76	114	116	105	77	59	59
Leaf Area	146	126	193	122	178	131	221	120	96	164	236	214	198	106	184

Table 4. A representation of *M. citrifolia* tree morphology, showing fruit volume (length X circumference) and leaf area (length X width / 2). Numbers represent the average of all trees measured at each site, and are expressed in cm^3 for fruit volume and cm^2 for leaf area. A zero indicates that there were no fruit on any of the trees sampled at the site.

Site 14 vs. Sites 6 and 12 Fruit Volume

Source of Variation	*MS*	***P-value***
Between Groups	3178.6	**0.015**
Within Groups	391.4	

Site 14 vs. Sites 10 and 11 Fruit Volume

Source of Variation	*MS*	***P-value***
Between Groups	10573.48	**.003**
Within Groups	391.38	

Site 14 vs. Sites 6, 12 vs. Sites 10, 11 Fruit Volume

Source of Variation	*MS*	***P-value***
Between Groups	5290.94	**.002**
Within Groups	606.90	

Sites 8, 9 and 14 vs. Sites 5,6 and 12 Fruit Volume

Source of Variation	*MS*	***P-value***
Between Groups	4134.72	**.004**
Within Groups	403.07	

Site 14 vs. Sites 6 and 12 Leaf Area

Source of Variation	*MS*	***P-value***
Between Groups	15053.3	**.038**
Within Groups	2768.1	

Site 14 vs. Sites 10 and 11 Leaf Area

Source of Variation	*MS*	***P-value***
Between Groups	29844.91	**.016**
Within Groups	3795.78	

Site 14 vs. Sites 6, 12 vs. Sites 10, 11 Leaf Area

Source of Variation	*MS*	***P-value***
Between Groups	15349.16	**.042**
Within Groups	4075.18	

Sites 8, 9 and 14 vs. Sites 5, 6 and 12 Leaf Area

Source of Variation	*MS*	***P-value***
Between Groups	28871.95	**.001**
Within Groups	2267.33	

Table 5. ANOVA results of the morphological data analyzed. Four total comparisons were conducted, analyzing both fruit volume and leaf area for each comparison. A p value of less than .05 indicates significant data to a 95% confidence rate.

M. citrifolia has a long history of Polynesian use and is frequently used in treatment of diabetes. Its uses also include being a dye plant, a famine food, and used in a myriad of medicinal preparations that range from treatments for head colds to rheumatic pain to ulcers (Whistler 1992). *M. citrifolia* has been shown to have anti-diabetic properties in rat studies (Olajide 1999) and is commonly used as a general health product due to its possible antitumour actitivy (Hirazumi et al. 1999). This value to the people, combined with the fact that *M. citrifolia* grows very rapidly, helps explain its popularity and abundance on the island.

Casuarina equisetifolia has been used by Polynesians since settlement for its strong wood qualities. Polynesians used the *C. equisetifolia* to make spears, clubs, tools, and fish hooks. Along with diabetes, it us used to treat coughs, asthma, and nervous disorders. *C. equisetifolia* is known to grow in poor soil conditions from the coast all the way up to high vegetation (Whister 1992).

Curcuma longa is thought to have originated in cultivation somewhere in Southeast Asia, and was brought to Polynesia with the first settlers. *C. longa* is often used as a main ingredient in curry, as a dye for cloth, and medicinally used to treat sores and rashes as well as diabetes (Whistler 1992). Curcumin, an ingredient found in *Curcuma longa*, has been studied for its cancer chemopreventive potential (Surh 1999).

Psidium guajava is a small tree most likely introduced to the islands of Polynesia by Europeans in the nineteenth century. It is traditionally used by Polynesians to treat digestive ailments such as dysentery, diarrhea, and stomachaches. It can also be used to treat small wounds and the fruit is used to make juices and jams (Whistler 1992). *P. guajava* has anti-diabetic properties and was shown to cause a glycemic decrease in rabbits (Roman-Ramos et al. 1995).

Cocus nucifera, or the coconut, is one of the most plentiful trees in the Polynesian islands. The fruit has been used for food since settlement of the islands, and is currently France's largest overseas export product (Petard 1998). Both the milk and the fiber are used in traditional Polynesian medicine, and the fiber from C. *nucifera* has been shown to cause a glycemic decrease in rats (Sindurani et al. 2000).

Cymbopogon nardus is a small plant found in many gardens in Polynesia. It has many traditional uses, including as a digestive aid, a diuretic, and an emmenagogue. In an oil form it is a highly effective mosquito repellent often used daily by Polynesians (Petard 1998).

Persea americana is a tree from South America brought to Tahiti by European explorers in 1846. In Polynesia it is an important food source, and its oil is also used as a digestive aid. *P. americana* has been studied for both its anti-HIV and anti-tumor properties (Oberlies et al. 1998), and is shown to have anti-diabetic properties as well (Petard 1998).

While modern research has just recently verified the bioactivity of many of these plants, Polynesians have been preparing and using these plants for as long as their culture has existed. Detailed preparations are included in Appendix A, but general trends can be seen throughout all of the treatments. The treatments used for diabetes are not difficult for a practiced, licensed healer to prepare.

The *C. equisetifolia* treatment that was observed being prepared is slightly labor intensive but not difficult to make. It involves picking a small bunch of fresh shoots from a *C. equisetifolia* tree, then mashing them up with a heavy rock and straining them through water or coconut milk. A healer was witnessed accomplishing this process in approximately thirty minutes. Other treatments mostly call for boiling the plant and making a drinkable liquid or tea.

While some of these recipes are easy enough for anyone to prepare, in Polynesian culture they are traditionally only prepared by healers. As reported by Mama Lucie, a healer is gifted with powers from God, and thus if the medication was self-prepared all of the medicinal qualities may not be present. This belief also allows all visits to healers to be free of charge, as the healer is only providing a service of God.

In general, the healers confirmed published statistics that suggest that diabetes is a large problem on the islands. Mama Lucie indicated that more than half of her patients she sees are treated for diabetes. All of the healers had multiple stories about patients with the disease. The healers also understood the need for lifestyle changes along with traditional treatment. Mama Lucie underscored the fact that treatment alone is not enough; patients with diabetes must also reduce their intake of sugar and abstain from alcoholic beverages. This data suggests that healers not only rely on traditional knowledge in their practice, but integrate modern scientific concepts as well.

Distribution

The distribution study showed that both *M. citrifolia* and *C. equisetifolia* were found in abundance around the island. With an average population of 19.1 *M. citrifolia* trees per kilometer and 52.6 *C. equisetifolia* trees per kilometer, abundance is high around the coastal road. The coastal road is easily accessible to residents and thus the needed plants are as well.

Examining the medicinal plant population is important when considering the specific needs of each recipe. Very few fruits are needed for the common *M. citrifolia* recipes. A large batch of medicine only requires four to five fruits, while healthy *M. citrifolia* trees can bear thirty fruits at one time. Due to the abundance of *M. citrifolia* trees on the island, a medicinal quantity is very easy to obtain.

The situation of the *C. equisetifolia* tree is similar as well. One *C. equisetifolia* recipe requires a handful of new shoots from the tree. The abundance of *C. equisetifolia* trees around the island, especially planted together in larger populations, indicates that there is always a source of new shoots. One healer was observed obtaining shoots of four medium *C. equisetifolia* trees being used as a property border in ten minutes.

While 31.4 percent of *M. citrifolia* trees and 30.3 percent of *C. equisetifolia* trees were considered private, this is a misleading statistic. Through personal experience, it was found that private populations are not always totally inaccessible. Oftentimes permission to use plants on private property is granted by the landowner upon asking.

Morphology / Ethnotaxonomy

The majority of interviews conducted with informants on the island of Moorea suggested that there were no varieties of *M. citrifolia*. However, on 7 November 2001, in an interview with Louis Boulaville, he recognized three different varieties of *M. citrifolia*. Mr. Boulaville's small plantation on his property consisted of the smaller Tuamotu variety, and did appear to have smaller fruits than observed elsewhere on the island.

The first ANOVA test compared Mr. Boulaville's plantation (Site 14) with Sites 6 and 12. Combining two sites together allowed for a more equal sample size, as the sample size at Mr. Boulaville's was slightly larger than elsewhere. Sites 6 and 12 were chosen because they were cultivated plantations as well and had similar growing environments in terms of shade and dispersion. The fruit at Site 14 appeared smaller than the fruit at Sites 6 and 12, and statistical data supported this observation. P values for both fruit volume and leaf area were significant at < .05.

Mr. Boulaville's plantation was compared to two different cultivated sites at Royal Tahiti Noni Plantation (RTNP) in the next test. RTNP consists of 20 hectares of *M. citrifolia* in a full-sun valley and exports juice product all around the world. It was chosen as the other cultivated site because being a worldwide exporter, having ideal growing conditions is of primary concern. The trees at RTNP were some of the most robust and healthy trees studied and there was a significant difference between the trees at Mr. Boulaville's and at RTNP for both fruit volume and leaf area.

Three groupings of sites were compared in the third test to look for possible variation. The first population was the Tuamotu variety at Mr. Boulaville's, compared with Sites 6 and 12, and with Sites 10 and 11. Significant p values for both fruit volume and leaf area suggest that there are three significant groupings of *M. citrifolia*. The Tuamotu variety found at Mr. Boulaville's was morphologically different than another population of cultivated *M. citrifolia*, which was different than the commercial grade population of RTNP. This test suggests that a third type of variety may be present at RTNP.

The fourth test attempted to group together populations that appeared to be similar to one another. Observations suggested that there were more than one site where the trees appeared to have smaller fruit and leaves like Site 14. Site 8 and Site 9 were grouped with Site 14 for the testing. Site 8 was a large, kept plantation that had very healthy trees, albeit somewhat smaller. Site 9 was a small population of exposed, full sun trees. These three sites were compared to the *M. citrifolia* found at Site 5, a large, un-kept (sauvage) site, Site 6, a smaller, cultivated plantation, and Site 12, a large, cultivated plantation. The p values for these tests were significant, suggesting that there is a significant difference in the morphology of these populations.

These tests show that there is a significant difference in both the fruit volume and leaf area of these tree populations. While the morphological difference in these plants could be explained by different growing environments, similar sites were selected in the ANOVA tests to eliminate this factor. Comparing data between two cultivated sites ensures that the amount of shade is standardized at zero percent. The variety hypothesis is further supported by interviews with Mr. Boulaville. Mr. Boulaville reported that *M. citrifolia* of the Tuamotu variety will always grow into a tree with smaller fruits, even in excellent growing environments. More research is needed to substantiate these claims, as well as more informants who agree with Mr. Boulaville. Genetic testing on possible varieties of *M. citrifolia* is recommended for future research.

The existence of different varities of *m. citrifolia* trees suggest many implications. It was also reported by Mr. Boulaville that oftentimes smaller fruits like the Tuamotu variety are desired for medicinal uses, while larger fruits like those from the Marquesas are desired for their juice production. If this claim is true, it would be beneficial for factories like the Royal Tahiti Noni Plantation to invest in *M. citrifolia* of the Marquesan strain. Some selective breeding might already be occurring as the trees at RTNP had significantly larger fruit than the other sites. Also, if smaller fruits were shown to be more beneficial for medicinal uses, healers could selectively choose the smaller fruits for use in their practice. Laboratory testing for differing bioactivity between possible varieties is recommended for future research.

Conclusion

A suitable system for the treatment of type II diabetes using traditional Polynesian medicine is currently in place in French Polynesia. Multiple plants are accessible on the island, and when prepared by skilled healers, the treatments are anecdotally evidenced to be effective. However, lifestyle changes are still important in combating the disease.

Varieties of *M. citrifolia* possibly exist on the island of Moorea. More research, including genetic testing and laboratory bioactivity assays, is suggested to validate the presence of these varieties. Continued ethnobotanical research is important in French Polynesia, as traditional knowledge may be dying out. Modern science must take this opportunity to learn all that it can in a non-invasive manner.

Acknowledgements

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Appendix A
Recipes and Preparations of Traditional Polynesian Treatments for Diabetes

This appendix serves to compile all the recipes and detailed directions for preparing treatments that were encountered during this study in Moorea, French Polynesia. All information presented here is done so with the consent of my informants.

Casuarina equisetifolia

Preparation:

Collect the young shoots of the *C. equisetifolia* tree. The shoots should be from five to ten centimeters in length. Gather enough to form a bundle approximately five centimeters in circumference. Only collect the young shoots that are still green and that come off the tree very easily. Wash them in water and dry the tips. Using a heavy rock, crush the tips until they are very soft and slightly broken apart. Wrap the soft tips in cheesecloth or gauze, then dip in a bowl of water and ring out. The water will turn an opaque green. Coconut milk can also be used instead of water.

Treatment:

Drink the medicine three times a day; morning, noon, and night. The medicine must be prepared before three PM or the tree will be too dry to produce enough juice. *Cymbopogon nardus* treatment can be used at the same time without side effects.

Cymbopogon nardus

Preparation:

Tear seven leaves of *C. nardus* off the plant. Fold the bottom of each leaf up towards the top and tie the leaf in a loose overhand knot. Add these seven leaves to three liters of water. Boil for ten minutes.

Produces a yellow colored liquid. Starting with three liters of water produces about two liters of medication.

Treatment:

Drink at least 1.5 liters of liquid every day.

Morinda citrifolia

Preparation:

Gather four large ripe fruits off the tree and place them in a jar. Let the jar sit for one week in the sunshine. The fruit will ferment and produce juice. Filter this juice through a cheesecloth or gauze. Bring juice to a boil then immediately remove from heat.

Treatment:

Drink everyday as a tea.

Persea americana* and *Psidium guajava

Preparation:

Take ten leaves of green *P. americana* and five new leaves of *P. guajava*. Add these leaves to six liters of water. Boil for fifteen minutes.

Treatment:

Drink six liters of medicine every other day. Depending on the severity of diabetes, medicine can be taken for a duration of ten days to one month.

A comparison of cultivation strategies for *Morinda citrifolia* in Moorea, French Polynesia

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ABSTRACT. *M. citrifolia* is a small tree traditionally employed by indigenous Polynesians for an assortment of uses including medicine, food, building materials, and dyes. It produces a fruit that has gained attention for its medicinal values by the industrialized world and is currently being cultivated more aggressively for export from Polynesia to the United States and Japan, among others. A comparison of three types of cultivation of *M citrifolia* was conducted considering insect diversity, soil characteristics, plant morphology, herbivory, and fruit yield on Moorea over a six week period to determine if the more commercial cultivation strategies offered higher risk of crop devastation or ecological damage. Plantation sites diplayed highest fruit yields, and least herbivory. Homegardens displayed higher soil pH, and lower soil moisture than others. Wild sites displayed a different morphology, possibly due to differences in management regimes. Insect diversity did not vary between cultivation strategies. It was concluded that the state of commercial agriculture on Moorea is not yet at an intense technological level and ecological and economic risks are not extraordinary. An agronomic status report is included.

Introduction

Polynesia is a broad term that describes the islands in the South Pacific within a triangle defined by Hawaii, Easter Island, and New Zealand inclusively. The Polynesians are the people who first inhabited these islands over one thousand years ago, and they came primarily from Papua New Guinea, bringing with them on long sea voyages their families, livestock, and useful plants (Lepovsky et. al. 1996). Many of the plants transported throughout Polynesia by Polynesians are still used and cultivated today. Medicinal and nutritional properties of these plants have caught the attention of the rest of the world, and some of the plants grown extensively throughout Polynesia such as coconut (*Coco nucifera*), and papaya (*Carica papaya*) have become the basis for an export economy in the South Pacific. A very recent addition to this list of traditional Polynesian cultivars of international economic importance is *Morinda citrifolia*.

Morinda citrifolia (Rubiaceae), also known as "beach mulberry," "Indian mulberry," Kuri, Noni, and Nono, is a shrub or small tree up to ten meters tall, distributed from India to Hawaii (Whistler 1992) that produces a fleshy, yellowish-white, very strong smelling fruit and is one of the "Pacific's most important medicinal plants" (Thaman 1993). Most literature states that it was probably introduced to Moorea by Polynesians, although Kirch (1996) reports finding pollen grains of *M. citrifolia* in soil cores taken from the Cook-Austral quasilinear volcanic chain well before human arrival there. Nono occurs in coastal vegetation, along streams, in the under story of forests, and as an early pioneer species in grassland and abandoned agricultural areas (Thaman, Manner 1993). Nono is also widely cultivated in home gardens, intercropped fields, and monocultures. Traditional Polynesian uses include wood production for construction, canoes, adze handles, stilts, and firewood; fruit production for medicinal properties in treating burns, abrasions, diabetes, stonefish stings, and general ailments, for stimulating effects, and as an emergency food supply; leaf production for treatment of juvenile vitamin A deficiency, as decorations, as compost, and to wrap food for cooking; and bark and root production for processing into red and yellow dyes (Thaman 1993; Nerney 1998 Boulaville, pers. comm.).

In 1993, farmers began planting Nono for commercial production, and in 1995, interest was taken in Nono production for export to industrialized nations as processed juice, hair care, and skin care products by a primarily American company called Morinda Inc.(McIlroy 2001, Nerney 1998). This interest created a trend toward large-scale plantation production and a shift in the economic dependence of Polynesian farmers as well as the ecology of agricultural areas.

In 1995, a large-scale Nono plantation was begun on Moorea with 3,000 plants producing 10 metric tons of fruits that year at a price of forty-six cents per kilogram (McIlroy 2001). Then, in 1997 Royal Tahiti Noni corp. opened a processing factory in Haapiti Moorea, which buys fruits for forty-two cents per kilogram. Royal Tahiti Noni makes a concentrated juice, which is shipped to the United States, Japan, and Canada for further processing into health juices that are sold for forty dollars per liter. Royal Tahiti Noni owns a twenty-hectare plantation of 10,000 Nono trees that produces fifteen percent of their total fruit intake. The rest of the three thousand kilograms of noni processed per month at the Haapiti plant comes from local farmers and fruit collectors on Moorea. Between Morinda Inc. and Royal Tahiti Noni, over ninety households on Moorea draw supplementary if not entire household income from sale of Nono fruit for export (Leite, pers. comm.). Thus, understanding the ecology of the noni plant and creating strategies for sustainable production is very important to French Polynesia and Polynesian farmers.

One common form of commercial cultivation is monocultural plantations (one species of crop plant grown throughout the entire plantation). Monocultural plantations offer the highest yield of one particular crop possible, as long as fertility can be maintained. Also, large monocultures offer high threats of crop devastation from unchecked herbivore or pest population growth. Over a longer period, soil fertility loss and soil loss to erosion become threats because the soil is not allowed a fallow (natural regeneration) phase to recover nutrients and organic matter. This requires many monoculturalists to seek soil fertility replacement through inorganic fertilizer application and pest protection through chemical pesticides and even genetic modification (Olasantan 1999; Howlett 1996).

An alternative to fertilizer application has been offered by many researchers (Norman 1995; Clarke 1993; Olasantan 1999; Howlett 1996) in which the desired crop is grown in association with other useful crops. These other crops are used as green manure, compost, nutrient cyclers, and protection from radiation, wind, erosion, etc. or as a cash or food crop, and this is called intercropping. One approach to intercropping is to mimic a natural system with the mix of plant species chosen. In the ideal system, natural processes are mimicked as well, such as nutrient cycling, competition between populations, etc. Indirect benefits of intercropping include diversification of herbivore niches such that the crop is afforded some protection from population outbreaks (Howlett 1996; Clarke 1993), and diversification of income for farmers affording greater economic security against crop failure (Thaman, Manner 1993; Olasantan 1999).

The purpose of this study was to compare the different commercial cultivation practices of *M. citrifolia* on Moorea considering insect diversity, soil quality, cost, and yield to reveal which practices are more ecologically and economically safe for farmers and for French Polynesia. Additionally, an agronomic status report was assembled in the style of Nerney (1998) and is reported here.

Materials and methods

Nine locations on Moorea were sampled for insect diversity, soil characteristics, and plant morphology (Figure 1). Three of these sites were in commercial size monocultural plantations, three were small-scale home gardens intercropped with banana (*Musa x paradisica*), papaya (*C. papaya*), breadfruit (*Artocarpus incisa*), coconut (*C. nucifera*), and acacia (*Leucaena leucocephala*). Three sites were wild or abandoned agricultural sites in which *M. citrifolia* had been planted and is harvested, but no other management occurs there. These "sauvage" sites often yield other crops such as banana, coconut, and acacia. Sites for the study and the agronomic status report were located by talking with local people and inquiring about land devoted to Nono production seen from the road.

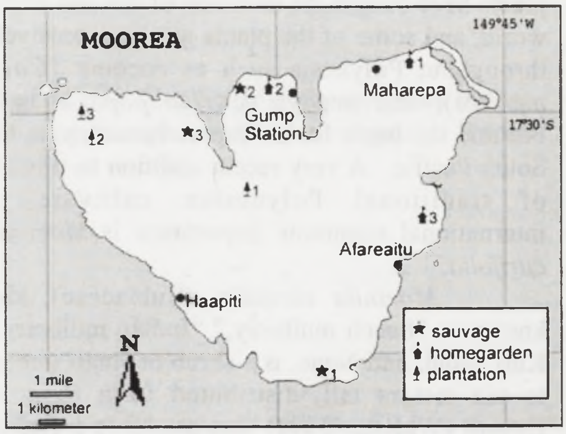


Figure 1. Nine study sites with index number denoted by 1,2, or 3, and cultivation strategy denoted by symbol.

In addition to cultivated fields, several natural populations of Nono were sampled for habitat characteristics and morphology data. These sites are described further by Glaser (2001), and analysis of them will be limited here.

Insect Diversity

In order to test for trends in insect diversity, all sites were sampled with pit-fall traps, sticky traps, and net sweeps. Analysis of insect data was performed by counting insects collected in sweeps, pit-fall traps, and sticky traps, and identifying them to their order. Identification of insects was performed by comparisons to a text (Borror et al 1998) and help from Elin Claridge and Dr. George Roderick. Comparisons were drawn between sites in each of the three categories described above considering number of orders of insect found, and number of individuals using analysis of variance and the Systat and Excel programs.

Six pit-fall traps were placed throughout each study site under Nono trees randomly, and left over two nights. Pit-fall traps were filled one-third of the way with slightly soapy water and were nine centimeters deep. Two additional pit-fall traps were placed in two exterior locations to each site as controls.

Five centimeter by twelve centimeter sticky traps were hung on one branch per tree, less than 10 cm from the trunk, at random heights, on six trees in each site over two nights. Two additional (control) sticky traps were placed in exterior locations to each site on vegetation other than *M. citrifolia* also at random heights.

Net sweeping was performed by inserting the first cluster of leaves on a branch into an insect net, and tapping the branch twenty times to knock insects off of the foliage and into the net. Four branches from each tree were swept at random heights and aspects, and four trees from each site were sampled.

Percent herbivory was recorded at each site by evaluating the percentage of each leaf that was missing, mined, or browned by insect activity. Eighteen leaves were sampled per tree. Four trees per site were sampled. Analysis of percent herbivory was performed through analysis of variance between the three cultivation strategies described above.

Soil characteristics

Soil samples were taken from each site according to procedures described by Sarrantonio et al (1996) and analyzed for percent moisture, bulk density, pH, rooting depth, and texture. Texture was characterized by the "feel method" described by Arshad et. al.(1996). Rooting depth was measured by coring the soil until coarse roots (>5mm) were absent and depth was then measured with a ruler. Samples for bulk density, moisture, and pH were collected from each site by randomly scooping soil from the top layer at four to six locations around the site and mixing these scoops together as described in Arshad et al (1996). Bulk density was calculated according to the equation:

$$BD = \frac{\text{dry weight of soil (g)}}{\text{Volume(cm^3)}}$$

Soils were dried in a microwave oven for five minutes. Percent moisture was calculated according to the equation:

$$\%M = \frac{\text{(wet - dry weight of soil)}}{\text{wet weight of soil}}$$

PH was measured with a hand held pH meter, and a soil solution of equal parts of soil and distilled water (Smith et al 1996, Lowery et al 1996).

Plant morphology

Leaf width, leaf length, number of leaves per cluster, space between clusters, fruit length, fruit circumference, fruit number, developmental stage of each fruit, height, and live crown ratio were measured for four trees per site. Leaves were sampled from the lowest branches on the tree and twenty-five leaves were sampled per tree. Leaf width and length were measured to the nearest centimeter at the widest and longest part of the leaf. Leaves were counted manually and recorded in number per cluster for five clusters per tree. Spacing between clusters (analogous to internodal length) was recorded for three to six branches per tree. Five fruits were sampled from each tree and length and circumference were measured at the largest part. Developmental phases defined in Petard (1986) were assessed for each fruit sampled. To standardize size measurements, phase one and five (youngest and oldest fruits) were disregarded. Number of fruits per tree was counted manually. Live crown ratio was estimated ocularly to the nearest ten percent and height was estimated ocularly to the nearest meter. Results were analyzed with analysis of variance and the Systat and Excel programs

Habitat characteristics

Shade, slope, and elevation were determined for each of the four trees sampled for morphology data. Shade was measured through ocular estimation to the nearest ten percent. Slope was measured using the clinometer gauge on a Silva Ranger compass. Aspect was determined with a compass to the nearest cardinal direction. Elevation was estimated for most sites as they were close enough to the ocean that sea level was visible.

Associated flora was recorded by observing plants growing within a one- meter radius of the four trees sampled for morphological data and unknown plants were brought to the lab, dried, and identified by comparison to a text (Florence 1997) and through the help of A. Hinkle.

Tree spacing was recorded by observing how far in each cardinal direction another Nono tree was growing. Any spacing over five meters was not recorded. Spacing was rounded to the nearest meter. Categories of spacing were then determined as 1-5:

1. one tree within 5m
2. two trees within 5m
3. three trees within 5m
4. four trees within 5m
5 four trees within 2m

Other information that could not be measured in the six week period such as age of plantation, yield of fruit, and management regimes (pruning, fertilizing, irrigating, etc) was found by interviewing owners and managers of the plantations and gardens. In some cases these data could not be found, and were deduced by observation and comparison with known sites.

Sites

Three categories were delineated according to management regimes, and are referred to hereafter as cultivation strategies. These were Sauvage, Homegarden, and Plantation (Table 1).

Sauvage

This cultivation strategy involves planting *M. citrifolia* on land already being used for cultivation of *C. nucifera, M. x paradisica, C. papaya* and others. The land is then left to produce fruits, and the only management that occurs is fruit collection. These sites have large amounts of dead biomass and leaf litter, many species of plants, and have cluttered understories. All sites in this category were at coastal elevation (5m) and had no slope.

site	strategy	pk	area	# trees	prior land use	fruit use	age	elevation	slope
1	sauvage	15.5 E	1 ha	60	coconut plantation	sold and consumed at home	3-5y	5m	0
2	sauvage	13.5W	1.5 ha	100	coconut plantation	sold and consumed at home	15 y	5m	0
3	sauvage	20 W	1 ha	30	coconut and banana cultivation	sold and consumed at home	3-5y	5m	0
4	home garden	3 W	2 ha	130	coconut plantation	sold to Haapiti factory	3 y	5m	0
5	home garden	13.2W	1 ha	100	breadfruit, coconut papaya garden	sold and consumed at home	5 y	5m	0
6	home garden	7.4 E	1 ha	45	coconut plantation	sold to Haapiti factor	1 y	5m	0
7	plantation	2 Interior	2 ha	500	Agricultural school growing	sold to Moinda Inc.	4.5y	40m	4%
8	plantation	24.3W	20 ha	10,000	Papaya platnation	processed at Haapti factory	3 y	40m	0
9	plantation	24.3W	20 ha	10,000	Papaya plantation	processed at Haapti factory	3 y	85m	20%

Table 1. Site descriptions by index number. Pk values correspond to distance in kilometers around the main road of the island from Viare. E denotes moving clockwise, W denotes counterclockwise, and interior refers to the interior road between Opunohu and Pao Pao. Fruit use refers to the consumption of whatever fruit is produced on the site. "Sold" refers to the Haapiti factory unless otherwise noted, and "consumed at home" refers to consumption as medicine, food, or any other home use.

Homegarden

This category involves plots of land in which some management is undertaken, usually pruning and clearing/burning of dead biomass, and *M. citrifolia* is grown in association with other crops such as *C. nucifera, C. papaya*, and *M. x paradisica*. It is called "homegarden" because these sites are usually located very close to the home of the owner, and the owner and their family or one hired gardener usually perform maintenance. All of these sites were located at coastal elevation (approximately 5m) and had no slope.

Plantation

This category describes the large-scale commercial plantations that produce a large quantity of fruit exclusively grown for export. Management of these sites involves clearing of the understory, soil preparation before planting sometimes including chemical fertilizers and pesticides, some irrigation, and large quantity harvesting by hired workers (Bartolemew, pers. comm.).

Results

Insect Diversity

Sticky traps revealed no statistically significant differences between cultivation strategies. Bark louse and ants were prevalent on every tree in similar proportions throughout all cultivation strategies.

cultivation strategy	site	D value	
sauvage	1	0.731	
	2	0.792	
	3	0.822	
homegarden	4	0.799	
	5	0.648	
	6	0.774	
plantation	7	0.799	
	8	0.861	
	9	0.632	
ANOVA	df	F ratio	p value
	2	0.17	<0.848

Table 2. Simpson Index diversity values for insects found in pit-fall traps and sweeps with analysis of variance results of the diversity value according to cultivation strategy. Simpson equation used: $D=1-\Sigma(p_i)^2$ where p_i=proportion of individuals of species i in the community.

Analysis of variance revealed no significant trends in insect diversity between cultivation strategies sampled by sweeping and with pit fall traps (Table 2). Number of species found within each category did not differ significantly from that between categories. A list of insects collected appears in appendix A.

Herbivory measurements revealed that sauvage sites are most significantly impacted by herbivory ($p<0.0001$) (Figure 2). Percent of leaf missing was not statistically related to cultivation strategy, but percent mined and percent browned were related in the same fashion to cultivation strategy as herbivory (their graphs were analogous to Figure 2). Percent mined increased as population density increased ($p<0.001$), and as moisture increased ($p<0.038$). Percent browned was not statistically related to either spacing or shade, but increased as spacing decreased ($p<0.171$) (Table 3).

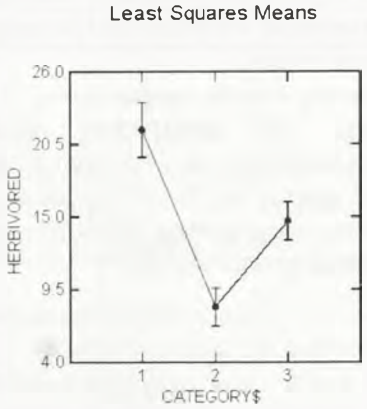


Figure 2. Percent of foliage effected by herbivory. Categories 1, 2, and 3 correspond to cultivation strategies sauvage, homegarden, and plantation respectively.

Soil characteristics

Soil pH varied significantly ($p<0.027$) between cultivation strategies with homegardens having the highest average of 8.01. Sauvage and plantation categories each averaged 6.9 on the pH scale (Figure 3).

Bulk densities ranged from .61 to .99 grams per cubic centimeter. A statistically significant correlation between cultivation strategy and bulk density cannot be drawn ($p<0.679$). However, sauvage and plantation sites shared similar values while homegardens showed slightly higher values (Table 4).

	Total Herbivory			Percent Missing			Percent Mined			Percent Browned		
	df	F ratio	p-value	df	F ratio	p-value	df	F ratio	p-value	df	F ratio	p-value
Cultivation strategy	2	**7.913**	**<0.0001**	2	1.612	<0.2	2	21.468	<0.0001	2	**3.495**	**<0.031**
shade	1	0.172	<0.679	1	2.206	<0.138	1	0.001	<0.976	1	0.155	<0.694
soil moisture	1	0.245	<0.621	1	2.403	<0.122	1	**4.329**	**<0.038**	1	1.563	<0.212
spacing	1	**5.759**	**<0.017**	1	0.251	<0.617	1	**11.19**	**<0.001**	1	1.876	<0.171

Table 3. Results of analyses of variance of percent total herbivory, percent of foliage missing, percent of foliage mined, and percent of foliage browned, grouped according to cultivation strategy, percent shade, soil moisture, and spacing. All analyses are one-way ANOVAs. Bold face values are statistically relevant.

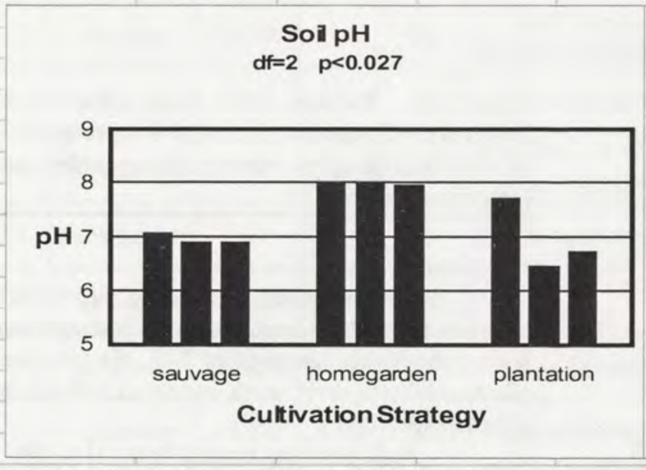
Soil textures are given in table 4. Homegardens tended to have the coarsest soil while plantation sites had the most clayey textures.

Percent moisture did not vary significantly between cultivation strategies ($p<0.159$) however an obvious trend was observed (Figure 4). Commercial plantations showed the highest soil moisture, sauvage sites displayed the most variance, and homegardens had the lowest values.

Rooting depths ranged from 13 cm to over 22cm. No statistically significant correlation could be drawn between cultivation strategy and rooting depth. The p value for the ANOVA comparing rooting depth according to cultivation strategy was <0.578.

	site	Texture	Bulk Density
sauvage	1	silty clay	0.6
	2	loamy sand	0.96
	3	sand	0.89
home-garden	4	sandy loam	0.98
	5	sandy loam	0.83
	6	sand	0.93
plantation	7	clay	0.97
	8	coarse silt loam	0.85
	9	clay loam	0.7
Bulk Density ANOVA	df	F ratio	p value
	2	0.413	<0.679

Table 4. Soil texture and bulk density values (g/cm^3) for each cultivation strategy and site. Analysis of variance results from bulk density values grouped according to cultivation strategy.



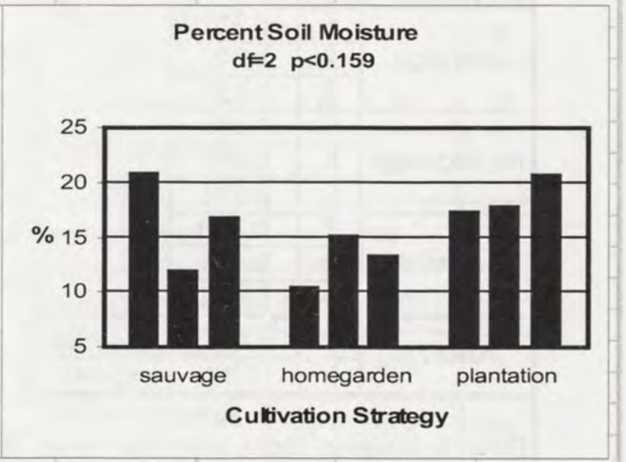


Figure 3. (left) Soil pH values for all sites according to cultivation strategy with results from analysis of variance between and within cultivation strategies.

Figure 4. (right) Percent soil moisture values for all sites according to cultivation strategy with results from analysis of variance between and within cultivation strategies.

Morphology

Leaf morphology data showed that for each cultivation strategy, leaf size differed significantly (p<0.0001) (Figure 5). Leaf length to width ratios were similar for each cultivation strategy. Leaf area was also shown to correlate directly with shade and soil moisture (Table 5).

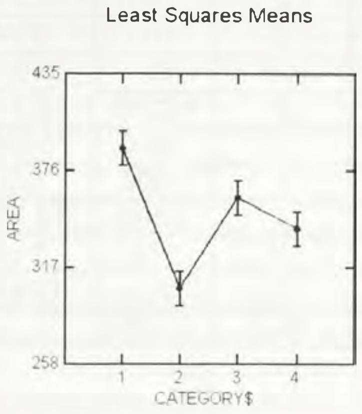


Figure 5. Leaf area values according to cultivation strategy. Categories 1, 2, 3, and 4 correspond with sauvage, homegarden, plantation, and natural populations respectively. P-value for this analysis was zero.

Leaf Area ANOVA results			
	df	F ratio	p value
cultivation strategy	2	16.71	<0.0001
shade	9	7.566	<0.0001
moisture	7	30.926	<0.0001

Table 5. Analysis of variance results comparing total leaf area between cultivation strategies, to percent shade, and to soil moisture.

Number of leaves per cluster was highest in sauvage sites averaging 5.5. Homegardens averaged 4.5 leaves per cluster while plantations averaged 5 (p<0.019). Spacing between clusters did not vary significantly between cultivation strategies (p<0.132).

Fruit size and number data are given in table 6. Sauvage sites displayed less fruit per tree than the other cultivation strategies, and plantation sites displayed larger fruits and more fruits per tree than the others.

Tree height did not correlate significantly between categories (p<0.126) and live crown ratio was shown to vary between categories (p<0.018) and to decrease with increase in shade (p<0.002) (Table 7).

Average values			
	length	circumference	# per tree
sauvage	5.71	14.03	5.00
homegarden	5.78	13.69	21.79
plantation	6.62	15.24	30.42
ANOVA results			
df	2	2	3
F ratio	6.679	7.659	25.34
p value	<0.002	<0.001	<0.0001

Table 6. Fruit size and number of fruits per tree data with results of analysis of variance within and between cultivation strategies.

Average Live Crown Ratio			
cultivation strategy	sauvage	homegarden	plantation
%	52.5	66.9	75.8
Live Crown Ratio ANOVA Results			
	df	F ratio	p value
cultivation strategy	3	3.634	<0.018
shade	9	3.656	<0.002

Table 7. Live crown ratio data for each cultivation strategy with results from analysis of variance between and within cultivation strategies, and according to shade.

Habitat characteristics

Associated flora observations revealed that home-garden sites had the same amount or more plant diversity than sauvage sites, while commercial plantations displayed little floral diversity. A complete list of plants associated with *M. citrifolia* is given in Appendix B. Most plants collected were from the *Asteraceae, Poaceae,* and *Fabaceae* families.

Slope and elevation were slightly variable, with most sites having no slope and coastal elevation. However, commercial plantations have been planted on steep slopes, up to twenty-five percent, and other *M. citrifolia* populations have been found on slopes of fifteen to twenty percent.

Shade varied between zero and one hundred percent with a correlation according to cultivation strategy (Table 9). Natural populations were found in the full range of shading.

ANOVA: Shade	
df	3
f ratio	704.173
p value	<0.0001

Table 9. Analysis of variance of shade values between cultivation strategies.

Agronomic status report

Cultivated populations are reported in figure 6. Statistics are given in table 10.

M. citrifolia is grown prolifically in yards and on roadsides and the fruit is often collected from natural populations and sold or consumed. Royal Tahiti Noni Corp. reports receiving fruits from ninety clients on Moorea, but only thirty on a regular basis (Leite pers. comm.). Most of those clients do not cultivate *M. citrifolia*, but collect it from natural populations. A comparison of the current status to the one from July 1996 (Nerney 1998) shows that area devoted to cultivation and number of cultivated trees on Moorea has increased since 1996. Five of the plantations reported that year are gone. The Agricultural School plantation (index #13) has grown from 118 individuals in 1996, to 500 individuals in 2001. The "Teuiau" plantation (index # 2) has shrunk significantly from 700 individuals in 1996 to just 100 in 2001. The biggest change in the status of noni cultivation on Moorea has been the creation of the twenty-hectare Royal Tahiti Noni plantation (index # 9) of ten thousand individuals. This plantation has more individuals than the total number of individuals reported in 1996.

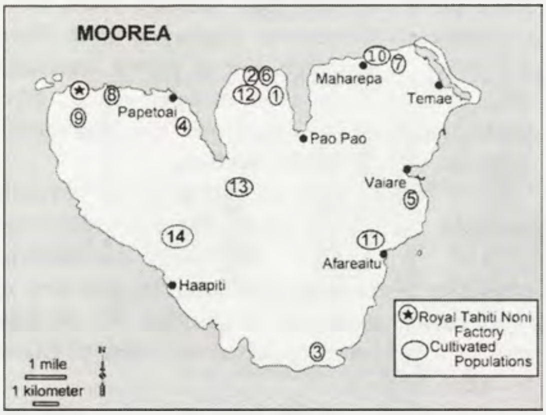


Figure 6. Locations of all known, cultivated *M. citrifolia* populations on Moorea, French Polynesia as of November 2001.

index #	size (ha)	# trees	pk
1	0.5	15	12.5 W
2	1	100	13.5 W
3	1	60	15.5 E
4	0.2	15	20 W
5	1	45	7.4 E
6	2	100	13.2 W
7	2	100	3 W
8	1.2	50	24.5 E
9	20	10,000	24.3 W
10	2	1600	3.6 W
11	2	1500	9 E
12	4	3000	13.2 W
13	2	500	2 int
14	1.5	300	33.5 W
total	40.4	17385	

Table 10. Results of *M. citrifolia* agronomic status report completed November 2001 on Moorea, French Polynesia. Pk refers to distance in kilometers from Viare along the perimeter road. E denotes moving clockwise around the island from Viare, W denotes counterclockwise, and int denotes the interior road between Opunohu and Pao Pao.

Discussion

The lack of insect diversity between cultivation strategies suggests that the risks of unchecked herbivore population growth in monotypic habitats in Moorea are still unknown. It was expected that plantation sites would show less diversity than the more vegetatively diverse sites. This could be true conceding the possibility of sampling error, or lack of consideration for other factors that effect insect diversity such as seasonality, food source, etc.

It is also possible that the plantation sites display similar insect diversity because the plantations on Moorea are not large enough to effect populations in the expected way. The largest plantation sampled was twenty hectares which is less than a fraction of the land area of most commercial farms in the United States in which the herbivore population explosion phenomenon is a serious threat.

Another important factor in consideration is the lack of chemicals used in this commercial agriculture in Moorea. Often commercial farms in the United States create a chemically artificial habitat that is perfectly suited to growing one crop while perfectly unsuited for many other organisms (mostly pests) to survive. Without creation of a chemically regulated system, populations may remain in balance naturally, as is the case on Moorea.

It has been considered that the nature of islands is so different from that of continents that trends we see in ecology on continents do not hold true on islands especially as small as Moorea. Insect diversity on continents is magnitudes larger than on islands, and this may contribute to the inability of a few insects to monopolize the monotypic food source presented in the commercial plantations on Moorea. It would also be interesting to consider the diversity values of native versus nonnative insects over many years to see which, if either, are surviving better in the commercial plantations, and perhaps will disproportionately develop the ability to exploit these artificial habitats. Unfortunately, a detailed, long-term, ecological assessment was not in the scope of this project and is here suggested for future research.

Herbivory observations revealed that sauvage sites are most strongly effected by herbivory, which is counter to expected results. With a diversity of vegetation, a diversity of insects was expected such that predators kept the populations of herbivores from damaging the crops. Instead, the homegarden and plantation sites had much less damage from herbivores suggesting that a clear and open understory with less available resources for insect populations (food, shelter, etc) has a greater control over insect population growth than natural predation.

Percent of foliage mined directly increased with soil moisture suggesting that the yet unknown mining insect prefers or requires moistness. Both browned and mined measurements increased as spacing decreased or population density increased. These results match with expectations as *M. citrifolia* occurs naturally in sandy soils, which generally show low soil moisture due to high infiltration rates, and do not grow in tight clusters unless planted close together. Recommendations from the Rural Development Services Agency of French Polynesia suggest a spacing equivalent to category four of this study (Garnier 1997), which is at the peak of mined and browned observations. Thus more research should be done to determine what the proper spacing should be to reduce the risks of damage by herbivory from these mining insects and unknown browning agent.

It should be noted that there have been no reports of any pest of the fruit of *M. citrifolia*, which is the commercially valuable product. This may lead to an unfounded disregard for the danger that foliage destruction can cause and should not be an excuse to ignore the risks of crop devastation by pest outbreak.

All soil parameters measured are a product of natural processes as well as human influence. As such, observations made about soil characteristics in each site are ambiguous. One important natural factor influencing soil measurements is slope. As slope increases, water flow is redirected from infiltration to runoff, and this effects soil erosion and plant nutrition. Soil texture is important in plant nutrition as texture determines availability of nutrients, water retention and infiltration, permeability to plant roots and shoots, and susceptibility to erosion by wind or water. Shade is also an important factor as solar radiation has large evaporative effects on bare soil (Aukerswald et al 2001).

Percent moisture observations show that the plantation sites have the highest soil moisture, which was expected, even though they have no shade, as they are irrigated. Their texture is more clayey than the other two cultivation strategies allowing them to retain water longer than more sandy soils. The sauvage category had slightly less sandy soil than the homegarden category, and had more biomass on the ground acting as mulch to hold in soil moisture, which is why it was expected to have the second highest soil moisture. Results supported the expectation that homegardens would have the lowest soil moisture, while irrigated plantations had the highest.

PH is also a very important factor in plant nutrition as it plays a role in nutrient availability as well as creates a habitat that can be hospitable to beneficial soil microbes or can kill them. The normal range of soil pH for agriculture is 5.5 to 8.3 while the preferred range is 6.5 to 7.8 (Harpstead et al 1997). While all cultivation strategies fell within the first range, the homegarden category averaged 8.01, which is above the optimal pH range. This can be explained by the high proportion of coralline sand composing the soil matrix in these sites, which is naturally basic. It is not shown that this relatively high pH has any detrimental effect on plant growth of *M citrifolia* and further research into the pH range for optimum growth is suggested for this economically important species.

Bulk density is a measure of the weight of dry soil according to volume. Thus, it indicates compaction if the value is high, and risk of wind erosion if the value is low. Average values of bulk density are around 1.3 grams per cubic centimeter, and fully compacted soil (without any air-filled pore space) is around 2.6 grams per cubic centimeter (Harpstead et al 1997). All observations fell below the average bulk density, but not by much. Thus, it can be suggested that there is no inflated risk of soil loss to wind erosion, or negative impact on plants due to compaction.

Leaf morphology data returned the expected results that leaf area is directly related to shade and soil moisture. Sauvage sites that had the highest amount of shade and a mid range value for soil moisture showed the largest leaves, while homegarden sites with mid range shade and low soil moisture had the smallest leaves.

In consideration of growth form, sauvage sites displayed more leaves per leaf cluster and longer distance between leaf clusters than the other cultivation strategies. This discrepancy in growth form could be the result of many factors such as growing season, available nutrients, water, light, etc. and future research is suggested here.

Tree height was found to be unrelated to any other parameters measured because *M. citrifolia* is extensively pruned by cultivators to ease in fruit harvesting and encourage foliar development.

Live crown ratio is an excellent indicator of plant health as a full crown indicates the highest possible photosynthetic production of the plant. It was found that plantation sites had the highest live crown ratios while sauvage sites had the lowest. Also, there was an inverse relationship found between shade and live crown ratio suggesting that the correlation found amongst cultivation strategies merely reflects the correlation of shade between strategies.

Measurements of leaf size and live crown ratio suggest that plantation sites have the most productive conditions for *M. citrifolia* growth as the leaves are not so large as to suggest a lack of sunlight, while the live crown ratios show the trees are full and productive. Sauvage sites, on the other hand offer larger leaves and lower live crown ratios suggesting the trees are stressed or lacking resources.

Fruit size and number per tree was the best indicator of productivity found and the results were as expected. Plantation sites had the largest fruits and the most per tree while sauvage sites had the lowest number and smallest fruits. It is possible that sampling ignored differing management in this case as the sauvage and homegarden sites are probably harvested more often than plantation sites because they are smaller in size.

A broad evaluation of all results is presented in table 11 in the form of a cost to yield comparison. Clearly the plantation strategy yields the highest profits in monetary terms, but when other benefits such as economic security and initial investment amount are considered, it becomes apparent that the sauvage strategy is the best way to get as much out of your land as possible with as little work as possible. The homegarden strategy offers a good compromise for the landowner who is willing to put some effort towards maintaining a crop without the risks associated with monocultural cultivation. Food is produced for the home as well as for sale to supplement the family income monetarily.

costs	plantation	home garden	sauvage
large initial investment	X		
maintenance costs	X	X	
land conversion	X		
yields			
profit	X	X	X
food		X	X
insurance against crop failure		X	X

Table 11. Cost and yield comparison of cultivation strategies discussed in this paper. Profit refers to monetary returns.

However, consideration for soil moisture and pH could increase the health of plants grown in this fashion on Moorea and possibly increase yields.

The follow up agronomic status report reveals that *M citrifolia* cultivation is intensifying on Moorea indicating that concern for the future of the noni industry is relevant and important. To draw larger conclusions than just Moorean agriculture, a larger survey must be performed.

Conclusion

A longer term, more in depth study could prove quite useful in the development and policy making process of the commercial agriculture industry in French Polynesia. It was shown here that suavage style management provided more herbivory and less fruits than the other strategies. Perhaps it should be said that production of *M.citrifolia* for export should not be performed in that manner in order to ensure land use efficiency. However, it should also be said that sauvage style management most closely mimics nature. The lower percentage of herbivory seen in the other strategies now could just be an effect of newness. That is, once an insect finds the under exploited niche (the uneaten leaves), it could harm commercial fields and devastate small scale farmers. This would harm small-scale farmers much more (by ruining their initial investment) than it would to keep noni production wild and have lowered yields.

Initial expectations regarding cultivation conditions were amended after preliminary observations because the commercial plantations of *M. citrifolia* are not typical of large-scale agriculture. The organic status and small size of the "noni" industry on Moorea makes it less environmentally impacting than the commercial farming industries in the United States and Europe. With time and encouragement, this industry could grow (as reported here) in French Polynesia and present higher investment and ecological risks such as destruction of native ecosystems through land conversion, loss of endemic species through loss of heterogeneity in human controlled agricultural systems, and crop/ profit devastation due to unchecked herbivore population growth and over dependence on one source of income. We should be cautious about the economic shift that agricultural intensification causes, and trepidatious about converting land from natural settings to commercial production as so little of it is left.

Globally, the diversity of product as well as the ecological diversity afforded by homegardening is a benefit that is not considered enough. Generally, the only profit considered is monetary. Analyses such as the one presented here in which ecological and risk assessments are valued equally with monetary profit are necessary in the literature to raise awareness of the instability of modern mechanized agriculture.

However, at this time the noni industry is not developed enough to know if it will detrimentally effect the standard of living and the ecology of French Polynesia. Considering the location of French Polynesia, the total land area, and the tourism industry, it seems unlikely that commercial agriculture will ever reach the sophistication of that in the northern hemisphere there, and concern is not urgent but monitoring should continue.

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Appendix: A - Insects associated with *M. citrifolia:* Order and number of different species in that order

- Homoptera - 11
- Hymenoptera - 8
- Aranea - 6
- Coleoptera - 3
- Collembola - 3
- Orthoptera - 3
- Neuroptera - 4
- Acari - 1
- Diptera - 3

Appendix: B - Flora associated with *M. citrifolia*

- *Nephrolepis cordifolia*
- *Stachytarpheta urticifolia*
- *Canthium barbatum var. barbatum*
- *Emilia tostergii*
- *Lantana camara*
- *Phymatoserus scolopendria*
- *Syzigium cuminii*
- *Passiflora edulis*
- *Poacaea members*
- *Ipomoea littoralis*
- *Wedelia trilobata*
- *Fabaceae members*
- *Asteraceae members*
- *Momordica charantia*
- *Digitaria setigera*
- *Coco nucifera*
- *Artocarpus incisa* (or *communis* or *altilis)*
- *Carica papaya*
- *Musa x paradisica*
- *Leucaena leucocephala*
- *Hibiscus tiliaceus*

Appendix: C – Personal communications

- Leite JE, manager of Royal Tahiti Noni processing factory, 9, Nov. 2001, Haapiti, Moorea, French Polynesia

- Bartholomew J, Assistant Director Lycee Agricole de Pao Pao, 15, Nov. 2001, Opunohu, Moorea, French Polynesia
- Boulaville L, Nono grower, 9, Nov. 2001, Opunohu, Moorea, French Polynesia
- McIlroy T, Nono grower, 8, Nov. 2001, Pao Pao, Moorea, French Polynesia

Ecological Growth Patterns and Distribution of *Dicranopteris linearis* on Moorea, French Polynesia

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ABSTRACT. The ecology of the mat forming fern *Dicranopteris linearis* plays an influential role in rainforest succession throughout the Old World tropics. Despite its ability to initialize succession and also inhibit forest regeneration, little is known about what factors influence its establishment, reproduction and maintenance. In this study, it has been suggested that *D. linearis* is a sun-thriving fern that requires shade for optimal germination of spores. After initial establishment in sheltered microhabitats, *D. linearis* tends to invest in vegetative spreading over large areas of available land. After *D. linearis* is established, it can dominate and exclude other plants from establishing. However, mature *D. linearis* growth was found to be inhibited by heavily shaded forests dominated by *Hibiscus, Pandanus, Inocarpus, and Mangifera*. Open forests of Pine and Acacia showed little or no exclusion of *D. linearis* in the understory. Suggestions of factors influencing the replacement of existing mats with forestland and also methods of initializing primary succession on recently disturbed sites have been made.

Introduction

Dicranopteris linearis is a thicket-forming native fern, widely distributed throughout the Old World tropics and subtropics, including Polynesia. Since Polynesian island settlement, *D. linearis* fernlands on colonized islands have expanded dramatically (Russel et al 1998). On the island Mangaia, Kirch (1996), found that *D. linearis* began to dominate the pollen spectra 1600 years ago, at approximately the same time of Polynesian settlement. Kirch further suggests that anthropological disturbances such as burning have allowed *D. linearis* fernlands to replace large amounts of indigenous forests.

D. linearis is known to quickly establish in a disturbed area, and with the right conditions arrest succession and develop into a climax species (Maheswaran & Gunatilleke 1988). In open canopy, oligotrophic, wet forests in Hawaii, monospecific *D. linearis* thickest >3m are a commonly found (Russell et. al 1998). Similar sites have been known to be *D. linearis* dominated for centuries (Dombois 2000). Mat forming ability, combined with clonal growth, shallow rhizomes, and marcescent leaves with low decomposability allow *D. linearis* to maintain exclusive dominance by physically restricting the establishment of competing plants and by controlling resources such as water and light (Russell et al. 1998).

D. linearis can be a pioneer species on primary successional sites such as landslides, burns, lava flows, and mountain ridges (Russell et al. 1998*)*. *D. linearis* has been viewed as a specialist in colonizing open canopy, wet, low -P sites (Russell et al. 1998). The fern is easily distributed by spores and once established, spreads vegetatively by stolons and rhizomes. The rapid growth of the fern can stabilize eroding soil, contribute organic matter (Dombois 2000), nutrients and moisture. In Hawaii, *D. linearis* has been shown to colonize harsh microenvironments uninhabitable by other endemic species (Russel et al. 1998). Russel et al. (1998) further suggest that *D. linearis* may play an important role in resisting exotic invasions by out-competing exotic species in recently disturbed areas.

D. linearis plays an important role in rainforest succession but little is known about the ecology and interaction of the fern with other species. Few studies have been done that examine the ecological conditions that contribute to the fern's exclusive domination of an area, and its effect on plant diversity and forest regeneration. Manipulative management of existing fern thickets has the potential to accelerate succession, encouraging the conversion of fern mats into indigenous forests. The fern's success as an indigenous colonizer in harsh environments suggests that it can be used to initialize succession of barren land into forests. As more ecological information is discovered, land managers can be informed of the benefits and hindrances of *D. linearis* to rainforest succession and conservation of native plant species.

Site, Materials and Methods

Four sites on Moorea, French Polynesia were chosen to study patterns of *D. linearis* growth.

- Site 1 is located above the Belvedere, at the beginning of the Three Coconuts Trail. The site consists of a 50 meter by 25-meter area dominated by thick mats of *D. linearis*. Further along the Three Coconuts Trail, a forest dominated by *Hibiscus tileaceus*, *Pandanus tectorius*, and *Inocarpus fagiferus* trees gradually replace the *D. linearis*. The site extended approximately 100 meters into the forest, until no *D. linearis* is found.
- Site 2 is located across from the Beachcomber hotel on the northeast side of Moorea. The site has minimal canopy cover and was burned approximately 3 years ago (Koehler, 1999). The less than 1 ha site has >50% bare ground exposed. Few plants are found on the site besides grasses, *Metrosideros collina* and some *D. linearis*.
- Site three is located below the Belvedere, .5 Km from the pig farm into the lower entrance of the Three Coconuts Trail. The site consists of a 100 by 50 square meter plot of planted *Pinus caribbea* trees, with an under-story dominated by *D. linearis*. Directly west of the pine plantation is a grove of *Albizia falcatoria* trees, approximately 100 by 200 square meters.The under-story is also dominated by *D. linearis*.
- Site four is located in between PK 19 and 20 South East of Haapiti. The site is approximately 150 by 75 square meters in size on an east-facing slope. The site consists of an open area heavily dominated by *D. linearis* and a forested area dominated by *Mangifera indica* , *Inocarpus fagiferus* and *Hibiscus tileaceus*. The site edge was determined to be 25 meters from the edge of *D. linearis* growth.

Germination study

The *D. linearis* spore germination study was conducted at site one above the Belvedere from 9/26/01 to11/14/01. Mature fronds containing spores of *D. linearis* were collected and processed. The fronds were spread out on sheets of paper and dried under a lamp for twenty-four hours. After drying, the released spores were collected. This was done until a total of 6 grams of spore material was collected. The spore material was mixed thoroughly.

Forty-five four-inch high dixie cups were filled two thirds full with all-purpose potting soil. A 2/10-gram spore material sample was evenly sprinkled over the surface of the soil. 3 milliliters of distilled water was evenly sprayed on top of the spores. A petri dish was placed on top of the cup to retain moisture.

Each set of ten cups was placed in areas with varying amounts of canopy cover at the Belvedere site. The first set was placed in an area

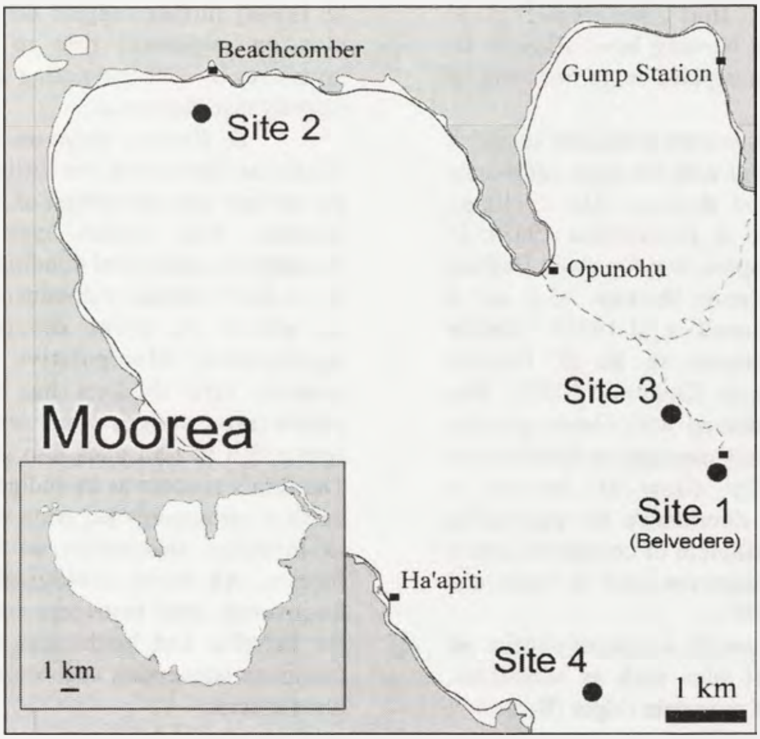

Fig.1 Map of *D. linearis* study sites on Moorea, French Polynesia.

where *D. linearis* was growing in direct sunlight with 0% canopy cover. The second set was placed in an area of 50% *Hibiscus tileaceus* and *Pandanus tectorius* cover, and a third was placed in an area of 95% *Hibiscus tileaceus* and *Inocarpus fagiferus* cover. The spores were allowed to germinate for 5 weeks undisturbed. After 5 weeks the presence of germinated spores was recorded for each individual cup.

Canopy cover/Diversity analysis

A canopy cover/plant diversity analysis of *D. linearis* growth patterns was conducted at all four sites. At each site, the edge of the *D. linearis* dominated area was determined, and a point randomly selected. The criteria for an edge was that *D. linearis* cover must be less than 50% and the canopy must be in transition from the *D. linearis* dominated mat. From the edge a random aspect and number of paces limited to 100 was chosen. A point was rejected if no *D. linearis* plants were present within 25 meters. From each viable point, the center of a one square meter plot was laid down on the chosen point, perpendicular to the chosen aspect. Canopy type and canopy cover was estimated with a spherical densiometer. Percent *D. linearis* ground cover was estimated within the plot. Number of other plant species present was noted. From this data, approximate canopy cover and *D. linearis* ground cover of each site was estimated by averaging the percentages. The numbers of other plants present on each meter plot was averaged.

Morphological study

A morphological study of the ratio of rachis to blade length was conducted at site 1 above the Belvedere, site 2 across from the Beachcomber and at site 4 near Haapiti. At each site, ferns were selected by starting in the approximate middle of a *D. linearis* dominated mat. A random aspect and number of paces was chosen from a random number table. The number of paces, limited to 200 was taken along the randomly chosen aspect to reach a point where *D. linearis* growth was present. For each live *D. linearis* plant in a 1.5-meter radius, rachis length from soil to the first live blade was measured, and the length of the remaining live leaf blade was recorded. At each site this was repeated until approximately 75 measurements were made.

Data analysis

Data was analyzed using Systat and Excel, 95% confidence intervals for all data were calculated. A chi squared test was conducted for the germination study, T-tests and one way anova tests were conducted for the analysis of the remaining study means.

Results

Germination study

After five weeks of germination at site one above the Belvedere, all three cover type plots contained cups with germinating spores. Twenty percent of the 0 % cover cups contained

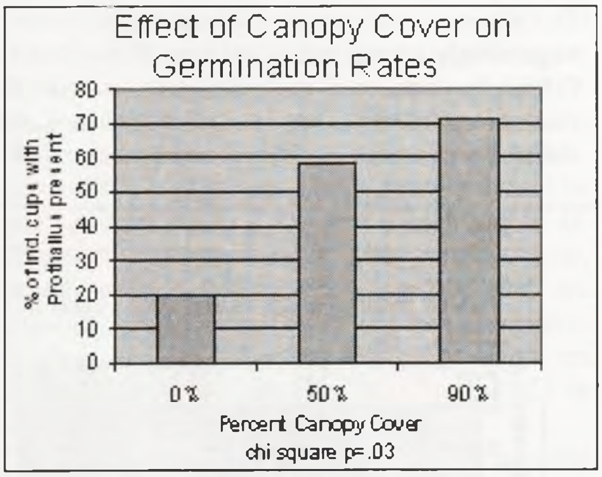

Fig. 2 Percentage of cups containing germinating spores of *D. linearis* in each three canopy cover percentages.

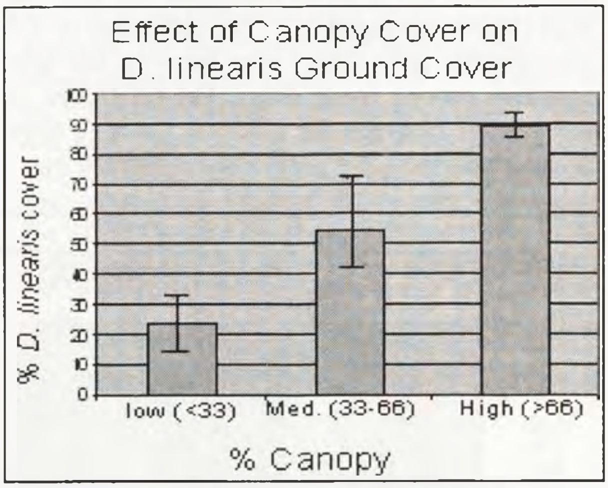

Fig. 3 Effect of various canopy covers on percent ground cover of *D. linearis*.

live spores. Fifty-eight percent of the cups in the 50 % cover plot and 71% in the 90% cover plot contained live spores. The difference in germination rates between the 0% and 90% plots is significant (p=.037).

Canopy cover/Diversity analysis

Various amounts of canopy cover in sites 1 and 4 showed significant differences in *D. linearis* ground cover. In the high canopy cover (>66%) areas, the mean D. linearis cover was 23.6%. The medium canopy (33%-66%) areas averaged 54.7% while the low canopy areas (<33%) averaged 89.4%. Using the t-test for significance, differences between the high and low sites (p=<.001) and the high and medium sites (p=.002) were significant.

The differences in % *D. linearis* ground cover in areas of high (>66%) canopy cover between site 1 and four, site 3 trees were all significant. In the high canopy cover of the native trees, there was an average of 16.4% ground cover of *D. linearis*. In the *Albizia falcatoria* canopy there was 42.0% and in the *Pinus caribbea* 79.4%. The highest differences in % *D. linearis* cover are between the native and pine covers (p=<.0001) and between the Acacia and Pine (p=<.0001). The difference between the Native and Acacia dominated plots were also significant (p=.004).

Results of the diversity analysis in sites 1 and 4 showed significant differences in plant diversity between varying amounts of *D. linearis* ground cover. The low cover (<33%) areas averaged 2 plant species per square meter, while the medium (33%-66%) averaged, 4.5 and the high (>66%) averaged 6.7. The difference of 4.7 plants/meter (p=<.0001) was between the low and high canopy cover sites. The difference in the average between the medium and high sites of 2.2 plants/meter (p=.001) and 2.5 plants/meter (p=.005) between low and medium sites are also significant.

Site 3 analysis and observations

The average ground cover of site 2 was 3.22% with a standard error of 1.272. The result of a 1999 vegetation survey of the same sight reported 0% ground cover of *D. linearis*.

D. linearis growth was found almost exclusively in ravines >.25m deep on site 3, with evidence of vegetative spreading by stolons and rhizomes.

Morphological study

The live blade/rachis ratio analysis of *D. linearis* plants in site 1, 2 and 3 showed significant differences among tall (>100cm) medium (50cm-100cm) and short (<50 cm) plants. Between the tall and medium sized plants p=.012, tall and short and medium and short both p=<.001.

Discussion

The results of the germination study suggest that optimum germination rates *of D. linearis* spores occur in shaded habitats. Shaded habitats tend to retain moisture and have less variation in temperature and humidity. This finding is consistent with current literature on *D. linearis*, and fern spores as a whole. Although *D. linearis* is a sun-demanding fern, initial germination requires shade and moisture due to spores' extreme susceptibility to drying.

At the recently burned site three, *D. linearis* appears to be establishing itself as a primary successor. Over a period of three years, *D. linearis* plants have established and begun to vegetatively spread out of ravines. Russell et al. (1998) has observed this phenomenon with *D. linearis on* Mauna Loa, Hawaii. Moisture and detritus accumulate in these cracks and allow

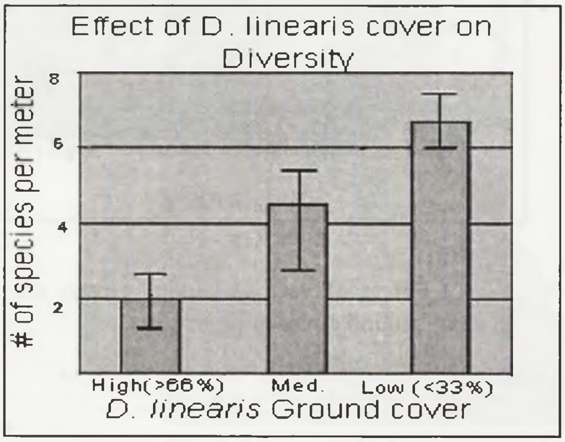

Fig. 4 Effect of *D. linearis* ground cover on plant diversity per square meter

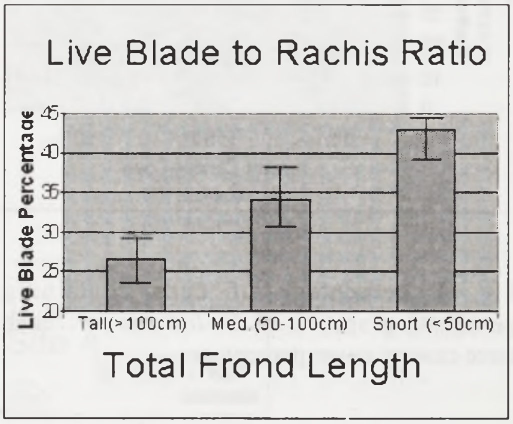

Fig. 5 Blade/ rachis ratio differences in relation to total *D. linearis* frond length

spores to germinate. Once established, rhizomatous growth enables *D. linearis* to grow in surrounding harsh habitats (Russell et al. 1998). With the proper conditions maintained, *D. linearis* can begin the succession of this site into a secondary succession.

The morphological study results relate the previous finding with a more general hypothesis; as *D. linearis* matures and establishes itself, more resources are allocated to vegetative spreading into surrounding habitats. The increase in rachis/blade ratio appears to enable *D. linearis* to form thick mats and dominate large areas. This trait may be an important morphological characteristic in preventing self shading and allowing *D. linearis* to exclude and dominate a site (Russell et. al 1998). This characteristic of the fern should be taken into consideration if *D. linearis* is to function as a primary successor.

Results of the canopy cover/diversity study suggest that dense, multi-layered native forest restrict *D. linearis* growth substantially; while introduced open forests do not dramatically restrict *D. linearis* understory dominance. As a result of *D. linearis* domination, understory plant diversity has been greatly reduced at these sites. Planting trees that form dense canopies may be a better choice than open forest trees for the succession of *D. linearis* mats into native forest.

If so desired by a land-owner, *D. linearis* sites can be converted into agricultural lands or returned to native forests. Dense *D. linearis* mats contribute a large percentage of above-ground net primary productivity while occupying only 14% live bio-mass (Russell 1998). For successful forest regeneration Maheswaran and Gunatileke (1988) suggest *D. linearis* should be allowed to contribute nutrients for several years. After this initial nutrient influx, secondary successional woody species should be planted after slashing down the thick mat of *D. linearis*. As observed in sites one and four, woody species that out-compete *D. linearis* are *Hibiscus*, *Pandanus*, *Inocarpus* and *Mangifera*. Studies on long-term restoration attempts on Moorea and other islands would be a useful in developing refined restoration strategies.

D. linearis eradication may be harmful to native species. As suggested by Russell et al. (1998), *D. linearis* can benefit native ecosystems by resisting the invasion of exotic species into newly disturbed habitat. Invasive plants can significantly influence ecosystem processes and consequently alter the function and development of native species (Russell 1998). In Hawaii, *D. linearis* has coexisted with endemic species for millennia (Hotchkiss & Juvik, unpublished data). *D. linearis*' ability to resist colonization of competing species can be utilized in the preservation of native species. With the knowledge of *D. linearis'* requirements and mechanisms for establishment, managers will be more equipped to prevent invasions and promote native species using this native fern

Suggestion for further study and refinement include reducing the error associated with the germination study. A more controlled growing environment and replication would make findings more significant. A continuous study looking at extended effects of burning and *D. linearis* growth. would contribute applicable knowledge for landowners. A final refinement to the morphological study, would be factoring in age of the *D. linearis* mat and correlating that with the blade/rachis ratio. This would shed light on plant resource allocation in relation to when the mat was established.

D. linearis' role in ecosystem succession should be further studied and understood in relation to land management. More knowledge about the process of succession involving *D. linearis* has the potential contribute management techniques that conserve native species and increase overall biodiversity in the new world tropics.

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A Survey of Macrolepidopteran Moths on Moorea, French Polynesia with a Preliminary Examination of Their Role as Pollinators

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ABSTRACT. Despite the unique biological opportunities presented by the island chains of French Polynesia, the biology of the area is poorly understood. Moths are among the many taxa with undocumented biodiversity. There is biological interest in documenting which moth species are present and how they interact with the plant community. I collected 161 macrolepidopteran moths at a range of elevations on the island of Moorea, French Polynesia to explore their diversity and study their role as pollinators. I assembled a pollen library of 27 common plant species and attempted to match these pollens with pollen grains that were removed from moths. Although identification to species was not possible with all of the moths that were collected, I grouped them into morphospecies by wing pattern; 35 morphospecies were found. There were more species found at higher elevations. The abundance of some species changed with elevation. The most abundant morphospecies at all sites were non-endemics that are widely distributed throughout the South Pacific. Many moths were found to feed on the invasive *Lantana camara* and some fed on plants in the Myrtaceae. This study suggests that the moth fauna on Moorea is dominated by non-endemics, but that there may be some relictual endemics, especially at the higher elevations. It also suggests that invasive plants have a considerable impact on the moth fauna. Future research on Moorean moths should focus on accomplishing a more comprehensive survey of moth biodiversity and examining the interaction of the moth fauna with native and introduced plants.

Introduction

Humans have drastically transformed the biology of French Polynesian islands in the last two millennia. The first major impact was the arrival of Polynesians more than 1400 years ago (Lepofsky et al. 1996). The second great impact was European colonization during the twentieth century. The transformation of the islands continues today as they become more developed and biological invasions alter the landscape (e.g. Meyer and Florence 1996).

Despite the unique biological opportunities presented by the island chains of French Polynesia, the biology of the area is poorly known. Moths are among the scores of large taxonomic groups that we know little about. Comprehensive surveys of moth diversity have been done in Hawaii (Zimmerman 1948) and on larger continental islands such as Australia (Common 1990), New Zealand (Hudson 1928), and Borneo (Holloway 1976). There have also been a few studies on smaller islands (e.g. Holloway 1977, Holloway 1990), but for most islands in French Polynesia, there is little more than a superficial examination (e.g. Paulian 1998) of the moth fauna since the Bishop Museum s entomological expeditions in the 1930 s (Adamson 1939).

From Darwin forward, biologists have used islands as natural laboratories to tease apart the intricacies of evolution. Each chance to explore undocumented biodiversity such as Moorea s moth fauna can lead to new evolutionary insights (e.g. Fleischer et al. 1998; Chown 1994). However, because human impact on the island is likely to result in the extinction of arthropod taxa (Hambler and Speight 1996; Dunlop 1989), it is imperative that we learn what we can from these organisms soon.

Furthermore, there is little in the way of conservation efforts in French Polynesia. As shown in Hawaii, a similar setting, documenting diversity is the first step to developing conservation goals for arthropods (Gillespie 1999). As an added benefit for studying moth diversity, Holloway (1984) suggests the use of moths as bioindicators on tropical islands. Kitching et al. (2000) effectively use moths as bioindicators in Australian rainforests. A better understanding of moth diversity in French Polynesia may allow moths to be used there to assess ecosystem health.

Beyond discovering what moth taxa are present on these islands, we need information on the moths role in the ecosystem to apply our knowledge in an evolutionary or conservation context. Moths play important roles as herbivores during their larval stage, as pollinators during their adult stage, and as food for predators and parasitoids throughout their life cycle (Scoble 1992). There is no information on how moths play these ecological roles on French Polynesian islands.

This study takes preliminary steps toward filling these gaps in our knowledge for the island of Moorea. Moorea is a high (1207 m) volcanic island in the Society Islands chain. It is 17 kilometers across and has 12,000 residents, most of whom live on the coast (French Polynesia Government Presidency 2001). Originally a conical volcano, the center of Moorea has collapsed and eroded out toward two bays leaving two connected central valleys surrounded by a ridge of high mountains rising up from the coast (Howel 1933). I surveyed macrolepidopteran moths at sites on the coast, in the valleys, and in the mountains to assess their diversity. (Macrolepidopterans are a traditional classification of moth families that include larger, easily-studied moths; this study focuses on them because little is known about their counterparts, microlepidopterans). On high volcanic islands, much of the insect diversity is found at the higher elevations (Peck 1999). This study compares the moth diversity of three regions of the island based on elevation coast, valleys, and mountains to see if moths in Moorea are consistent with this trend.

This study also includes an examination of the moths capacity as angiosperm pollinators. Adult lepidopterans feed almost exclusively on angiosperm nectar and, with coleopterans, dipterans, and hymenopterans, are responsible for most angiosperm pollination. Moths often pollinate plants with pale, fragrant flowers (Kevan and Baker 1999). The extinction of moth species due to habitat loss or competition with invasive moths could negatively impact plant diversity on Moorea, especially for rare plants (Spira 2001). To discover which plants moths were feeding on in Moorea, I compared pollen grains that were taken from the mouthparts of collected moths to pollen grains taken from local plants. Like the moths, published pollen libraries are available for floras in Hawaii and larger continental islands (e.g. Selling 1946; Moar 1993), but none is available for islands in French Polynesia. Thus, this study includes a small pollen library created with plants collected in Moorea.

Methods

Study Location

All moths and plants were collected at nine sites at a range of elevations on the island of Moorea, French Polynesia (149...50'W, 17...32'S) during 19 collection expeditions between 28 September and 15 November 2001. To aid in the analysis of distributional data for the moths, I divided these collection sites into three regions: those in the coastal areas of Moorea, those in the mid-elevation valleys, and those on mountain ridges and peaks (Figure 1).

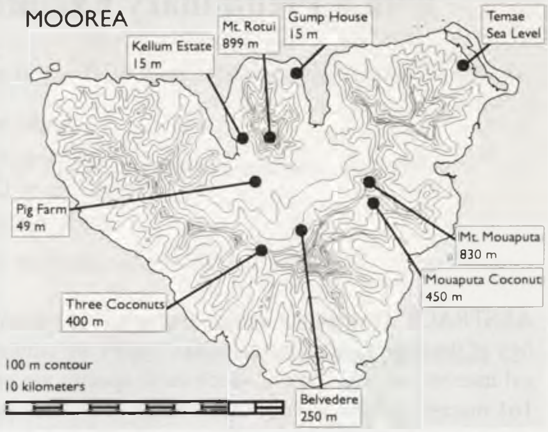

Figure 1. Topographical map of Moorea showing collection sites and their elevations. Geographic coordinates of collection sites:

Coast			
Temae	149...46.34'W	17...28.48'S	0 m
Gump House	149¡49.60'W	17¡29.44'S	15 m
Kellum Estate	149¡50.90'W	17¡30.87'S	15 m
Valleys			
Pig Farm	149¡50.12'W	17¡32.24'S	49 m
Belvedere	149¡49.59'W	17¡32.44'S	250 m
Mountains			
Three Coconuts	149...50.52'W	17...32.83'S	400 m
Mouaputa Coconut	149...48.21'W	17...31.85'S	480 m
Mt. Mouaputa	149...48.20'W	17...31.58'S	830 m
Mt. Rotui	149...49.70'W	17...30.75'S	899 m

Moth and Flower Collections

Moths were collected after dark (1900-2300) with a blacklight. A white sheet, ~2 x 3 m, was suspended between two supports with a rope, the blacklight hanging in the upper center. I caught as many different species as possible. Moths were usually caught on the sheet, but occasionally with a net. All moths were frozen overnight and pinned for storage. One moth of each macrolepidopteran species had its wings spread for identification.

Moths were identified to superfamily, and family if possible (Borror et al. 1992; Mandy Heddle, personal communication). Also, if possible, moths were identified to species using Paulian (1998). The remaining unknown moths were grouped into morphospecies by wing pat-

Location	Morphospecies	(Super)Family	# Collected	Known Range
Coast:	Chasmina tibialis	Noctuoidea	15	Unknown
	Gnathothlibus erotus	Sphingidae	4	India, South Pacific, Australia
(Tamae: Sea Level	Unknown # 71	Noctuoidea	4	Unknown
Gump House: 15 m	Unknown # 8	Noctuoidea	3	Unknown
Kellam Estate: 15 m)	Macroglossum hirundo	Sphingidae	2	Southeast Asia, South Pacific, Australia
	Unknown # 7	Noctuoidea	2	Unknown
	Agrius convolvuli	Sphingidae	1	Europe, Asia, Australia
	Mocis trifasciata	Noctuoidea	1	Indonesia to Fiji, Northern Australia
	Simplicia caeneusalis	Noctuoidea	1	India to Australia
	Spodoptera mauritia	Noctuoidea	1	International agricultural pest
	Thalassodes pilaria	Geometridae	1	Unknown
	Unknown # 39	Noctuoidea	1	Unknown
	Unknown # 72	Noctuoidea	1	Unknown
Valleys:	Thalassodes pilaria	Geometridae	7	Unknown
	Simplicia caeneusalis	Noctuoidea	5	India to Australia
(Pig Farm: 49 m	Chasmina tibialis	Noctuoidea	5	Unknown
Belvedere: 250 m)	Chrysodeixis chalcites	Noctuoidea	4	International agricultural pest
	Gnathothlibus erotus	Sphingidae	2	India, South Pacific, Australia
	Unknown # 19	Noctuoidea	2	Unknown
	Unknown # 53	Noctuoidea	2	Unknown
	Unknown # 60	Noctuoidea	2	Unknown
	Macroglossum hirundo	Sphingidae	1	Southeast Asia, South Pacific, Australia
	Unknown # 1	Noctuoidea	1	Unknown
	Unknown # 3	Geometridae	1	Unknown
	Unknown # 4	Noctuoidea	1	Unknown
	Unknown # 22	Geometridae	1	Unknown
	Unknown # 26	Geometridae	1	Unknown
	Unknown # 41	Noctuoidea	1	Unknown
	Unknown # 52	Noctuoidea	1	Unknown
Mountains:	Chrysodeixis chalcites	Noctuoidea	17	International agricultural pest
	Chasmina tibialis	Noctuoidea	11	Unknown
(Three	Cleora sp.	Geometridae	8	Unknown, but native to French Polynesia
Coconuts: 400 m	Simplicia caeneusalis	Noctuoidea	8	India to Australia
Mouaputa	Gnathothlibus erotus	Sphingidae	7	India, South Pacific, Australia
Coconut: 480 m	Thalassodes pilaria	Geometridae	7	Unknown
Mt. Mouaputa: 830 m	Macroglossum hirundo	Sphingidae	4	Southeast Asia, South Pacific, Australia
Mt. Rotui: 899 m)	Unknown # 19	Noctuoidea	4	Unknown
	Unknown # 21	Noctuoidea	3	Unknown
	Agrius convolvuli	Sphingidae	2	Europe, Asia, Australia
	Unknown # 20	Noctuoidea	2	Unknown
	Unknown # 53	Noctuoidea	2	Unknown
	Hippotion celerio	Sphingidae	1	Worldwide
	Ophiusa coronta	Noctuoidea	1	India, Pacific Islands, Australia
	Unknown # 1	Noctuoidea	1	Unknown
	Unknown # 22	Geometridae	1	Unknown
	Unknown # 26	Geometridae	1	Unknown
	Unknown # 29	Geometridae	1	Unknown
	Unknown # 42	Noctuoidea	1	Unknown
	Unknown # 44	Noctuoidea	1	Unknown
	Unknown # 45	Noctuoidea	1	Unknown
	Unknown # 52	Noctuoidea	1	Unknown
	Unknown # 69	Noctuoidea	1	Unknown
	Unknown # 70	Noctuoidea	1	Unknown

Table 1. Macrolepidopteran moths were collected at nine locations on Moorea. Some species were identified using Paulian (1998); unknown species were grouped into morphospecies by wing pattern and their identification numbers follow the labels in the actual collection. The moths are grouped according to what region of the island they were collected at: the coast, the valleys or the mountains. The collection locations that were in each region are listed with their elevations. The number collected of each species is shown because it roughly corresponds to the abundance of each species. If possible, the known range of each species is shown.

tern and given identification numbers; they are labelled this way in the collection which is in the Essig Museum of Entomology at the University of California, Berkeley. For species that were identified, their global ranges were determined if possible (Common 1990; Nielsen et al. 1996; Swamiappan and Balasubramanian 1979; Mandy Heddle, personal communication).

Flowers, especially common flowers that were likely to be moth-pollinated, were collected on several expeditions. Flowers from 10 plant species were collected in the field at several sites:

Fagraea berteriana: Mouaputa Coconut
Gardenia tahitensis: Gump Station
Lantana camara: Belvedere
Leucaena leucocephala: Belvedere
Merremia sp.: Three Coconuts
Rubus rosifolia: Three Coconuts
Spathodea campanulada: Mouaputa Coconut
Spathoglottis pacifica: Three Coconuts
Stachytarpheta urticifolia: Belvedere
Tecoma stans: Belvedere

For 17 additional plant species, pollen was obtained from mounted herbarium specimens from a collection of common plants on Moorea established in 1992-1993. This collection is kept at the Gump Research Station on Moorea.

During all moth collections, I made casual observations of nearby flowers to see if moths could be seen to feed at them.

Pollen Preparation and Analysis

Pollen was removed from moths and mounted in glycerine jelly (10 g gelatin, 70 ml glycerine, 60 ml water). I dipped a pin in warm glycerine jelly on a microscope slide then dabbed the proboscises and other mouthparts of the moths. I repeated this several times. The slides were then covered, left to dry overnight and sealed with nail polish if pollen grains were found on them.

Pollen from plants was treated by acetolysis before being mounted on slides. Anthers or pollen masses were first separated from other plant material. They were then hydrated for 10 min in 3 ml water using a few drops of detergent if they were dry specimens from the herbarium collection. The pollen was washed in glacial acetic acid before acetolysis. 1 ml of acetolysis fluid (90% acetic anhydride, 10% sulfuric acid) was added to the decanted pollen mass. The pollen was shaken in test tubes and placed in boiling water for 10 min. It was then centrifuged and washed in glacial acetic acid and then water. Finally, a concentrated solution of pollen was mixed with glycerine jelly on a slide. The slides were allowed to dry overnight and then ringed with nail polish.

To match pollens with each other, digital pictures were taken of all unique pollen grains at as many angles as possible on all plant and moth pollen slides. The pictures were then placed into a single computer graphics file to compare pollen shape and sizes. The microscope slides from which the pictures are taken are at the Jepson Herbarium at the University of California, Berkeley. I examined how many different kinds of pollen were found on individual moths, on each species, and at each collection site. I then looked at the pollen from the moths to discern which plant families the pollens were from, and if possible, matches to specific local plant species for which I had pollen grains.

Results

Moth Collections

I collected 161 macrolepidopteran moths in Moorea. I divided them into 35 morphospecies: 25 noctuoids, 6 geometroids, and 4 sphingids. I identified 12 species by wing pattern using Paulian (1998); the remaining species were grouped into unknown morphospecies. Table 1 (previous page) shows the moth assemblages found at each of the regions on the island: coast, valleys and mountains. Table 2 shows how many expeditions were within each region and shows how many moth individuals, morphospecies, and unknown morphospecies were found in each.

Region	Number of collection expeditions	Number of moths collected	Number of morphospecies found	Number of unknown morphospecies found
Coast	5	37	13	5
Valleys	3	37	16	10
Mountains	6	87	24	14

Table 2. Summary of moths collected in three regions of Moorea.

Plant	Moth species	Location and elevation
Unknown species in Myrtaceae	*Gnathothlibus erotus*	Kellum Estate (15 m)
	Unknown 53	Belvedere (250 m)
Lantana camara	*Macroglossum hirundo*	Gump Station (15 m)
	Chrysodeixis chalcites	Belvedere (250 m)
	Thalassodes pilaria	Mt. Rotui (899 m)
	Unknown 69	Belvedere (250 m)

Table 3. Plant-pollinator associations in Moorea.

At all sites, the most abundant moths, most of which I was able to identify with Paulian (1998), were non-endemic species that are widely distributed across the South Pacific and often much of the southern hemisphere. Some are international agricultural pests (Table 1). There are several species that are abundant at all elevations. *Chasmina tibialis*, a white noctuid, was the most frequent visitor to the blacklight during the collections and may have been the most numerous macrolepidopteran on the island. *Gnathothlibus erotus* was the most abundant sphingid and was found at all elevations; *Macroglossum hirundo* was also abundant at all elevations. At the valley and mountain sites not the coastal sites *Chrysodeixis chalcites*, *Thalassodes pilaria*, and *Simplicia caenseualis* were abundant.

Plant-Pollinator Associations

Pollen was found on 51 of 161 moths (32%). Two associations between moths and plants were found (Table 2). The pollen library I created to identify the pollen found on moths is in Appendix 1.

Pollen from *Lantana camara* (Verbenaceae), a common introduced flower at all elevations, was identified on four moth species (Table 2). Except for an unidentified noctuid moth, all of these species were observed to feed on *L. camara* in the field at the Belvedere. *L. camara* pollen was taken off of moths from all three regions of the island. Two moths had pollen from a plant that is a member of the Myrtaceae. This might be *Metrosideros collina* or *Syzigium malaccense* on Moorea.

Multiple kinds of pollen some unidentified were found on all of the moth species in which pollen was found on more than one individual. This indicates generalist feeding habits. The exact numbers of plant species these moths were feeding on is unclear. The moths determined to be generalist feeders were *Thalassodes pilaria*, *Chrysodeixis chalcites*, *Chasmina tibialis*, *Macroglossum hirundo*, *Simplicia caenseualis*, and two unknown noctuids (60 and 19).

Discussion

Moth Diversity on Moorea

Although 35 macrolepidopteran morphospecies were found, there are doubtless more on Moorea. Even the final collection expeditions yielded unseen species. Furthermore, the validity of the morphospecies based on wing pattern is questionable (Holloway et al. 1987), and I suspect that some of the species identified using Paulian (1998) are incorrect. While this study has done much to elucidate a fauna that was previously unknown, it is a small step toward a complete taxonomic understanding of moths on Moorea.

The numbers of morphospecies and unknown morphospecies that were found increased from coast to valleys to mountains (Table 2). This may be because I had more successful collection expeditions in the mountains: twice as many macrolepidopterans were caught there. However, there was only one more expedition in the mountains than on the coast, and more than twice as many moths were caught. Also, although three expeditions were taken in the valleys and five on the coast, the same number of moths was caught. This suggests that the moth fauna at the higher elevations on Moorea is more diverse and likely to contain biologically interesting endemic species.

I did not rigorously sample for moth abundance, but the number of moths of each species that were collected at each site roughly corresponds to their abundance at that site. The numerical dominance of non-endemic moth species that are widespread or cosmopolitan many of which are probably introduced is an indication of the strong effect humans have had on the biology of Moorea.

Although some species appear at all elevations, the data suggest a disparity between the coastal sites and the valley and mountain sites. The valley and mountain sites have more species in common than either has with the coastal sites. None of the unknown species from the coastal sites are found in the valley or mountains and vice versa. And some species found at all elevations demonstrate the disparity through abundance. It would be interesting to examine whether this is because of the natural differences associated with elevation or because of the more intense human impact on the coast.

Moth-Plant Interactions

The number of moths that feed on *Lantana camara* is evidence of the impact that invasive species have had on Moorea. *L. camara* is an international weed with an international effort to control it. It is a popular subject of biocontrol research (Broughton 2000). Its attractiveness to pollinators no doubt helps make it a successful invader. Also, these moths may be successful because they take advantage of *L. camara* as a food source. The introduction and spread of *L. camara* and other invasive plant species may have indirect negative effects on the native flora by competing for their pollinators.

The moths that feed on the Myrtaceae have unknown significance. Of the two most likely candidates, *Metrosideros collina* is a charismatic native tree, important to insects throughout the Pacific and *Syzigium malaccense* is a Polynesian introduction (Whistler 1991). It would be useful and interesting to discover the rest of the plant-pollinator relationships among the moths on Moorea. The web of relationships is no doubt complex and will be difficult to uncover. This study shows that there are many generalists, and some of the most interesting links to uncover are those between rare plants and their pollinators where the chances of discovery are slim.

Future Research

This project s value would increase with more labwork that was not possible due to time constraints. I could identify all of the moth species collected, possibly including even the microlepidopterans and determine, if possible, their origins and status on the island: native, recent introductions, or long-established introductions. Because the pollen taken from the moths was not treated by acetolysis, matches with the plant pollen grains were not possible for most of the 51 moths on which pollen was found. The cytoplasm in the pollen grains usually removed by acetolysis hid the features necessary for identification. With proper pollen preparation procedures and comparisons to existing pollen libraries, possibly supplemented by more collections on Moorea, the pollen found on moths could be identified. To better understand the moth fauna on Moorea, more extensive collections should be done to collect uncollected species and get abundance data.

The level of human impact on Moorea has made most if not all taxa a mixture of native and introduced species. Understanding the interaction between introduced and native moths with introduced and native plants through pollination is an excellent way to gauge how human impact via species introductions has affected Moorea s biology. This should be a goal for moth and plant research on the island. It would paint a picture of how intact or how shattered the ecology of the island is and how it compares to what may have been before European and before any human impact. This will help shape conservation policies in the future.

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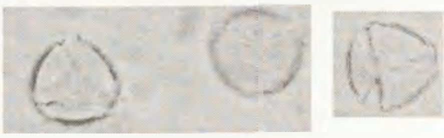
Conyza bonariensis
Asteraceae

Emilia fosbergii
Asteraceae

Spathodea campanulada
Bignoniaceae



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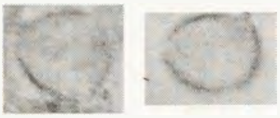

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Casuarinaceae

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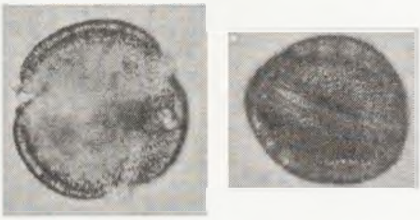

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Convolvulaceae

Chamaesyce atoto
Euphorbiaceae

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Lecythidaceae

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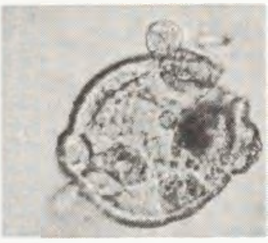
Loganiaceae



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Malvaceae

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Myrtaceae

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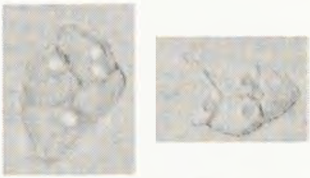

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Orchidaceae

Rubus rosifolia
Rosaceae

Gardenia tahitensis
Rubiaceae

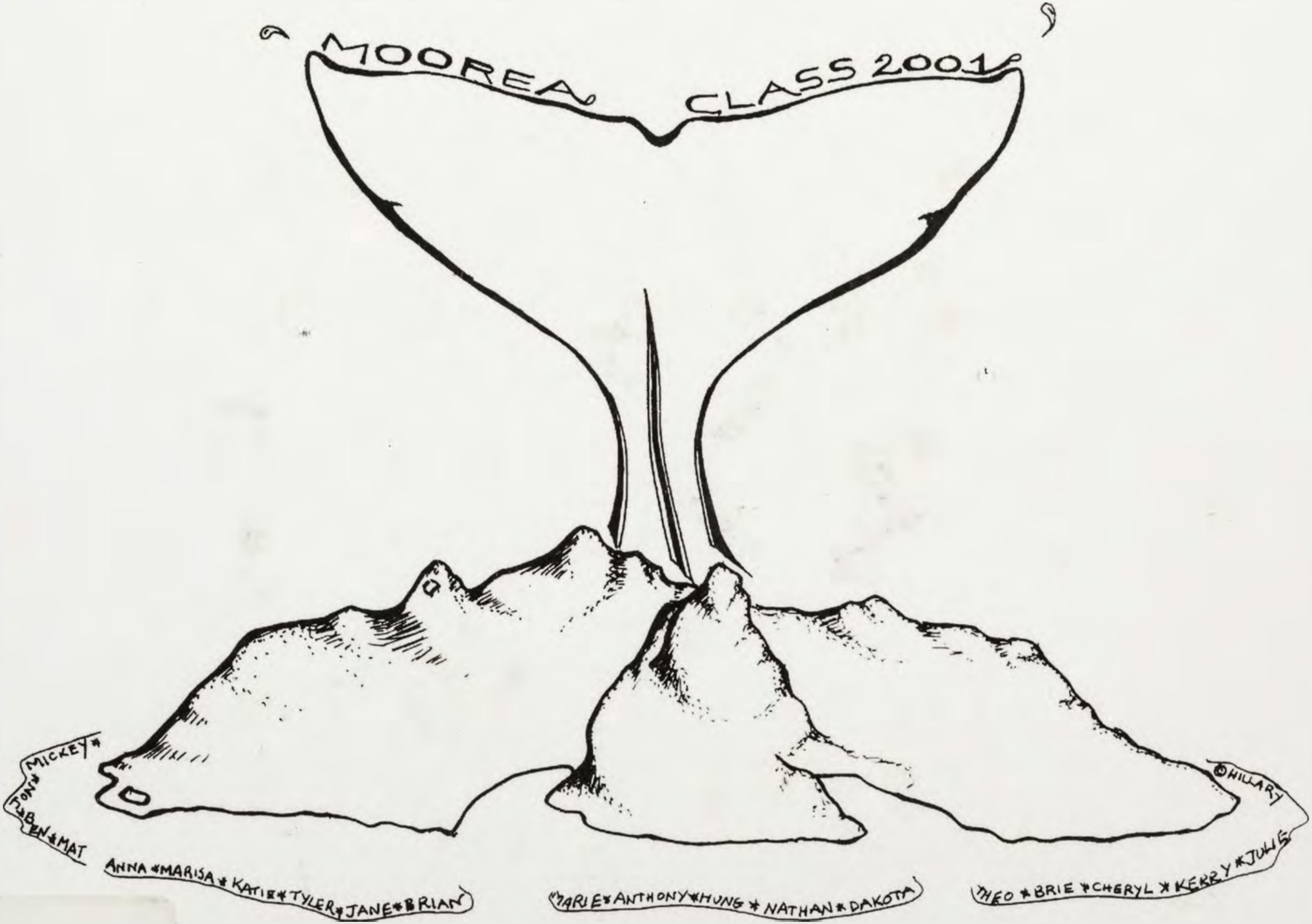
Dodonaea viscosa
Sapindaceae

Commersonia bartramia
Sterculiaceae

Lantana camara
Verbenaceae

Stachytarpheta urticifolia
Verbenaceae

Appendix 1. Pollen grains from some common plants on Moorea, French Polynesia. Where possible, pollen grains are shown from multiple angles. The magnification is 400 x. All pollen grains were prepared by acetolysis.


MOOREA CLASS 2001
MICKEY * JON * BEN * MAT
ANNA * MARISA * KATIE * TYLER * JANE * BRIAN
MARIE * ANTHONY * HUNG * NATHAN * DAKOTA
THEO * BRIE * CHERYL * KERRY * JULIE
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