Environmental Science Policy and Management 107
and Integrative Biology 158

The Biology and Geomorphology of Tropical Islands

Student Research Papers, Fall 2000

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Book cover: The Red-Tailed Tropic Bird painted by Sydney Parkinson on Lieutenant James Cook's first circumnavigation of the globe from 1768 to 1771 in H.M. Bark *Endeavour*. The specimen was obtained at Palmerston Island, west of the Society Islands. Parkinson joined Cook's expedition at the invitation of the ship's naturalist Joseph Banks, and painted and sketched largely botanical specimens, but also zoological ones, coastlines, tattoos, canoes, villages, and Cook's fort at Point Venus, adjacent to Matavai Bay, Tahiti. Parkinson later died crossing the Indian Ocean in 1771, the victim of dysentery contracted at Batavia in the Dutch East Indies. The original painting is housed in the Natural History Museum (London).

The Tropic Bird, *Phaethon melanorhynchos,* is common at Moorea. It flys high above the Gump Research Station, hardly flapping its wings while on speedy passes over Cook's Bay. Occasionally, it dives to the Bay to feed on fish. The long, narrow red tail feathers were highly valued by the Polynesians living in the Society Islands.

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Above: The north shore of Moorea, French Polynesia, from Mt. Rotui, showing the barrier reef with Cook's Bay to the right, and the north slope of Mt. Rotui in the forground. The Gump Research Laboratory is on the left shore of Cook's Bay at the lower edge of the fringing reef. Photo taken by Aaron Wallace on the trail to the top of Mt. Rotui.

The 2000 Moorea Class

On Tahiti with Moorea in the distance

From left to right: Back row: **Jae Pasari, Aaron Wallace, Paul Roberge, Brent Mishler (Professor, IB), Matt Epstein, Sharifa Gulamhussein, Hilary Street, Jamison Lipps (visitor), Sean Askay, Neil Davies (Gump Research Station Research Director), Audrey Aronowski (GSI).**
Middle row: **Aimee Betts (GSI), Galen Peracca, Julie Ekstrom, Chicory Bechtel, Jia Hu, Rhea Muchow, Sarah Rieboldt (GSI).**
Middle row seated: **Ken Peer, Lorraine Cheng, Brian Meux, Shele Poetker, Catherine Chan, Leah Goldstein.**
Front row, lounging: **Celina Yong, Israel King, Jere Lipps (Professor, IB).**
Not yet arrived in French Polynesia: **Jamie Bartolome (Professor, ESPM), Vince Resh (Professor, ESPM, and Director, Gump Research Station).**

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Cook's Bay, Moorea, from the science laboratory, Gump Research Station.
Photograph by Jack Penkethman

The Moorea Class 2000

Introduction

The Moorea Class is a special experience in island biology and geology for undergraduates at Berkeley. Its purpose is to make undergraduate students into professional natural scientists through the design and implementation of their own original research project. Perhaps the best part of the course is the fantastic setting for the course on Moorea, the sister island of Tahiti, in French Polynesia. Many unique research opportunities exist there, from the native flora found only at the highest elevations to the reef biota sounding the island below sea level. Berkeley students and faculty are indeed fortunate that Richard P. Gump donated land and facilities to the campus years ago, so that these experiences could be available to undergraduate and graduate students.

Two departments at Berkeley--Environmental Sciences and Policy Management in the College of Natural Resources and Integrative Biology in the College of Letters and Science—give the course at the Richard P. Gump Biological Research Station on Cook's Bay, Moorea. A team of faculty and Graduate Student Instructors from both departments teaches the course. Some of the team gives lectures at Berkeley while others provide instruction and assistance on Moorea. In fall 2000, four faculty took part in the field instruction for two or three weeks each at different times, and three GSIs lived and worked with the class for the entire nine weeks.

The course has been immensely successful since its inception in 1991. Over 160 students have taken the course, done research on their own original projects, presented their results in the annual Symposium on the Biology and Geomorphology of Tropical Islands, and have written scientific papers published in this series of books. These books have been widely distributed to libraries and personnel at Berkeley, on Moorea and Tahiti, as well as elsewhere. Many of the students' papers have also been published in regular scientific journals.

The Moorea course combines many aspects of any natural history research project--scientific preparation, logistic planning, equipment and supply selection, and the development of an individual research problem. It is a complete research experience, not unlike that of professional scientists. The course begins in Berkeley with 3-1/2 weeks in Berkeley absorbing the science background and figuring out the logistics and supply needs of the class. Then the entire group of 21 students, several faculty and three Graduate Student Instructors moved with much excitement to Moorea. After a week of scientific field trips on Moorea and Tahiti, students then developed their own scientific project. Although science is important, safety, logistics, cost, equipment and supplies are critical aspects of each project that requires detailed planning by the students as well. For the next eight weeks, students pursue their own projects on Moorea, returning to Berkeley to prepare an oral presentation at the annual Symposium and the written report contained in this volume.

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

Acknowledgments

Any field expedition is enormously complex and the Moorea course is no exception. We thank the students, faculty, Graduate Student Instructors, and our colleagues at Berkeley and on Moorea who made the 2000 class hugely successful. We all appreciated and remember with wonderful feelings the good times, camaraderie and excitement we shared with the students. Those of us in the field with the students also learned a good deal from each of them, as they developed and explored their own research.

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

On Moorea, we also are grateful to many people. We thank our colleague Dr. Neil Davies, manager of the UC Berkeley Gump Research Station on Moorea and the Station staff, who kept the facilities operating so efficiently and who made our stay so very pleasant. Frank and Hinano Murphy of Moorea and Tahiti, and many other Mooreans, provided much assistance and interaction. Lastly, Professor Vincent Resh of the College of Natural Resources and Director of the Gump Station for his encouragement, assistance and complete support of what we did in Moorea, even as he served as a co-instructor. Gordon and Betty Moore have continued to support the Gump Research Station, making it one of the best field laboratories in the tropics.

The faculty thanks Audrey, Sarah and Aimee, our GSIs, for their service. They had a large responsibility for the care and guidance of the class, and they performed excellently.

Without all this help of such various kinds, our students would not have had this fantastic experience that they can remember for the rest of their lives. They have been changed forever!

Jere H. Lipps, Professor, Integrative Biology. Course organizer.
Jamie Bartolome, Professor, ESPM
Brent Mishler, Professor, Integrative Biology
Vince Resh, Professor, ESPM
Audrey Aronowski, Graduate Student Assistant, Integrative Biology
Aimee Betts, Graduate Student Assistant, ESPM
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Factors Affecting the Distribution on Amphidromous Gobies (Teleostei: Gobiidae) on Moorea, French Polynesia.

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ABSTRACT. Five species of amphidromous gobies live in the streams systems of Moorea, French Polynesia. Despite numerous studies conducted on the taxonomy of Indo-Pacific gobies, very little is known about their microhabitat preferences and with-in stream distributions. Increasing development and construction on Moorea might create stream barriers that prevent juvenile gobies from returning to the upper reaches of streams after their marine larval stage. Because the five Moorean freshwater gobies have differing abilities to climb obstacles (using their mouth and pelvic-fin sucker) and have a variety of microhabitat preferences and feeding strategies, more detailed information on their with-in stream distributions is needed to assess the threat of stream barriers. I conducted goby surveys throughout the Opunohu Valley on the north side of Moorea. Data on substrate type, amounts of algae, leaf detritus, surrounding terrestrial environment, pool dimensions, elevation and water temperature was collected at each site in hopes to determine which factors may affect distribution. Three species of goby (*Stiphodon elegans, Awaous ocellaris* and *Stenogobius genivittatus*) were all restricted to lower elevations and had significant positive correlations with some or all of the following: amounts of algae, silt, leaf detritus, water temperature and pool dimensions. *Sicyopterus taeniurus* was universally distributed and showed no significant correlations with any of the tested variables. *Sicyopterus pugnans* showed unexpected negative correlations with the amount of algae, leaf detritus and presence of Tahitian chestnut trees (genus: *Inocarpus*). Two stream barriers were encountered at high elevations; however, they did not seem to affect the distributions of the two species present at high elevation: *S. pugnans* and *S. taeniurus*.

Introduction

Among the thirteen families of stream-dwelling fish found in French Polynesia, amphidromous gobies (Gobiidae) are the most diverse with fourteen species in six genera (Marquet 1997). There is much taxonomic confusion in the family Gobiidae (Watson 1995; Nelson *et.* al 1997) and most research conducted on amphidromous gobies has focused on clarifying relationships among species and genera. However, a few scientists such as Kido (1996, 1997) and Nelson *et. al* (1997; Nelson 1999) are working on aspects of goby biology and natural history.

All stream dwelling gobies known to Pacific islands feature an amphidromous life history; adults spawn in freshwater, the larvae develop in the ocean and eventually return to freshwater to mature (Dotu & Mito 1955; Iguchi & Mizuno 1990). Gobies are also characterized by having a modified pelvic fin sucker that allows them to grasp the stream substrate and sometimes climb out of water on wet rocks (Ford & Kinzie and Ford 1982; Schoenfuss *et. al* 1997). Amphidromous gobies also lack an air bladder, allowing them to conserve energy by resting on the stream bottom instead of continually swimming against the current like other stream fish (Ryan 1991). Ryan suggested that these features have contributed to the success and radiation of gobies in tropics.

The expansion of human development across many Pacific islands poses numerous threats to natural stream populations. Reduction of water quality and quantity, riparian habitat destruction due to road construction and the introduction of exotic species are threats suggested by Nelson & Eldredge (1991). A study conducted in Guam by Nelson (1999) showed that nertid gastropods and several fish species (without the special climbing adaptations found in gobies) were unable to surmount barriers like dams and reservoirs. Nevertheless, the gobies were found at lower densities above the barrier than below.

Although the island of Moorea is relatively undeveloped compared to Tahiti (with a human population of about 10,000), development and construction up into the river valleys may be preventing the native stream fauna from moving upstream. Examples of such barriers are bridges, dams, weirs and irrigation systems. As upstream migration is an essential phase in goby development, these barriers might affect their natural distributions.

The islands of Moorea and Tahiti are home to five species of freshwater gobies, more than are found on any other French Polynesian island (Marquet 1997). Even though all these gobies are equipped with a pelvic

fin sucker, their abilities and tendencies to climb are different (Watson 1995; Nelson *et. al* 1997; Smith & Smith 1998). *Sicyopterus taeniurus* juveniles on Moorea use their mouth and sucker to scale formidable obstacles such as the Afareaitu Waterfall, which has a vertical rock face over one hundred meters high (personal observation). *S. taeniurus* adults do not traverse such large obstacles, but continue to use their suckers to grasp rocks in the swift currents of high elevation streams. However, *Stenogobius genivittatus* is unable to overcome large barriers (Nelson *et. al* 1997) and is usually restricted to the lower reaches of streams (Marquet 1988).

Before the effects of barriers on Moorean gobies can be understood, there must be an understanding of how their morphology and natural biology interact with the environment. What are the habitat preferences for these gobies? Which environmental factors affect their distribution? How does their biology interact with these factors? What are the species' distributions? How can this information be used in understanding the effects of barriers on streams?

In an attempt to answer some of these questions I conducted a series of stream surveys throughout the Opunahu River Valley. This is Moorea's largest watershed system and feeds into the Opunahu Bay. My study's primary goal was to investigate the relationships between environmental factors and goby distribution. I also wanted to look at how these variables are interrelated. Any formidable natural or man-made barriers encountered were also noted in the hopes of discovering if differential goby presence occurred above and below the obstacle.

My working hypotheses were organized around three categories of environmental factors: 1. Goby distributions are most effected by the physical attributes and abiotic factors of the stream. 2. Goby distributions are most effected by in-stream biotic factors. 3. Goby distributions are most effected by biotic factors outside the stream. My null hypothesis states that none of the documented factors in my study affect goby distribution.

Methods and Materials

Opunohu River Valley

The Opunohu River is located on the north side on Moorea. It extends from the base of the eroded volcanic rim on the interior of the island out to the Opunohu Bay (Figure 1). The river system has four major perennial tracks and one major seasonal tributary. Only three of the four perennial flows were large enough to sample thoroughly. These forks (labeled A, B and C) were surveyed from October 27th to November 10th, 2000 (Figure 2).

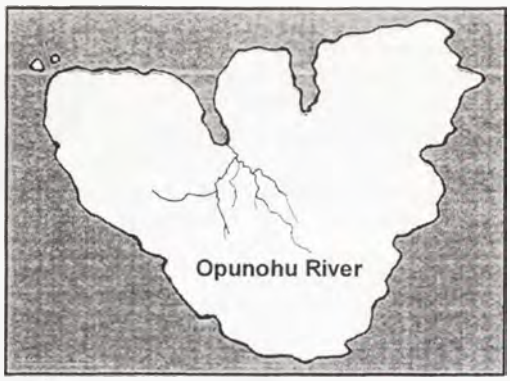


Figure 1: Map of Moorea and the Opunohu River

Fork A is the farthest east, having its source below the Belvedere lookout point. It flows north until meeting with Fork B at the valley floor. The areas I surveyed on Fork A were characterized predominately by *Inocarpus* spp. forest. The forest floor is heavily covered with dead leaves; ground vegetation is often sparse, consisting mostly of ferns. At medium and high elevations the airspace above the stream is fairly open, with a high canopy; the stream is usually narrow with deep cascading pools.

Fork B is the middle branch of the Opunohu, lying off to the west of Fork A. It is characterized by dense *Hibiscus* forest and dense ground vegetation, mostly ferns. At middle elevations the river is very wide and shallow with open airspace above the flow. At high elevations the stream travels through dense *Hibiscus* thickets and were often difficult to traverse.

Fork C is the farthest west in the Opunohu Valley. Similar to Fork B, its forest is composed of *Hibiscus* with some Ironwood, bamboo and the infrequent *Inocarpus* spp. tree. The forest floor is moderately covered with dead leaves and ferns. The stream is narrow at low elevations, where it is often muddy, shallow, and filled with grass-like water plants. At middle elevations the stream is mostly covered by thick *Hibiscus* thicket. At high elevations below the dam the stream has abundant airspace under the canopy, and is wide with many boulders. There were also sections with no canopy cover, unique among all other middle and high elevation areas of the Opunohu River. Above the dam at very high elevations the stream is densely covered by *Hibiscus* thicket and features dense ground vegetation.

Selecting survey sites

I conducted a series of stream surveys at 35 sites on the Opunahu River. To detect a species distribution gradient along the streams, I chose a systematic sampling method. I surveyed at five sites per kilometer at 200-meter intervals. The beginning and ending sites (and any other sites where geographic landmarks were

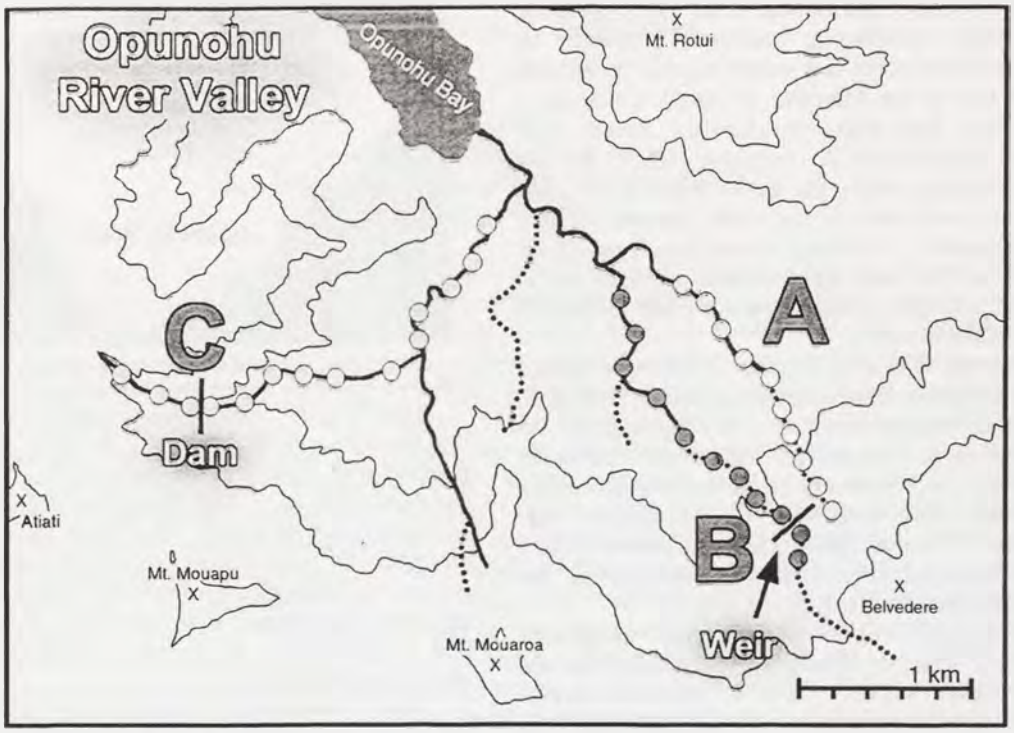


Figure 2: Map of the Opunohu River Valley. Forks A-C are labeled and survey sites are designated by circles. The Dam on Fork C and the Weir on Fork B are marked.

visible) were pinpointed on a topographical map by compass triangulation. In order to measure the 200 meter distances, I measured my pace-length on typical rocky stream terrain. My pace length was about 2 steps per meter, thus I walked about 400 steps in-between sites. Upon traveling 200 meters, I looked for the nearest stream-pool deep enough to snorkel. The minimum pool depth used for surveys was about 20 to 30 centimeters. Any extra distances traveled to suitable pools were recorded and considered when mapping the sites.

I was concerned that sampling only in stream pools would bias my results, as some gobies might prefer other microhabitats. Frequent snorkeling in the shallow runs and riffles was too difficult for efficient observation. However, I preformed several "dives" in shallow water to test my pool bias. I always found the same species present in adjacent pools, riffles and runs, although their relative densities varied. Nelson *et. al* (1997) performed out-of-water visualization surveys in all three stream flow types in Micronesia. Although the densities for each species varied from pool to riffle to run, each species was found in all three. Based on Nelson's results and my observations I felt comfortable only collecting data from pools and using this as a representation of species present in the immediate area.

Surveying techniques

As approaching objects easily frighten gobies, I walked around the target pool and entered from downstream. I snorkeled in each pool for ten minutes. Counting the absolute numbers of individuals was often difficult because the same fish might swim past me several times during the survey. Thus I did my best to estimate numbers, but concentrated more on species presence or absence. Focusing on species presence or absence is also supported by results from Nelson *et. al* (1997). They found goby densities were different in each stream flow type (pool, riffle, run). Thus, sampling only in pools could give biased density results. I focused on identifying adults or sub-adults, as identifying juveniles was often difficult.

After the ten minutes of stream observation, I took data on a suite of environmental variables. I recorded the time of day and weather at each site. I also measured water temperature with an alcohol thermometer, elevation by using a topographical map, percent tree cover by visual observation, pool dimensions with a metric transect tape, and substrate composition and the characteristics of the surrounding terrestrial environment by visually estimation.

Substrate composition was the variable of primary interest to me. My preliminary observations suggested that it was the predominate factor affecting species

distribution. Aspects of goby feeding biology also support this idea. They are often algal or invertebrate feeders that scrape food from stream rocks and/or forage through sediment by filtering silt and fine sand through their gills (Kido 1996a, 1996b, 1997).

I used the Wentworth particle size scale as the basis for the characterization of stream substrates. A concentric circle diagram with particle classifications was created on waterproof paper and taken into the stream for measurement. For simplicity I compressed Wentworth's classifications into five categories: boulders, both large and small cobbles, all sizes of pebbles, granules/course sand and fine sand/silt. In pools that contained several microhabitats (such as only cobbles/pebbles at one end, and silt/mud at the other) I noted where each species was found.

I noted leaf detritus as approximate percent cover on the pool bottom. I recorded the amount of algae present on rocks with a 4-tiered scale. A value of "four" represented a thick, soft layer of algae covering 75 to 100 percent of the substrate; "three" was a thick, soft layer of algae covering between 25 and 75 percent of the substrate; "two" was a thick, soft layer on only a few rocks; "one" was a very thin layer of algae that coated substrate rocks, that fish would have to be scraped off to be removed.

I also wrote down general descriptions of the terrestrial plant densities and types (including scientific names of predominate species if known) for the characteristics of the "surrounding environment." Because most of the habitats around the streams were homogeneous, I did not define an observation radius around the stream. However, I took note if a different habitat type was nearby (such as a meadow beyond the riparian stream vegetation).

Equipment used

I used dive booties, a long sleeve rash guard, and a long leg, short sleeve wetsuit (3mm/5mm) for warmth during surveys as the water temperature ranged from 21 to 25 deg. C. I also used a mask and snorkel and a clipboard with waterproof data sheets for observation and data recording. Small fishnets and zip-lock bags were often brought to capture gobies for later photography. I used a 30 meter transect tape for measuring stream dimensions, an alcohol thermometer for temperature readings, and a topographical map and a surveying compass for mapping sites.

Species identification

Comparisons and identificatrions were made by using Marquet's 1988 doctoral thesis. I later consulted Mike Moser at the University of California at Berkeley and examined other articles to confirm my identifications.

Extensive field observation in the Opunahu, Afareaitu and Haapiti River Valleys along with the temporary collection of live specimens improved my identification abilities. Because three of the six species of gobies on Moorea are strongly sexually dimorphic (and these distinctions were not completely clear at the time of surveying), detailed information on the physical appearance of observed gobies was taken down in my notes.

I repeatedly witnessed rapid pigmentation change in both *Sicyopterus taeniurus* and *Sicyopterus pugnans*. I also saw some contrast changes in the pigmentation of *Stenogobius genivittatus* and *Awaous ocellaris*. Marquet (1988) noted these rapid changes in coloration and intensity while was transporting gobies from the field. Coloration and pattern change in *Sicyopterus stimpsoni* (from Hawaii) during mating and courtship displays was extensively documented by Fitzsimons *et. al* (1993). The fifth goby species, *Stiphodon elegans*, did not exhibit these changes. *Sicyopterus* individuals often altered the intensity of the sub-orbital stripe and also changed the intensity of stripes and spots on their sides in response to human and fish interaction. This combined with sexual dimorphism in both *Sicyopterus* species and *Stiphodon elegans* along with the presence of two color morphs in *S. pugnans* (orange and blue) made initial identification difficult. In all there were about 15 different looking gobies, representing the five species. My field identifications were extended beyond the species level to include all these color morphs and sex differences.

Juveniles were often difficult to identify. Brown, non-descript juveniles were frequently found in great numbers in all stages of the streams. Although *Sicyopterus* spp. juveniles are reported as the far majority in stream biomass counts (Marquet 1988) they were only included in data if easily recognized as such.

I constructed a freshwater tank with several chambers and circulating water to allow for the observation and photography of collected specimens (Figure 3). Live specimens were placed into a small, clear rectangular box with metric contrast rulers for scale (Figure 3). A digital camera was used to take pictures of the live gobies to aid in identification upon returning from Moorea. Figures 4-8 feature some photographs of the six goby species found on Moorea.

I also developed a species key for Moorean gobies including pictures, discussion of color and sexual morphs, pigmentation changes and anatomical characteristics. This guide is available at the Gump Research Station on Moorea and through the course instructors of *Biology and Geomorphology of Tropical Islands* course at the University of California at Berkeley.

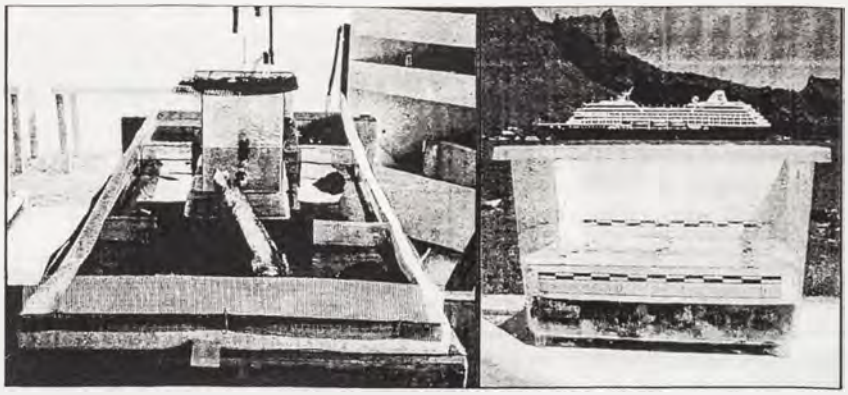
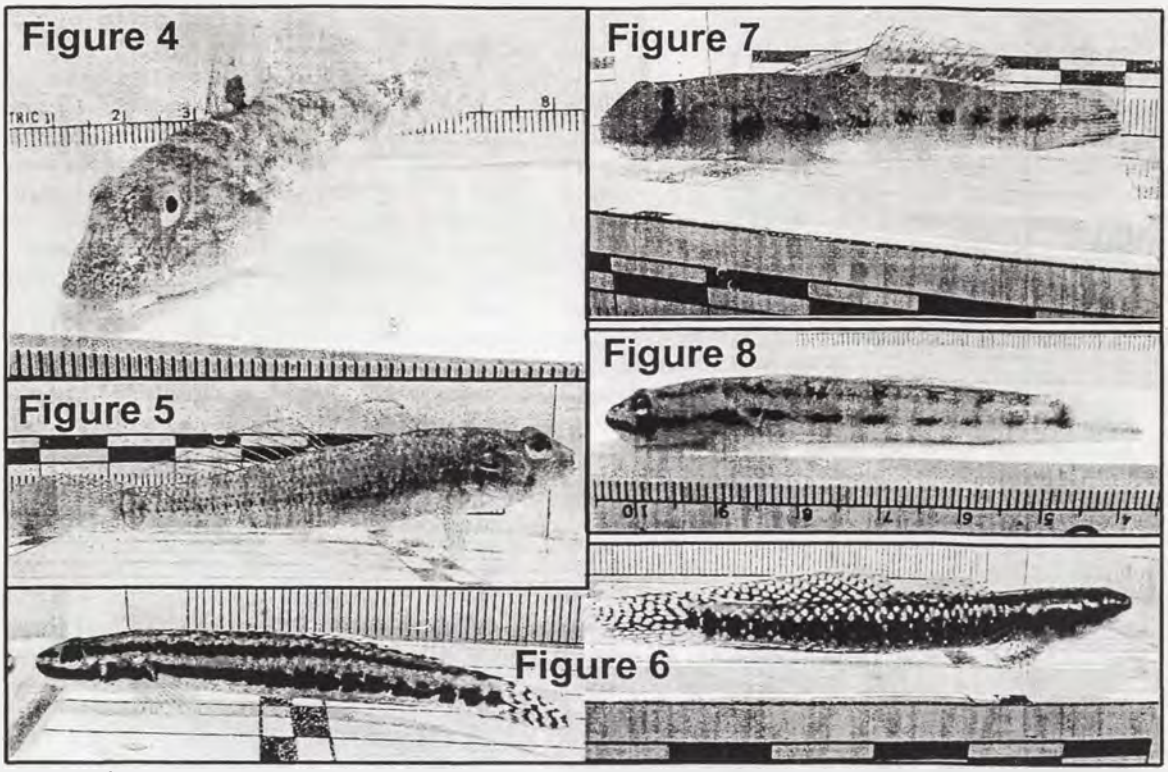


Figure 3: Picture of the fish tank (left) and the photography chamber (right).



Figures 4-8: Pictures of the five species of Moorean gobies. **Figure 4:** *Awaous ocellaris.* **Figure 5:** *Stenogobius genivittatus* **Figure 6:** *Stiphodon elegans (female left, male right).* **Figure 7:** *Sicyopterus taeniurus.* **Figure 8:** *Sicyopterus pugnans (blue form female)*

Methods: Data Analysis

Environmental variables vs. environmental variables

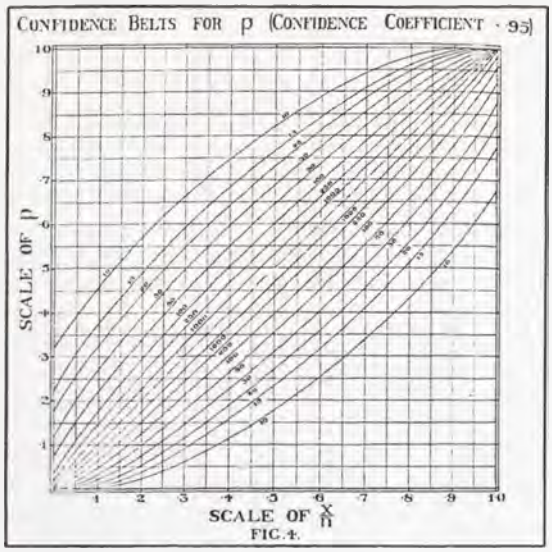
Concerned that variables might scale with one another, I performed regression analyses on all combinations of variables.

Species distributions vs. environmental variables

My study's primary focus was the relationship between species distribution and environmental variables. I used a grouping t-test to analyze this data. I assumed separate variances for the data sets.

Of the terrestrial factors measured, I chose to investigate the presence of *Inocarpus* spp. trees as an influencing factor. For evaluating the presence of *Inocarpus* spp. and the amount of algae on the substrate versus species I used binomial distribution analysis. This method allows for the comparison of data that includes arbitrary number scales and presence or absence (such as the four levels of algae and *Inocarpus*

spp. data). This method allowed me to compare the ratio of gobies in one category to the ratio of gobies in another category. Pearson's Confidence Belt chart (1934) (Figure 9) was used to calculate 95% confidence intervals.



Figures 9: 95% confidence belts used for binomial distribution analyses. A sample size of n=30 was used to conservatively approximate values for my sample size of

Species presence vs. species presence and the effect of sampling time and weather

I have also considered inter-species interactions as a cause for observed distributions. I used binomial distribution analysis to calculate the relationship between different species' distributions. I was also concerned that the time of day and weather might affect the species of fish observed. Again, I used binomial analysis to compare which species were observed during three time blocks equally spanning the range of sampling times (10am to 6pm). Weather categories were divided into rain, cloudy and sunny.

Results

Environmental variables vs. environmental variables

Fork A of the Opunohu differed dramatically from Forks B and C in several ways. This fork uniquely featured a universal presence of *Inocarpus* spp. and high amounts of algae and leaf detritus regardless of elevation. Thus I decided to separate them while conducting correlation and regression analyses. By doing these I was able to identify several significant relationships that were specific to Fork A and Forks B/C and that would have otherwise cancelled each other out. Only relationships with p values less than 0.05 are reported. A complete record of correlation, R^2 and p values is summarized in Table 1.

Fork A: Elevation was significantly related to water temperature and slit. Length was significantly related to amount of algae. Percent canopy cover was significantly related to amount of and silt.

Fork B and C: Elevation was significantly related to water temperature, algae, leaf detritus, length, and silt. Pool length was significantly related to algae, leaf detritus, silt and water temperature. Volume was significantly related to silt and leaf detritus.

Species distribution vs. environmental variables: t-tests

There were several factors that did not significantly relate to species distributions. These were the percent of canopy cover, and the amount of bedrock, boulders, cobbles and pebbles in the streams.

Elevation had a very significant relationship with *Stiphodon elegans, Stenogobius genivittatus,* and *Awaous ocellaris.* These fish were more likely to occur at lower elevations; their p-values were all equal to 0.00. Water temperature had significant relationships with these same three species such that they were more often found in warmer waters; their p-values were: 0.018, 0.012, and 0.02. Larger pool dimensions (width, length and volume) related to *Stiphodon elegans, Stenogobius genivittatus* and *Awaous ocellaris* significantly ($p \leq 0.05$), except width for *S. elegans.* Larger amounts of granules and course sand significantly related to *S. elegans* (p=0.053). More silt was present at sites with *S. elegans* (p=0.031), *S. genivittatus* (p=0.046) and *A. ocellaris* (p=0.084). Less leaf detritus related to *Sicyopterus pugnans* (p=0.057) and more leaf detritus was related to *S. genivittatus* (p=0.038).

Table 2 shows all the t-test results including the value of t, degrees of freedom and p. Only significant or near significant results are shown.

Species distribution vs. environmental variables: binomial distributions

In order to clarify the use of binomial distribution analysis consider the following example:

Of the 35 sites I sampled at, 11 had *Inocarpus* spp. trees. *Sicyopterus pugnans* occurred only twice at these 11 sites. Hence the ratio of *S. pugnans* found at *Inocarpus* spp. sites is 2/11 (0.18). Of the 24 sites that did not have *Inocarpus* spp., *S. pugnans* occurred 19 times, a ration of 19/24 (0.79). Using Pearson's Confidence Chart with a sample size N=30 (slightly more conservative than the actual sample size of 35) the 95% confidence intervals are 0.8-0.37 (for the 0.18 ratio). The confidence interval for the other ratio (0.79) is 0.6-0.91. The confidence intervals for both categories of *Inocarpus* spp. do not overlap; hence these ratios are significantly different. Thus it seems

FORK A

elevation vs.

	water temp	algae	leaf det	length	per cover	silt
corr	-0.772	-0.067	-0.004	-0.415	0.545	-0.617
R^2	0.596	0.004	0	0.172	0.297	0.38
p	0.009	0.854	0.992	0.223	0.103	0.058

length vs.

algae	leaf det	silt	water temp
0.628	0.506	-0.04	0.437
0.395	0.256	0.001	0.191
0.052	0.135	0.917	0.206

volume vs.

silt	leaf det
0.218	-0.24
0.047	0.546
0.546	0.505

per. cover vs.

algae	silt
0.668	-0.897
0.446	0.805
0.035	0

FORKS B and C

elevation vs.

	water temp	algae	leaf det	length	per. cover	silt
corr	-0.442	-0.699	-0.441	-0.655	0.328	-0.468
R^2	0.195	0.489	0.194	0.429	0.107	0.219
p	0.027	0	0.027	0	0.11	0.018

length vs.

algae	leaf det	silt	water temp
0.795	0.431	0.673	0.426
0.632	0.186	0.454	0.676
0	0.031	0	0.034

volume vs.

silt	leaf det
0.736	0.569
0.542	0.324
0	0.003

per. cover vs.

algae	silt
-0.023	-0.116
0.001	0.014
0.913	0.579

Table 1: Correlation, R^2 and p values for results of correlation and regression analyses performed on environmental variables versus environmental variables.

	Sicyopterus pugnans			Sicyopterus taeniurus			Stiphodon elegans			Stenogobius genivatattus			Awaous ocellaris		
t-test results	t=	df=	p=	t=	df=	p=	t=	df=	p=	t=	df=	p=	t=	df=	p=
Elevation							7.569	30.3	0	6.699	32.9	0	7.796	28.5	0
Water temp.							-2.653	15.7	0.018	-2.774	19	0.012	-2.542	18	0.02
Granule/sand				no significant			2.13	13.2	0.053						
Silt				relationships			-2.603	8.3	0.031	-2.473	6.3	0.046	-1.931	9.5	0.084
Leaf detritus	2.011	21.2	0.057							-2.543	7.1	0.038			
Width										-2.721	6.4	0.032	-2.691	10.1	0.022
Length							-4.462	8.5	0.002	-3.303	7.1	0.013	-4.362	9.6	0.002
Volume							-2.51	9.4	0.032	-3.605	6.1	0.011	-3.675	9.1	0.005
Binomial dist.	abs/little	pres/lot	95% sig	abs/little	pres/lot	95% sig	abs/little	pres/lot	95% sig	abs/little	pres/lot	95% sig	abs/little	pres/lot	95% sig
Inocarpus	0.6-0.9	0.08-0.37	yes	no significant					no						
Amount algae	0.60-0.91	0.2-0.58	yes	relationships			0.04-0.27	0.25-0.64	yes	0.0-0.13	0.25-0.64	yes	0.07-0.34	0.25-0.64	yes
S.pugnans	x	x	x				0.03-0.22	0.21-0.59	yes						
S. taeniurus				no significant											
S. elegans	0.31-0.7	0.72-0.96	yes	relationships			x	x	x	0.01-0.18	0.47-0.83	yes	0.05-0.28	0.59-0.9	yes
S. genivatattus							0.04-0.27	0.68-0..94	yes	x	x	x	0.08-0.36	0.52-0.86	yes
A. ocellaris							0.03-0.24	0.5-0.85	yes	0.03-0.24	0.31-0.7	yes	x	x	x
time sampled	no relationship			no relationship			no relationship			no relationship			no relationship		

Table 2: Results for t-tests and binomial distributions analyses conducted on environmental variables versus species distributions, species distributions versus species distributions and time versus species distributions. Only significant or near significant results ($p \leq 0.05$) are reported.

that *Inocarpus* spp. has a negative impact with the presence of *S. pugnans*.

Inocarpus spp. was only significantly related to *S. pugnans* such that the goby occurred more frequently at sites without *Inocarpus* spp. *S. elegans, S. genivittatus* and *A. ocellaris* were all significantly related to higher amounts of algae. *S. pugnans* was significantly related to lower amounts of algae. Binomial distribution analyses, including rations and 95% confidence intervals are summarized in Table 2.

Species distributions vs. species distributions: binomial distribution

S. pugnans was significantly related to the presence of *S. elegans*. The presence of *S. elegans* was significantly related to the presence of *S. pugnans*, *A. ocellaris* and *S. genivittatus*. The presence of *A. ocellaris* was significantly related to the presence of *S. elegans* and *S. genivittatus*. The presence of *S. genivittatus* was significantly related to the presence of *S. elegans* and *A. ocellaris*. *S. taeniurus'* universal distribution created no relationships with the distribution of other species when using binomial analysis. This data is summarized in Table 2.

Species presence vs. sampling time and weather

None of the five goby species showed statistical significance when compared to the three time blocks chosen to test the effect of sampling during different parts of the day. Weather also showed no relationship with species presence or absence.

Discussion

Environmental variables vs. environmental variables

In order to understand why so many of the environmental variables had significant relationships with species distributions, it is important to consider what interactions they have within themselves. Dependent relationship between variables could cause variables to scale with one another, confounding the results of species distributions.

Many of the environmental variables correlated with each other with significant p-values. This makes it possible (if not probable) that only a few of these variables are actually responsible for affecting a species' distribution. For the purposes of analysis, Forks B and C were separated from Fork A because they had very different environmental characteristics.

Low elevation pools featured larger dimensions (length and volume specifically), warmer water, thicker coats of algae, larger amounts of silt and leaf detritus compared to high elevations pools. Fork A was an exception in that large amounts of algae and leaf detritus were found at almost all elevations. Long pools (length vs. other variables) in Forks B and C significantly correlated with silt, leaf detritus and algae and water temperatures. Long pools in Fork A only had larger amounts of algae. High volume pools in Forks B and C had more silt and leaf detritus.

These relationships can be logically explained by the dynamics of watershed systems. Silt, leaf detritus and other debris build up at low elevations as a result of normal flow and sedimentation. Larger volumes of water flowing in the lower portions of rivers necessitate larger dimensions to accommodate the flow. Larger flows of water on flat terrain form larger pools with slower flow rates. Also, wider stream flows allow a separation in forest canopy, allowing more sunlight to reach water. The combination of large pools with slow currents and more sunlight encourage the growth of algae, as they receive more energy for photosynthesis and are not washed away by fast currents.

However, higher percentage canopy cover in Fork A was correlated to more algae but less silt. This relationship is not as clear, because I would suspect less canopy cover and more sunlight would increase algae amounts. I do not think that canopy cover directly relates to silt; rather I think that it is an artifact of other intermediate factors that scale with elevation and canopy cover.

With so many of the environmental factors scaling with one another, it is hard to say which ones are more likely to be responsible for species distributions. I was not comfortable using the highest p-values to imply a hierarchy of importance, so I turned to each species' specific characteristics. I applied aspects of each species' biology towards the results. Information on feeding morphology and diet, physical abilities and limitations, and behavioral observations such as predator avoidance proved very useful in interpreting the results.

Factors not affecting distribution:

Among sites with and without each species of goby there was no significant difference between the average values of canopy cover and larger substrate elements (pebbles, cobbles and boulders). A recent study by Nelson *et. al* (1997) that investigated microhabitat preferences in Micronesian gobies showed that the observed density of gobies was weakly negatively correlated with canopy cover. Nelson explained this by suggesting that more direct sunlight to the streams allowed for more algae growth, the staple of most freshwater goby diets.

The same study also showed a positive correlation between the overall percent of hard substrate (Wentworth pebbles and larger) and the densities of gobies. He supports this result by suggesting that the gobies' feeding biology (often adapted to scrap algae off of rocks) made sites with higher amounts of hard substrate more abundant in food.

Despite our opposite findings, I feel that my results do not contradict Nelson's conclusions. I measured the presence or absence of fish, not their density. Hence density differences were not compared to variables statistically. By using species presence and absence I had hoped to quantify which factors severely affect goby distributions. Thus density changes would not show up in my analyses.

The following sections are species-specific and incorporate the environmental factor results and aspects of their biology for explanation.

Awaous ocellaris

Watson (1995) describes members of the genus *Awaous* as diurnal bottom-dwellers. *Awaous guamensis* (a close relative of *A. ocellaris* common to Hawaii and other Pacific island groups) has been observed diving into the substrate when threatened, leaving only the eyes exposed. I have witnessed this myself in *A.* ocellaris and actually caught my first specimen by pinning it under the sediment with net. At night they are reported to sleep under the substrate in a similar fashion. This aspect of their biology supports the weakly significant relationship observed with silt ($p=0.084$). "Substrate diving" might be a predator avoidance system that requires soft substrate for execution. The distribution on silt on the Opunohu is shown in Figure 10a. A picture of *Awaous ocellaris* is shown in Figure 4.

Watson also describes *Awaous* spp. as primarily eating algae and detritus; their guts also frequently contain large amounts of silt and mud along with an occasional crustacean, gastropod or worm. The source of sediment in the gut relates to their feeding strategy, which Watson describes as "taking large amounts of substrate and filtering it through the gill chamber." Periodically these fish are observed "chewing" which may be mastication performed by the pharyngeal teeth. I have witnessed both these behaviors in the field.

Kido (1996b) conducted morphological studies on *A. guamensis* where he suggested feeding mechanisms as related to mouth structures. He describes *Awaous* as having "carnivorous" feeding features like sharp bony teeth adapted for "picking and biting" invertebrates. He also described the elaborate gill raker system that allows *Awaous* to forage through sediment (same process as described by Watson) for smaller invertebrates. However, he also noted that *Awaous* had a very long convoluted gut allowing for the processing of plant materials. This was confirmed when he examined guts contents to find mostly algal material and a few invertebrates. This supports my results coupling *A. ocellaris* with high amounts of algae and silt. These two environmental factors seem involved with aspects of their feeding biology. Figure 10b shows the distribution on algae in the Opunohu.

In another article Watson (1991) describes *Awaous* spp. as living mostly in freshwater up to elevations of 1000 meters, but occasionally seen in brackish waters. I did not find any above about 20 meters. *A. ocellaris* was strongly correlated with low elevation. This is also reflected by the relationship with warmer water temperatures (which occur at lower elevations) but I have not come across any literature suggesting temperature preference. Figure 10c shows the temperature gradient for the Opunohu.

Marquet and Galzin (1992) described the distribution of *A. ocellaris* on Tahiti and Moorea as being limited to the two sections of the "cours inferieur" (defined as areas of little current – on the coastal plain). The first section is the "estuaire" (estuary, or area affected by saltwater) and the second is the "amount" (area affected mostly by freshwater). They also describe the species as being in "eaux stagnates littorals" (meaning – stagnant pools off the main flow). My distributions agree with Marquet and Galzin's observations. Figures 10a-d show the distributions of environmental variables. Figure 11 shows the distribution of *A. ocellaris* on the Opunohu.

However, Nelson (1999) lists *Awaous guamensis* as being able to pass dams ad reservoirs. Nelson *et. al* (1997) describes gobies of the genera *Awaous, Stiphodon and Sicyopterus* being able to climb waterfalls. Perhaps elevation and large pools sizes are not directly responsible for *A. ocellaris'* distribution (as they have been reported higher up). The exclusive presence of silt, heavy algae and leaf detritus at low elevations limits their assent. Given a larger, longer river with silt, algae and leaf detritus higher up, *A, ocellaris* might thrive. The extent of large pools in the Opunohu is shown in Figure 10e.

Binomial distribution analysis showed significant relationships between *A. ocellaris*, *S. elegans*, and *S. genivittatus*. Although many gobies were observed engaging in aggressive behavior (mostly *Sicyopterus* spp.) I sampled in several pools that had all five goby species. Therefore I do not suspect that these results are due to interspecies interactions. Rather I think that similar biological requirements and habitat preferences among them drive this relationship.

Stenogobius genivittatus:

Kido (1996b) described their feeding morphology as adapted for both algae and invertebrates. The fused serrated upper teeth are adapted for scraping algae off rocks, where as the fleshy gill rakers make for efficient sediment foraging (which I have witnessed). However Kido also discovered that almost 9% of gut contents from *Stenogobius (hawaiiensis* in this case) was leaf detritus. Whereas Kido did not believe that this consumption of detritus was for nutritional purposes, he suggests that it is a result of feeding in areas of high

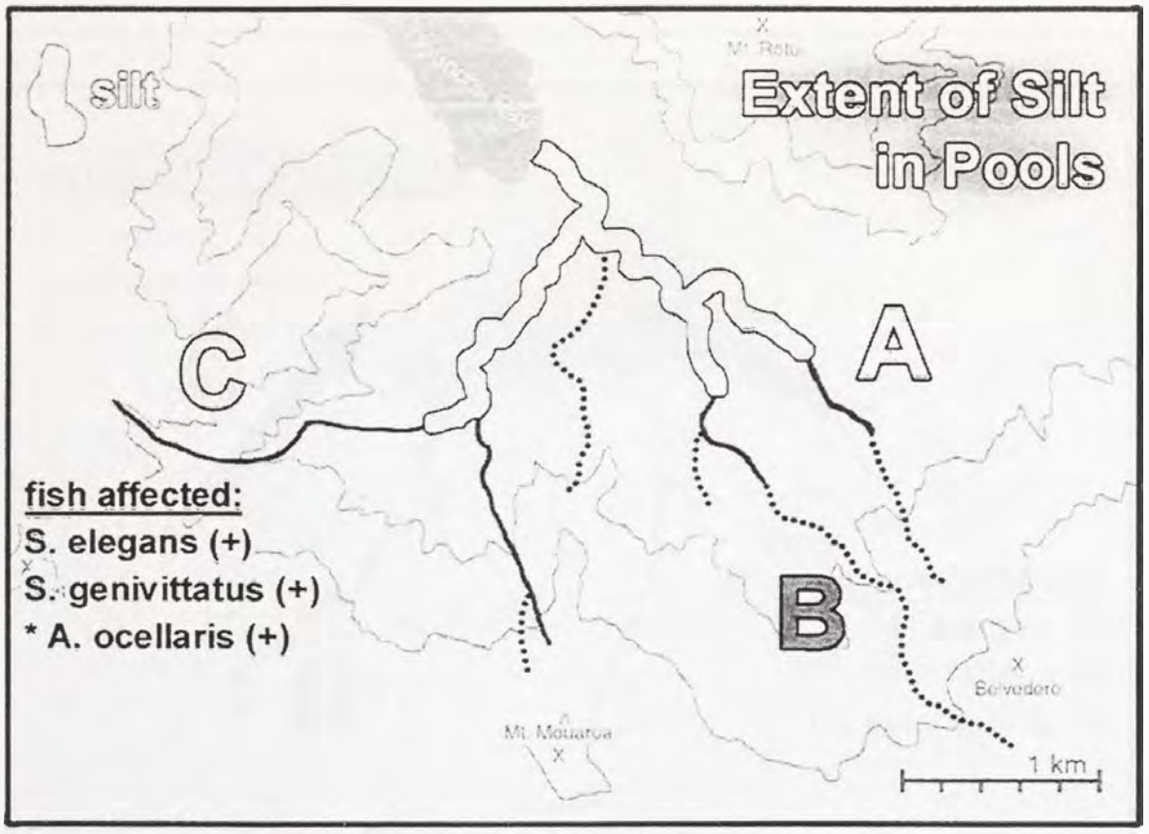


Figure 10a: The distribution of silt in the Opunohu River.

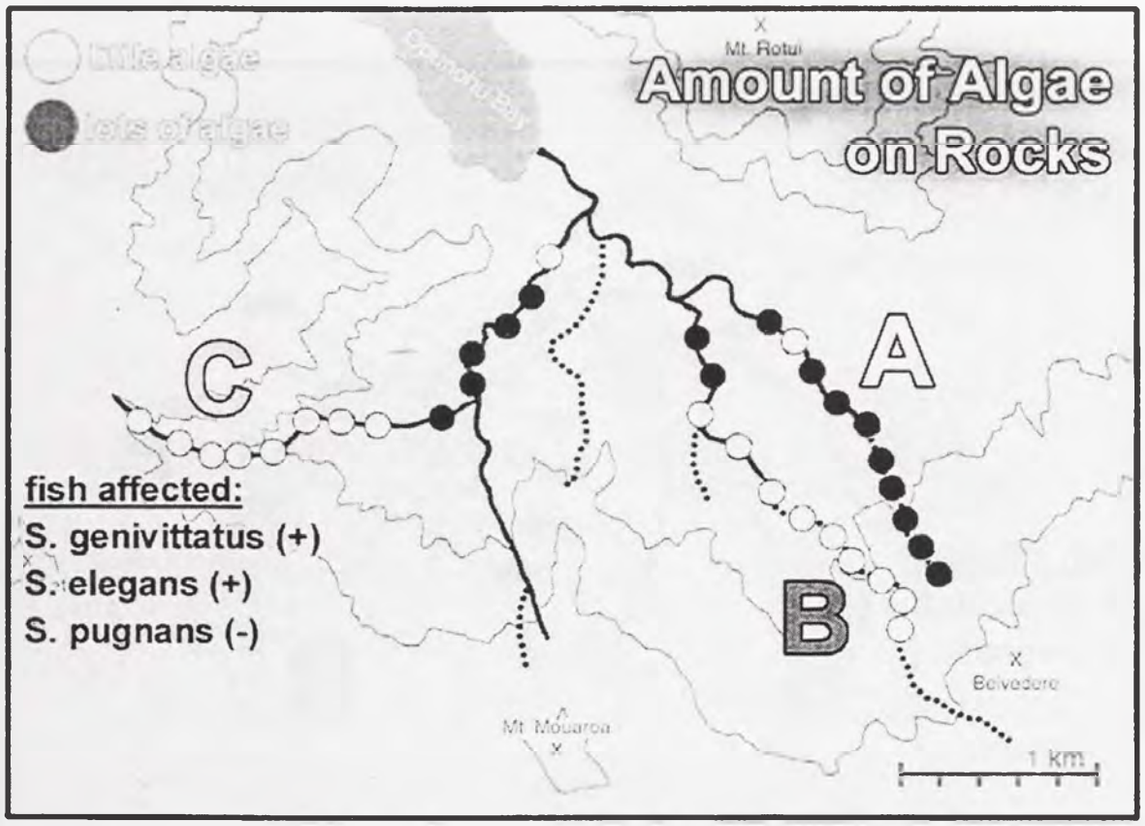


Figure 10b: Simplified expression of the amount of algae in pools in the Opunohu River. The 4-tiered scale has been reduced to a 2-teir scale for this diagram.

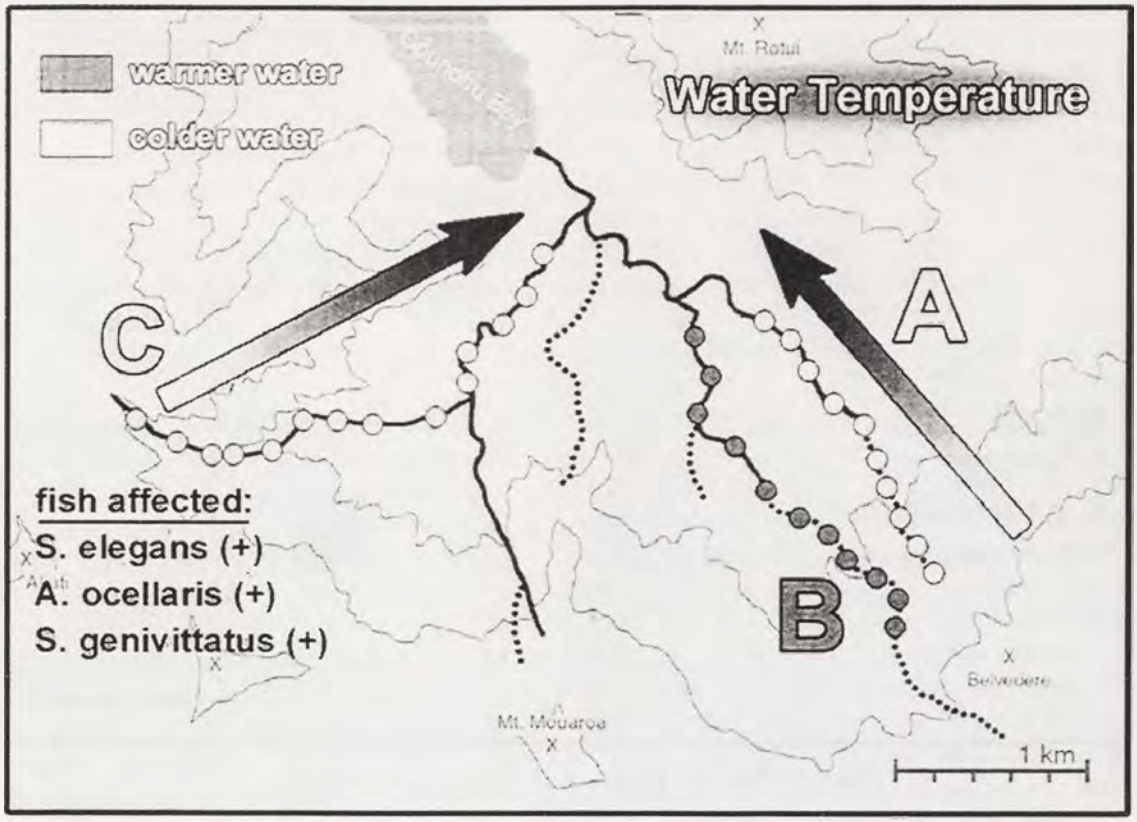


Figure 10c: A general temperature gradient map of the Opunohu Valley.

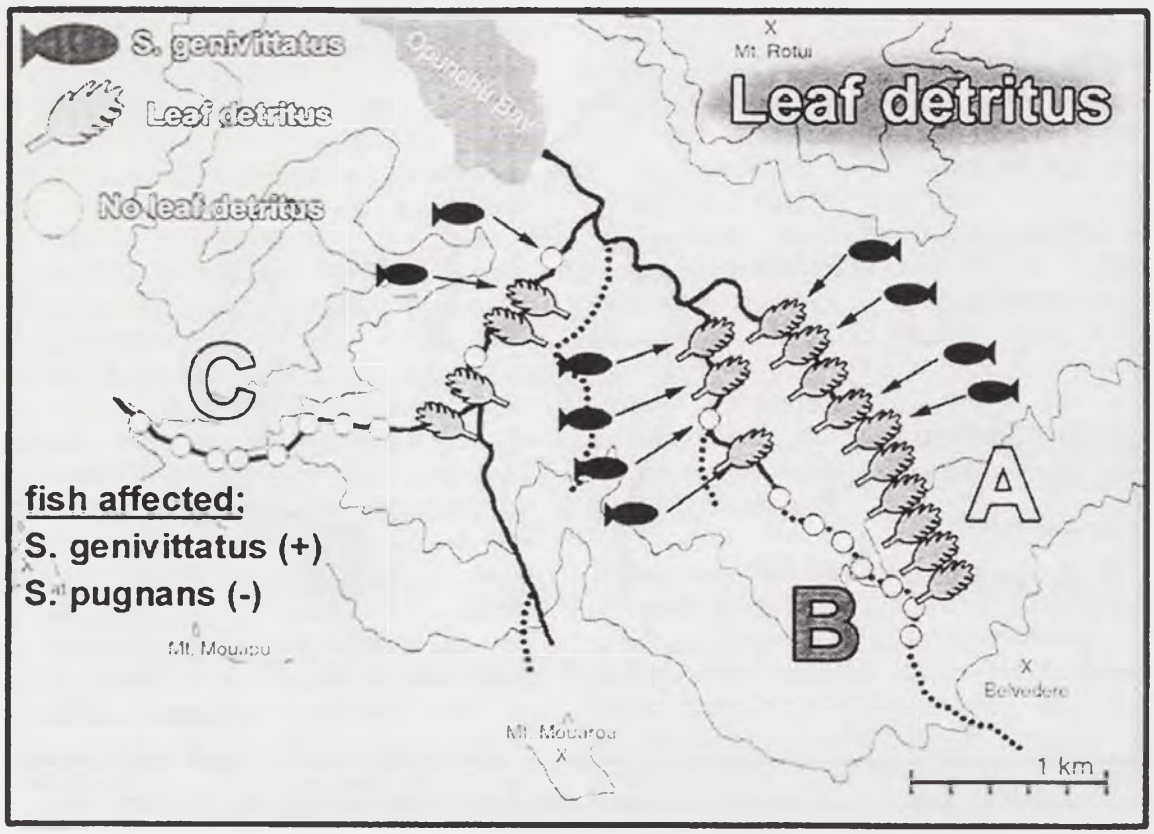


Figure 10d: The presence of leaf detritus and *Stenogobius genivittatus* in the Opunohu River.

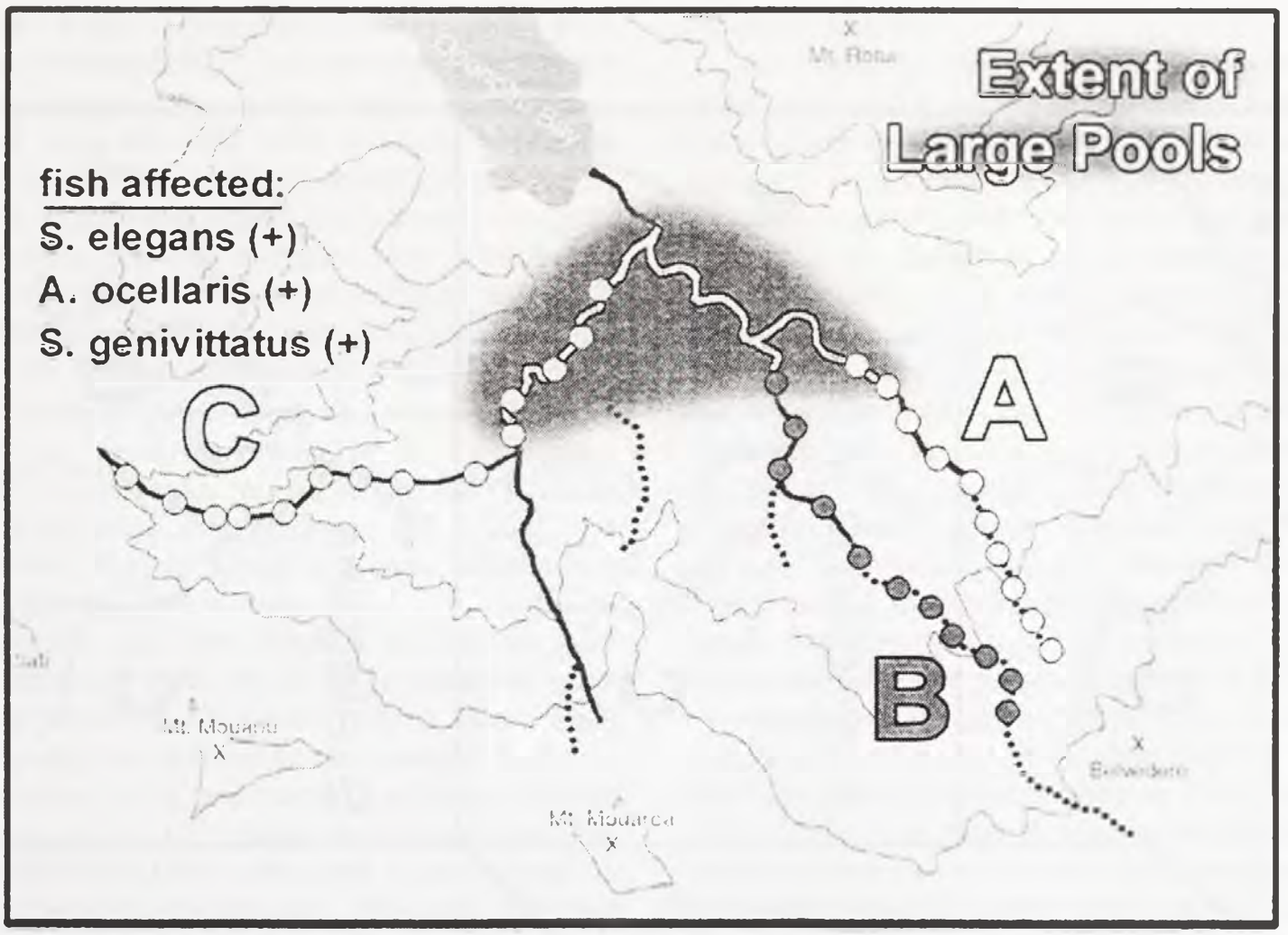


Figure 10e: The distribution of "large" pools in the Opunohu River.

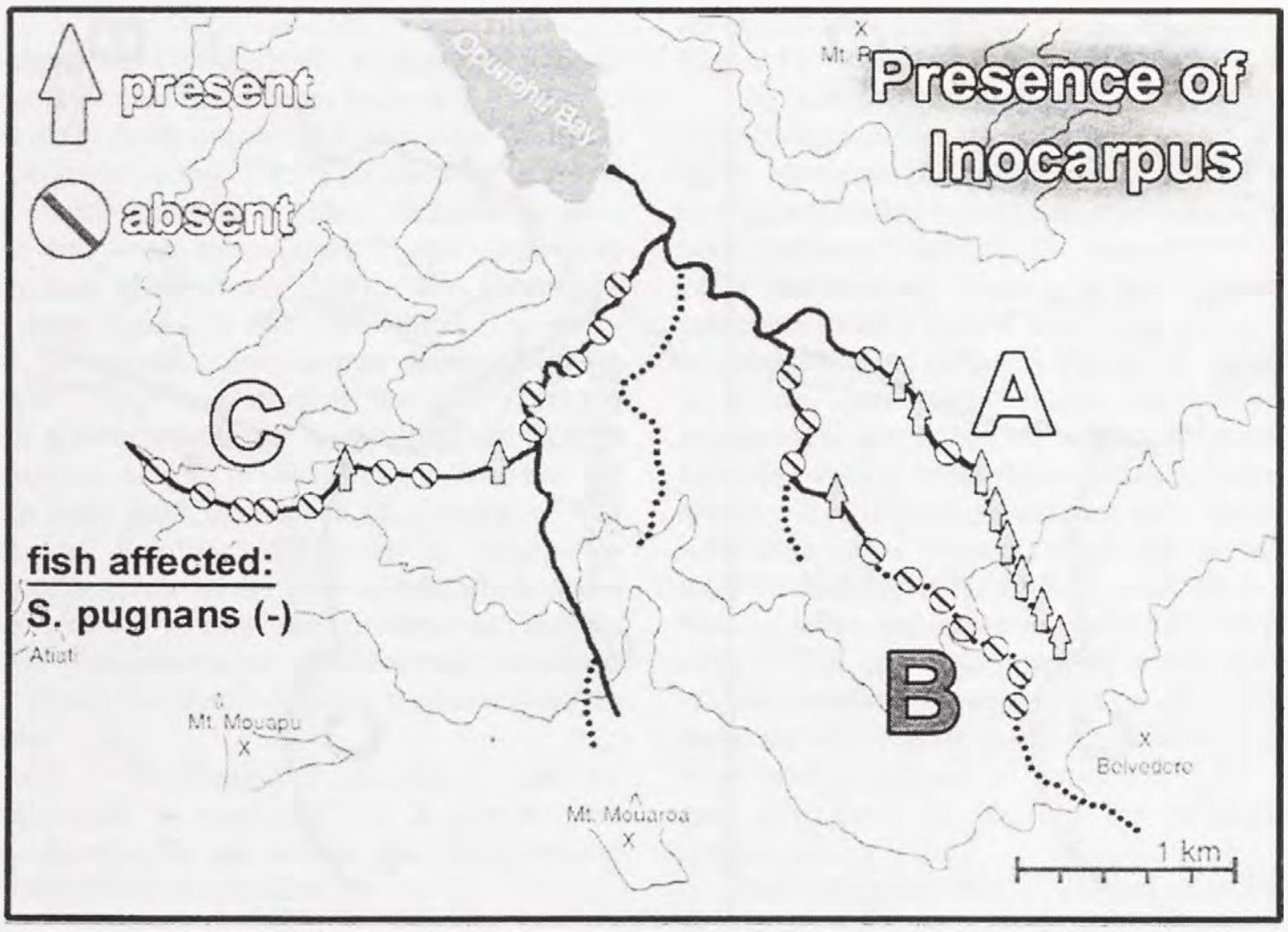


Figure 10f: The presence of *Inocarpus* at the sites studies on the Opunohu River.

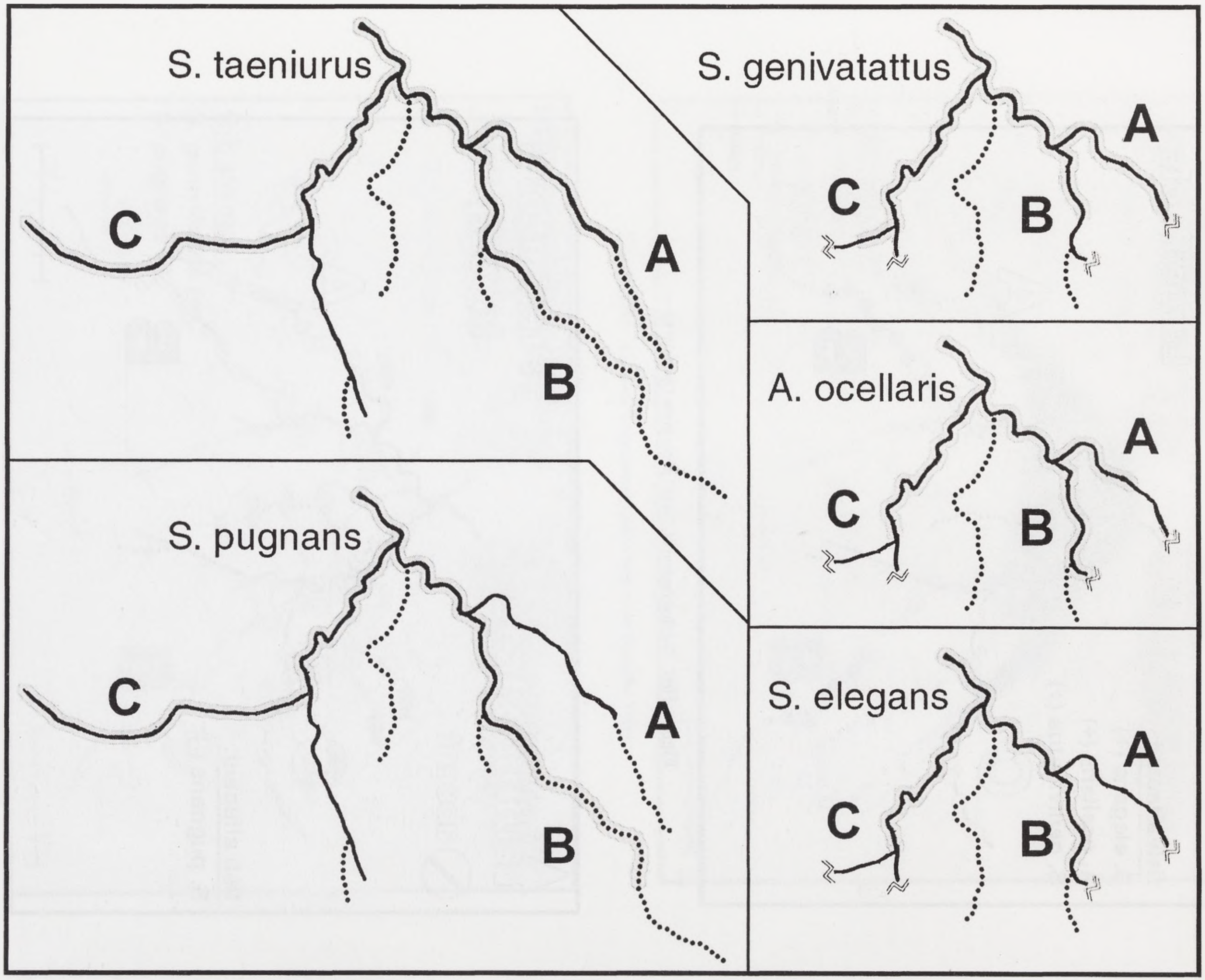


Figure 11: The stream distributions for the five species of gobies son Moorea.

leaf detritus. With *S. genivittatus* closely related to *S. hawaiiensis* (Watson 1991) it is reasonable to suggest that these observations could apply to both. *S. genivittatus* was the only goby to have a positive significant relationship with leaf detritus, which agrees with Kido's suggestion. The distribution on leaf detritus on the Opunohu is shown in Figure 10d. A picture of *Stenogobius genivittatus* is a shown in Figure 5.

The strong relationship with silt and algae also agree with its feeding strategies of sediment foraging and algae consumption. I have also witnessed what I believe to be "sediment diving" in *S. genivittatus*. Individuals would seem to come out of nowhere (probably from under the substrate) as I floated over during surveys. Figure 10a and 10b show distributions for silt and algae in the Opunohu.

Stenogobius spp. are reported as having poor climbing abilities (Nelson *et. al* 1997) compared to the other goby genera. Watson (1991) states that *Stenogobius* spp. is limited to the coastal plains in freshwater, often living near marine conditions. Watson also mentions in his 1991 paper that through personal communication with Gerard Marquet, *S. genivittatus* on Tahiti are limited to the estuary. However, Marquet goes on to state that on Moorea they can move well inland up to the bases of waterfalls. Although, Marquet was not speaking about the Opunohu (because there is no waterfall in the valley) my results suggest that they are limited to the lower elevation areas nearer to the ocean.

In Marquet and Galzin (1992) the distribution on *S. genivittatus* is limited to the same areas as *A. ocellaris*: the coastal plain (both estuary and freshwater portions) and in stagnant pools. *S. genivittatus* exhibited significant relationships with elevation, pool dimensions and water temperature. Figure 10c shows the temperature gradient and Figure 10e shows the extent of large pools in the Opunohu . The fore-mentioned biological traits support some of these relationships. In this case, it seems that poor climbing ability and habitat preference is limiting the goby's range. However, as with *A. ocellaris*, I believe that the relationship with pool dimensions is a result of silt, algae and leaf detritus building up in large, low elevation pools nearer to the river mouth, not a direct correlation with *S. genivittatus'* distribution. Figures 10a-d show distributions of environmental variables. Figure 11 shows the distribution of *S. genivittatus* on the Opunohu.

Binomial distribution analysis showed relationships with *A. ocellaris* and *S. elegans*. As mentioned above I do not believe that these are the results of interspecies interactions.

Stiphodon elegans:

According to Watson (1995) *Stiphodon* spp. are found only in the high gradient streams of volcanic islands. It is usually collected in swift waters where the substrate is cobbles, pebbles and gravel. It is sometimes captured near boulders or backwaters. They are usually not known to climb waterfalls. Although other researchers have found *Stiphodon* spp. at high elevations, surmounting obstacles like dams and reservoirs (Nelson *et. al* 1997; Nelson 1999) I found that their distribution got no higher than that of *A. ocellaris* and *S. genivittatus*. *S. elegans* demonstrated significant relationships with elevation, water temperature, and amounts of granules/sand, algae, silt and pool dimensions - all low elevation traits for the three forks of the Opunohu. Figures 10a-e show the distributions for the above environmental variables in the Opunohu. Figure 6 shows a picture of *S. elegans*.

Marquet and Galzin (1992) also noticed this limited distribution on a fish often called a "mountain goby" by other researchers (Watson 1995; Nelson *et.al* 1997). They describe *S. elegans* as limited to the freshwater portion of the river with weak current (on the coastal plain) up to the "cours moyen" (defined as areas with medium current flow with a grade of less that 10%. I cannot speculate why this goby (the smallest in Moorea) does not reach the higher elevations they are reported to do in other island group like Palau and Pohnpei of Micronesia (Nelson *et. al* 1997). The distribution for *S. elegans* is shown in Figure 11.

As with *A. ocellaris* and *S. genivittatus*, feeding biology might cause the relationships seen with silt and algae. However, in my preliminary surveys I witnessed *S. elegans* feeding by scraping thin coats of algae off of hard substrates (and in a few instances of eating algae off of leaf detritus). Pools with high algae content have substrates coated with a soft layer that can easily be scraped off with a finger. Therefore, the relationship with silt and algae might not reflect feeding preferences, but rather an artifact of some unknown variable scaling with these variables (like elevation scales with silt, pool dimensions, etc.) However, pools with high algae content might be so rich in food content, that gobies do not have to spend as much time feeding when compared to pools with only thin algal coats. This could also explain why I did not see *S. elegans* feeding in algae rich pools. Unfortunately, there are no feeding morphology or diet studies that have been completed on *Stiphodon* spp. Therefore I can only leave the reasons for its distribution to speculation.

One difference that *S. elegans* exhibited from its sympatric low elevation gobies (*A. ocellaris* and *S. genivittatus*) was its absence from Fork A. Although none of these three species were found very far up Fork

A, *S. elegans'* absence might have special implications. I will leave the discussion on the special conditions of Fork A to the section on *S. pugnans* as this species has a pronounced distribution anomaly in Fork A.

As expected from my earlier argument, binomial distribution analysis showed relationships with *A. ocellaris* and *S. elegans*. However *S. elegans* also had a significant relationship with *S. pugnans*. In the field I never witnessed interactions between these species. I have also found no scientific literature describing interspecies interactions between *Stiphodon* spp. and *Sicyopterus* spp. Most of *Sicyopterus pugnans'* distribution is well above that of *S. elegans*. So this result is one of random chance, or some unknown interaction between the two species.

Sicyopterus taeniurus:

Found at all but five of the thirty-five sites in the Opunohu, *S. taeniurus* is the most versatile of Moorean freshwater gobies. Individuals are found at all the highest elevation sites on all three forks. Marquet (1988) described this species as reaching the highest parts of streams, frequently scaling waterfalls to do so. I assume he was referring to the juveniles, as I have never witnessed adults climbing rocks out of the water. This nearly universal distribution in the Opunohu led to the absence of any significant relationships to the environmental variable or presence of other species. Figure 11 features the distribution of *S. taeniurus* in the Opunohu. Figure 7 shows a picture of *S. taeniurus*.

Sicyopterus pugnans:

Unlike its close relative *S. taeniurus*, *S. pugnans* demonstrated significant relationships with a several environmental factors. The presence of *Inocarpus* spp., algae, and leaf detritus all had significant relationships with the absence of *S. pugnans* ($p \leq 0.05$). These results were unexpected until I looked at each of the Opunohu forks separately. *S. pugnans* is found virtually everywhere, except for Fork A. Fork A is different from Forks B and C in that it is surrounded entirely by *Inocarpus* spp. forest (instead of *Hibiscus*). The data for Fork A also shows that leaf detritus and very high amounts of algae were found at nearly every site, regardless of elevation. Figures 10b, 10d and 10f show the Opunohu distributions for algae, silt and *Inocarpus* spp. Figure 8 shows a picture of *S. pugnans*.

Sparse ground vegetation, low tree density and many dead leaves characterized the *Inocarpus* spp. forests in which I worked. This is contrasted by *Hibiscus* forest, which had relatively high ground vegetation (ferns), mostly dead branches and logs, and high tree density. With little scientific work on *Inocarpus* spp. in the literature I found it difficult to explain why *S. pugnans* seems to be avoiding this fork entirely. However, *S. taeniurus* was found in lower densities in Fork A than B and C, so it may or may not be affected by the same variables discouraging *S. pugnans*. The distribution for *S. pugnans* is given in Figure 11.

I have developed one hypothesis as to how the effects of *Inocarpus* spp. might affect *S. pugnans*. Both eucalyptus and pine trees are known to acidify the forest soils. This acidity is transmitted via fallen pine needles or leaves, and results in the lack of acid tolerant ground vegetation on the forest floor. A similar process might be in place in Moorea. *Inocarpus* spp. might be altering the chemistry of the soil and stream water (either directly with fallen leaves or by run-off). This chemical change might either directly affect *S. pugnans*, or it might affect the algal species present in the stream.

Marquet (1988) found that although *S. taeniurus* and *S. pugnans* feed on the same algal species, the main component of their diets is different. Stomach contents from *S. taeniurus* contained *Navicula ignota* – 25.86%, *Nitzschia paleacea* –15.59%, and *Gomphonema parvulum* – 6.46%. He also noted that they eat larger algal species like *Synedra lancelata* – 5.7%. *S. pugnans* had a diet of primarily *Navicula ignota* – 43.84%. Perhaps this preference for *N. ignota* is responsible for the distribution difference: *N. ignota* might be discouraged from growing in Fork A due to the *Inocarpus* spp. effects. If the *Inocarpus* spp. prevents the growth of certain species, or promotes the growth of another unpalatable species that out competes *S. pugnans'* algal staple, this interaction might be resolved. The presence of high algal levels all throughout Fork A also supports the notion that a different algal flora might be present.

The negative relationship between leaf detritus and *S. pugnans* might be indirect as mentioned above (by changing the stream/soil chemistry) or it might be a more direct deterrent; although I have no suggestions for a direct mechanism for deterrence.

A study by Friberg *et. al* (1996) investigated the effects of six tree species on the algal communities of New Zealand streams. Their findings showed that leached chemicals (such as tannins and other phenolics) from five tree species inhibited the growth of certain algae. The chemicals leached from the sixth tree actually stimulated algal growth. Another study by Chergiu *et. al* (1997) showed that leaching chemicals from oleanders and willows significantly reduced the amount of dissolved oxygen in water samples. The results from both these studies could be applied to thinking about the *Inocarpus* spp. effect. If these trees changed the algal diversity of Fork A, or reduced the amount of dissolved oxygen in the water, the absence of *S. pugnans* might be explained.

Whatever the exact cause for such a relationship, it is undoubtedly there. I think that additional research

projects could easily discover the cause by investigating the special characteristics of *Inocarpus* spp. forest streams compared with *Hibiscus* forest streams. As mentioned before, *S. elegans* was also not found in Fork A, and might be under the influence of similar factors.

In regards to *S. pugnans'* distribution in the other forks, did not seem to be impeded by any factor. At high elevations in Fork C *S. pugnans* were the dominant adult goby present. *S. taeniurus* juveniles were also abundant, but adults were found more infrequently than *S. pugnans*.

Binomial distribution analysis for *S. pugnans* showed a relationship with *S. elegans*. As mentioned above I do not have any suggestions for potential inter-species interactions between these two gobies.

Discussion of Hypotheses

Although my results are somewhat confounded by the scaling and redundancy of environmental factors, all three of my working hypotheses were correct for certain species.

For *Awaous ocellaris* aspects of its feeding biology and possible predator avoidance behavior suggest that the presence of silt and algae are important in-stream biotic components of its preferred habitats. However, I was unable to eliminate the significant relationships with large pool size, elevation and water temperature. These might be contributors to silt and algae, but might also directly reflect habitat preferences of *A. ocellaris*. Thus hypotheses 1 and 2 cannot be rejected.

Stenogobius genivittatus was also under the influence of silt and algae, and probably has similar feeding and predator avoidance strategies as *A. ocellaris*. However, *S. genivittatus* was the only goby to significantly relate to leaf detritus. This is supported by Kido's 1996b study. Again, hypotheses 1 and 2 cannot be rejected.

Stiphodon elegans had weaker scientific background to support its affinity for the same in-stream biotic variables that seem to influence *A. ocellaris* and *S. genivittatus*. This may also be a result of habitat preference for silt and algae. Pool dimensions, and water temperature are other possible considerations. In addition, *S. elegans* might be subject to the same unknown factors that *S. pugnans* undergoes in Fork A. There also remains the question of its significant relationship with *S. pugnans*. Perhaps species interactions are at work here. Hence, for *S. elegans* Hypotheses 1, 2 and 3 cannot be rejected.

Sicyopterus taeniurus was universally distributed on the Opunohu, and hence did not relate to any variables. So it seems that we can reject all three hypotheses for factors affecting its distribution.

S. pugnans presents the most interesting results. Here it seems that terrestrial biotic factors might be at work (perhaps in addition to the in-stream biotic factors algae and leaf detritus – but this may be a secondary effect of the *Inocarpus* forest.). So hypotheses 2 and 3 cannot be rejected.

Barriers on the Opunohu

Only two man-made barriers were found on the Opunohu River system. The first was a weir found at on Fork B at high elevation (see Figure 2 for map location and Figure 12 for weir picture). This weir is used to collect drinking and irrigation water for the Opunohu Valley. It was relatively new, and its construction seemed ecologically sound. I witnessed juvenile gobies climbing up the smooth surface of the weir, and saw numerous gobies in the collecting pool just above the weir. As *Sicyopterus taeniurus* and *Sicyopterus pugnans* were the only gobies found at that high elevation, and because I found adults above the weir, I felt that it posed no serious ecological threat to gobies.

However, I did find a formidable barrier at high elevation on Fork C. Figure 2 shows the dam's location and figure 13 is a picture. This water-collecting dam also drains stream water for drinking and irrigation use. However, it was much older than the Fork B weir, and seemed to pose a substantial threat to upstream migration. Almost all the water coming down from the upper part of Fork C was diverted into the pipes. Little more than a weak, continuous trickle was allowed to flow outward. The outflow was a large diameter pipe that was blocked completely from the dammed side with a piece of plywood. The only water escaping was forced through the small cracks around this piece of wood. The water then poured into the rather stagnate pool below the dam. No gobies were visible in the lower pool, and from my estimation, it was not possible for gobies to surmount the barrier through the outflow pipe or over the dam structure as it was too dry. Fork C remained extremely dry for quite a distance below the dam until other minor tributaries boosted flow.

Gobies were found farther below the dam, and to my surprise, above it also. In the water collecting pool of the dam there were numerous *Sicyopterus* spp. juveniles and a few adults. As juveniles must have come from the ocean, they must have found a way over this barrier. Looking at the rock wall and the sides of the dam, it was evident that during heavy rainfall the dam overflowed (as no lichens or moss grew below the top of the dam). I think that at these times of heavy flow, gobies are able to climb the dam, and continue upstream. In fact, Fork C above the dam had some of the highest concentration on adult *s. pugnans* adults I have seen anywhere in the Opunohu.

Thus is seems that the only barriers on the Opunohu posed no great threat to the only two species of gobies that reach high elevation elevations.

Figure 12: Pictures from the Fork B weir. Note the smooth design of the weir allowing gobies to pass (right and enlargement) and the gobies found just above the weir.

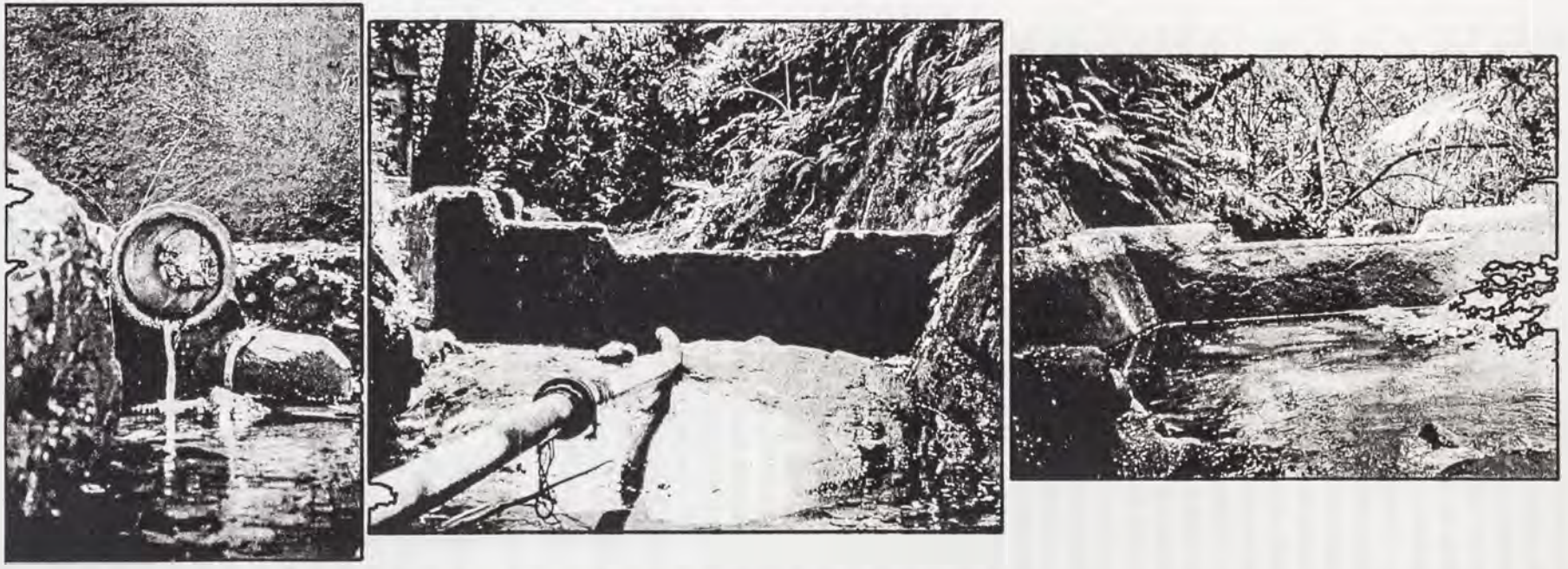


Figure 13: Pictures from the Fork C dam. Note the small outflow (left) the front of the dam (center) and the back of the dam (right). On the left side of the back of the dam the lower growth limit of moss and lichen is visible – indicators that the water level frequently rises above the spillway level and into the pool below.

However, the Opunohu River Valley is also one of Moorea's most prestine, and I believe that the possibility of lower elevation blockage on other streams is high.

Application to studying other barriers

The results of this study show that three species of gobies in Moorea (*Stiphodon elegans, Awaous ocellaris,* and *Stenogobius genivittatus*) are present only at low elevation. Hence using them as an indicator of stream blockage anywhere in middle or high elevations is unpractical. *Sicyopterus taeniurus* is a champion climber, noted for overcoming huge waterfalls (Marquet 1988) and hence its use as an indicator is also a poor choice. Perhaps *Sicyopterus pugnans* is a good intermediate, with its far-reaching distribution, but lower tendency to climb large barriers. There also still remains the question of the *Inocarpus* spp. interaction.

I think that other good indicators of stream blockage are other Moorean stream fish without climbing adaptations. Using their distributions along with that of the better-equipped gobies might add depth to barrier studies.

Suggestion for future research

Future studies on the distributions of tropical freshwater gobies would benefit from a few improvements on my study's design. Taking density data on species in all three stream flow types (run, riffle and pools) would allow regressions to be applied to environmental barriers, and would provide more resolution to the results. Like the Nelson *et. al* study (1997), this improvement would aid in understanding how certain variables affect the relative density or gobies, not just their presence or absence.

Including survey data on non-goby fish species, gastropods and crustaceans could also expand this study's breadth. This would allow for a better understanding of how barriers affect stream fauna as a whole.

As for non-distribution related studies on Moorean gobies, I believe there are many. I have witnessed extensive territoriality and aggression both within and between species. Gobies seem to be very protective of their feeding zones, and will aggressively fend off intruders.

There is also the subject of color and pigmentation changes. As documented by Fitzsimons *et. al* (1993), *Sicyopterus stimpsoni* exhibits mating and mood color changes. I believe the same is true for both Moorean *Sicyopterus* species. After capturing or even chasing a goby with a net I would notice marked changes in coloration, such that I was often unsure if it was the same fish I had originally sought out to capture. With the aid of underwater photography and filming, this could prove to be a very interesting field of study.

Conclusions

Moorean stream are potentially threatened by the construction of barriers preventing the upstream migration of many animals. Gobies studied in the Opunohu River Valley show relationships to physical characteristics stream pools, and biotic factors both in and out of the stream. *Stiphodon elegans, Awaous ocellaris* and *Stenogobius genivittatus* all seems to prefer larger, low elevation pools with lots of algae and silt. *Stenogobius genivittatus* also seems to prefer the presence of leaf detritus. *Sicyopterus taeniurus* has universal distribution on the Opunohu, and seems to be affected by no external factors. *Sicyopterus pugnans* exhibits an interesting relationship with the *Inocarpus* forest around one of the river's forks. It seems that the presence of this tree, and the abundant algae and leaf detritus found all the way up this fork are somehow discouraging *S. pugnans* from continuing up what might be expected as favorable territory.

Although insights into the feeding and behavioral biology of gobies have been helpful in sorting through significant relationships between gobies and environmental factors, I have not been able to determine scientifically conclusive causations. Therefore I have made suggestions as to how these aspects of their biology might be related to the distributions seen on the Opunohu River.

Using this information as groundwork, I believe that future studies on the barriers to upstream migration of gobies and other stream fauna can help prevent the restriction of natural animal distributions.

Acknowledgements

I would like to thank the professors and graduate student for their dedication to the IB 158 course. I would also like to thank Mike Moser at UC Berkeley for being a wonderful resource for my work on the Moorean gobies. I would also like to thank Mike for his dedication to the Biology 1B field section that sparked my interest in field biology three years ago, and is why I am completing this study today.

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Effects of Secondary Forest Growth on Soil Organic Carbon in Moorea, French Polynesia

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ABSTRACT. Changes in land use alter soil C stocks, thus affecting global carbon budgets. Rapid conversion of forest to pasture or agriculture land in the tropics often leads to decreases in soil organic carbon. Secondary forest growth has been shown to increase SOC stocks in depleted crop and pasture soils. Soil samples from maintained and abandoned coconut plantations were collected from 0-10 cm and 10-20 cm depth to determine and compare soil organic carbon (C) and nitrogen (N) contents. Abandoned plantations with thirty years of secondary forest growth showed a 19 % decrease in C concentrations and an 18% decrease in N concentrations compared to maintained plantations. Total forest C and N stocks (kg/m²) to a depth of 20 cm both decreased by 23% compared to maintained plantation sites. These results contradict previous assumptions that soil organic carbon storage improves after abandonment of pasture or agriculture to secoondary forest growth.

Introduction

World carbon budgets have become a subject of study due to the increase in atmospheric carbon released every year from the burning of fossil fuels. In particular, it has become important to quantify the stocks and residence times of carbon in various sinks, such as the air, ocean, vegetation, and soil. Reasonable accounting has been done for atmospheric and ocean sinks. Quantification of carbon storage in land vegetation and soils, however, is more difficult to determine due to the variability across landscapes, as well as shifting land use.

Conversion of primary forest to pasture and agriculture use in the tropics has increased over the last several decades. Tropical soils comprise 27% of the world's land area, and contain 21% of total world soil organic carbon (SOC) (Schlesinger 1977). With carbon stocks this large, widespread land use changes throughout the tropics can affect global carbon budgets.

Research has shown that tropical soil organic carbon stocks respond to land use changes in a multitude of ways (Batjes & Sombroek 1997; Guggenberger & Zech 1999; Neill *et al.* 1997; Raich 1983; Silver 2000; Trumbore *et al.* 1995). Conversion of primary tropical forest to pasture has resulted in increased SOC (Neill *et al.* 1996). Forest conversion to pasture and crop has also resulted in a loss of SOC (Brown & Lugo 1990). Subsequent abandonment of pasture and agriculture land to secondary forest growth has resulted in a replacement of SOC after 50 years (Brown & Lugo 1990).

Coconut plantations (*Cocos nucifera)* that were planted in the early 1900s in Moorea are increasingly abandoned as profits from coconuts decrease. Many plantations were abandoned 30-40 years ago and have grown into secondary forests. I hypothesized that C and N concentrations and pools would differ between maintained and abandoned coconut plantations. I expected to see increased C and N concentrations in the abandoned plantations. Additionally, based on < 12 year turnover rates in surface soils (Trumbore *et al.* 1995), I expected to detect differences in the top 20 cm of the soil profile

Methods

Study sites

This study was conducted on Moorea, French Polynesia (17°30'S. 149°50'W) from September 16 to November 16, 2000. In order to study changes in SOC due to changes in vegetation, other soil forming factors at each site, such as parent material, climate, slope, aspect, elevation, and plantation age, were kept as uniform as possible (Jenny 1941).

All sites receive 1750-2350 mm of seasonal rainfall per year and have a mean annual temperature greater than 22.7° C (ORSTOM 1993). Plantations are on west to southwest (235-288°)

facing backslopes of 10-20°, at elevations of 20-40m. All soils are derived from basaltic colluvium.

Having met these parameters, I paired three maintained coconut plantations with three abandoned plantations (Figure 1), for a total of six sites (Table 1). Plantation age was determined by interviewing landowners. All six plantations were 70-90 years old. The three forested plantations had been abandoned for approximately 30 years. *Wedelia trilobata*, grasses, sedges, and exotic weeds provided low ground cover in maintained plantations. Abandoned sites were dominated by secondary forest growth of *Hibiscus tiliaceus*, *Syzigium* sp., as well as bracken ferns.

For the rest of the paper, maintained and abandoned plantations will be referred to as plantation (P) and forest (F) type sites, respectively.

Field sampling

Even maintained coconut plantations require little maintainance. Thus, alterations that would affect C or N concentrations in other types of plantations, such as fertilization, or extensive burning, were not a concern. However, when a tree is cut or falls down, the stump is burned *in situ*. In order to avoid C associated with burning in my samples, I sampled multiple points in each site, never sampled where there had been a recent burn, and looked for signs of charcoal in my samples.

Samples were collected from five random points within the inner 900 m^2 of each site. At each site I excavated four 30x30x25 cm^3 pits, and one deeper pit measuring 30x30x35 cm^3. From all five pits I collected volumetric bulk density and root cores from 0-10 cm and 10-20 cm depth. In sites with high rock contents, I cored until obstructed by rock, removed the rock, recorded and subtracted the rock depth from the core depth, and continued coring.

Where full 10 cm cores couldn't be taken due to rocks, I collected samples of unknown volumes of soil from full 0-10 and 10-20 cm increments, to be later used for determination of C and N. In the deep pit, I collected soil samples of unknown volume from 20-30 cm for the purpose of determining C and N concentrations at greater depths in the profile. These measurements were later abandoned in the interest of time.

Laboratory analyses

Soil bulk density was determined by weighing volumetric cores, then drying a subsample to constant weight at 105°C to determine water content and dry weight of the total sample. The remaining soil was air-dried and sieved through 2-mm mesh to be used for C analysis later. Root cores were washed over a 2mm sieve to remove soil from the coarse root fraction. Roots were dried at 65°C until constant weight.

Rock content was determined by estimating percent cover of the greater than 5 cm fraction across two walls of the pit. Percent of smaller rock fragments (2mm-5cm), which were less detectable by eye in the profile, was determined by sieving soil through 2mm mesh. Soil pH was determined using a 2.5:1 solution of deionized water and air-dried, sieved soil.

Soil C and N concentrations from three samples per site were determined by combustion on a CE Elantech CN Analyzer. Total soil C and N pools were determined by multiplying percent concentration by soil bulk density, then correcting for approximate rock fraction (> 2mm) of the 10 cm depth increments. Dry root weight was divided by two to yield C weight per volume (Schlesinger 1997).

Data analysis

Dependent variables of pH, C and N concentrations, C and N stocks, and root C content were plotted against independent variables of site pairs (1, 2, and 3), depths D1(0-10 cm) and D2 (10-20 cm), site types plantation (P) and forest (F), and interaction between site type and depth. For normally distributed and equally variable data I used ANOVA. I tested for unequal variances using Bartlett's test. The non-parametric Wilcoxon Rank Sum test was used for data with unequal variances. All data was normally distributed with the exception of root C data, which normalized under a log transformation.

Results

All values are reported as means ± one standard error of the mean. Differences are reported as significant for p values < .05.

Bulk density

Bulk density cores varied between 4 and 10 cm depth in all sites except for one maintained (site 5) and one forest site (site 6), in which most of the cores were taken to a depth of 10 cm. Failure to obtain full 10 cm- deep cores mainly occurred due to rock interference. High variation in core depth made it difficult to compare bulk density between site pairs, site type, and depth. C and N pools were calculated using average bulk density values from sites 5 and 6 for plantation and forest sites, respectively. I used 0.83 g/cm^3 for plantation

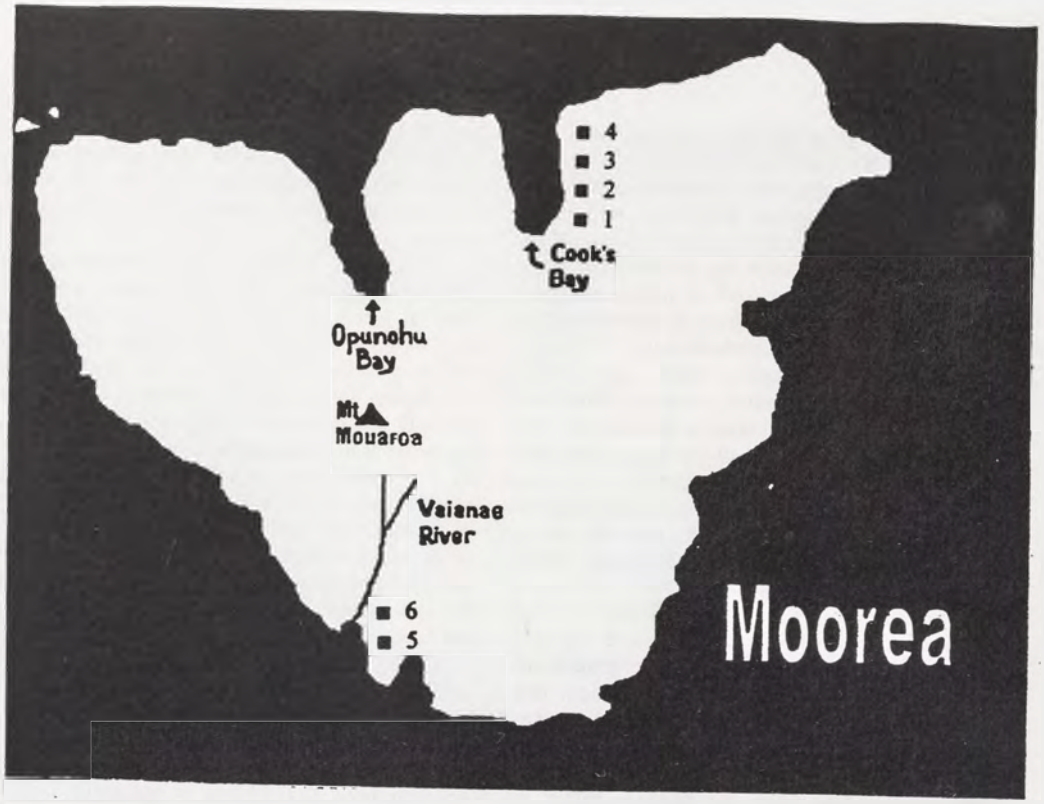

Figure 1. Map of site locations on Moorea.

Pair	Site #	Type	Location	Vegetation
1	1	P	p.k. 8.4*;	*Cocos nucifera;* grasses
1	2	F	p.k. 8.3*	*C. nucifera; H. tiliaceous; Syzigium sp.*
2	3	P	p.k. 7.7*	*C. nucifera;* grasses
2	4	F	p.k. 7.6*	*C. nucifera; H. tiliaceous*
3	5	P	p.k. 21.5**	*C. nucifera;* grasses; sedges;
3	6	F	p.k. 21.3**	*C. nucifera; H. tiliaceous; Syzigium* sp.

Table 1. Site information. P= plantation site; F= forest site; P.k. = Pointe kilometrique (kilometer mark).
* North of the airport (p.k. 0)
** South of the airport

soil bulk density and 0.78 g/cm³ for forest soil bulk density.

C and N concentrations

Carbon percent is significantly higher in plantation (3.51 ± 0.29) than forest (2.86 ± 0.30) sites (Figure 2). Percent C decreases with depth in both F (D1 = 3.90 ± 0.15; D2 = 1.82 ± 0.28) and P (D1 = 4.17 ± 0.38; D2 = 2.84 ± 0.31) sites. There is no interaction between site type and depth, nor a significant difference between site pairs.

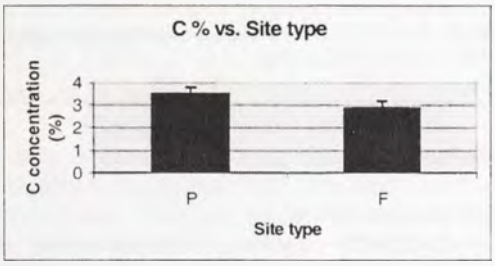

Figure 2. Decrease in C% between plantation (P) and forest (F) sites. P = 3.51 ± 0.29 (mean ± std err); F = 2.86 ± 0.30.

C and N are highly correlated in both P (r^2= 0.90, p<.0001)and F sites (r^2= 0.95, p<.0001), thus N trends are similar to C trends. Plantation soils (0.33 ± 0.03) show significantly greater (Figure 3) N concentrations than forest soils (0.27 ± 0.02). N decreases with depth in F sites, but shows no significance in P sites. When forest and plantation values are pooled, N concentration shows a decrease with depth (p<0.0001). Pair 3 soils contain significantly higher N concentrations (0.35 ± 0.04) than pair 1 (0.27 ± 0.03) or pair 2 soils (0.28 ± 0.03) (Figure 4). Pair 3 was located on a different part of the island than the other two pairs, which were located within approximately the same 3 square kilometer area.

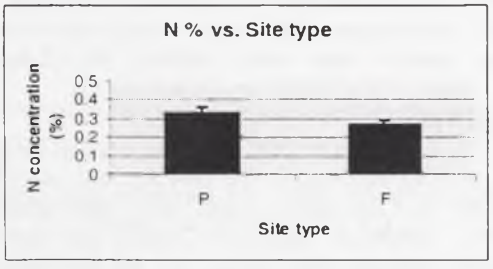

Figure 3. Decrease in N% between plantation and forest sites. P = 0.33 ± 0.03; F = 0.27 ± 0.02

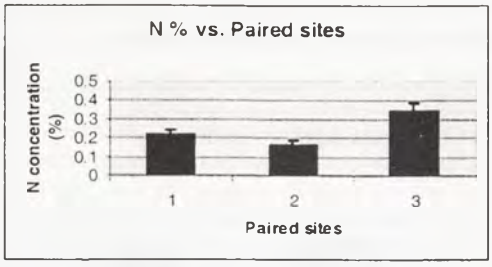

Figure 4. Difference in N% between site pairs. Pair 1 = 0.27 ± 0.03; Pair 2 = 0.28 ± 0.03; Pair 3 = 0.35 ± 0.04

C and N pools

Soil C and N pools are calculated by multiplying C and N concentrations times bulk density, then subtracting rock fraction. Average bulk density values from site 5 (0.83 g/cm³) were used for all plantation stocks and from site 6 (0.78 g/cm³) for all forest stocks. Plantation SOC stocks are higher (2.35 ± 0.18 kgC/m²) than forest stocks (1.80 ± 0.20 kgC/m²) to a depth of 20 cm (Figure 5), representing a difference of approximately 0.17-0.93 kgC/m². C stocks decrease with depth in both plantation and forest sites. Plantation soils store 2.79 ± 0.23 kgC/m²in the 0-10 cm interval and 1.90 ± 0.18 kgC/m² in the 10-20 cm interval. Forest values are 2.46 ± 0.18 kgC/m² in the 0-10 cm interval and 1.15 ± 0.19 kgC/m² in the 10-20 cm interval. C pools show no significant difference between site pairs.

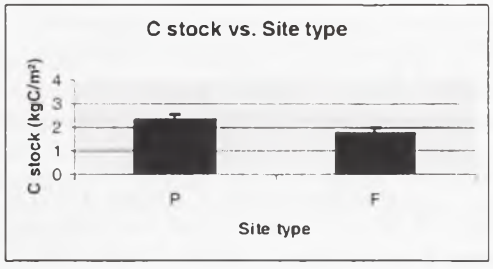

Figure 5. Decrease in C stock (kgC/m²) between plantation and forest sites. P = 2.35 ± 0.18; F = 1.80 ± 0.20.

N stocks in plantations are also higher (0.22 ± 0.02 kgN/m²) than in forests (0.17 ± 0.02 kgN/m²) to a depth of 20 cm, representing a difference of .01-.09 kgN/m² (Figure 6). N stocks decrease with depth in both plantation and forest sites. Forest values are 0.22 ± 0.01 kgN/m² in the 0-10 cm interval and 0.12 ± 0.02 kgN/m² in the 10-20 cm interval. Plantation soils store 0.25 ± 0.02 kgN/m² in the 0-10 cm interval and 0.19 ± 0.02

kgN/m² in the 10-20 cm interval. N pools show no significant difference between site pairs.

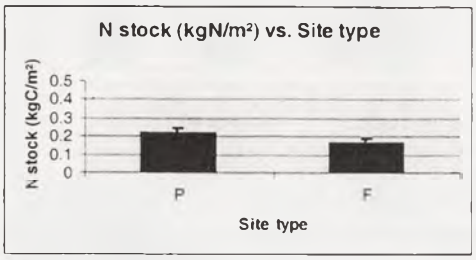

Figure 6. Decrease in N stock between plantation and forest sites. P = 0.22 ± 0.02; F = 0.17 ± 0.02 kgN/m².

C/N ratios

C/N ratios decrease with depth (D1 = 11.20 ± 0.32; D2 = 9.07 ± 0.47) in forest sites (Figure 7), but show no significant changes with depth in plantations. C/N ratios do not differ between plantation and forest, nor between paired sites.

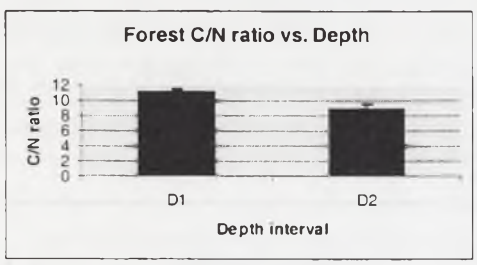

Figure 7. Decrease in C/N ratio with depth in forest sites. D1 = 11.20 ± 0.32; D2 = 9.07 ± 0.47.

Root C

Log-transformed root C content within 0-10 and 10-20 cm interval show no difference between P and F sites. Within F sites, root C (kgC/m²) decreases with depth (D1 = 2.39 ± 0.14; D2 = 2.13 ± 0.15). Within P sites root C also decreases with depth (D1 = 2.37 ± 0.09; D2 = 1.94 ± 0.15).

Soil pH

Mean soil pH is significantly higher in forest (5.09 ± 0.06) than plantation (4.79 ± 0.12) sites (Figure 8). Differences between the three site pairs are significant as well. Both F and P sites from pair 2 show the highest pH. Soil pH does not significantly differ with depth in either P or F sites, and no interaction was found between site type and depth.

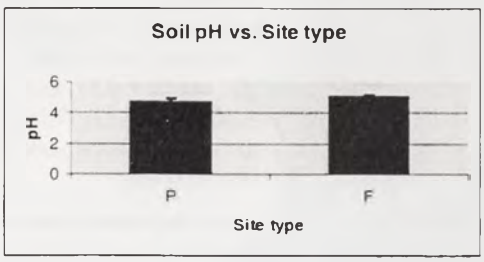

Figure 8. Lower soil pH in plantation sites than forest sites. P = 4.79 ± 0.12, F = 5.09 ± 0.06.

Discussion

C and N concentrations

Increased carbon concentrations in maintained plantations concur with previous findings that grass ecosystems can effectively contribute to and maintain significant carbon content, at least in surface soils (Neill *et al.* 1996). In such cases, annual fine root biomass turnover was a major contributor to high pasture SOC in surface soils. In other studies, however, higher SOC has been found at depths greater than 1m in forests due to deep root C cycling (Trumbore *et al.* 1995). I did not measure changes in fine root biomass in this study, nor did I investigate deep soil C cycling. Thus I have no mechanism to explain the measured decrease in surface SOC under secondary forest growth.

The common correlation found between C and N concentrations in nature easily explains the higher N percent in plantations. Additionally, plantation N concentration values were mainly raised by the P site from pair 3. This site contained a variety of unidentified herbaceous plants, some of which may act as nitrogen fixers. It showed higher N concentrations than plantations in pairs 1 and 2, which appeared to consist of only one or two grass species that were unlikely N- fixers.

C and N stocks

Estimates of SOC stocks in tropical forest and savanna ecosystems are 10.4 kgC/m² and 3.7 kgC/m² to 1 m depth, respectively (Schlesinger 1977). However, tropical C stocks are highly variable, ranging from 2.3 to 21.7 kgC/m² within primary tropical forests alone (Moraes *et al.* 1995). I found that although C concentrations decreased by 19% under secondary forest growth, C stocks decreased by 23%.

N stocks similarly decreased by a greater amount (23%) than N concentrations (18%) from plantation to forest. This follows closely with other studies that have shown parallel decreases in N where decreases in C occurred (Hughes *et al.* 1999; Matzek 1999).

The higher decrease in stocks than in concentrations for both C and N is associated with higher rock content in forest soil profiles. Rocky plantations are harder to maintain, are subsequently abandoned, and become sites of secondary forest growth. Thus, when calculating soil C stocks, rock content further reduces the lower storage capacity of secondary forest soils.

C/N ratios

Higher C/N ratios are associated with woody vegetation. Forest floor organic matter typically contains both leaf litter and wood from fallen trunks. The high density of woody debris at the surface accounts for a higher C/N ratio in the 0-10 cm fraction of the forest soils. Grass biomass consists of lower C/N ratio, and thus exhibits a less distinguishable difference with depth.

Root C

Coarse roots to a 20 cm depth decrease more rapidly in plantation than forest sites. However, the combination of high density finer grass roots with the lower density, but larger, *Cocos nucifera* roots in plantation soils make root C content differences between forest and plantation sites undetectable. Differences between site type may have been detectable if fine roots (<1mm) were compared instead of the coarse root fraction I measured. Coarse root C content, unlike fine root biomass, is not incorporated as soil organic carbon annually; it contributes to belowground C storage over longer timescales.

Soil pH

Low soil pH can be attributed to a high amount of organic matter commonly associated with forest ecosystems. Forest soils generally yield lower pH than grasslands, at least in surface soils, due to the acidic nature of decomposing leaf litter. However, soil pH trends differed in this study, suggesting that the use of ammonium instead of nitrate by plants, or increased leaching of base cations may play a larger role.

Conclusion

Secondary forest growth in plantations that are abandoned due to high rock content results in a loss of carbon storage in surface soils. This contradicts previous studies that suggest C and N stocks increase following abandonment of pasture or agriculture to secondary forest. However, it supports other findings that land quality plays an important role in soil carbon dynamics.

Although we see losses in soil carbon stocks with secondary forest growth, overall ecosystem C stocks may be higher than in maintained plantations due to greater C storage in aboveground biomass. Future research should focus on more comprehensive look at ecosystem C cycling, including losses from soil respiration (CO_2), differences in fine root biomass, aboveground C stocks, and deeper soil C studies.

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Dynamics of Coarse Riparian Detritus in a stream in Moorea

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ABSTRACT. The standing crop, or input, of coarse particulate organic matter (CPOM) was measured in three different streams on Moorea, French Polynesia. This study was conducted during the end of the dry season and in the beginning of the rainy season. Measurements were taken on two consecutive days at one week intervals. Results were divided into two categories, one day and six day accumulation periods. Each of the streams studied differed in canopy cover, in the composition of the surrounding vegetation and in the daily rainfall received. At all three sites, significant relationships were found between discharge and collected leaves of *Hibiscus tiliaceus*. Significant statistical relationships were also found between discharge and other different types of detritus. No significant relationship was found between the discharge and the total biomass collected for any of the three streams. In one site, relationships were found between canopy cover and biomass collected. In all three sites the amount of *Hibiscus tiliaceus* was found to increase as the canopy cover increased. During rain, stream discharge increased by up to 1500%. Stream discharge and precipitation were measured independently and were found to correlate after statistical analysis. These results suggest that precipitation affects the stream discharge. Finally, the comparison of one day and six day accumulation data for each site suggested that the stream benthos has a maximum capacity for retention of CPOM. Results imply that the proposed maximum capacity is reached in one day or less.

Introduction

The lush riparian vegetation is a constant source of coarse particulate organic matter (CPOM), in tropical streams (Resh et al 1990). Leaves enter the streams all year round and serve as nutrients to many organisms. Resh et al (1995) found that the discharge patterns of many tropical island streams are characterized by frequent spates generated by torrential rainstorms, which dislodge and transport benthic detritus downstream, reducing retention time and degree of processing.

After a reduced stream discharge before rain, retention time of riparian detritus is expected to be greater due to reduced flow in the streams. Fragments of leaf litter tend to accumulate in pools or slow reaches of streams, sometimes producing accumulations rich in microbiota, and that anaerobic decomposition often occurred within the thick layer of debris. Floods scour the stream beds, transporting fragments further downstream and into the ocean, where released nutrients contribute to algal productivity in the estuary, with particulate matter being directly consumed by estuarine organisms (Archer, 1983). Resh et al. (1995) found that variable discharge has considerable habitat impact, particularly in smaller streams.

Larned (2000) commented that patterns of retention, transport, and processing of riparian detritus in stream are closely related to discharge. Reduced stream discharge during droughts may increase retention time of riparian detritus relative to normal flows.

Benthic detritus is often used as an indication of nutrition level in streams. Detritus may be categorized into leaves, stems, fruit, flowers and miscellaneous detritus such as leaf fragments, lichens and bryophytes.

This study was designed to examine the relationship between the CPOM and rainfall in a group of Moorean streams, French Polynesia. The main goals of this study were: a) To determine the relationship between the weight of riparian detritus and the amount of rainfall, b) To investigate the effect of stream discharge on the detritus collected, c) To examine the effect of canopy cover on the benthic detritus, d) To examine the effect of rainfall on discharge, e) To observe and quantify the variation of benthic detritus over time and f) To sort the CPOM into categories.

Methods

Studies on CPOM were carried out from 26 September to 17 November, 2000. Three streams on Moorea, French Polynesia were studied. The island of Moorea consists of a volcanic core surrounded by coral reefs. Moorea has approximately 20 river basins and they are arranged radially around the island.

For this study data were collected for the following variables; precipitation, biomass collection, discharge, canopy cover and leaf cover. The period of data collection included four weeks of the dry season followed by two weeks of the rainy season.

Figure 1. Map depicting study sites.

Study Sites

Site 1 - Opunohu River (lat 17°32'25.2"S, long 149°49'36.5"W), is accessed via a short trail approximately 70 meters east of the Belvedere lookout parking lot. The site is approximately 335 meters above sea level, and is 4 km from the ocean. The average slope covering the three plots at this site is 35°. The canopy is 2.5-5 m above the stream. The studied stream is one of the sources to the Opunohu River. This study site was not in an inhabited area.

Site 2 – Afareaitu River (lat 17°32'34.7"S, long 149°48'27"W), the site is 12 meters from the Afareaitu Cascade. The site is approximately 2.5 km from the ocean. The canopy was 4-6 m above the stream. The average slope covering the three plots at this site is 25°. There are no houses bordering this river. One plot had no canopy cover (see table 1).

Site 3 – Unnamed river at Haapiti (lat 17°32'58.4"S, long 149°52'52.5"W). The site examined was 90 meters from the mouth of the river. The canopy was 7 to 10 meters above the stream. The stream has no canopy cover 300 meters upstream from the site. The sides of the stream were populated and farms are located above it.

Table 1. Description of canopy cover according to site and plot.

Site	Plot	Canopy cover (%)
1 Opunohu	1	63
	2	81
	3	87
2 Afareaitu	1	0
	2	37
	3	90
3 Haapiti	1	47
	2	41
	3	79

The dominant riparian trees in all sites are *Hibiscus tiliaceus* and *Inocarpus fagiferus*. These trees are evergreen and are the source of most of the riparian detritus. *Hibiscus* is a low growing tree with large, heart shaped, flexible leaves. *Inocarpus* has oval shaped. *Artocarpus altilis* has pinnate leaves over 16 inches in length, this tree bears green fruit (bread fruit) three times a year. *Angiopteris evecta* is fern whose 9-12 feet long fronds are fixed to a 7 feet thick rough stem.

Precipitation

Precipitation data, for all three sites, was provided by Meteo France, of Tahiti. Meteo France has seven sites of precipitation data

collection on Moorea, three of which coincide with the sites used in this study.

Biomass Collection / Detritus Level

The three sites were selected to represent different areas of the island. The Opunohu site and Afareaitu site are on the wet side of the island and Haapiti is on the dry side of the island. For each of the three streams three plots were selected. Using non-toxic permanent markers, and flags these plots were laid out exactly at the same place for each measurement during the entire 6 week study. The first plot at each site was chosen randomly and the second and third plots were defined downstream, at 20 and 40 meters respectively. The plots were rectangular in shape, each plot had an area of 10 square meters.

One day and six day accumulations of detritus were collected on a weekly basis at each site. For example, at the Belvedere site collections were made on Mondays and Tuesdays. This allowed a one day accumulation from Monday to Tuesday and a six day accumulation from Tuesday to the following Monday.

Effectively all CPOM was removed at each collection. Every leaf, stem, flower, and piece of fruit were collected inside each plot. The detritus was brought back to the UC Gump station lab where it was separated by species and color (green or brown). The detritus was then dried under the sun for 24 hours followed by oven drying for a further 48 hours at 60°C to 70°C. The dried material was then weighed.

In order of abundance the following types of detritus were collected: leaves from *Hibiscus tiliaceus* and *Inocarpus fagiferus* (these two accounted for 99% of all CPOM collected), leaves from *Artocarpus altilis* and *Angiopteris evecta*, *Hibiscus tiliaceus* flowers and *Inocarpus fagiferus* fruit. Stems were not removed from leaves. The frequency of branch appearance was low and all size branches were removed from the plot. Branch weight was not recorded. Branches were not present at every collection.

Discharge

Discharge was measured by recording the time taken for a floating object to travel a predetermined distance. Using a transect tape, the average cross-sectional area was determined for the length of the stream being used to measure discharge. Discharge was then calculated in m^3/s. Discharge measurements were made during each collection. One reading was made at each site, that value was used for all three plots.

Canopy Cover

Canopy cover was taken as constant for the duration of the study.

Flower Count

At each collection, flowers were counted and categorized by species, color and state (fresh or brown).

Leaf Cover

The leaf coverage was estimated using a quadrat before each collection was made.

Results

Regression analyses were performed to determine the correlation between: 1. Discharge and biomass, *Hibiscus*, *inocarpus*, ground cover and precipitation. 2. Precipitation and biomass, *Hibiscus*, *inocarpus* and ground cover. 3. Canopy cover and biomass, *Hibiscus*, *inocarpus*, and discharge. These analyses were applied to the data for all sites combined and also for each site individually. One day and six day accumulation data were treated separately.

Discharge

For all three sites (combined) discharge was found to correlate with the weight of *Hibiscus* leaves (corr. = -0.309, $p = 0.023$). Discharge also correlated with leaf cover for the Belvedere site (corr. = -0.633, $p = 0.015$). These sets of data both show a negative correlation suggesting that as discharge increases the amount of detritus in the streams decreases. Average discharge was found to increase during the last two weeks of this study. Specifically, there was a 480% discharge increase for the Belvedere site, an 800%

increase for the Afareaitu site and a 1500% increase for the Haapiti site.

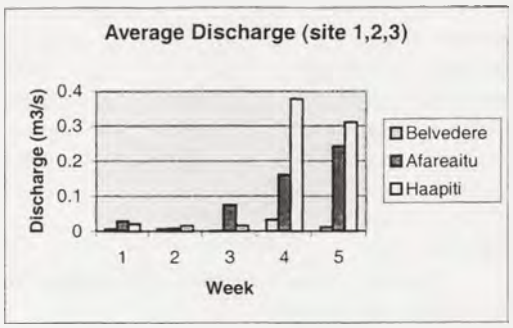

Figure 2. Histogram of average discharge per week, for each of the 3 sites.

Precipitation

Using the precipitation data, from all three sites combined, a significant correlation between precipitation and the weight of *Inocarpus* collected was found (corr. = -0.469, p = 0.006). As precipitation increases the weight of *Inocarpus* decreases. Anomolous data was collected. On one occasion a precipitation level of 37 mm at the Belvedere site resulted in no detritus collection. On one day a precipitation level of 46.2 mm at the Afareaitu and Haapiti sites gave precipitation data positively correlated with the weight of *Hibiscus* (corr. = 0.803, p = 0.009).

Discharge and Precipitation

Regression analyses on discharge and precipitation on all sites combined and on the Afareaitu site alone showed a positive correlation of (corr. = 0.556, p = 0.001) and (corr. = 0.911, p = 0) respectively. Individually the Belvedere site and the Haapiti site did not show significant relationships.

Canopy Cover

A significant relationship was found between canopy cover and *Hibiscus* leaves for the combined sites. The correlation was found to be 0.51 with a p-value of 0.025. At Afareaitu, canopy cover was found to correlate with the total biomass, the correlation value was 0.76 with a p-value of 0.002. Negative correlations between canopy cover and total biomass and also between canopy cover and *Hibiscus* were found at the Belvedere site. The correlation between canopy cover and biomass and between canopy cover and *Hibiscus* were -0.384 and -0.269 respectively.

Detritus Level

The weight of leaves accounted for more than 99% of the detritus input to the study areas. *Hibiscus tiliaceus* and *Inocarpus fagiferus* were the dominant riparian trees. *Inocarpus fagiferus* accounted for 42% and *Hibiscus tiliaceus* accounted for 57% of the total detritus collected. In all three sites the average weight of detritus was found to decrease towards weeks 5 and 6 (figure 3). This pattern coincides with the data for rainfall.

Sorting of detritus into green and brown categories was not useful, it was found that the change of color of green leaves was rapid. Collected green leaves turned brown in about a day. The weight of green detritus were therefore added to the weight of brown detritus.

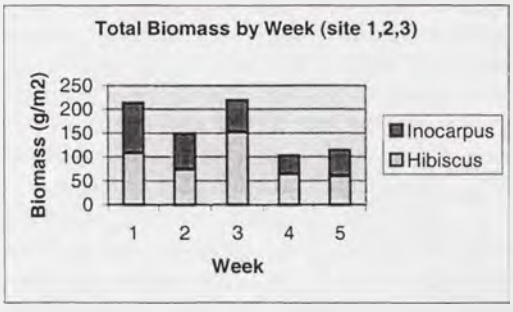

Figure 3. Histogram of the total biomass, combined for all of the three sites, per week.

Flower Count

Although by sight the flowers in the streams were found to correlate with the amount of detritus picked up during the same collection, flower dry weight was not used for data analysis. Flower count was found to be

more reliable than flower dry weight due to the weight loss of the *Hibiscus tiliaceus* flower during drying. The wet weight of one flower was equal to the dry weight of nine flowers. These masses were all less than one gram.

Leaf Cover

At the Belvedere site and at the Afareaitu site the percent leaf cover in the streams was positively correlated to the dry weight collected (corr. = 0.62, corr. = 0.73) respectively. The combined data for the three sites did not show a significant correlation with leaf cover (corr. = 0.19), this is due to the factors that govern leaf cover on the ground. Visual comparison of all the three sites indicates that they differ significantly in their canopy cover, in the detritus they can retain and in the rainfall they receive. A three way ANOVA was made on the three sites showed no significant variance.

	One day	Six days
Minimum	0	0.06
Maximum	17.87	16.71

Table 2. Maximum and minimum weight of detritus after one day and six day accumulations at the Belvedere site on week 2.

Discussion

Discharge

The statistical results obtained for all sites show a negative correlation between discharge and the weight of detritus collected in the streams. This finding demonstrates that as discharge increases the amount of detritus in the stream decreases. This can be explained by the increased speed of the water moving down the stream, which has more capacity to remove the detritus. Reduced stream discharge during droughts may increase retention time of riparian detritus relative to normal flows. In addition, water stress in riparian vegetation and subsequent leaf abscission may increase detrital leaf input to streams (Melick 1990).

Precipitation

Precipitation affects the level of benthic detritus, as precipitation increases less detritus is found in the plots. This effect is similar to the effect discharge has on the detritus level. An unexpected positive correlation was found between precipitation and *Hibiscus*. This finding suggests that other factors may affect the amount of detritus found in streams. The shape and the benthos of the plots also has an effect on leaf retention. This is illustrated in the correlation between leaf cover and biomass (corr. = 0.19, combined sites 1,2,3). This low correlation suggests that as the amount of biomass increases the leaf cover on the floor does not necessarily increase, field observations show that specific shapes of the benthos retain many leaves juxtaposed on each other keeping a low visual leaf cover on the floor but increasing the amount of detritus. These findings suggest that the shape of the plot is a factor that might influence the effect of precipitation on detritus.

Discharge and precipitation

Discharge directly correlates with precipitation, as precipitation increases discharge also increases. The response of discharge to precipitation is immediate, a greater discharge is noted on the same day as higher precipitation is measured. A data collection made on the fifth week at Afareaitu showed a 300% increase in discharge from $0.01 m^3/s$ to $0.3 m^3/s$ when precipitation increased from 8.65 mm to 46.2 mm. This correlation gives support to the validity of the chosen method for measuring discharge.

Canopy Cover

The statistical results obtained for all sites combined demonstrate that as canopy cover increases, CPOM also increases. This finding supports the observations of leaf abundance made during field work. This outcome would be expected as a result of direct litter fall from the canopy above. Two negative correlations were found at the Belvedere site between canopy cover and total biomass and canopy cover and *Hibiscus* leaves. These two values suggest that detritus level does not necessarily increase with canopy cover. These results

(suggest) that canopy cover above each stream is not the only factor that influences the detritus level. An important observation was made at Afareaitu where there was no canopy cover, there was on average one gram of CPOM per collection. This shows that with no canopy cover the plot still acquires CPOM. Therefore detritus in the streams does not come entirely from direct litter fall, but could come from floor flooding, upstream input or other sources.

Negative correlations between canopy cover and total biomass and also between canopy cover and *Hibiscus* were found at the Belvedere site. This unexpected result may be explained by the many other factors that control the amount of benthic detritus. One of which is the surrounding vegetation. Canopy cover only describes the density of leaves and branches directly above the plot. The number of tree species and their abundance around the plot also influences the input of detritus. At the Haapiti site no *Artocarpus altilis* trees were directly above the plots but there was an average of 3 *Artocarpus altilis* trees found within 3 meters of two of the three plots.

The method of measurement used does not adequately account for the great difference in mass of *Artocarpus altilis* leaves as compared to the *Inocarpus* and *Hibiscus* leaves. In this study an average *Artocarpus altilis* leaf was sixty times heavier than an *Inocarpus fagiferus* leaf and fourty times heavier than an average *Hibiscus tiliaceus* leaf. This fact means that each *Artocarpus altilis* leaf had a more significant influence on the data than if it had been treated as a single leaf in an analysis conducted according to leaf number. Using leaf number data may not be a valid technique either as the larger leaf of the *Artocarpus altilis* may occlude the space that would be occupied by many smaller leaves of the other species.

Leaf Cover

The riparian benthos differs from plot to plot and each plot has its own characteristics. For example at the Belvedere site one of the three plots contains an elevated *Inocarpus* tree root structure, this special condition facilitates retention of leaves in a pack. The leaves on the basaltic benthos do not have this obstacle and are constantly subjected to current.

Leaf cover was found to be unrepresentative of the total biomass of the stream. It did not show significant correlation with the total dry weight of the detritus and it would therefore be misleading in determining the weight of CPOM. However, it was useful in understanding the dynamics of the riparian detritus. Using the weight of detritus and its percent coverage, it is possible to differentiate structure of the benthic floor of the stream. A high level of detritus but a low leaf cover percentage suggests the presence of stacks of leaves.

An additional finding was made while observing these leaf stacks. A maximum capacity for retention of detritus is observed. The comparison of one day and six day accumulation data for each site suggested that the stream benthos has a maximum capacity for retention of CPOM, results imply that the proposed maximum capacity is reached in one day or less (Table 2).

Conclusion

In summary, this study has demonstrated that precipitation affects the dynamics of riparian detritus. The weight of detritus was found to decrease as precipitation increased during the study, as discharge increases and finally as canopy cover decreases. These factors are all involved in influencing the amount of benthic detritus amassed. Rainfall was also found to affect discharge as time progresses. Furthermore, a maximal retention capacity was observed and also that only one day was required to reach this maximum.

Future studies could be carried out during the dry season (from March to October). This proposed study would ensure that the data would be representative of the dynamics of the riparian detritus.

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Changes in Territoriality with Food Supplementation in a Land Crab, *Cardisoma carnifex*, on Moorea, French Polynesia

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ABSTRACT. In *Cardisoma carnifex*, a land crab abundant on Moorea, French Polynesia, individuals occupy and defend separate burrows and will often defend areas surrounding burrows. The resource they are thought to be maximizing is food, mainly I the form of overhanging hibiscus flowers and leaves. Food supplementation was conducted at four different levels: 60-100g, 100-140 g, 140-180g, and 180-220g. Territoriality showed no changes despite food supplementation. Individuals were not found to be feeding in close proximity to one another and no evidence of decreased aggression was observed. However, it was discovered that burrow placement correlated with a greater amount of natural food fall. It was also discovered that there was food preference for hibiscus flowers over green and yellow leaves. Size proved to be a factor in the ability to obtain food and larger crabs were able to feed at the exclusion of smaller ones. Therefore it is concluded that the burrows *of C. carnifex* are maintained for multiple purposes other than accessibility to food.

Introduction

Territoriality in animals emerges when there is competition for a defendable resource (Brown 1964). In many territorial species, food supplementation will lead to a decrease in competition and thereby, a decrease in territoriality. When this occurs, members of the same species may be seen in unusual proximity while foraging, due to decreased aggressive interactions. This has been shown in many different animals from the Blue-throated hummingbird, *Lampornis clemenciae* (Powers and McKee 1994) to the Meadow vole, *Microtus pennsylvanicus* (Fortier and Tamarin 1998). However, there are often multiple reasons to maintain territories. In cases where territories are maintained for breeding or for raising young, territoriality will not completely disappear regardless of resource supplementation. In a study of Rock Pipits, *Anthus petrosus*, (Askenmo et al. 1993) it was determined that "the breeding territorial system is much more rigid than predicted by existing theories".

Many members of the order Crustacea have been found to be territorial. For instance, the ocypodid crab, *Ilyoplax pusilla*, maintains intricate territory defenses such as baracade-building and burrow plugging for access to food and mates (Wada 1993). These behaviors have been reported in other ocypodid crabs such as *Ilyoplax ningopensis* (Wada et al. 1998). Territoriality has been well researched in the generas *Uca, Iloplax* (Wada 1993) *Pachygrapsus* (Bovbjerg 1960), and *Cardisoma* (Foale 1999).

In the terrestrial land crab, *Cardisoma carnifex*, individuals dig burrows, which are occupied and defended from other individuals. Areas surrounding the burrows may also be defended as well. These burrows suggest some type of resource partitioning. The resources that are being maximized might include shade, proximity to the ocean, mating, and access to food mainly in the form leaf and flower fall form overhanging hibiscus trees.

By increasing food availability in a certain population of *Cardisoma carnifex*, I am testing to see if territoriality decreases. I also interested to see if more crabs feed closer together with less incidents of fighting during food supplementation. This would indicate a decrease in territoriality despite an increase in potential interactions. I will also compare burrow placement with different environmental gradients, which may elucidate what resources *C. carnifex* is trying to maximize in the spatial distribution of their burrows. I also would like to test for food preference for hibiscus leaves or flowers in *C. carnifex*. In the case of the null, food supplementation and other environmental gradients will not change behavior or spatial distribution of crabs and burrows and preference in food is absent.

Land crabs have enormous effects on litter turnover and have a large effect on microbial organisms. They play a central role in the ecology of an area by controlling the rate of detritus processing and renewal of nutrients (Green et al. 1999). Cardisoma carnifex is no exception (Alexander 1979). Studies on behavior of Cardisoma have been limited mainly to *Cardisoma hirtirpes* (Foale 1999) and *Cardisoma guanhuni* (Staus and Mayer 1999). Much of the research conducted on *C.carnifex* is restricted to physiological studies (Morris et al.

1996). This study aims to elucidate some of the behavioral aspects of *C. carnifex*, an ecologically important organism.

Methods and Materials

Natural History

Cardisoma carnifex (Herbst 1794) is common in all parts of French Polynesia. The range of these crabs is restricted to the Indo-west Pacific extending from South Africa to Japan (Poupin 1994). *Cardisoma carnifex* construct burrows clustered in groups above the high tide line (Smith 1990) in sandy soils. Individual burrows are dug to the water table and can be up to 2 meters in length (Tarvd 1999). The crabs are crepuscular and must return to the ocean to reproduce. Reproductive cycles are timed by the new and full moon but *C. carnifex* has reportedly unpredictable spawning habits and no extensive breeding migration occurs (Foale 1999). Their diet includes fallen leaves, fruits and flowers and occasionally they have been known to eat small mammals and carrion (Woods and Boutelier 1985). They are also suspected of digesting microflora from the surface of sediment grains and leaves. The genus *Cardisoma* is known to be the least tolerant of dry conditions of the land crabs and for *C. carnifex* burrows must be maintained in an area with ground water year round and the species seems to need regular immersion in water (Bright and Hogue 1972; Burggren and McMahon 1988). In Moorea, these crabs are found by roadsides, in coconut plantations and under hibiscus groves, always close to the ocean's edge. They are a food source for residents in many parts of French Polynesia.

Study Site

The study was conducted from October 3, 2000 to November 7, 2000 on the property perimeter of the Richard B. Gump South Pacific Research Station owned by the University of California, Berkeley in Moorea, French Polynesia (Figure 1). Moorea, located at 17°30's and 149°50'W, is apart of the Society Island archipelago and lies 25km northwest of Tahiti. The research station is located in Cooks Bay on the northeast portion of the island. The study plot was located approximately 100 meters south of the Library, 150 meters north of the pk11 marker and was bordered on the east by ocean and the west by the main highway (Figure 2). The station for most of the year does not maintain the study site

A study plot was chosen based on the merits of accessibility, visibility, and abundance of crab holes. A 6 X 6 meter plot was delineated and all crab burrows were mapped including all holes on the borders (Figure 3). There were approximately 63 holes on the plot.

Recording of Observations

Cardisoma carnifex is very sensitive to human intrusion and will quickly retreat in burrows if human presence is sensed. However, with care and patience, observation of these crabs is possible with seemingly little effect on crab behavior. For all observation intervals the observer was seated on a ladder or a chair placed where the entire plot was visible. Observation occurred during daylight hours between 9am and 6:20 pm. During an observation period, the paths of all individual crabs were drawn on the burrow map. Where one crab crossed the path of another crab the area of intersection was approximated and was called the " path overlap". The total value of all "path overlap" was calculated for each observation period. "Path overlap" represented the potential for interaction among the crabs. A twine grid was used to approximate spatial scale.

Territorial behaviors such as fighting and dominance were recorded. A fight was considered by the observer to have taken place when one crab chased another crab and/ or uses its claw to attach and pinch it's opponent. Fighting interactions were brief and each separate chase and/or attack was denoted as a separate fight. Fighting was recorded under two sub categories: Fighting for territory or fighting for food. These categories are differentiated by the presence of food and by an attempt by one or both of the crabs to obtain it. A fight motivated by territory defense occurred when there was trespassing in the absence of food with no obvious attempt to gain food.

Dominance in feeding was recorded when one crab was able to feed at the exclusion of other crabs. Common dominance in feeding behaviors was characterized in two ways. "Big over small" represents a behavioral event when a larger crab wins over a smaller crab. "Small over big" represents the opposite case. Dominance in feeding can be demonstrated in the following ways: 1) one crab will win the leaf due to speed and agility 2) one crab will be allowed to feed by the passivity of the surrounding crabs. Dominance in this case is defined by the ability to feed. This data was used later in a chi-squared test to see whether size affected the ability to feed.

All new burrows that appeared on the study site were added to the master burrow map. The date the hole appeared or disappeared was recorded. The movement of crabs on the plot was later tested using a chi-squared test to see if more crabs moved onto the plot after food supplementation started.

Data was recorded for one week without food supplementation to establish a baseline of behavior. Observational periods

Figure 1. Map depicting the Richard B. Gump South Pacific Research station on the Island of Moorea, French Polynesia

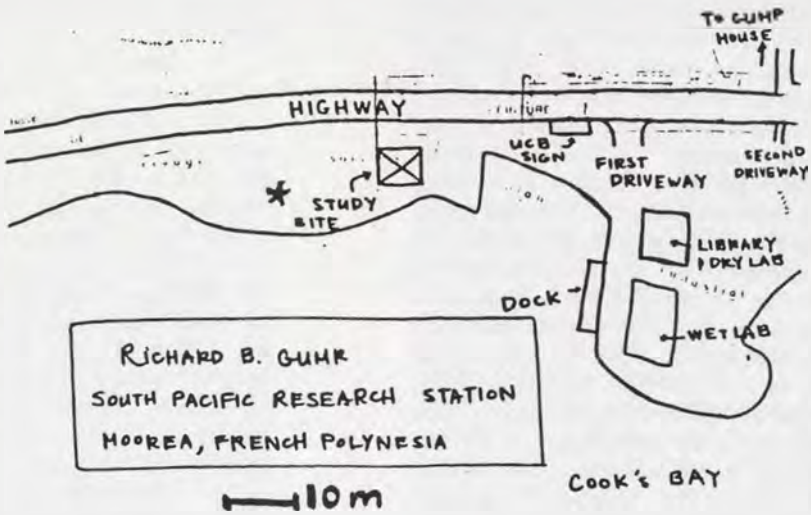

Figure 2. Map depicting the study site on the property of the Richard B. Gump South Pacific Research Station.

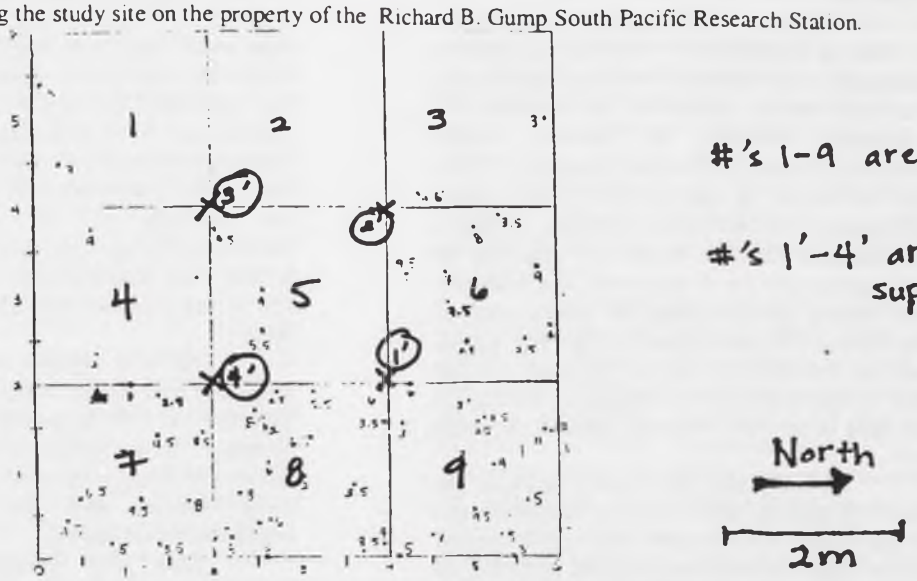

Figure 3. Map depicting crab burrows, zones of burrow placement, and food supplementation areas.

during this time were conducted at all times during the day. The times with the greatest amount of "path overlap" were chosen because of the increased crab interactions. Only data from that time period was used to make comparisons between observations collected during different food supplementation levels.

Burrow placement

To determine the effects environmental gradients had upon the spatial distribution of crab burrows, the study plot was divided into 9 sub-plots, each 2 by 2 meters in size (Figure 3). Natural food fall rate, shade, and proximity to water was determined for all of these zones. Crab holes were counted using the earliest crab map as to avoid complications of possible crab burrow movement due to food supplementation.

Food fall rate was defined as the total number of leaves and flowers that fell naturally during an observation period of one hour. Natural leaf or flower falls was recorded with "x" marks in red or blue on the burrow map. The rate of natural food fall on each sub plot was plotted against the number of crab burrows using Systat's linear regression program.

Shade from overhanging trees was drawn on the burrow map between the hours of 11a.m.- 4p.m. This time period was chosen because it included the hottest parts of the day and therefore, represents the crab's greatest susceptibility to water stress. Averaging the percent of cover generated a Shade Index for each sub plot. A linear regression using Systat was used to see if shade affected the number of holes on each sub-plot.

To evaluate each sub-plot's proximity to water a ranking system was used. A number from "1" to "3" was assigned to every subplot where "1" represents the closest to the ocean and "3" is the farthest. This gave a general picture as to whether placement of burrows was influenced by the proximity to ocean.

Food supplementation experiment

Crabs were observed mainly to be feeding on hibiscus vegetation that would drop from the overhanging canopy. During supplementation, food was made available to the crabs in the form of hibiscus leaves and flowers collected outside the plot. There were 3 categories of food provided: green hibiscus leaves, yellow hibiscus leaves, and flowers. Food was set out at four points in the plot chosen for symmetry to ensure that food was spaced evenly throughout the plot (Figure 3). Prior to supplementation food was weighed and the same number of leaves and flowers were set at each point. The overall weight of the food at each point was also measured so it would fall within a weight range. This was done so the same amount of food was distributed at each of the four points in the plot. After the food was placed in the designated areas the researcher would retreat to the observing station and observe for approximately one hour. At the end of the hour all supplemental leaves and flowers remaining were recorded.

Food supplementation occurred at different levels. The amount of food provided at each point was 15-25 g (consisting of 2 green leaves, 2 yellow leaves, 2 flowers), 25-35g (3 green leaves, 3 yellow leaves, 3 flowers), 35-45g (4 green leaves, 4 yellow leaves, 4 flowers), and 45g-55g (5 green leaves, 5 yellow leaves, 5 flowers). Food supplementation was conducted for at least five days at one level before moving on to another level. The supplemental increase in food by number of pieces was 31% -78%. These different levels of food augmentation, were conducted in succession to each other with no period of delay. Crabs tend to take an entire leaf or flower to their burrow before consuming it. Therefore it seemed reasonable to deal with number of pieces rather than actual grams.

Linear Regression using the Systat program was employed to see if there was any trend between food supplementation and "path overlap" or food supplementation and fighting. All averages were from approximately the same time of day.

To test for food preference a chi squared test was performed to see if flowers, yellow leaves and green leaves were consumed without preference.

Results:

General Observation

Anywhere from 6 to 36 crabs were observed during the observation periods. During food supplementation there appeared to be many more crabs above ground at one time where before food supplementation crab appearance was more staggered in time. The time it took for the crabs to reemerge from their holes generally decreased throughout the study either because of decreased sensitivity on the crabs part or increased skill on the observer's part. The general territory of a crab was confined to the crab's actual burrow while some crabs would defend the area just outside and others permitted other crabs to walk over the opening of their burrows. There was only one crab per burrow. On the few occasions that I did witness another crab go down a burrow that was inhabited by another crab, it was quickly chased out. Only under extenuating conditions, i.e. an intruder, would a crab enter some other crab's hole.

Data from seventeen hours of observation without food supplementation was graphed on a bar graph to try to determine if there was a time the crabs were more active (Figure 4). It seemed reasonable to assume that the greater the amount of path overlap area, the greater the movement of the crabs. The midpoint of observation periods was found and was then rounded to the nearest hour. The number that resulted was graphed against the amount of path overlap. The following graph illustrates that the crabs were the most active in a 24h day, between 15h to 18h. All observations following this period were done between these times.

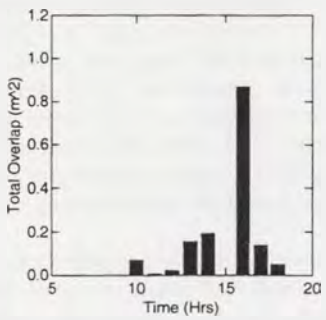

Figure 4. Bar graph depicting Crab Activity

I wanted to investigate the relationship between size and the ability to feed. I used a Chi squared test, or a goodness of fit test, to test the null hypothesis that size is not a factor in the ability to feed. If the null hypothesis is correct the number of times a larger crab was able to feed exclusively would be the same number of times a smaller crab could feed exclusively. This proved not to be the case (X^2=12.75, p< .05) and, in this case, it is three times as likely that a larger crab will feed exclusively.

	Big/Small	Small/Big	sample size
Observed(f)	37	12	
Expected(f')	24.5	24.5	
			49

Figure 5. Chi squared chard for size related dominance

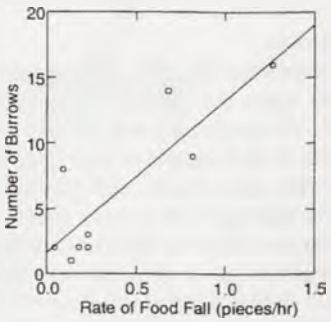

Figure 6. Linear regression with significant correlation between the number of burrows and the rate of food fall.

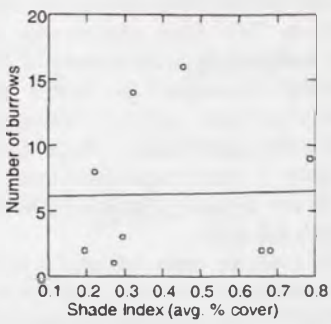

Figure 7. Linear regression depicting no correlation between the number of burrows and the Shade Index

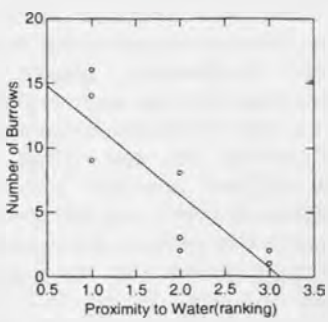

Figure 8. Positive linear regression showing the correlation between the number of burrows and the proximity to the ocean

Burrow placement is affected by the natural food fall rate of an area and the proximity to the ocean but not by the amount of shade an area receives.

Figure 6 depicts a positive correlation between the natural food fall rate an area receives and the number of crab holes in that area. I performed a linear regression with food fall as the independent factor and the number of crab burrows as the dependent factor and obtained p values of .003 and R^2 values of 0.753. Figure 7 is a linear regression that was completed using the number of crab holes as an independent variable and the Shade Index as the dependent variable. The results showed that shade was not an influence on the placement of crab burrows (p value = .692, R^2 value= .024). Figure 8 depicts a linear regression run using the number of holes as the dependant variable and the proximity of the ocean as the independent variable giving significant results (p=.003, R^2=.747). This indicates that proximity to the ocean is a factor in the placement of burrows.

Food Supplementation

Territoriality, path overlap, and changes in new burrows were unaffected by food availability. A linear regression was run with the level of food supplementation as the independent variable and total fighting rate as the dependent variable statistically insignificant values were obtained (p=.471 , R^2 =.026). When fighting was divided into two different components, fighting over food (p=.164, R^2=.529) and fighting for territory (p=.337, R^2=.302), the results were insignificant. Both linear regression analyses used fighting as the dependent factor and food supplementation as the independent factor. A linear regression using path overlap as the dependent variable and food supplementation as the independent variable was completed showing no correlation (p= .287, R2=.008). Please refer to figures 9 and 10 for scatterplot graphs of these results.

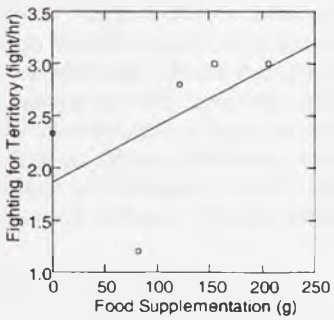

Figure 9. Scatterplot graph showing no correlation between fighting for territory and the amount of food available.

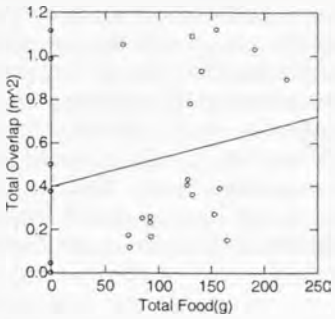

Figure 10. Scatterplot graph showing no correlation between path overlap and food availability.

It was expected that the crabs would be feeding in closer proximity to each other due to decreased territoriality during food supplementation. This means an increase in the potential for agonistic behavior but a decrease in agonistic behavior, a negative correlation. This proved not to be the case. When path overlap, a measure of potential for agonistic behavior was compared to fighting, a measure of agonistic behavior the results were a positive correlation(p=.004, R^2=.583). A linear regression using path overlap as a dependent variable and territoriality as an independent variable was completed to obtain these results.

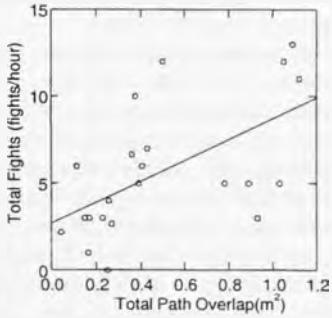

Figure 11. Scatterplot graph showing positive correlation between fighting and total path overlap

New burrows did not increase at a greater rate with food availability as expected (X^2=. 114, p<. 05). This was shown with a chi-squared test in which the null hypothesis, that the rate of appearance of new holes was not affected by food supplementation, was tested (Figure 12). The null hypothesis was not rejected and therefore no increase in new burrows occurred due to food supplementation.

I used a chi-squared test to validate food preference. The chi-squared analysis tested the null hypothesis that there was no food preference and the same amount of green leaves, yellow leaves and flowers were consumed. This is not the case (X^2=41.586, p< .05) and crabs eat more flowers than or yellow leaves. There are twice as many green leaves eaten as yellow leaves. This shows that there is a bias towards flowers and green leaves.

	Green leaves	Yellow leaves	Flowers
Observed (f)	66	34	110
Expected (f')	70	70	70
n= 210			

Figure 12. Chi squared chart showing food preference for flowers and green leaves.

Discussion

Observations

The crabs are the most active in the evening between 15h and 18h in a 24h day. This is consistent with the crepuscular cycle that has been reported for them (Roy Caldwell personal conversation) on Moorea. Being more active in the evenings and mornings may be an important adaptation to heat and water stress as they have low tolerance of dry conditions.

Dominance in *Cardisoma carnifex* is dependent on size. The chi squared analysis showed that the occurrence of a big crab being able to feed at the exclusion of a small crab was not equivalent to the number of events in which a small crab feed exclusively. In fact three times the number of events in which the larger crab was successful was observed. It was observed that a firm grip on a food item contributed to successful claim of the item (i.e. dragging it underground), as did speed and agility in maneuvering the item into the hole. Agility and food maneuvering may possibly be learned which explains why larger, older crabs were able to feed faster than smaller, younger ones.

Burrow Placement

To determine what resource *Cardisoma carnifex* is trying to maximize in the spatial distribution of its' burrows, several environmental gradients were inspected. They were: Shade, proximity to ocean and food fall. While shade didn't prove to be a factor for burrow placement, food fall and proximity to the ocean were determinants. Shade was probably not a determinant in the spatial distribution of burrows because the burrow itself is an escape from heat stress and water loss. The burrows may be deep enough to avoid any threatening effects of the sun. Proximity to the ocean does positively correlate with the number of burrows, however, this data may be biased by the placement of the plot. The plot did not extend from the roadside all the way to the ocean, which would be important in determining the spatial scale of crab burrows with consideration to the proximity of the water. The ocean, however, is important to *C. carnifex* both reproductively and for osmotic regulation. The strong statistical correlation of natural food fall with the number of burrows indicates that the spatial set up of crab holes is heavily dependent on food. However, if food were a resource the crabs are trying to maximize as the data shows, one would expect an increase in food to decrease territoriality. This did not occur.

Food Supplementation

Fighting, path overlap and a change in the appearance of new burrows did not change with food supplementation. This suggests that an increase in food did not change the territorial behavior of *Cardisoma carnifex.*

This leads us to assume some other factor other than optimization of food is influential in territoriality of *Cardisoma carnifex.* When a chi-squared test was completed with the movement of crabs after supplementation it was shown that there was no increase in new burrows after supplementation. This lends evidence for another factor, besides food availability, in determining spatial situation and crab territoriality. The burrows of *Cardisoma carnifex* are very important for escape from predators. It was observed on one occasion a rat carrying a crab whose limbs were missing. Rats were observed commonly running through the area and crabs would retreat to their burrows in response, which interestingly they did not do for birds. The burrows enable the crabs to reduce predation by allowing them to remain either underground or close to the burrow, in many cases right on top of the opening, to feed. Many crabs remained underground until a food item, a leaf or flower, fell. By sensing the vibrations

from the fall, several crabs closest to the item would appear and race to the fallen food item. This happened repeatedly and it was a challenge to get the crabs to recognize the supplemented food as food. Without the vibrations that accompany other falling food, many pieces of food were passed over. Many crabs would respond to a fallen colored pencil but not to static hibiscus leaves and flowers.

The burrow also might have served as an enemy free zone, a possibility supported by the fact that virtually no crabs went into another crabs' burrows. The establishment of territories may serve as one way to avoid intraspecific aggression. Many of the fights among *C. carnifex* were terminated by the disappearance of one crab into its burrow. No pursuers were ever observed to follow the other fighting crab. It was common, however, to observe one crab stick it's claw into the other crab's hole after a fight. Displays were not common but witnessed. *Cardisoma carnifex* assumes a position in which both claws are lifted facing the threat. Threat displays were commonly the response the trespassing of one crab into another crab's territory. Aggressive behaviors only evolve when they prove not to be fatal. In this case, burrows allow for aggressive displays to exist without fatality.

Burrows also allow *C. carnifex* to partition resources. A crab's burrow acts as a " stash", an area where the food is safe from other crabs. Other crabs will pursue a crab with food until it is dragged down the burrow, out of sight. A crab will also drag its food down its burrow and immediately resurface to claim another piece of food. This insinuates that once a piece of food has disappeared down a burrow, the burrow's inhabitant has unlimited amount of time and access to the food item. In addition, crabs seldom ate their food above ground. If piece of food was too large to drag down the burrows it was not eaten. For example, there was a branch that somehow was broken from a tree that remains untouched. In this case the burrow seems to be an efficient, non-fatal way to divide food resources.

It is interesting to note when one inspects the graphs surrounding territoriality and fighting that when food availability is compared with fighting in the presence of food it not nearly as insignificant as when total fighting is analyzed. In fact, except for an outlier and small sample size it seems as if the results might prove to be significant (R^2=. 529). It is possible that an increase in food would increase the fighting over food. It is also interesting to note that when the amount of food available is compared to the fighting for territoriality the results, although insignificant, have a negative trend. This, also affected by an outlier and a small sample size, might suggest that with more data collection increased food availability may decrease fighting over territories.

Another possibility for the lack of change in behavior in spite of food supplementation may be errors in the study itself. The distribution of food was spaced evenly throughout the plot but not calibrated to the density of the crabs. This leads to another point, if food supplementation was not weighted for crab density, in areas of high crab density and the highest territoriality, crabs received less food than crabs of less densely populated areas. Therefore, the amount of food supplemented may not have been adequate to provoke behavioral changes. The amount of supplementation was calculated by pieces of food and not grams. Lastly the sensitivity of these crabs to outside observers may impact the results of this study.

Food preference was investigated using a chi squared and it was shown that *Cardisoma carnifex* does have a preference for a type of food. Out of the three types of food supplemented in equal amounts, hibiscus flowers were chosen twice as often as, green hibiscus leaves. Lastly, yellow hibiscus leaves were the consumed in the smallest percentage. Although the gram amounts of each category were not the same, the number of pieces was identical. The fact that entire pieces and not portions of food were dragged away implies that the number of pieces of food is more important than the grams of food available. Flowers may have been selected for because they are on the average lighter, easier to carry, easier to recognize and may be of more nutritional value. The optimal foraging theory states that the balance between costs and benefits must be so that the benefits of foraging, the food, outweigh the costs of foraging, the energy of foraging. It may be that a flower is a good balance between the costs and benefits of a foraging crab. It was noticed that crab were more successful in holding and dragging away flowers than leaves. Flowers were also in continuous supply naturally during the course of the study, which may indicate them as being a reliable food source. It is interesting to note that almost twice as many green leaves were taken than yellow leaves although the same amount was supplemented. Green leaves heavier than yellow leaves so the selection for them is at an extra cost in terms of energy for the crabs. It may be that the green leaves provide more nutrition and are energy efficient. Fresh water, also being in shortage for the land crabs may influence *C. carnifex*'s choice for green leaves, which contain more water.

Conclusion

Burrow placement suggests that population density is limited both by the amount of food available and the amount of space available close to the ocean's edge. These are, most probably, physiological constraints that determine the spatial distribution of *C. carnifex*. The density of *C. carnifex* can reach large numbers, over 3,600 individuals per hectare in Aldabra (Alexander 1979), and may have ecological significance.

Land crabs are responsible for leaf litter turnover rates and regulating nutrient cycling (Green et al. 1999). They affect everything from the regeneration of plants to the populations of microbial organisms and insects. In addition, the burrows of *C. carnifex* also create unique habitats for different organisms. Insects, mosquitoes, and certain vectors of diseases have been known to breed in the standing water of *C. carnifex* burrows (Goettel et al.1981). *C. carnifex* is also a food source for the inhabitants of Moorea and it's surrounding islands. Regulation of densities of these crabs will prove to be important for the human community in years to come.

As more is discovered about the effect crabs have on the human community it will be more useful than ever to know standard ecological and behavioral knowledge surrounding these ubiquitous creatures. Further areas of investigation in areas of behavioral ecology are suggested. I hope further food supplementation experiments will be conducted with greater amounts of food manipulation. I also think further investigation into the social biology of *C. carnifex* should be conducted. Placement of female and juvenile burrows as compared to adult male burrows may help elucidate why these burrows are maintained. Laboratory manipulations may be useful in inspecting issues of territoriality.

Development of human communities especially on the coast of Moorea threaten *C. carnifex* habitat. With so little known about its' ecological role and behavior, this practice is potentially dangerous.

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Behavioral and physical responses of damselfish (*Stegastes nigricans*) to stream run-off in Moorea, French Polynesia

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ABSTRACT. Fish and other animals can be used in several ways to assess environmental health, such as in species composition, diversity, species richness, and behavioral analyses. My study in Moorea during October and November 2000 sought to discern a pattern in behavior differences of the Dusky Gregory damselfish (*Stegastes nigricans*) between experimental and control study sites to find their responses to organic input in the coastal habitat. I hypothesized that the fish would have higher intraspecific attacks in areas with more algae. The higher amount of algae in an area increases the available food so fish density would increase. Coastal morphology, proximity (within 50m of stream mouth) to shoreline, stream width, and presence of *Stegastes nigricans* were used to select the streams. Water sample tests revealed higher nitrate and ammonium in areas at stream outflows relative to their control sites. Samples of algae taken from the Dusky Gregory damselfishes' territories weighed more at stream sites. Behavior observations of feeding and aggression were conducted in five-minute periods on 16 to 25 fish at each site. Paired samples t-tests showed that the intraspecific aggression at stream sites were higher than control sites, which did support the hypothesis. The other behaviors physical aspects noted, such as interspecific aggression, feeding, individual length, and territory size, had no significant difference at stream sites when compared with control sites. There was no significant difference in geographical location on the island for any aspects observed in this study. Therefore, the stream influence does reflect a change in the intraspecific behaviors of the Dusky Gregory damselfish.

Introduction

Anthropogenic pollution is of great concern to the coastal communities throughout the world. These areas contain some of the world's more prosperous fisheries and other economic reinforcements such as tourist resorts and agriculture. Stream run-off of fresh water and organic sediments affect near-shore ecosystems including coral reef communities. It is crucial to understand a coral reef ecosystem in order to protect and manage it effectively (Farnworth & Golley 1974). To assess impacts of stream outflows, some scientists and environmental analysts have studied the sediment deposition, algae diversity, and the presence/absence of certain species. The habitats of these species are often affected by a change in nutrient flow from streams. Some fish can be used as an indictator of environmental health (Garrison 1999). My project looks at damselfish (*Stegastes nigricans*) to determine their behavioral and physical responses to stream outflows on Moorea.

In Hawaii Fitzsimons and Nishimoto (1999) found that fish behavioral data can be a better indicator of stream health than species richness. Indicator species may be chosen because it is common in a certain type of habitat. The presence and absence of this chosen indicator may not be due on environmental health, but instead a slight change in habitat type. Fish behavioral studies, on the other hand, can assess the fishes' reactions to a changed environment.

The Dusky Gregory damselfish (*Stegastes nigricans*) is one of several types of "farmer fish" that protect algae growing on a substrate, usually on coral. This area provides habitat, food, protection, and spawning sites (Bartels 1984). The Dusky Gregory damselfish is found through the South Pacific and Indian Oceans. Several studies have been done on them regarding their behaviors on aggression, territoriality, feeding, and recruitment (Glynn and Colgan 1988; Letourner 2000; and Shima 1992). Letourneur (2000) found a positive relationship between the length of individual *S. nigricans* and the size of their defended territories. Shima looked at the difference between *S. nigricans* off the Tiahura motu and Temae motu on Moorea to see if their geography influenced their territoriality and behaviors. Species richness of intruders increases as the distance of aggregations increased from shore (Shima 1994). Territory defense energy (referred to in this report as "chase and flex") of *Stegastes nigricans* does

not depend on their territory sizes (Shima 1992). The Dusky Gregory protects its territory unless a more suitable habitat is available or their territory is taken over. Because of their stable living arrangements, these fish are easily used to monitor behavior.

In this study I looked at how the behaviors, individual lengths, and territory sizes of the Dusky Gregory damselfish are affected by stream outflows. The hypothesis was that a difference in territory size, aggression, feeding behaviors, and territoriality existed between *S. nigricans* occupying areas affected by stream mouths and *S. nigricans* occupying areas not affected by stream outflows. The stream outflows were assumed to release nutrients to the coastal marine waters that would increase the algae growth in the Dusky Gregory territories. I predicted that with more available food, in turn, the damselfish would decrease defense of food resources. This was tested by observing the fish at sites close to stream outflows, where houses and small farms were upstream. I also chose streams on three sides of the island to see if any physical or behavioral differences of the damselfish existed geographically.

Methods

Study Selection

Sampling of water and observations of fish took place within 50 meters of hightide line in lagoons on three sides of the island of Moorea, French Polynesia (17° 30' S, 159° 46' W). The Dusky Gregory damselfish is abundant in Moorea's fringing lagoon in areas where there was suitable habitat (sand and coral rubble and *Porites* sp. heads were the substrate that dominated the shorelines in Moorea that were not in bays).

In selecting study sites, many stream outflow areas were too shallow for the fish. Several requirements were necessary in selecting a site: presence of stream outflow, the presence of *Stegastes nigricans* within 50 meters of the high tide line, accessible shore, tunnel width under the road from 210cm to 550cm, and flat shoreline (not a bay or inlet). These requirements were necessary to keep the sites as consistent as possible for statistical replication.

Stream mouths in bays and inlets were avoided because I assumed the outflows of all near-by streams would mix. Two streams on three sides of the island were chosen with an adjacent site, assumed to be un- or less affected by the stream outflow (Figure 1). The experimental sites were chosen because they were assumed to receive chronic freshwater input from rainfall.

Area 1 – Area 1 encompasses Experimental and Control Sites A and B that were located on the north side of Moorea. Experimental Site A is located 10m east of PK 12. Substrate at experimental site A was silt past 50m out. The aggregation observed was 51m west of this point. The stream flowing into the experimental site had one house upstream and no agriculture or pastures.

Experimental Site B was located 50m west of PK 14 at the stream adjacent to the eastern border of the Sheraton Resort and Spa. This hotel was under construction for more than the past eight months and was assumed to influence the habitat of the Dusky Gregory damselfish. There were eight houses and no agriculture or pastures upstream.

Area 2 – Area 2 includes Experimental and Control Sites C and D that were located on the west side of Moorea. The stream outflowing to experimental site C had seven houses upstream. The stream outflowing to experimental site D had 10 houses along the stream. Coconuts lined approximately 150 meters of the stream. This area was close to the town of Haapiti but did not appear to be influenced by any pollution other than erosion and household waste.

Area 3 – Area 3, located on the east side of Moorea, accounts for Experimental and Control Sites E and F. Experimental and control sites E were between PK 5 and 6 near Afareiatu. There were six houses and a coconut grove (approximately 1500 squared meters) in along the stream. This stream was 310cm wide. The experimental and control F sites were between PK 5 and 6. There were four houses along the stream and a small pasture with 4 horses.

	area	PK location	width, cm
A	1	15m E of PK12	530
B	1	50m E of PK 14	210
C	2	75m S PK 34	440
D	2	15m N PK 35	410
E	3	btwn 12-13PK	310
F	3	btwn 5-6PK	290

Table 1. Experimental site locations and stream widths

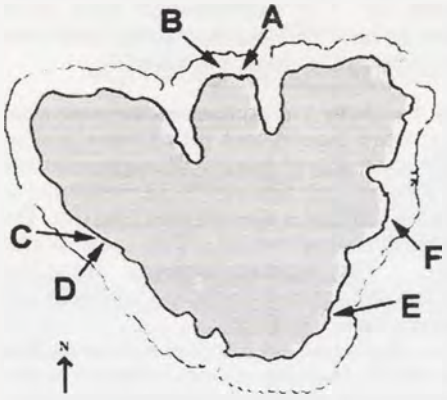

Figure 1. Map of Moorea with study sites marked. Area 1 is A and B; Area 2 encompasses C and D; and Area 3 includes E and F.

The experimental sites were located directly out from the stream mouth at the first presence of the damselfish, while the control sites were located 50m up current (Fig 2).

I observed fish consistently from 12:00pm until 3:00pm to avoid behavioral changes influenced by the time of day.

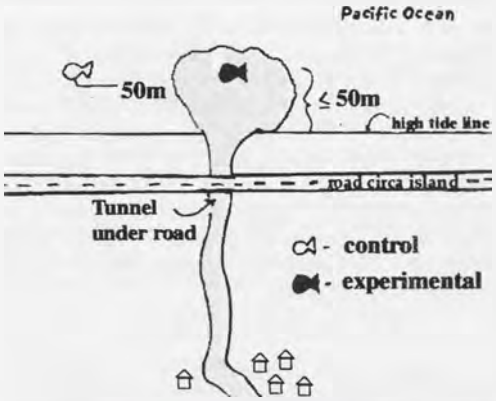

Figure 2. General diagram of experimental and control site geography relative to stream outflow, shore and one another. Gray indicates estimated freshwater influence.

Data Collection

I observed Dusky Gregory damselfish at the first aggregation sited from the stream mouth perpendicular to the shoreline (referred to as both "experimental site" and "stream site" throughout the paper). I measured the distance from the effluent mouth to the first territory where I began observations of individuals.

For each 5-minute observation I noted an individual fish's chase and flexes, length, and bites of algae (see Behavioral and Physical Factors of Observed Fish section for description of each). I had a trained (by me) assistant to help in the data gathering. We simultaneously observed the same aggregations but took notes on different fish so as to decrease necessary field time and increase the gathered data. To avoid disturbance of the fish and behavior modifications, I swam no closer than 1.5m to the individual being observed.

After each five-minute observation of the individual I noted the total length of individual and size of its territory. I measured the length of the observed individual by comparing it when it is in front of or in between coral heads and then measured the length between the noted area. The territory size was measured by noting the area protected by the individual in two dimensions (where it consistently fed without being chased away, the area it protected from intruders, and circled throughout the five minutes observed). I gathered this information for at least 16 fish at each study site. If there were not enough potential individuals to observe (due to lack abundance or visibility) then I observed individuals at a neighboring coral heads. At the end of each observation period, I gathered samples of algae-covered coral from each control and stream site to bring to the lab to weigh the dried weight.

Abiotic and Environmental Factors

Amount of algae in gardens -- I chiseled a small chunk of dead coral covered with algae from the aggregations I observed. In the lab, I scraped algae from 1 square centimeter of the coral with a razor blade and dried it in the drying oven on 60 Celsius for 12 hours. I weighed the algae cover from one square centimeter from each experimental and control site at a U.C. Berkeley laboratory with a one-thousandth of a gram precise scale.

Water sampling -- I took water samples at each study site and its control site on November 1, 2000 on a rainy day so that streams would have freshly outputted stream water. The samples were taken at approximately 50cm depth near the aggregation area where I observed the fish. I tested for nitrate, nitrite, phosphate, ammonium, pH, and temperature. Litmus paper was used to test for pH. A LaMotte test kits were used to test for phosphate, ammonium, nitrate, and nitrite. Salinity tests were not available.

Behavioral and Physical Factors of Observed Fish

I compared several aspects of *Stegastes nigricans*, such as territory size, length of individuals, intraspecific and interspecific aggression (chases), and bites of algae per five-minute interval. These aspects were statistically analyzed according to their geography (stream vs. control with paired-sample t-tests, three sides of the island using ANOVA), environmental and abiotic characteristics (farm size, stream width, nutrients presence), and behavioral and physical aspects.

Chase and Flexes -- I divided the *Stegastes nigricans'* intraspecific and interspecific aggressive acts into three categories to gather data on chase and flexes of the individual fish. The categories represent three types of aggression that I have observed in the damselfish. The same method was suggested by Barlow (Per. comm.) and used by Vallejo (1998): 1) spine flex); 2) short chase; and 3) long chase.

The "spine flex" is when the observed fish flexes its spines without simultaneously chasing the intruder. The "short chase" is when the fish moves towards an intruder to scare it off but does not go beyond the space that the intruder was occupying when the chase began. The "long chase" is when the observed fish leaves the territory to chase the intruder away. I noted the occurrence of each with tally marks on a dive slate.

Following the idea of weighting actions according to the amount of energy used as was suggested in Barlow's Modal Action Pattern (1968), I multiplied spine flexes by 1, short chases by 2 and long chases by 3 (Vallejo 1998). These were added together to account for total chase and flexes on same species and different species. The sums were compared with length of individuals, circles, leavings, and feeding behavior, within stream and control sites. These actions multiplied by their coefficients are referred to as "chase and flex" in this paper.

The amount of circles the observed fish made along the boundary of its territory in the 5-minute period were also noted.

Length of individuals -- I used a centimeter ruler attached to my dive slate that I held up as close as possible to the fish to estimate its length to the nearest centimeter. To double-check my estimates of length I waited until the fish is next to a piece of coral and I noted its location and then measured the area on the coral that the observed individual covered (technique from Barlow).

Bites of algae -- I tallied the number of bites on algae per five-minute intervals while observing each individual. I differentiated between bites taken in the observed fish's territory and outside its territory.

Results

Analysis of data was computed with the statistics program SYSTAT.

Abiotic and environmental factors

Results of nutrient and pH tests and temperatures of all sites are in Table 2. Minimal differences resulted from the nutrient tests done on each site and its control. Nitrate and ammonium were clearly higher in experimental sites relative to the controls, while pH, temperature, nitrite (ppm) and phosphate (ppm) did not vary according to experimental and control sites. Paired sample t-test was done to compare nitrate contents and the p-value was 0.011 (Fig 3).

	NO3- ppm		PO3 ppm		pH		NO2- ppm		NH4+ ppm		temp (C°)	
Site	exp	control	exp	control	exp	control	exp	control	exp	control	exp	Control
A	1.1	0.55	0	0	7	7	0	0	0.1	0	27	27
B	1.1	0.275	0	0	7	7	0	0	0	0	27	27
C	1.1	0.55	0.5	0.5	7	7	0	0	0.2	0.1	28	27.5
D	0.825	0.55	0.5	0	7	7	0	0	0	0	27	27.5
E	0.825	0.825	0	0	7	7	0	0	0.1	0	26.5	26.5
F	1.1	0.55	0.5	0.5	8	7	0	0	0.1	0	26.5	26

Table 2. Abiotic results from water samples at control and experimental sites of *Stegastes nigricans*

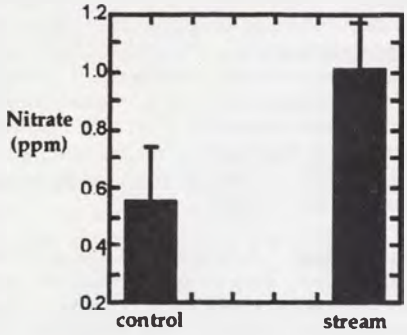

Figure 3. Bar graph of average nitrate at experimental vs. control sites of where *Stegastes nigricans* were near stream mouths and in waters not affected by stream outflows

No nitrite was found in the water samples taken. An average of ammonium in experimental sites was 0.083 ppm and in controls was 0.017 ppm (p= 0.025). The pH values in water samples were all neutral (7) except for at the experimental F site (8).

There was no significant linear regression of distance of first *S. nigricans* presence in relation to stream width (p= 0.249). The one square-centimeter of defended algae in five of the six stream outflow sites was higher than adjacent control site (Table 3) except for site B. However, the difference between control and experimental sites was not significantly different (p= 0.291).

Proximity of aggregations to stream mouths was tested using regression with the width of the stream under the road (p=0.243, r-squared=0.313).

SITE	experimental	control
A	0.0391	0.0119
B	0.0171	0.0349
C	0.0439	0.0399
D	0.0672	0.0629
E	0.0243	0.0094
F	0.0139	0.0034

Table 3. Grams of algae in territories of observed *Stegastes nigricans*

Behavioral and physical results

Table 4 summarizes data (averages) of behavioral and physical factors. Analyses of most observed fish behavior and physical aspects (length, territory size, chase & flexes, and bites of algae) revealed no statistically significant differences between experimental and control sites. Amongst behavioral and physical factors, regression showed a significant negative relationship between length and territory size (p= 0.01269, r-squared= .03117). Regression was not significant for interspecific defense energy and the length of individual *S. nigricans* (p= .648, r-squared= 0.033).

The difference between the amount of intraspecific chase and flex for intruders was statistically significant at the control sites when paired to their experimental sites (p=0.025). However, according to the paired samples t-test, no significant relationship exists between stream and control sites for the interspecific chase and flexes (p=0.939). A regression of intraspecific chase and flex dependency on algae weight revealed no significant relationship (p= 0.226, standard error= 0.042). No significant relationship was

found in a regression between bites of algae within the observed fish's territory and the algae weight (p= 0.150; r-squared= 0.195).

	length	territory size	interspecific chase & flex	intraspecific chase & flex	bites
probability	0.633	0.951	0.939	0.025	0.475
mean control	8.691	2460.999	2.702	1.335	10.132
mean stream	8.829	2498.974	2.623	2.342	12.189
mean difference	-0.138	-37.974	0.079	-1.007	-2.058
SD difference	0.666	1450.229	2.409	0.776	6.534
standard error	0.272	592.054	0.983	0.318	2.667
df	5	5	5	5	5

Table 4. Paired sampled t-tests of control vs. experimental sites for the observed fish's behavioral and physical averages

Geography

I did an ANOVA with "area" as the factor on several variables. Intraspecifc chase and flexes was not stastistically significant according to the side of island, however, chase and flexes in area 3 were relatively lower than areas 1 and 2. The bar graphs of means of the chase & flexes according to sites on the island are in figure 4.

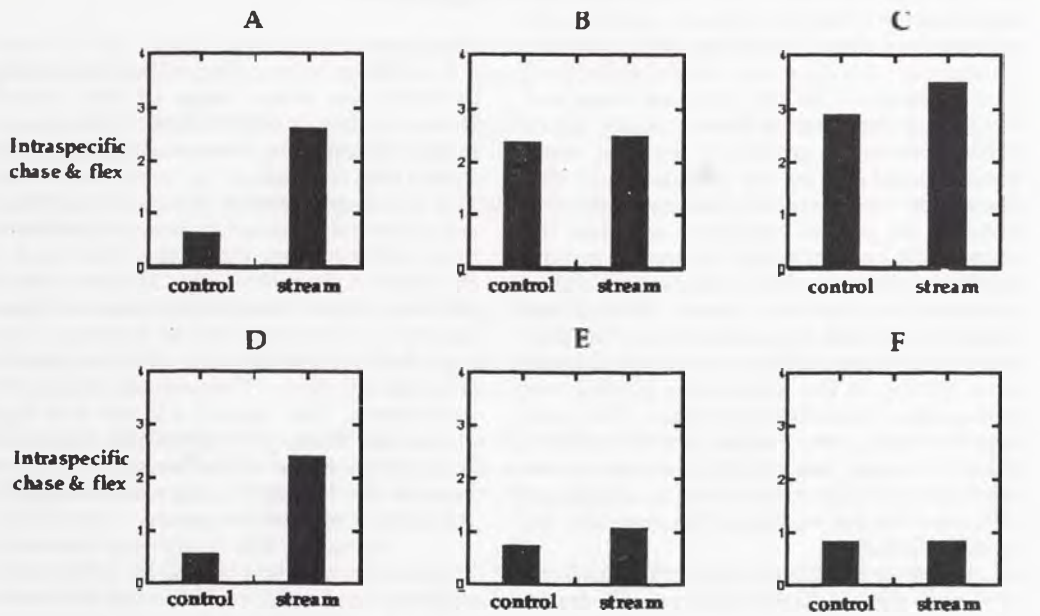

Figure 4. Bar graphs of intraspecific chase and flexes divided into the sites studied on Moorea (see figure 1 for reference to sites A-F)

Discussion

Abiotic and Environmental Factors

Minimal differences resulted from the nutrient tests done on each site and its control. Nitrate was the only nutrient that was clearly higher in experimental sites relative to the controls. T-test was done for the nitrate comparison and I found the p-value to be less than 0.05. Nitrate and phosphate increase plant growth and therefore were assumed to

be affecting the algae "farms" of the damselfish. Although higher weight of algae was found at stream outflow territories, the difference between control and experimental sites was not enough to be statically significant in a paired-samples t-test.

Because no salinity meter was available, the higher nitrate at stream outflows was used to indicate stream influence. Nitrate in the open Pacific Ocean is nearly zero (Millero and Sohn 1992) and water sample tests of several streams in Moorea indicate the presence of nitrate (Harbaugh 2000). Stream E water sample tests did not reveal a difference in stream sites relative to its control. This may be because the experimental received very little stream flow impact. However, this area had higher ammonium concentrate in the water than the control site, so it was assumed to be influenced by the outflow.

Behavioral and Physical Factors

Of the observed fish behaviors, only the intraspecific chase and flexes sum was significantly different between control and experimental sites. This difference is positively related with the algae and nitrate differences. One explanation for the increased chase and flexes is a response to the increased algae. With more algae growth in the area, more food is available so the population of fish increases. The increased density of the fish reduces the size of territories and thus the damselfish are constantly trying to expand their territories. This leads to higher intraspecific chase and flexes. When I was observing the fish it appeared to me that their territories were smaller at the experimental sites relative to the control sites but this was not apparent in statistical analysis. With more nitrate causing more algae growth, one also would assume there to be a change in the feeding amount but there was no significant difference in the feeding of the observed fish at each territory.

A hypothesis for the lack of differences in aggression and feeding behavior is that I chose streams that were not increasing the water nutrients significantly enough to change the algae growth in the territories of *S. nigricans*. I would have expected the stream width to have a significant influence on *Stegastes nigricans'* distance from the stream mouth. Perhaps the width of streams under the road was not adequately representative of the maximum outflow.

Another possibility for the lack of difference between the stream and control data is that the experimental sites may have had more bites by intruders but they were not accounted for other than when the observed damselfish was chasing an intruder away. I did not take notes on the number of bites intruders took from the observed fish's territory. This is suggested to denote for future behavioral studies of this species.

Using raw data rather than averages of each control and experimental site, regression analysis shows that length and territory size are negatively correlated which is in contrast to Letourneur's study suggested of *S. nigricans* in the Indian Ocean (2000). The p-value was 0.01269 (r-squared= .03117). This may be due to poor estimation of territory size. Some boundaries of territories seemed huge when a fish moved around a lot without being attacked. Other fish seemed to have very small territories when they made very little action since territory size was defined by movements, intruders and the observed fishes casual grazing around a certain area in the aggregation.

Geography

There were no significant differences between the three sides of the island. However, area 3 (sites E and F) had much lower intraspecific chase and flexes at the stream sites compare to the other stream sites. This is probable because they had very little, if any, influence from the streams. The widths of these outflows were the smaller than most of the other experimental sites studied. Site B did have higher intraspecific chase and flexes because a construction site for building a new hotel had been at the base of the stream for over six months. This created more of a disturbance. The control site also had high chase and flexes intraspecifically because it was still in range of the stream's influence because the natural current was changed by the building of hotel bungalows.

Although this study was successful, there were a few drawbacks in the data collection and analyses. I focused on the fish above 4 cm long and I was restricted by visibility of fish so therefore *S. nigricans* on the exterior of aggregations were most observed. The population of each study site was not noted because it was impossible to account for all individuals that were hiding and those that were defending interior territories that I could not swim close to without them hiding. Perhaps the populations of fish were higher at the control sites relative to the experimental sites but this was not tested for. Interspecific

chase and flexes regressed with length of individual observed fish was insignificant (p= 0.648). This may be because intruders of different species sometimes avoided observed fishes territories because I was swimming right next to them.

Conclusion

Although there is a considerable amount of agriculture on the island of Moorea, which results in eroded soils and organic wastes that flow out to the coastal waters, the island is still relatively environmentally unharmed. As the island population and tourist visitations increase, so will development and agriculture. This will eventually lead to a problem for terrestrial and marine habitats. The increased intraspecific chase and flexes at stream sites is a result of the outflow of nutrients to the area. This should be studied at other more nutrient rich outflows, such as the one in Cook's Bay at the Pineapple Juicery stream. With more algae, one would assume the territories would be smaller and there more severe impacts at more impacted stream outflows. Hopefully these fish will endure the habitat alterations of the future as agriculture and development increase.

Acknowledgements

I would like to first express gratitude to my parents for supporting this trip so that I could increase my knowledge of my two passions of tropical fish and fieldwork. I must thank Rhea for snorkeling at my side for hours to watch the damselfish with me. I appreciate observational help also from Lorraine, Jae, Chicory, and Shele. Thank you also to the professors and teaching assistants whom supported my project.

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Habitat preference of the polychaete *Gastrolepidia clavigera* on the holothuroid *Bohadschia argus* in Moorea, French Polynesia

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ABSTRACT. Aspects of the symbiotic relationship between the polychaete worm, *Gastrolepidia clavigera*, and the holothuroid, *Bohadschia argus*, were examined on a plot in front of the Richard B. Gump Biological Research Station on Cook's Bay in Moorea, French Polynesia. Aspects of this relationship were also studied in aquarium tanks at the aforementioned station. This study compared the presence of *G. clavigera* on *B. argus* in the day versus the night. It also looked at the behavioral preference of the two different color morphs (light and dark) of *G. clavigera* for the two different color morphs (light and dark) of *B. argus*. No significant difference was found between the presence of *G. clavigera* in the day versus at night. There was, however, a higher number of *G. clavigera* and *B. argus* found in the night than during the day. This is most likely due to the nocturnal behavior of *B. argus*. The aquarium studies demonstrated that neither morph of *G. clavigera* showed any significant preference for one morph of *B. argus* over the other. This implies that while *G. clavigera* has a preference for its host, it doesn't have a preference for its host's color.

Introduction

The number of intimate interactions between different species of organisms living together is almost limitless. Such symbiotic relationships vary from temporary contact to metabolic dependence of one species upon another (Vernberg 1974). The host represents the environment of the symbiont, and the symbionts have evolved adaptations to these environments in the same manner that free-living forms have adapted to their physical and biotic environment (Vernberg 1974). Within the past few decades more and more attention has been directed toward an understanding of the complexity of these relationships, and some of the most exciting work has been with marine organisms where such relationships are particularly common (Vernberg 1974). One such marine symbiotic relationship occurs between the sea cucumber *Bohadschia argus*, and the polychaete worm, *Gastrolepidia clavigera*.

Bohadschia argus, a common species of holothuroid (sea cucumber), can be found throughout a large region of the tropical and subtropical world, inhabiting mainly shallow water reef and rubble habitats. The localities of *B. argus* are the Seychelles to Australia; New Caledonia; New Guinea; Indonesia; Malaysia; Philippines and southern Japan (Sipadan Island, Borneo; Manado, Indonesia) (Gosliner et al. 1996). Like all sea cucumbers, *B. argus* is a deposit feeder: the tentacles surrounding the animal's mouth pass sand and mud into the oral cavity, and the small amount of organic material contained is digested (Buchsbaum et al. 1987). *B. argus* is found in two different color morphs: a gray body with purple spots and a yellowish ventral surface (photo 1), and a brown body with orange spots and a brown ventral surface (photo 2). I will frequently refer to these two morphs as light and dark, respectively. While these two different color morphs are quite distinct, both morphs of *B. argus* can be found in the same environment on the same substrate.

Numerous animals have been found living in, on, and around *B. argus* (the harlequin crab, *Lissocarcinus orbicularis*, and the pearl fish, *Carapus* sp., to name a couple) (Gosliner et al. 1996). One of those organisms, *Gastrolepidia clavigera*, is a polychaete worm that makes its home on the surface (in particular, the ventral surface) of *B. argus* (photo 3). This species is a commensal worm that is found living symbiotically with 13 species of tropical holothuroids from two families (*Stichopodidae* and *Holothuridae*) (Britayev and Zamishliak 1996). The localities of *G. clavigera* are South Africa; Madagascar and Mozambique to Australia; Malaysia; Philippines and Marshall Islands (Sipadan, Borneo; Batangas, Luzon, Philippines) (Gosliner et al, 1996). *G. clavigera* tends to mimic its host in terms of morphology (Britayev and Zamyshliak 1996; Gibbs 1969; Martin and Britayev 1998), so it is found in essentially two different color morphs on *B. argus* – yellowish and brown. Like *B. argus*, I will frequently refer to these two morphs as light and dark, respectively.

Photo 1. Light color morph of *B. argus*.

Photo 2. Dark color morph of *B. argus*.

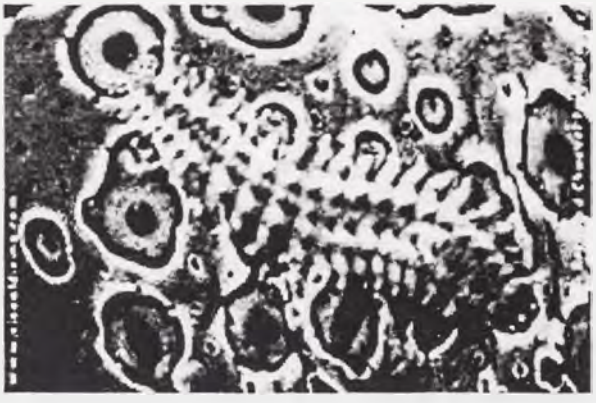


Photo 3. Light color morph *G. clavigera* on light color morph *B. argus*.

Although it is legitimate to say that the relationship between *B. argus* and *G. clavigera* is a symbiotic one, to specify what kind of symbiotic relationship is occurring is a difficult matter. In this paper I am terming the relationship between *B. argus* and *G. clavigera* a commensal one, thus implying that the interaction is defined as positive for *G. clavigera* and neither positive nor negative for *B. argus*. However, it is important to note that it is uncertain as to whether this symbiotic relationship may be more closely defined as a parasitic relationship (Martin and Britayev 1998), *B. argus* being the host and *G. clavigera* being the parasite.

The idea that *G. clavigera* may be a parasite comes about because of the question: how does *G. clavigera* mimic the color of its host? To answer this question, two more questions must be asked first: does the observed color pattern of *G. clavigera* come about due to the host influencing the developing polychaetes, or does a commensal population consist of several "sub-populations," genetically pre-adapted to each appropriate host species (Martin and Britayev 1998)? (With the exception of the experimental demonstration of the trophic origin of the color pattern in the parasitic syllid *Branchiosyllis oculata* (Pawlik 1983), there are no specific studies on this particular aspect of the arrangement of symbiotic associations (Martin and Britayev 1998).) If the answer is yes to the former question, then how does this host influence take place? Is there an

intrinsic metabolic reaction of the symbiont to the different stimuli provided by the different hosts? Or are the symbionts taking pigments directly from the hosts, i.e. feeding on it? If the second answer is correct, then the associations involved should be re-evaluated as being closer to parasitisms (Martin and Britayev 1998). Because no one has answered these difficult questions, to call *G. clavigera* a parasite would be somewhat misleading. The dilemma in applying one definition to the relationship taking place between these two invertebrates arises due to the difficulties of applying experimental methods to such a complex network of interactions (Martin and Britayev 1998). Strict boundaries cannot be easily drawn between various types of associations. In practice, it is difficult to infer unequivocal demonstrations of benefit (be it reciprocal or not) from the analysis of an association or from the existing literature (Martin and Britayev 1998).

Whether or not the relationship between *B. argus* and *G. clavigera* is commensal or parasitic, a symbiotic relationship is occurring. The next question that arises is why does this symbiotic relationship exist in the first place? Is *G. clavigera* living on *B. argus* (in particular, on its ventral surface) for protection and/or access to food? The fact that *G. clavigera* is usually located near the anterior or posterior ends of its holothurian host's body, quickly hiding in the oral or cloacal openings when threatened (Britayev and Zamyshliak 1996) leans strongly toward the idea that *G. clavigera* is living on its host primarily for protection. With this idea in mind, one can ask if *G. clavigera* avoids its predators as much as possible by hiding during the main hours the predator hunts. After performing preliminary observations in the water of Cook's Bay on the island of Moorea in French Polynesia, I noticed that there appeared to be more worms around at night versus in the day. This led me to my first hypothesis:

1.) There is a significant proportional difference between the presence of *G. clavigera* on *B. argus* in the day versus at night.

In regards to why this would occur, my best educated guess was that predation avoidance during daylight hours led to less *G. clavigera* being found on the surface of *B. argus*. Predation on *G. clavigera* has been documented. In studies done by Britayev and Zamishliak (1996) it was observed that worms washed away during collecting were eaten by coral fishes. In addition, their studies pointed out that predators probably attacked these commensals under natural conditions too. This first hypothesis gives rise to another idea.

With the same predation avoidance concept in mind, one would assume that the color morph of *G. clavigera* would mimic the coloration of *B. argus*, as mentioned previously. Since polychaete relocation of one host to another has been demonstrated (Britayev and Zamyshliak 1996; Gibbs 1969), one would also assume that when forced to move, *G. clavigera* would choose a *B. argus* host with the same coloration. Thus my second hypothesis is:

2.) If given the choice, *G. clavigera* would attempt to choose a *B. argus* host that matched its coloration.

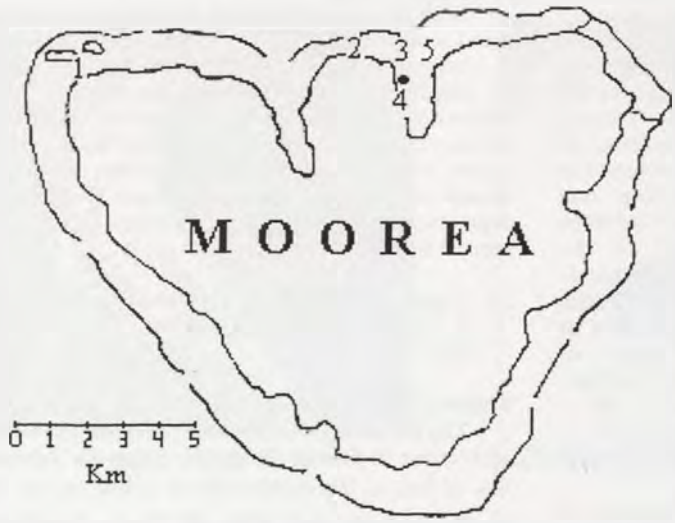
Materials and Methods

The following studies took place on the island of Moorea in French Polynesia (map 1). Moorea lies 25 km to the northwest of Tahiti, at 17° 30′ South latitude and 149° 50′ West longitude. Triangular in shape, with 61 km of coastline, Moorea – about 1.2 million years in age – is an ancient volcanic system today considerably eroded (Galzin and Pointier 1985). The island is sheltered by a barrier reef which delimits a lagoon, 500 to 1,500 meters wide. The reef front is intersected by 12 passes corresponding to the principal valleys and bays. There are two bays located parallel to each other on the north side of the island – Cook's Bay and Opunohu Bay. Moorea enjoys a warm, oceanic, tropical climate (25° to 30° C). The tides are semi-diurnal. Low tide occurs between 5:00 a.m. and 8:00 a.m., and 4:00p.m. and 6:00 p.m. (Galzin and Pointier 1985). The physico-chemical characteristics of the lagoon waters are highly variable, in function with the climatic conditions, although they do not differ strongly between the various lagoons of the island. The average values of temperature are 24 - 27°C in winter and 29 – 31°C in summer. The average salinity is 35 – 36 g/l (Galzin and Pointier 1985).

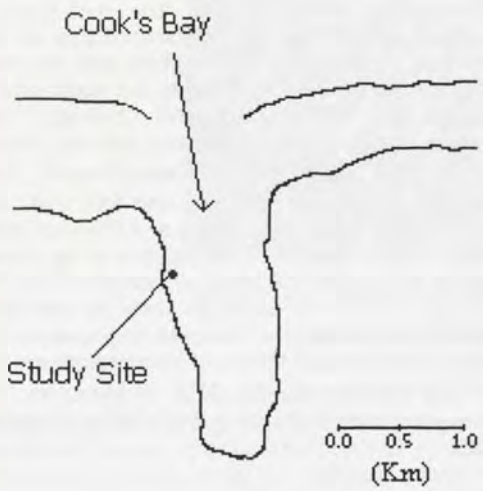
Field Study

Location

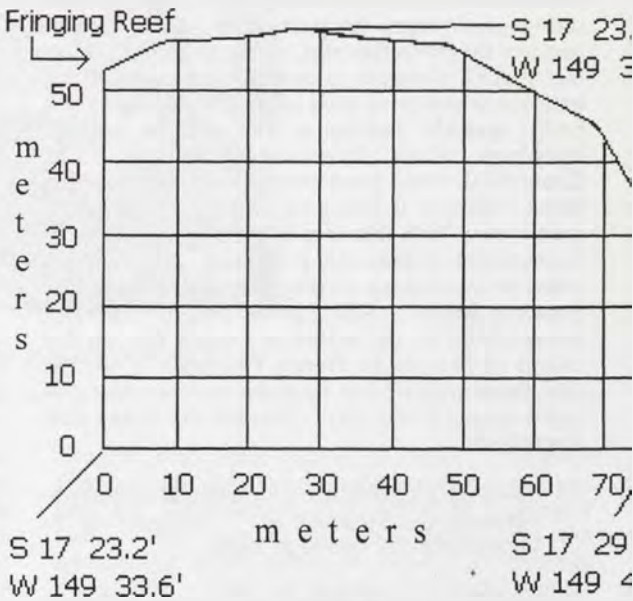
The observational studies on the presence/absence of the two color morphs (light and dark) of *G. clavigera* on the two color morphs (light and dark) of *B. argus* took place on the northwest side of Cook's Bay directly in front of the Richard B. Gump Biological Research Station at South 17° 24.4′, West 149° 33.0′ (maps 1 and 2). All data was collected between October 6 and November 2, 2000.



Map 1. The island of Moorea, including the study site and the collecting sites.



Map 2. Cook's Bay on Moorea including the study site.



Map 3. Grid map of the study site. Each corner of a square corresponds to a flag location.

Setup

A grid map was set up using a GPS unit and transect tapes (map 3). The plot measured approximately 80 meters by 50 meters. A flag Was placed in rows every 10 meters across the entire plot (via snorkeling). Depth of the plot varied from .75 meters to 3 meters. The seafloor was composed almost entirely of sand and dead and live *Porites* coral heads.

Sampling

Data collection took place by swimming along each row of flags across the plot while scanning at least 5 meters on both sides of each flag line so the entire surface area of the plot would be accounted for. During data collection, whenever a previously unobserved *B. argus* was found, it would be scarred on its dorsal surface using a surgical scalpel (Reichenbach 1999). Types of scar designs included numbers, letters, and symbols. Whenever a *B. argus* came into view, it would be searched thoroughly for *G. clavigera*. If *G. clavigera* were present, the number and color morph of the *G. clavigera* would be recorded. Whether or not any *G. clavigera* were found, the time, location, color morph, and scar design of all *B. argus* would be recorded. Data was collected both during the day (sunrise to sunset) and night.

Lab Experiment

Location

The experimental studies looking at the preference of *G. clavigera* for the two different color morphs of *B. argus* took place in the flow-through seawater system in the Dr. and Mrs. Gordon Moore Field Laboratory at the Richard B. Gump Biological Research Station. All experiments took place between October 19 and November 8, 2000.

Setup

In the laboratory portion of this study, four *G. clavigera* (two light morph and two dark morph) were placed in a central tank. Attached to the central tank via clear tubing were 3 exterior tanks. One exterior tank contained a light morph of *B. argus*, one a dark morph of *B. argus*, and the other was a control tank containing no organisms. Water flowed into the central tank solely through the exterior tanks and the only drainage came from the central tank (photo 4). The four *G. clavigera* were then free to move to the exterior tanks via the tubing attaching them to the central tank. This experiment was run five times, each experiment lasting 3 days. After each experiment was completed, the animals would be returned to the seawater and the tanks drained and cleaned.

Collecting Sites

Animals used in this experiment were collected from 5 different sites on the island (map 1).

Data Analysis

Field Analysis

The data collected during the field portion of this project were analyzed using T-tests (two sample assuming unequal variance and two sample assuming equal variance). P-values were considered to be significant if they were less than or equal to 0.05.

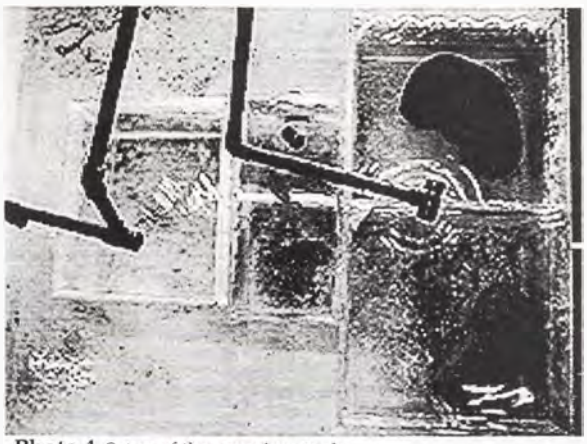


Photo 4. Setup of the aquarium tanks.

Lab Analysis

The data collected during the experimental portion of this project was analyzed using T-tests (two sample assuming unequal variance and two sample assuming equal variance). In addition, Chi squared tests were also run. As with the T-tests, Chi squared p-values were considered to be significant if they were less than or equal to 0.05.

All T-tests were analyzed using Microsoft Excel. Chi-squared tests were calculated manually.

Note that all n values signify the number of sampling periods, not the number of organisms sampled.

Results

Field Study

No significant difference was found proportionally between the presence of *G. clavigera* on *B. argus* in the day versus at night (0.25±0.05, n=11 and 0.24±0.05, n=8, respectively) (p-value=0.85) (figure 1). An increase from day to night in the number of *G. clavigera* (2.5±0.58 to 3.1±0.67, n=11 and n=8, respectively) (figure 2) was observed but wasn't found to be significant (p-value=0.46). There was, however, a significant

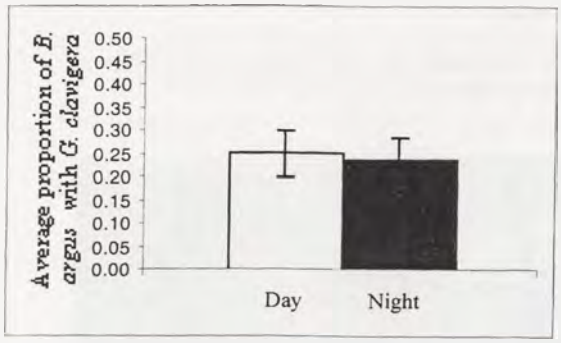


Figure 1. Average proportion of G. clavigera found on *B. argus* in the day versus at night.

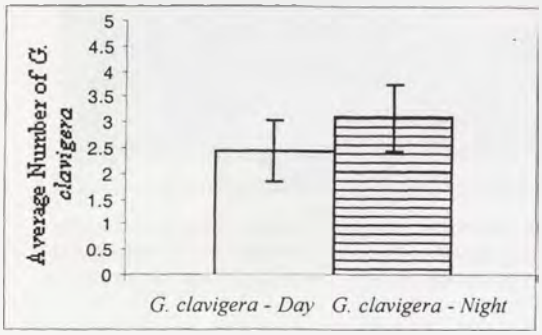


Figure 2. Comparison of the presence of *G. clavigera* on *B. argus* in the day versus at night.

Lab Experiment

Of the 20 *G. clavigera* (10 light morph *G. clavigera* and 10 dark morph *G. clavigera*) used in the lab experiment, 50% went to a tank with a *B. argus* in it, 45% stayed in the central tank, and 5% went to the control tank. Of the *G. clavigera* that chose an outside tank, there was a significant difference between the average proportion of *G. clavigera* going to the control tank versus going to a *B. argus* tank (0.05±0.05, n=5 and 0.95±0.05, n=5, respectively) (p-value=1.367E-6, Chi squared p-value = 0.05-0.10) (figure 6). Of the dark morph *G. clavigera* that chose a tank with *B. argus*, there was no significant difference between the average

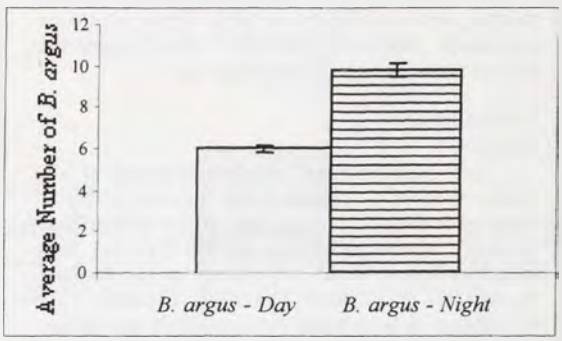


Figure 3. Comparison of the presence of *B. argus* in the day versus at night.

increase from day to night in the number of *B. argus* (6.1±0.16 to9.9±0.29, n=11 and n=8, respectively) (p-value=0.0018) (figure 3).

Among those *B. argus* documented on more than one occasion, 76% of them showed no change in the quantity of *G. clavigera* found on them.

Among those *B. argus* with *G. clavigera* found on them, there was a significant difference between the average proportion of dark morph *B. argus* hosting dark morph *G. clavigera* and light morph *B. argus* hosting dark morph *G. clavigera* (0.98±0.02, n=16 and 0.17±0.17, n=6, respectively) (p-value=0.005) (figure 4). There was also a significant difference between the average proportion of dark morph *B. argus* hosting light morph *G. clavigera* and light morph *B. argus* hosting light morph *G. clavigera* (0.05±0.04, n=16 and 0.83±0.17, n=6, respectively) (p-value=0.004) (figure 5).

proportion of dark morph *G. clavigera* going to a dark morph *B. argus* tank versus a light morph *B. argus* tank (0.33±0.33, n=3 and 0.67±0.33, n=3, respectively) (T-test p-value=0.52, Chi squared p-value=0.25-0.50)(figure 7). Of the light morph *G. clavigera* that chose a tank with *B. argus*, there was no significant difference between the average proportion of light morph *G. clavigera* going to the dark morph *B. argus* tank versus the light morph *B. argus* tank (0.60±0.24, n=5 and 0.40±0.24, n=5, respectively) (T-test p-value=0.58, Chi squared p-value=0.99) (figure 8).

Discussion

The results obtained in the field study demonstrate that there is no significant difference proportionally between the presence of *G. clavigera* on *B. argus* in the day versus at night (figure 1). However, the observed (though not significant) increase from day to night in the number of *G. clavigera* (2.5±0.58 to 3.1±0.67, n=11 and n=8,

respectively) (p-value=0.46) (figure 2) and the significant increase from day to night in the number of *B. argus* (6.1±0.16 to 9.9±0.29, n=11 and n=8, respectively) (p-value=0.0018) (figure 3) could imply that the increased

However, even though dark *B. argus* hosting dark *G. clavigera* and light *B. argus* hosting light *G. clavigera* is the most common occurrence, the fact that variation is occurring is an important matter. This variation provides insight into the lab experiment and hypothesis #2.

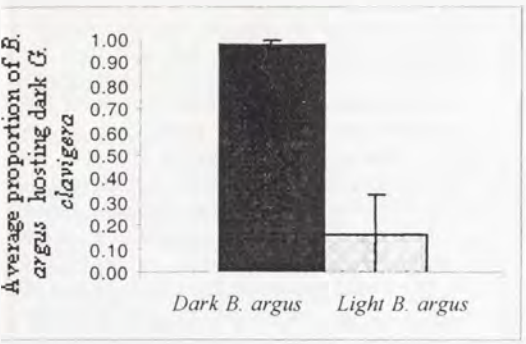


Figure 4. Of those *B. argus* hosting *G. clavigera*, the average proportion of dark morph *B. argus* hosting dark morph *G. clavigera* versus light morph *B. argus* hosting dark morph *G. clavigera*.

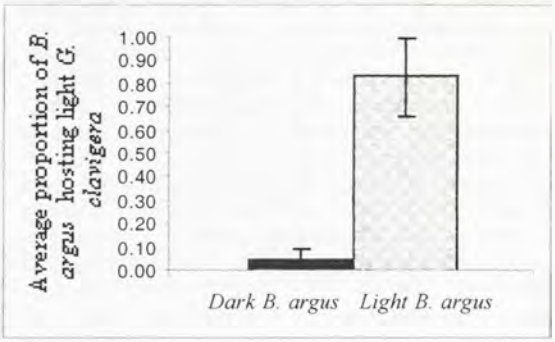


Figure 5. Of those *B. argus* hosting *G. clavigera*, the average proportion of dark morph *B. argus* hosting light morph *G. clavigera* versus light morph *B. argus* hosting light morph *G. clavigera*.

observation of *G. clavigera* at night was not due to an increased percentage of *G. clavigera*, but rather due to an increased number of *B. argus*. This nullifies hypothesis #1 (there is a significant proportional difference between the presence of *G. clavigera* in the day versus at night). The increased number of *B. argus* found at night is likely due to the probability that it has a daily rhythm in its feeding frequency related to light levels. This nocturnal feeding behavior has been observed among many other sea cucumbers in the genus *Holothuria*, including *Holothuria scabra*, *Bohadschia vitiensis*, *Bohadschia bivittata*, (Massin 1982; Yamanouchi 1956) and *Holothuria tubulosa* (Crump 1965). As Yamanouchi pointed out (1956), the species belonging to the same genus bear similar daily activity rhythms, thus supporting the idea that B. argus is a nocturnal feeder.

The significant difference between dark morph *B. argus* hosting dark morph *G. clavigera* and light morph *B. argus* hosting dark morph *G. clavigera* and vice versa (figures 4 and 5) supports the basic idea that the coloration of *G. clavigera* usually corresponds to host coloring (Britayev and Zamyshliak 1996; Gibbs 1969). The idea that this color congruency is due to predator avoidance is still a probable one.

The lab experiment demonstrated that if given the choice between a control tank with no organisms in it versus a tank with *B. argus* in it, *G. clavigera* would favorably go to a *B. argus* tank (while the Chi squared p-value was just over 0.05, this can be attributed to the small sample size – a definite trend was still observed as demonstrated by the low T-test p-value of 1.367E-6). This implies that some form of host recognition is occurring. In regards to how *G. clavigera* can recognize *B. argus*, a plausible answer is that certain chemical cues released by or from the host to the surrounding water explains host recognition behavior in polychaetes (Davenport and Hickok 1951). Since water was only flowing from the outside tanks into the central tank (where the *G. clavigera* were located), any chemical scent given off by *B. argus* would have to pass into the central tank before being drained out (photo 4). Since *G. clavigera* could not see *B. argus* or touch *B. argus* through the tanks, chemical cues are a likely alternative. It is important to note that 45% of the *G. clavigera* stayed in the central tank. A possible reason for this phenomenon is that some *G. clavigera* were too stressed by the sudden change in environment to move at all. Yet since these *G. clavigera* neither moved into the control tank nor the *B. argus* tanks, I have chosen to deem their response as a neutral one, thus not being positive or negative in regards to hypothesis #2.

The fact that there was no significant difference in preference for *G. clavigera* (light or dark) to choose

a particular morph of *B. argus* (figures 7 and 8) implies that while *G. clavigera* has a preference for its host, it doesn't have a color preference. This could be due to the fact that in some cases commensals respond positively to several taxonomically close hosts (Martin and Britayev 1998).

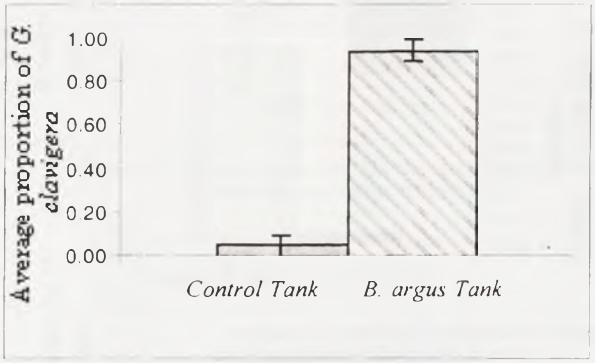


Figure 6. Of the G. clavigera that moved to an outside tank, the average proportion of G. clavigera going to the control tank versus a B. argus tank.

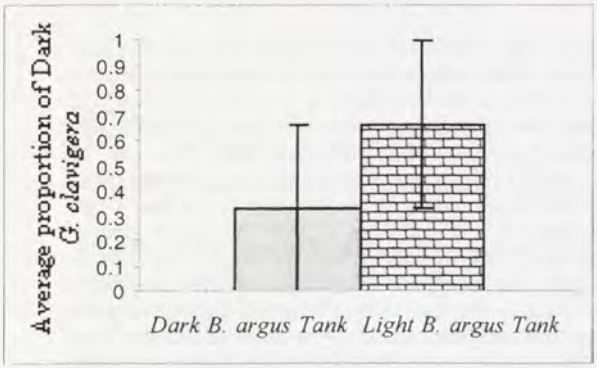


Figure 7. Of the dark morph *G. clavigera* going to a *B. argus* tank, the average proportion of dark morph *G. clavigera* going to a dark morph *B. argus* tank versus a light morph *B. argus* tank.

predator, *G. clavigera* would flee its host. Another possible reason arises due to the fact that several polynoid worms have shown strong territorial behavior, fighting against other conspecifics (Devaney 1967; Dimock 1974; Britayev 1991). It has been suggested by Martin and Britayev (1998) that territorial behavior may be generalized to most symbiotic polychaetes with regular distributions (*G. clavigera* included). In addition, the fact that *G. clavigera* occurs in isolated heterosexual pairs (Britayev and Zamishliak 1996) further supports the idea that competition could be occurring (mate competition in this case). Moreover, there is no indication that the species living in heterosexual pairs are not as aggressive as the species living alone (Martin and Britayev 1998). Two possibilities arise from the negative intraspecific interactions generated by territorial behavior: the death of one of the competitors or its relocation to another host (Martin and Britayev, 1998). Given these reasons, movement of *G. clavigera* between *B. argus* is likely to take place.

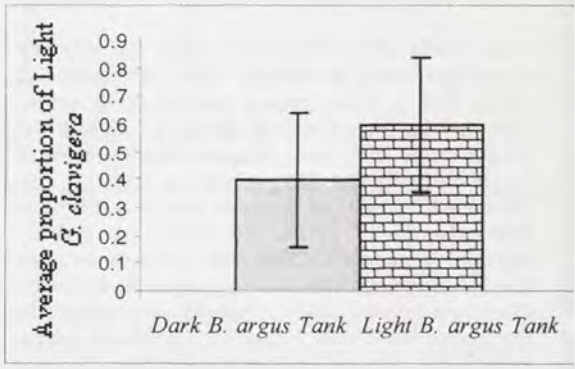


Figure 8. Of the light morph *G. clavigera* going to a *B. argus* tank, the average proportion of light morph *G. clavigera* going to a dark morph *B. argus* tank versus a light morph *B. argus* tank.

This suggests a possible biochemical similarity between the hosts, with their cues having the same or very similar chemical composition (Martin and Britayev 1998). Seeing that light morph *B. argus* and dark morph *B. argus* are the same species, this is most likely the case. This would explain why, in the wild, there are a small amount of light morph *G. clavigera* found on dark morph *B. argus* and dark morph *G. clavigera* found on light morph *B. argus*.

Given this information, one must ask, why would *G. clavigera* need to move in the first place? One possibility is that to avoid a

Based strictly on my lab data, when this happens, *G. clavigera* seems to have no preference in choosing a light or dark *B. argus*, so variation will occur. One must also take into consideration that unlike in an experimental tank, in the ocean *G. clavigera* may have to travel far greater distances to get to a new *B. argus*. So, to be particular about which color morph to pick may not be advantageous to the health of the *G. clavigera*. Even though *G. clavigera* is sacrificing less camouflaging ability when on a *B. argus* of a different morph, to continue to search for a *B. argus* of a similar color morph may be even more of a risk. Although not many light *G. clavigera* were found on

dark *B. argus* nor were many dark *G. clavigera* found on light *B. argus*, the fact that 76% of all *B. argus* documented more than once showed no change in the number of *G. clavigera* found on them suggests that this occurrence does not happen often. So one would not expect to see as much variability of *G. clavigera* on *B. argus* in the field when compared to the aquarium conditions. Thus hypothesis #2 (if given the choice, *G. clavigera* would attempt to choose a *B. argus* that matched its color morph) has been legitimately refuted.

Conclusion

This study examined the commensal relationship between the polychaete worm, *Gastrolepidia clavigera*, and the holothuroid, *Bohadschia argus*. In particular, it investigated the difference between the presence of *G. clavigera* on *B. argus* in the day versus at night, and the possible variation in preference of the two different morphs of *G. clavigera* (light and dark) for the two different morphs of *B. argus* (light and dark). Although neither hypotheses 1 or 2 proved to be correct, there was sufficient evidence to support why both hypotheses were false.

In regards to hypothesis #1, although there was no significant proportional difference between the presence of *G. clavigera* on *B. argus* in the day versus at night, there was a significant difference in the number of *B. argus* found in the day versus at night. The numerical difference most likely arises due to the idea that *B. argus* is primarily a nocturnal feeder, thus it is more easily found at night. Given the higher number of *B. argus* there is likely to be a higher number of *G. clavigera*, while the proportion of *G. clavigera to B. argus* still stays the same in the day versus at night.

In regards to hypothesis #2, no preference was demonstrated by either light or dark morph *G. clavigera* for light or dark morph *B. argus*. Both morphs of *G. clavigera* preferred both morphs of *B. argus* with roughly equal intensity and had practically no preference for the control tank containing no *B. argus*. This suggests a likelihood that *G. clavigera* has a special ability to sense *B. argus* (possibly chemoreception), yet it may not be able to differentiate between the two different color morphs. This approach may be advantageous in the wild where, when forced to move, the cost of searching for alternate *B. argus* may be greater than staying on a *B. argus* of a different color morph, even though camouflage ability (thus predator avoidance) would be limited.

Future work studying the symbiotic relationship between *G. clavigera* and its host species needs to be carried out in order to gain a better understanding of these rather complex relationships. If this study were to be repeated, I would suggest using a larger sample size for both the field experiment and the lab experiment. This would aid greatly in obtaining significant results. I also recommend a study looking more in depth at the color variability of *G. clavigera*. In particular, I would suggest a genetic versus behavioral study. One could look at possible genetic differences between the different color morphs of *G. clavigera* and compare that to the possible ability of *G. clavigera* to change color morphs when moved to a *B. argus* (or other host species) of a different color morph than its own. The genetic and behavioral data combined could provide valuable insight into whether the relationship between *G. clavigera* and its host species (*B. argus*, in particular) is a commensal one or a parasitic one. There is a rather extensive list of questions still not answered regarding the symbiotic relationships associated with polychaetes. Any future studies examining these relationships would likely provide more insight into this intriguing, yet widely unexplored, field.

Acknowledgements

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Invertebrate communities of *Inocarpus fagiferus* tree holes on Moorea, French Polynesia

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ABSTRACT. I sampled the detritus and water of *Inocarpus fagiferus* tree holes and the ground beneath the trees on Moorea, French Polynesia, to test for distinctness between hole and ground meso and macro fauna communities, and to test the theory that tree holes are functioning as ecological islands. Tree holes did not function like islands in terms of MacArthur-Wilson island biogeography models, as no correlation was found between tree hole size or height and species richness, diversity, taxonomic composition, or similarity to the ground of the tree hole's fauna. Species diversity was polynomically correlated with soil moisture, with the highest diversity found at intermediate soil moistures between 50% and 60% water, by weight. Soil moisture was on average lower in the ground than holes, making tree holes distinct microhabitats. Community and soil moisture differences caused mean diversity to be significantly higher in the ground than tree holes. Rainfall patterns further reduced the diversity of soil organisms in the dynamic environment of tree holes that could hold pools of water. Populations established during a drier period were diminished when a pool and aquatic community developed, and then recolonized by the more mobile soil and leaf litter species when the pool dried up. No significant difference in distribution between tree holes and ground was seen in taxonomic groupings of mites or insects, as most common species had a broad tolerance to soil moisture level. Water-filled tree holes, in contrast were distinct from soil communities and were important habitat for insect larvae.

Introduction

Species' distributions are strongly influenced by their range of tolerance to environmental factors. Communities develop through the interactions between organisms with similar environmental tolerances, so that the community composition is influenced by both biotic and abiotic factors. Habitat selection may further occur by organisms seeking to optimize each environmental factor, making microhabitats important within a larger habitat (Huhta 1996).

Theories of island biogeography (MacArthur and Wilson 1967) emphasize the importance of factors other than the environmental conditions and biotic interactions in influencing the community that develops on islands. The number of species found on an island is considered to be a function of island size and distance from the mainland. Larger sized islands theoretically can support more species, having a greater range of habitats and being able to support larger more stable populations. Proximity to mainland influences the probability of an island's colonization. The actual species composition will be influenced by organisms' ability to colonize over the barrier surrounding the island. According to the MacArthur-Wilson theory of island biogeography (1967), the dispersal ability limits the composition on the island, and the number of species on the island reaches an equilibrium based on the size of the island and its distance from the mainland. Subsequent researchers have described isolated ecological communities, like trees, mountain tops and pools as functioning like islands (Seifert 1975; Brown 1971; Janzen 1968)

Tree holes are crevices on the trunk and between branches of trees that can accumulate leaves, twigs, and water, and develop a detritus based community as the organic material is decomposed. In the context of islands, tree holes may be thought of as islands varying in size, distance from ground, and environmental characteristics, and the surrounding soil can be compared to the mainland as a source of colonizers. Further, tree holes show a broad range in leaf litter content and in soil moisture, from dry habitats to water-filled pools, potentially representing several different microhabitats. As habitat islands, tree holes may be a good model for studying community development and composition in terms of environmental factors of the microhabitat and its size and isolation.

Inocarpus fagiferus groves are found in valleys around the island of Moorea. The trees have buttress root structures and fluted trunks that lead to the formation of tree hole crevices. I analyzed the meso and macrofaunal composition of *Inocarpus fagiferus* tree holes by measuring environmental characteristics of tree holes, tracking the changes in tree hole communities over time, and investigating differences in ground communities and tree hole communities. I hypothesized that 1) Tree hole moisture and leaf litter thickness are correlated with species diversity and community

composition. 2) Tree hole communities change through time as soil moisture changes. 3) Tree holes are microhabitats with faunal communities distinct from ground communities in composition and diversity. 4) Tree holes are ecological islands, with the number of species in the tree hole correlated positively with hole size and negatively with hole height.

Figure 1. Map of study site location, Moorea, French Polynesia.

Materials and Methods

Study Sites

I chose three *Inocarpus* groves on Moorea, an island in the Society island chain of French Polynesia, as study sites (Figure 1). Site One was a 25 X 50 meter grove, at 230 meters elevation, located at the Belvedere in Opunohu valley. The ground had a 1-3 centimeter covering of *Inocarpus* leaves. The forest had an under story of ferns and young *Inocarpus*. The second site was at a higher elevation, 270 meters, at the Belvedere, along the Three Cocos trail. The grove was approximately 45x50 meters, with my sample area on a sloping hill's ridge. The edge of the grove had an under story of ferns. The third site was at Afareaitu, on the east side of the island, at low elevation. The site was rockier and on a steeper hillside than the other sites, with the ground heavily covered by hibiscus branches that have washed down from trees above. The sites were chosen as having *Inocarpus* forests with trees large enough size that a variety of tree hole sizes and types. All groves were bordered by hibiscus trees, nearby streams, and on hills. The spectrum of holes and soil sampled was increase by choosing sites on different sides of the island, with different rainfall patterns.

Sampling

At each site I first surveyed the large *Inocarpus* trees with holes, categorizing holes by size: small, medium, and large, and three main types : woody, moist, or with a pool. Small holes were less than 10x10 cm in surface area, and large holes greater than 20x20cm. Woody holes were made up of fine particles of decomposing wood from the tree's trunk, a mass of roots from the tree, and usually many decomposing arthropods. Most woody holes were sheltered by the tree trunk so received little direct rainfall. The moist category included holes that were to shallow or too irregularly shaped to hold a pool of water, and holes that were small or exposed to air enough that water had evaporated and no pool was present at the time of the survey. Holes with a pool had at least a .5cm depth of water above the detritus. The survey only included holes I could sample from, within 2 meters of the ground. I randomly chose holes from within each combination of categories to sample from, sampling 12 holes from site one, nine from site two, and eight from Afareaitu.

The first sampling day, October 10 at the Belvedere site one, I removed samples with a shovel, dug down to approximately 3 cm, and removed soil and leaf litter from an 8x8 cm area of the ground and up to ¼ of the material in a tree hole. On further collection days I used a soil core sampling method. The method was modified so that I would collect a smaller sample with a proportion of leaf layer to soil more representative of the holes and ground. This enabled me to better compare samples and to do a thorough job counting and identifying invertebrates by spending more time on a smaller sample. I removed samples from the center of tree holes and the ground .5m from the base of the tree, below the hole, with a ¾ inch piece of PVC pipe. I took a core to a depth of 3 cm, or to the bottom of the hole in shallower holes. Pools, very moist holes, and one small narrow hole could not be sampled with the pipe, so a shovel was used to remove an equivalent volume of material from the hole. Additional water was sampled from pools with one draw of a turkey baster. Samples from October 10 were subsampled to obtain a volume more comparable to that taken in the core samples.

Sampling days were spaced out to reflect changes in tree holes as the weather changed from the dry season to the onset of the rainy season. I sampled each hole and the ground below the tree at the Belvedere Site One on October 10, 2000, October 19, and November 7.. Belvedere Site Two was sampled once, on October 24, and Afareaitu on October 12, October 27. October 10 and 12 were after several days with a few hours of rainfall. Little rainfall occurred between October 11 and 29, so samples taken between the dates were done in a dry period. October 17th had a short drizzle, which would have made holes and ground not at their driest when sampled. Starting on October 30th were several days of hard rainfall, so final samples were taken during a rainier period.

Hole environment

For each hole I measured the average depth from the bottom of the hole to the brim, and the average length and width. I also measured the hole's height above the ground, the depth of the leaf litter layer, the depth of the more decomposed organic matter beneath the leaf litter, and the depth of water above the soil for holes with pools.

To calculate soil moisture, I recorded the wet weight of each sample, and then placed the sample in the drying oven for a minimum of 12 hours at a temperature between 70 and 85 degrees Celsius, until constant weight of the samples was obtained. I recorded the dry weight and percent water in the sample. After drying, I estimated the percent volume in the sample of leaves, twigs, and roots.

Faunal composition

I counted and identified all invertebrate species larger than .3mm in samples using a hand sorting method, under a dissecting microscope. The method was chosen to allow for an inclusion of a large size and species range of organisms, and a large number of samples. I laid the soil sample out in a thin layer on a petri dish, and scanned all material under a dissecting microscope at 7.5 power. I used dissection picks to pull apart all clumps of dirt and all decomposing twigs, and to look at the surfaces of all leaves in the sample I sketched, recorded characteristics, and collected species I could not identify. I spent a minimum of 20 minutes scanning each sample, or until all material was looked examined.

Data analysis

Tests used data from the first sampling session at each site to avoid problems of pseudoreplication. Other sampling dates were used to look at changes through time. I calculated the species diversity of each sample with the Shannon-Weaver Diversity Index: $H=\text{Sum} -(p_i \ln p_i)$.

Regression analyses were done with height, surface area, and volume as independent variables. Regression analysis was also done with soil moisture, pool height, and leaf litter layer as independent variables. Regressions, and further tests, were run with the dependent variables of species diversity, species richness, total number of individuals and species, and number of mites, uropodina mites, cryptostigmatid mites, collembola, insect larvae, annelids, nematodes, six common mite species, two common collembola species, and nematodes.

One-way ANOVA tests, and Wilcoxon Ranked Sum test for nonparametric data were used to look for changes over time between the three sampling sessions at the Belvedere site. Sampling date was the independent variable and the dependent variables listed above for the regression analyses were again used.

A Paired t-test, or Wilcoxon Test for nonparametric paired data was run for comparing the tree holes with the ground below them, for each dependent variable (listed above). Tests were run separately for my three hole types; woody, moist, and pool.

Results

Community characteristics

In tree holes and the ground, I categorized 138 species based on their general morphology. The majority of arthropods belonged to the order Acari and Collembola, with prevalent cryptostigmatid mites and mesostigmatid mites of the groups Uropodina and Gamasidae, and collembolids of the suborders Isotomidae and Sminthuridae. Two Isotomidae were abundant, one with a reduced furca and more common in the soil, and another smaller and more active. Annelid species were found, with Enchytriadae most abundant, and found in environments ranging from low moisture to pools of water. Nematodes also represented a large proportion of individuals found, even with only ones longer than .3mm included in my count. Less common groups included four myriapod species and two microgastropod species. Other insect species included ants, coleoptera, coleoptera and dipeteres larvae, and thysanura. Water-filled tree holes contained aquatic communities with dipteres larvae, including mosquito larvae, copepods, ostracods, flatworms, annelids, and nematodes. Similar factors controlled distribution of multiple species, as seen in the positive correlation of the number of springtails to number of mites in samples ($R^2=.29$, $p<.0001$). Community species richness was positively correlated with the number of individuals ($R^2=.71$, $p=.0001$).

Soil moisture

For ground samples, I found a positive linear relationship between percent water and the number of species ($R^2=.49$, $p=<.0001$), individuals($R^2=.46$,$p=.0001$), and diversity ($R^2=.34$, $p=.002$) (Figure 2). Ninety five percent of the ground samples were under 65% water. For the second Belvedere sampling session, at the most dry period, ground soil moisture levels ranged only from .22 to .39, and the correlation of species with soil moisture was not evident. For holes, in contrast, regression analysis for the effect of soil moisture on species richness and diversity showed a quadratic relationship ($R^2=.67$, $p<.0001$), with lower diversity and species richness found in dry holes and holes with higher soil moistures higher than the moisture

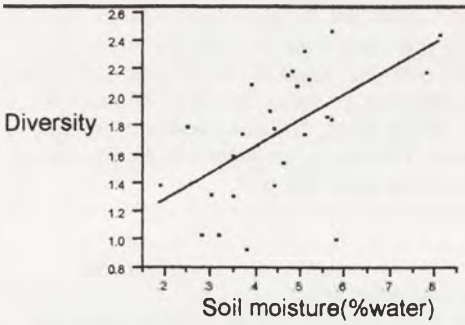

Figure 2: Regression of species diversity and soil moisture for ground samples, including the first sampling session at each site. R^2= 34, p= .002

level usually found in the ground. The curve had a maximum between 50% and 60% as optimal soil conditions for high diversity (Figure 3).

The same trend with hole soil moisture was found for the number of collembola in the sample (R^2=.28, p=.0002), and the number of mites (R^2=.25, p=.0005). Other taxonomic groupings had organisms with distributions within an intermediate range of soil moisture, though not enough individuals were seen in samples to display the polynomic trend.

The second Belvedere sampling session was during the most dry period, with soil moisture in the ground ranging only from .22 to .39, and species richness showed no correlation with water.

Leaf layer thickness

Tree holes ranged in leaf litter thickness of zero to ten centimeters above the hole. The layer thickness did not significantly fluctuate with time, before or after rainstorms. The leaf layer thickness was positively correlated with species richness in non-water filled tree holes (R^2=.22, p=.03), but not in water filled tree holes. The percent of leaves in my core sample was positively correlated with species richness for ground samples (R^2=.22, p=.015) and non-water filled holes (R^2=.24, p=.02). No correlation was found between the thickness of the leaf layer in holes and the soil moisture.

Changes through time

Soil moisture showed a greater fluctuation through time in the ground than in tree holes (Figure 4). Soil moisture of tree holes fluctuated between the three sampling days, though no significant difference was found. The lack of rain around the October 19th sampling day did not have a significant effect on all holes, with differences in hole size, leaf layer, and exposure to sun causing some holes to loose more moisture than others. Soil moisture of the ground in contrast was significantly lower on October 19th than October 10th or 17th (p<.0001).

Species richness of tree holes significantly differed between the three sampling days at the Belvedere (p=.049). Species richness was significantly higher on the first sampling day, October 10, than October 19 during the drier period, with a positive value in a Tukey test. The ground in contrast had similar species richness found in the October 10 and 19, and significantly lower species richness on November 7. Species diversity did not significantly differ with rainfall changes in ground or tree holes.

Annelids and one collembola species were significantly more abundant on the first then second sampling day (p=.04 and .03 respectively).

Monitoring of pools showed their temporary nature. All pools dried up at some point within the sampling period, having no water layer above the soil for an aquatic habitat. Hole communities subsequently lost aquatic species, at least at the top layer that was sampled, and a drier habitat became available for soil organisms. Two holes at Afareaitu had pools the first sampling date and not the second. In the 15 days between them, one cryptostigmatid mite colonized one hole, and 2 species, including an oribatid mite, were found in the other hole. Species usually found in pools were not found in my samples of the dried pools on October 19. These holes had lower species richness and diversity than other holes and the ground, which would have contributed to the polynomic relationship between species and water.

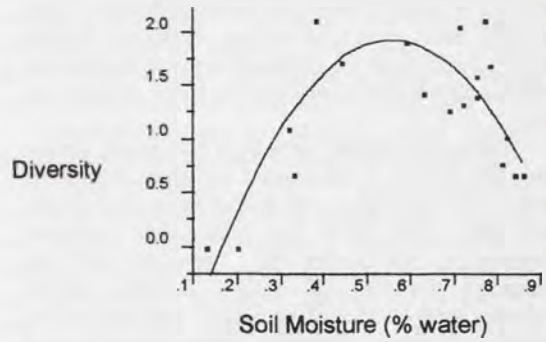

Figure 3. Second order polynomial regression of soil moisture (percent water) and species diversity of non-water filled tree holes, on the first sampling date at each site. Diversity = -1.9811 + 13.9974 water - 12.5204 water^2. R2=.695. p<.0001.

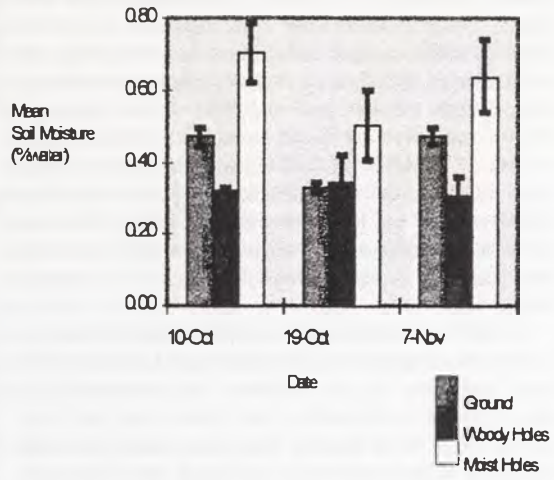

Figure 4. Changes in soil moisture through time at samples from Belvedere site one. For ground, n=12, for woody holes n=2, for moist holes n=6.

Differences between holes and ground

Soil moisture was significantly different between tree holes and ground, with a paired *t*-test $p<.0001$. Separating samples by hole type, woody tree holes were not significantly different in soil moisture with the ground, instead having a positive linear correlation ($p=.03$, $R^2=.93$) with more moist holes above more moist ground.. Other non-water filled tree holes were significantly more moist than the ground, $p=.003$, and a mean difference of .22. In the two cases where tree holes were drier than the ground sample, one hole was extremely small, and the other very rocky, shallow, and exposed to sun.

Fifty-nine species were found only in holes, of which 10 were only in pools, and only 13 were found in more than one hole sample. Thirty-seven species were found only in the ground, of which 14 were found in more than one sample from the ground. Twenty-five mite species were found only in holes, and 19 species only in the ground. Five collembolan species were only in holes, and four only in the ground. Species were not found in enough samples to draw conclusions on species as hole or ground specialists or on species' preference for holes or ground

Species richness and diversity was significantly higher ($p=.008$, $p=.002$) in ground than tree holes, in a paired t-test for the first sampling session at each site.

Holes in the moist category, most environmentally similar to the ground, were not significantly different in species richness from the ground beneath them ($p=.86$), but diversity in holes was lower than ground diversity ($p=.03$). Comparison of moist holes and ground did not give significant results for taxonomic groupings of species. The number of collembola in the tree hole was positively correlated with the number in the paired ground sample ($p=.005$, $R^2=.45$), indicating that the same factors are influencing ground and hole population levels.

Woody holes were not significantly different from the ground in any factors, likely due to a small sample size. More insect species other than collembola ($p=.09$) were found in the woody hole environment. Isopoda was only found in woody holes, and thick trails of ants were often seen running through the woody hole environment.

Pools had a dramatically different community than ground, containing no soil mites or insects. Organisms found in pools, and not ground included ostracods, copepods, flatworms, annelid species, and mosquito and other dipteres larvae. Nematodes and enchytriadae were in common between the environments. Pool communities were characterized by a few insect larvae and then huge populations of the smaller aquatic organisms, while the ground had a more even distribution of individuals of different species, with populations not reaching such high numbers or densities.

Island characteristics: hole size and height

The regression of hole volume, surface area, and height with the number of individuals, species richness, species diversity, and difference in species richness between ground and tree holes showed no significant correlation in water filled or non-water filled tree holes. No correlations were found for taxonomic groupings of species. One effect of hole size was that larger holes with pools maintained the pools farther into the dry period of sampling than smaller pools.

Discussion

Soil moisture and leaf litter thickness impacted the diversity, species richness, number of individuals, and to some extent the composition of the community that developed in a particular microhabitat. As environments with high soil moisture levels, tree holes offered unique microhabitat characteristics from the ground. My results lead support to the importance of environmental characteristic in determining community properties, with little evidence of more general theories of island biogeography being related to the case of tree holes.

Soil Moisture

The correlation between soil moisture and species richness may be related migration abilities of organisms

choosing optimal habitats. Vertical migration in response to moisture and temperature has been documented in soil organisms including mites and springtails (Butcher Snider and Snider 1971). At low soil moisture, organisms moved deeper into the soil to moister soil levels, so that fewer organisms were found in the top three centimeters that I sampled with my core. The extent of vertical migration to avoid desiccation is limited by the size of organisms and the decreasing pore size of the soil at deeper layers (Wallwork 1967), so that further mobility by way of horizontal migration to the more favorable, more moist microhabitats may have occurred (Huhta 1996). A few sample cores at lower levels showed a low count of organisms, so that the limited vertical migration may have influenced horizontal migration that led to eventual colonization of tree holes.

The polynomial relationship of species richness and diversity with soil moisture indicates that many species have overlapping ranges of tolerance and optimal levels for soil moisture between 55% and 60% water. The high water conditions of holes are likely less optimal for species adapted to the ground's lower soil moistures. The maximum of the curve indicates that, at least during the period sampled, high soil moisture rarely limits ground populations adapted to the normal range of ground soil moisture of up to around 65%. Hole soil moistures were often found outside of this range, so that fewer species could tolerate the high water conditions, and possible flooding of the spaces in the soil needed for movement and breathing. Habitat preference for optimal soil moistures would lead to more individuals and likewise more species found in areas of soil moisture around 60%. The response to soil moisture shows community composition being structured by similar responses to environmental factors.

The high moisture levels in low diversity tree holes may indicate pools that were recently flooded, devastating the populations of soil organisms when a pool formed. For more moist holes, less time has past since flooding, so less time has been available for soil organisms to recolonize after the pool dried out. Frequent flooding of holes would make it difficult for populations to become established in the hole, and make the environment less preferential because it is more prone to flooding.

Numerous studies have shown the importance of soil moisture in soil communities, with the number of collembola and mites positively correlated with soil moisture through space and time (Joose 1981; Mir 1994; Badejo 1993; van Nieuwenhuizen et al. 1994). My data showed similar results at low soil moistures.

Leaf layer thickness

In addition to soil moisture, the depth of the leaf layer correlated with diversity in non-water filled tree holes. The presence of leaf layer increased the habitat diversity within a tree hole. Habitat availability has similarly been found as an important factor determining island species richness (Seifert 1975). In the tree holes, the leaf litter layer provided an additional habitat layer to be used by leaf litter dwelling species, and the humus layer below provided habitat for humus dwelling species. A thicker leaf layer provided habitat for more leaf dwelling individuals, and likewise species, and may have protected organisms from desiccation by increasing the humidity level.

Leaf litter layer in contrast showed no relationship to hole diversity in pools. Srivastava and Lawton (1998) used leaf litter as an indicator of productivity in phytotelmatid communities, and found that leaf litter correlated with an increase in species, possibly because there was a lower risk of holes drying out. My study did not find a similar relationship because I included smaller organisms that were not able to selectively choose their habitat, in contrast to ovipositing mosquitoes in the previous study.

Other factors

Biotic factors such as prey distribution and the influence of earthworms on the soil environment can influence the community composition and properties (Salmon and Ponge 1999). Moist tree hole's lower diversity yet higher species richness than the ground's may be evidence of differing biotic interactions in holes and ground. The lower diversity of holes indicated that some species were more dominant in holes, possibly reaching higher population in response to hole biotic factors like increased prey availibility, less competition, or less predation. Seifert (1975) found that phytotelmata of greater area had reduced evenness, as populations of common species were more likely to increase before many new species colonized. Similar effects may be occurring in tree holes, where in an optimal environments, increase in populations of the common species will occur at a greater rate than the arrival of new species migrating into the better environment.

Changes through time

In the time of year of sampling, the transition from the dry to wet season, organisms in holes were faced with problems of flooding during rainy periods, while organisms in ground were more at risk of desiccation during dry periods. The community that develops in a tree hole would reflect the range of soil moisture

through time. Lower species richness in dry periods may be a consequence of vertical migration. The effect of the dry period around October 19th may have been greater on the ground communities than hole communities, as species richness of holes returned to its original level after the dry period, but species richness of the ground further dropped. Desiccation may have reduced population sizes in ground samples, or organisms may have modified their life history habits for the drier period by vertical migration or reduction in birth rate (van Nieuwenhuizen et al. 1994). Other studies have shown changes through time to be in response to population fluctuations of species in response to their breeding cycles rather than the environment (Badejo 1993). The decline in collembola between sampling days may be explained as the natural population fluctuation.

Differences between holes and ground

I found tree holes and ground to differ in soil moisture and leaf litter layer, so that the presence of holes in the forest increased microhabitat diversity. The spectrum of tree hole environmental characteristics, with the hole's size, shape, orientation on the tree, and exposure to the sun relating to its water and leaf holding qualities, further increased the microhabitats available. The distribution of species between holes and ground may be reflective of species' ranges of tolerance to environmental conditions of the microhabitat.

A lack of significant difference in species composition of ground and tree holes may be because the species I sampled were from upper soil and leaf litter environments. The environments are more exposed to temperature and moisture fluctuations than lower ones, so that the species found in the upper layers would more likely be tolerant of the range of environmental conditions of tree holes and ground, and have developed ways of dealing with them. They would not, then, show a preference for holes based on these characteristics. Upper layer species are generally more mobile in non-soil environments than species in lower layers, and more capable of withstanding migration to tree holes. Furthermore, the ground farther from the base of the *Inocarpus* tree may represent a more distinct environment and community than where I did my sampling, in areas influenced by the tree and its root system.

In looking for species preference or specialization for ground or tree holes, and environmental characteristics, I was only able to analyze the distribution of the common species that occurred in multiple samples. Their commonness may have been a consequence of tolerance of a wide range of environmental characteristics, including those unique to tree holes, so that few trends were identified. Out of the large number of rare species found only in tree holes or ground in my samples, it is possible that some species have a preference for tree holes and others limited to the ground environment, though more data collection is needed to determine if this is found.

Contrasting to organisms that disperse to the woody and moist hole environments, are the organisms in pools. Larvae are hole specialists, with dipteres larvae requiring stagnant pools, like tree holes, for development, and specifically select oviposition sites based on factors like canopy cover and the time duration of the pool (Becker 1992). Other organisms in pools may be transported to holes by wind and rain, as they are not found in the soil environment.

Tree holes as islands

The lack of correlation of hole height and volume with species richness gave little support to the consideration of tree holes as islands. Holes did not act as islands for the organisms I studied because of their high dispersal abilities. Hole height, in the two meter above ground range that I studied, did not act as an important barrier to species distribution. Island biogeography theory would predict a lower number of species in higher tree holes because only a limited number of organisms could disperse there (MacArthur and Wilson 1967), but differential dispersal ability was not seen in tree hole organisms to the extent that it would affect species richness or composition of the hole. Sampling from higher tree hole heights may provide better insight on dispersal ability of soil and tree hole organisms. Dispersal mechanism, whether by climbing the tree, or being moved by wind, water, or other animals may also be an important consideration.

A further important difference between holes and islands is the unstable, changing nature of tree holes. In water-holding tree holes, communities are continually wiped out and then reestablished, and so have only a limited time for development between heavy rainstorms. The organisms found in holes are the species with adaptations to survive environmental fluctuations and the good disperses and colonizers, without time being available for chance colonizations by many other species.

My data showed no relationship between hole volume or surface area with the number of species. For other systems it has been hypothesized that a larger area would provide enough resources for more functional groups, have greater microhabitat availability, support larger, less extinction-prone populations of species, and be a larger target for dispersal (March and Bass 1995). The relationship may not be present in the tree hole system because of the high dispersal ability of the

organisms under study, moving to optimal microhabitats as a standard life history strategy of many mites and collembola (Wallwork 1967). With individuals easily and frequently moving between ground and holes, populations would not go extinct. Furthermore, my constant volume sampling of a portion of the tree hole rather than the entire hole made my sampling method differ from other studies that have found correlations between habitat area and species richness, with the relationship: Species richness= $C*Area^z$, where C and z are constants (Macarthur and Wilson 1967). My data showed that the number of species per 8.6 cm^3 sampled did not increase with hole volume, but as the hole environment is heterogeneous, the number of species in the sample may not be reflective of the number in the entire hole. For example, some species are found more commonly at the edge than center of the hole (Ferguson 2000). The positive linear relationship between the number of individuals and the number of species suggests, however, that if more total individuals are in a large hole, more species may actually be found there.

Other studies have found phytotelmata like water filled tree holes to have some similarities to islands. Seifert (1975) found species area relationship to apply to *Heliconia* inflorescences, water filled habitats, as islands of varying area, although I found no relationship in water filled tree holes. Frank and Lounibos (1987) found that water-filled tree holes resemble islands for lower organisms, with colonizers that are not selective and colonize sequentially. For higher organisms, in contrast, a rapid arrival of all selective, specialist biota is seen, not resembling island communities. These concepts also apply to the non-water filled tree holes I sampled. Like on islands, most organisms in tree holes were not hole specialists. Looking at colonization of dried pools showed further island-like characteristics, with sequential colonization, species richness increasing through time, and communities changing as new species arrive and other populations die out. Trends in the distribution of selective insects depositing larvae, in contrast, were unlikely to be consistent with island biogeography theory. Ideal environments in holes for oviposition were most likely chosen highly selectively using characters like height, volume, and shade (Lounibos and Machado-Allison 1982).

Conclusion

Tree holes function as microhabitats within the forest soil community. Differences between tree holes and ground diversity support the concept of microhabitat selection by soil animals through horizontal migration. Diversity was highest in areas with soil moisture within an optimal range for numerous species, so that high diversity was a product of environmental conditions. Preference for the environmental conditions of tree holes may lead to tree hole specialists, while colonization ability restricts access to tree holes for some lower soil dwelling species. Tree holes may function like islands in their sequential colonization by soil and leaf litter species although island biogeography theories did not apply to tree holes because of their temporary nature, fluctuating between distinct aquatic and soil communities, and the strong influence of the environmental characteristics of the habitat on the community. Further research into the function of tree holes as microhabitats and islands is needed, including investigations of colonization processes, hole changes through a longer time scale, studying a broader range of hole heights, and classification of fauna.

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Distribution and desiccation tolerance of the filmy fern genus *Trichomanes* in two stream valleys on Moorea, French Polynesia

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ABSTRACT. Three native fern species within the *Trichomanes* genus were found growing in *Inocarpus fagiferus* groves on Moorea, French Polynesia: *T. bipunctatum, T. humile,* and *T. tahitense* (a new species record for Moorea). In Opunohu and Afareiatu stream valleys, six field sites were chosen based on elevation and substrate availability for epiphytic or lithophytic growth. Percent cover for fern species and forest canopy cover were measured at each site. Abiotic factors were tested to assess distribution patterns: valley location, altitude, substrate, aspect, and height from forest floor. For the desiccation experiment, fern fronds were left to dry for a twenty-day period. Rehydration response rates of the fern fronds were observed and recorded. Results for the distribution study showed significantly more mean percent cover of *T. bipunctatum* in Opunohu than Afareiatu Valley. At high elevations, there was significantly less mean percent cover of *T. bipunctatum*, and more of *T. tahitense*. The average cover of *T. humile* was higher on boulders; *T. tahitense* cover was higher on bark substrate. There was no significant relationship between fern cover and aspect. There was significantly high cover of *T. bipunctatum* on top of boulders. High percent cover was measured of *T. tahitense* at the base of trees. *T. humile* and *T. tahitense* percent cover depended significantly on canopy cover above rocks and trees. Results for the desiccation experiment showed that all three species survived for twenty days after being dehydrated, and *T. tahitense* exhibited the fastest rate of moisture loss.

Introduction

The filmy ferns (Hymenophyllaceae) are an incredibly diverse family. At least six hundred species are found in both the Old and New World tropics today (Iwatsuki 1979; Brownlie 1977). Filmy ferns also claim a large geographic range, with genera in India, Japan, Melanesia, Polynesia, and the Americas (Maxon 1924). In rainforests, these delicate ferns may be found hanging from trees epiphytically, or growing terrestrially (Madison 1977). Their tiny fronds are adapted to flourish in shady, mesic environments.

Systematic debates dominate filmy fern literature because of their extremely diverse morphology and plasticity. Classically, the Hymenophyllaceae has been divided into the two genera, *Trichomanes* and *Hymenophyllum*. The distinctions between the two are based on morphological differences of their collection of spores, or sori (Copeland 1933). *Trichomanes* tend to have a cylindrical shaped covering over the sorus called an involucre, or indusium. In contrast, *Hymenophyllum* possesses a bi-valved involucre. Although Morton in 1968 acknowledged Copeland's bigeneric system, he revised the family further into six genera and numerous subgenera. In 1977, Iwatsuki found a diverse range of soral morphology, reporting eight distinct genera. More recently, Dubuisson in 1997 preformed a cladistic analysis based on morphological data for *Trichomanes* and *Hymenophyllum*. Current DNA research shows exciting phylogenetic relationships supporting monophylly for *Trichomanes* and *Hymenophyllum* (Pryer et. al 2000) (Appendix A).

Even so, research studies on the ecology of the Hymenophyllaceae are almost non-existent! In the South Pacific, the first formal collection of ferns from Tahiti occurred in 1922 (Maxon 1924). Copeland in 1932 published a more extensive key of the fern species found in the Society Islands. However, upon review, I found this key neither dichotomously arranged, nor very descriptive of the *Trichomanes* genus. On the tropical island of Moorea, (17° 29' 20 S, 149° 49'30 W) only four species within the genus *Trichomanes* have been formally documented: *T. bipunctatum, T. humile, T. caudatum,* and *T. dentatum* (Copeland 1932). Nothing is known about their distribution or role in forest ecosystems. The purpose of my study was to 1) determine distribution of species within

the filmy fern genus *Trichomanes*, and 2) measure frond dehydration and rehydration rates on Moorea.

Hypotheses Tested

Investigating a plant's spatial distribution in the field is the first step in understanding its role in tropical ecosystems (Lobban, 1997). As I hiked around the different stream valleys in Moorea, I observed a variety of environmental factors that could influence *Trichomanes* growth in the wild: valley location, elevation, substrate for epiphytic growth, aspect on substrate, vertical height from forest floor, and moisture response. I then proceeded to test the following hypotheses:
1) There is more fern percent cover in the wetter Opunohu than Afareiatu Valley 2) There is more fern percent cover at higher elevations 3) Species percent cover differs on rock versus bark 4) Direction does not effect percent cover on different substrates 5) There are vertical stratification patterns for species percent cover on different substrates 6) Species percent cover depends on canopy cover and 7) Fern fronds desiccate and rehydrate at different rates over time.

My research was conducted in three parts: 1) a general field survey to find and identify filmy fern species in different valleys, 2) account for abiotic factors which may effect *Trichomanes* distribution, 3) test desiccation tolerance thresholds of these species.

Materials & Methods

Field Survey

I collected filmy ferns samples for species identification from Opunohu, Paopao, Afareiatu, Paoroa, and Toto Valleys. Specimens were prepared using a plant press and sent to the University of California, Berkeley Herbarium (Dr. Alan Smith) for formal identification. Due to the fact that I had limited time (2 months) to determine patterns in filmy fern distribution, I decided to sample within two stream valleys: Opunohu Valley and Afareiatu Valley. Opunohu valley is found toward the interior of Moorea, and is more sheltered from oceanic influences such as high winds, salt spray, and mild temperatures. Afareiatu Valley on the other hand, is located on the eastern side of Moorea, and may be more prone to maritime influences (Figure 1).

Figure 1. Map of Moorea, indicating valleys sampled and field sites.

Three *Trichomanes* species were identified during my survey: *T. bipunctatum*, *T. humile*, and *T. tahitense*. *T. bipunctatum* was found growing epiphytically on *Inocarpus fagiferus* trees, and on basaltic boulders. It is dark green in color, and has an average frond length of 8 cm, winged stipe, and triangular-lipped involucre. *T. bipunctatum* often curls and turns a darker color during dry spells in the forest. *T. humile* is a lighter green color, has an average frond length of 4 cm, and possesses a cylindrical involucre. This species was also found growing on similar substrate and exhibited similar response to moisture as *T. bipunctatum* in the field. *T. tahitense* has small 1.5 cm dark green, peltate fronds. It was found growing appressed to various trees and rocks as well. Preserved specimens of my collection from Moorea and Tahiti can be found at the University of California, Berkeley Herbarium.

Site Selection

Sites 1 through 3 were located in Opunohu Valley, and sites 4 through 6 were in Afareiatu Valley. Sites were selected in each valley to represent high (~200m), medium (~100m), and low (~0m) elevations. Each site was also chosen based on occurrence of filmy ferns, upper story dominant *Inocarpus* trees, boulders, and close proximity to streams (Figure 1, Table 1).

Table 1. Description and location of study sites. Sites 1-3 are in Opunohu Valley, sites 4-6 are in Afareiatu Valley.

Site	Altitude (m)	Location/Description
1	180	60 meters downhill on Marae trail from Belevedere outlook; dry stream bed ran through the center of an older *Inocarpus* grove along the main fork of the Opunohu River, mostly fern understory
2	80	100 meters north-east and perpendicular to the French Agricultural School along an Opunohu River tributary, old *Inocarpus* grove many trees growing with roots and buttresses submerged by stream
3	40	20 meters south-east from fence marking trail on Mari Mari Kellum's property stream on right hand side of grove, Opunohu Valley
4	130	Trail head at pka 8.5, Afareiatu Valley on the East Cascade tributary fork, uphill, approximately a 40 minute hike into *Hibiscus sp.* forest, site along trail 6 meters south of main cascade; site mostly older *Inocarpus* trees, first collection of *T. tahitense* on bark
5	80	Trail head at pka 9, Afareiatu Cascade Trail, site on left when hiking uphill (first 80 meter elevation reading on altimeter); a young and drier (?) *Inocarpus* grove
6	30	Along same trail as site 5, a tributary from the Afareiatu Cascade, older grove (first 30 meter elevation reading)

Distribution Study

Trichomanes species were sampled within Opunohu and Afareiatu Valleys to determine trends in elevation and species abundance, as well as substrate preference and distribution. Sites were found on foot using an altimeter for high, medium, and low elevations (Whittaker 1970). Using transect tape, a $300m^2$ plot was measured for each site. I sketched the general landscape, mapping the ten largest boulders and the largest *Inocarpus* trees present. Boulders were mapped only if they possessed a diameter between 1.5 to 4 meters. *Inocarpus* trees were selected if they had an estimated trunk diameter greater than 0.5 meter. Boulders and trees were then marked using ribbon. A random number table was used to choose six trees and six boulders within each plot.

Fern species percent cover was measured to estimate if environmental factors at my sites effected the spatial growth distribution of *Trichomanes* in *Inocarpus* groves. I used a $400cm^2$ quadrat, with a grid of twenty squares, to sample both substrate types. The quadrat was placed at vertical heights 2m, 1m, and at the base of an *Inocarpus* trunk. Percent cover was recorded *for T. bipunctatum, T. humile, T. tahitense*, moss, lichen, bare bark or rock, and other ferns. I repeated this technique on the North, East, South, and West directions on each replicate tree using a compass. A similar method was applied to boulders, where high, medium, and low heights were measured for the four directions on each boulder. Ocular percent canopy cover over each tree and boulder was also measured and recorded.

Various statistical methods were used to understand the distribution of *Trichomanes* in the wild. Two-sample t-tests were implemented on my data to compare the average percent cover of the species found against geographic location (valley), elevation, substrate for epiphytic growth (basaltic rock or bark), aspect, height from forest floor, and percent canopy cover above the ferns. I also used Two-way Analysis of Variance (ANOVA) tests to assess whether *Trichomanes* percent cover is influenced by the different abiotic factors mentioned above.

Desiccation Experiment

I dehydrated fern fronds from *T. bipunctatum, T. humile*, and *T. tahitense* to assess 1) how long each species could last without direct moisture and 2) how long it took for fronds to curl up after rehydration. Ten 1 gram samples of each filmy

fern species were collected on October 20th from the 3-Coconut trail (elevation 270m) in Opunohu Valley. All samples were collected from random *Inocarpus* trees, with the bark attached. Rhizomes were carefully separated from the bark, and 1 gram of each fern species was placed in ten open petri dishes. Samples were left to dry for twenty days between 10/25-11/10.

Frond response after rehydration was also tested during these twenty days. "Response" was defined as when 2/3 of the fronds in a petri dish remained flexed, and full of water. A change in response, then, was when the fronds began to curl and dehydrate once more. Two replicates of 1 gram samples for each species were sprayed randomly with 4 grams of deionized water every four days. Data were recorded on days when the samples were watered. I observed fronds at ½ hour intervals using a dissecting scope over the course of three hours. I then created three categories describing fern frond response to water: 1) initial response, 2) response; then curled up, and 3) no response. Observations of these categories were recorded for each of the 3 filmy fern species. Finally, a linear regression analysis was used to determine whether species percent cover was dependant on frond response after water rehydration.

Results

Field Survey

Fifty-five *Trichomanes* specimens were collected and pressed between 9/18-10/5 from different valleys on Moorea. Voucher specimens were sent back to the University of California, Berkeley for formal identification. Other species of ferns found growing epiphytically in association with *Trichomanes* species were also noted and collected. Furthermore, filmy fern fronds were preserved from Moorea and Tahiti with Drierite, to be used for DNA analysis to help build genetically based phylogentic trees of the two genera *Trichomanes* and *Hymenophyllum*.

I also attempted to identify my filmy fern specimens using Pteridophytes of the Society Islands (Copeland 1932), and found it neither dichotomously arranged, nor very descriptive of the *Trichomanes* genus. Upon returning to Berkeley, I measured average fern frond length for the specimens collected, and described each *T. bipunctatum*, *T. humile*, and *T. tahitense* in depth. Finally a dichotomous identification key was created for *Trichomanes* on Moorea, which I modified from Copeland's key (Appendix B).

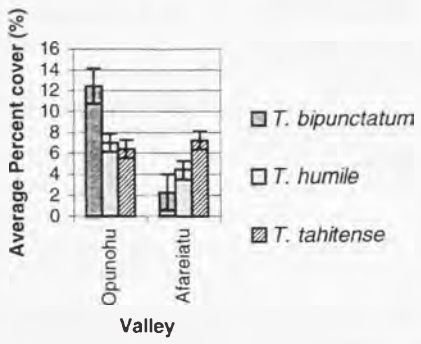
Distribution Analysis

The following environmental factors were analyzed against percent cover of *Trichomanes* species, and percent canopy cover measured: 1) valley, 2) elevation, 3) substrate (rock or bark), 4) direction (North, East, South, West), and 5) vertical height from forest floor.

Valley

A two-sample t-test was conducted on the three *Trichomanes* species sampled on Moorea, grouped by stream valley. *T. bipunctatum* has a significantly higher mean percent cover in Opunohu Valley ($\bar{x}$ =12.4%, n=432) than in Afareiatu Valley ($\bar{x}$=2.3%, n=432, p=0.003). Mean percent cover for *T. humile* was not significantly different between the two valleys (Opunohu: $\bar{x}$=7.0%, n=432; Afareiatu: $\bar{x}$=4.4%, n=432, p=0.141). Similarly, mean percent cover for *T. tahitense* was not significantly different between the two valleys (Opunohu: $\bar{x}$=6.4%, n=432, Afareiatu: $\bar{x}$=7.2%, n=432, p=0.646) (Figure 2).

Figure 2. Comparison of *Trichomanes* percent cover between Opunohu and Afareiatu stream valleys (p=0.003).

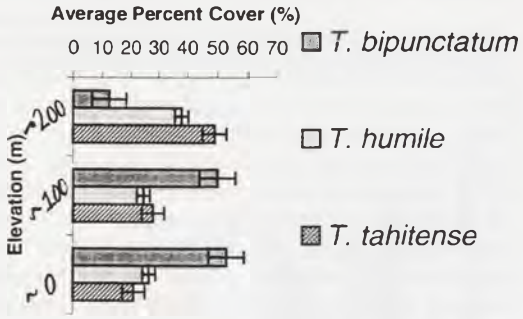


A two-way analysis of variance (ANOVA) was run with canopy cover as the dependant variable against valley, site grouped across valley, and substrate. A post-hoc Tukey multiple comparison method was performed to assess which means were significantly different from each other (Weiss 1999). Opunohu Valley had a significantly higher mean canopy cover than Afareiatu Valley (p=0.033, R^2= 0.359 std.err.=±0.032).

Elevation

A two-way (ANOVA) was conducted to determine a whether fern species percent cover was influenced by elevation. The categorical values used were valley, sites grouped across valleys, and substrate. A post-hoc Tukey multiple comparison method was performed to account for important interactions between means. Average percent cover of *T. bipunctatum* was found to increase significantly from high to low altitudes ($p=0.053$, $R^2=0.44$, std.err.=$\pm$0.013). Although *T. humile* had a relatively high average cover at high elevations, there was no significant difference across elevations ($p=0.099$, $R^2=0.49$, std.err.=$\pm$0.003). However, *T. tahitense* had the greatest mean percent cover at higher elevations, and significantly lower mean cover at low elevations ($p<0.001$, $R^2=0.66$, std.err.=$\pm$0.002) (Figure 3).

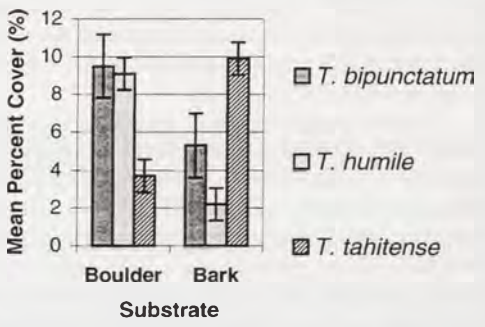
Figure 3. Comparison of *Trichomanes* percent cover along an elevational gradient. *T. bipunctatum* ($p=0.053$, R squared=0.44), *T. humile* ($p=0.099$, R squared=0.49), *T. tahitense* ($p<0.001$, R squared 0.66).



Substrate

Another two-sample t-test was conducted on the three *Trichomanes* grouped by either rock or bark. There was no significant difference in means between substrates for *T. bipunctatum* (rock: $\bar{x}$= 9.5%, n=36; bark: $\bar{x}$=5.3%, n=36, $p=0.214$). There was significantly more *T. humile* found on boulders than on bark (rock: $\bar{x}$=9.1%, n=36; bark: $\bar{x}$=2.2%, n=36, $p<0.001$). However *T. tahitense* showed a significantly higher mean cover on tree bark (rock: $\bar{x}$=3.7%, n=36; $\bar{x}$=9.9%, n=36, $p<0.001$) (Figure 4).

Figure 4. Comparison of *Trichomanes* percent cover on rock versus bark substrate. *T. bipunctatum* ($p=0.214$), *T. humile* ($p<0.001$), *T. tahitense* ($p<0.001$).



The results for a two-way ANOVA Tukey test for valley, site, and substrate against canopy cover revealed a relationship between trees and high canopy cover ($p<0.001$, $R^2= 0.359$, std.err.=$\pm$0.032).

Direction

A two-way ANOVA was run against direction for the three fern species in question, and also with total canopy cover. There were no significant differences between aspect (North, East, South, West) and mean species percent cover for all three species. Likewise, there was no significant difference between aspect on substrate and total canopy cover.

Vertical Height

Another two-way ANOVA was conducted to determine mean species cover difference in height from forest floor. Here, the site was nested within valley, and height was determined on either rock or bark substrate. *T. bipunctatum* had a significantly higher mean percent cover at the top of boulders than on trees ($p=0.054$, $R^2=0.62$, std.err.=$\pm$0.014). *T. tahitense* was found to have a higher mean percent cover at the base of trees than on boulders($p=0.042$, $R^2=0.48$, std.err.=$\pm$0.005).

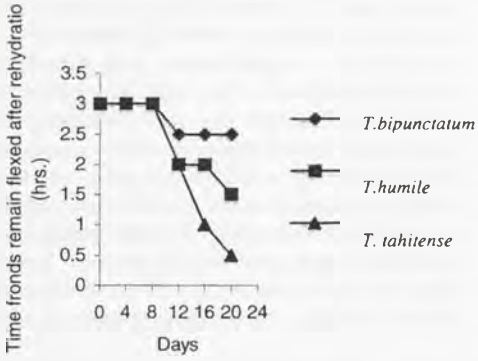
Canopy Cover

A linear regression was conducted to assess whether total mean species cover depended on total percent canopy cover. *T. humile* total cover depended significantly on total canopy cover ($p=0.086$, $R^2=0.041$). *T. tahitense* total cover also depended significantly on canopy cover measured ($p=0.011$, $R^2=0.090$).

Desiccation Analysis

I ran a linear regression test to determine whether species percent cover was dependant on frond response after rehydration. All three species responded significantly: *T. bipunctatum* ($p=0.058$, $R^2=0.750$), *T. humile* ($p=0.058$, $R^2=0.050$), *T. tahitense* ($p=0.047$, $R^2=0.781$). All three species responded to the final water treatment after the twenty day experiment (Figure 5).

Figure 5. Frond response behavior after rehydration treatment over time was graphed. Note that all the species responded to the twenty day treatment, indicating a desiccation survival rate of at least twenty days. *T. bipunctatum* (p=0.058, R squared=0.75), *T. humile* (p=0.058, R squared=0.75), *T. tahitense* (p=0.047, R squared=0.78).



Discussion

Field Survey

After surveying the various watersheds, I observed filmy ferns growing epiphytically up to 3 meters high on the upper story dominant *Inocarpus* trees. *T. bipunctatum* and *T. humile* were also frequently found growing up to approximately 4 meters high on *Hibiscus tiliaceus, Syzigium sp., Metrosideros sp.*, and other native rainforest trees (Brent Mishler). Epiphytic filmy ferns were not observed growing on the invasive tree *Miconia calvescens* at my sites. This may be because *Miconia* is a new invader, and native filmy ferns have not had a chance to establish themselves epiphytically. I also did not find *T. dentatum* and *T. caudatum* growing in the wild (two out of the four species which were formally reported to occur on Moorea). This may be because my sampling took place during the dry season, and these particular species have a different growing period. Also, these species may not occur in *Inocarpus* groves, so I was unable to account for them in my study. A new species of *Trichomanes* was discovered for Moorea, *T. tahitense*, a fern with delicate, peltate fronds growing appressed to both rocks and trees. It was found growing ubiquitously in shady, moist habitats in the stream valleys surveyed. *T. tahitense* has been reported on other islands, such as Tahiti, and on other Society Islands as well (Maxon 1924).

Distribution Study

Forest ecosystems, by their very definition, are composed of the intimate interactions between species and their environment (Whittaker, 1970). The first step in understanding these interactions is testing the abiotic factors that may or may not influence species distribution. For this reason, I chose to investigate valley location, elevational gradients, substrate for growth, vertical height from forest floor, and moisture.

Water in particular, is a crucial limiting factor for the success of ferns in the wild. Throughout most of the world, fern spores need damp conditions for germination. Male fern gametes are flagellated, and must swim through water to find the egg. These gametes do not possess a protective covering like higher vascular plants. Consequently, dry conditions could potentially hinder growth, or kill the gametes (Fosket 1994). Shady conditions then are critical for *Trichomanes* growth. In designing my experiment, I assumed that moisture and shade are critical limiting factors for *Trichomanes* species (Madison 1977). These factors were tested when I measured canopy cover in the field, and ran my desiccation experiment. So the effects of water and shade should be kept in mind as I discuss the following distribution results for the filmy fern species sampled.

Trichomanes bipunctatum

There was a significant difference in species percent cover between valleys for *T. bipunctatum*. Higher mean cover in Opunohu Valley may have been a result of geographic location. Opunohu Valley is found toward the interior of Moorea, and behind a series of volcanic ridges (Figure 1). In general, higher cloud cover usually shrouds the interior of young volcanic islands like Moorea, contributing to more precipitation inland than on

the coast (Lobban 1997). We can infer that Opunohu Valley provided a more suitable moist habitat for filmy fern growth. There was also a higher mean canopy cover in Opunohu Valley. This may be due to older grove age, resulting in larger, fully crowned *Inocarpus* trees. Higher canopy cover could also contribute to a lower light environment suitable for fern growth.

My data suggests that *T. bipunctatum* is able to withstand a drier range of field conditions. This hypothesis is reinforced by elevation, substrate, height, and desiccation data. As altitude decreases with elevation, temperature increases, creating drier environmental conditions at lower altitudes (Whittaker 1970). I found that species percent cover changed significantly between elevations, with less *T. bipunctatum* cover at higher elevations and increased cover at lower elevations. *T. bipunctatum* was also found growing on the top of boulders, which may receive higher sunlight intensity than the forest floor (Whittaker 1970). Also, the top surfaces of boulders in rainforests tend to loose moisture the fastest during and after rainstorms. I observed water to course down into crevices toward the boulder's base. This may create a more xeric microclimate at the top than at the base of boulders. Finally, *T. bipunctatum* lost the least amount of water over time after rehydration. In comparison to the *T. humile* and *T. tahitense*, *T. bipunctatum* dried out the slowest, indicating a larger range of desiccation tolerance. Data for this species would be more conclusive if I could have made direct precipitation readings for sites at each elevation, and measured moisture directly from my substrates. Additionally, I did not have a way to directly measure shade over boulders and beneath trees. A shade meter would be helpful in the future.

Trichomanes humile

Valley location was not a significant factor in determining *T. humile* distribution. This may be due to either low sample size, or the fact that my sampling was conducted during the dry season, and overall species percent cover was low. More stream valleys on Moorea need to be explored and sampled within to find a difference between watersheds. Mean species cover did not differ between sites, again suggesting either low sample size, or that more elevations would have to be accounted for to find a difference in cover. My data proposes no noteworthy difference in vertical height from forest floor. However, there were more *T. humile* found growing on boulder substrate than bark substrate. This may suggest substrate preference regardless of shade. I was unable to distinguish or separate individual fern plants in the field; they grew in mats with tangled rhizomes. However more field tests must be conducted on individual plant density instead of percent cover to account for abundance and preference. *T. humile* also depended significantly on canopy cover. However, this test does not tell us if *T. humile* depends significantly on high canopy cover or low.

Trichomanes tahitense

As with *T. humile*, valley location was also not a significant factor influencing *T. tahitense*. This may be because due to time constraints, I was only able to sample during the dry season. At my 200m sites, there was significantly more *T. tahitense* cover than at lower sites. This may be due to higher precipitation at higher elevations in general in tropical montane forests (Lobban 1997). More percent cover of this fern was found on bark, toward the base of *Inocarpus* trees than on rock substrate. Usually the base of trees provides a very moist microclimate for the organisms living there. During tropical storms, stem flow down large tree trunks and buttresses also contributes to a moist environment at tree bases. These conditions are ideal for *T. tahitense* growth, and may be one reason why this particular species is found in shady, mesic environments on Moorea.

Desiccation Experiment

Results from my desiccation experiment revealed that all three *Trichomanes* species were able to live after a drying period of twenty days. This indicates that although fronds may survive without water, they probably gather humidity from the atmosphere. My experiment could be improved if I were able to measure evapotranspiration rates of fern stomata at different time intervals. *T. tahitense* seemed to dry out the fastest within the 3 hour period after rehydration. A confounding factor in my experiment was that I could not account for the humidity in the laboratory, which may have been drier than the actually habitat where *Trichomanes* species live.

Conclusion

T. bipunctatum, *T. humile*, and *T. tahitense* all grow either epiphytically or epilithically on the island of Moorea. They grow as thick, continuous rhizome mats on boulders and trees in *Inocarpus* groves. No filmy fern species were found growing

terrestrially. This may be because typically, most *Trichomanes* have adapted their rhizomes to fulfill an epiphytic niche. Competition for space and resources may also play a role, but these ecological factors still need further research. Abiotic environmental factors were tested to assess their influence on three *Trichomanes* species. A significant relationship was found between *T. bipunctatum* cover and valley, elevation, substrate, height, canopy and rehydration rates. Results suggest that *T. bipunctatum* may be able to tolerate more xeric microclimates than *T. humile* and *T. tahitense*. Significant trends were found with *T. tahitense* cover and elevation, substrate, vertical stratification (height from forest floor), canopy cover, and rehydration rates. This suggests *T. tahitense* is the most sensitive to limiting factors of the three fern species, and tolerates more mesic microclimates. *T. humile* was found more on boulders and depended significantly on canopy cover. However, there was no conclusive evidence to suggest a trend in moisture or shade for percent cover of *T. humile*. In the field, I was unable to separate individuals from populations of fern species. Rhizomes were often intertwined making it virtually impossible to tell ramets and gamets apart. Hence, no conclusions can be drawn regarding niche differentiation, or species preference. Further studies are needed to assess fern frequency and diversity for a more descriptive distribution of *Trichomanes*.

Future Research

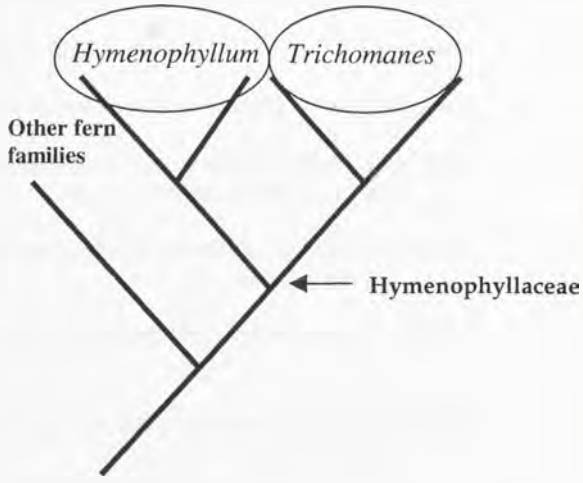
On Moorea, more valleys need to be explored for filmy ferns, especially at higher elevations. Mapping and sampling within these valleys can help draw a more accurate picture of *Trichomanes* distribution. The same experiments need to be conducted during the wet season on Moorea for species cover and canopy cover. To better understand the role of moisture in filmy fern distribution, it is important to find an effective method to measure moisture content on basaltic boulders versus *Inocarpus* bark in the field. This could help with understanding moisture availability for filmy ferns. Different types of substrates such as *Hibiscus tiliaceus*, or fallen tree trunks, need to be examined for future distribution studies. Furthermore, vertical zonation of epiphytes is often a result of the relative abundance of propagules of each species (Odum 1959). It would be interesting to account for the abundance of sori and average number of spores per frond on individual plants. Finally germination experiments of sporophytic and gametophytic life phases, as well as observation of gemmiferous propagation would help us understand filmy fern growth cycles in the wild.

Acknowledgements

Many thanks to Professors Brent Mishler, Jere Lipps, Jamie Bartholome, TAs, Dr. Alan Smith, Mari-Mari Kellum, Israel King, Galen Peraca, Lorraine Cheng, Aaron Wallace, and the entire Moorea class of 2000.

Appendices

Appendix A. Recent genetic methods using rbcL sequencing indicate that the genera *Trichomanes* and *Hymenophyllum* are indeed monophyletic. The following phylogenetic tree is simplified from Pryer et. al in 2000, and depicts the relationship between the two genera.



Appendix B. During my study, I found it difficult to identify the different filmy fern species I collected in the wild. In part, my frustration stemmed from an out of date key, which was vague and not dichotomously arranged. I decided to create a key specific to Moorea for the Hymenophyllaceae. The following is my modified version of the key found in Pteridophytes of the Society Islands (Copeland 1932).

Revised Key for the genus *Trichomanes* (Hymenophyllaceae) found on Moorea, French Polynesia

Rhizome creeping
- Stipe winged, fronds ranging from dark to light green
  - Fronds peltate, minute, average frond diameter 1.5 cm, often found at the base of trees and on boulders ..... *T. tahitense*
  - Fronds pinnatifid or pinnate
    - With specialized margin, no false veins, light green fronds with average frond length from base of stipe to tip 4 cm ..... *T. humile*
    - With sub-marginal false vein, dark green fronds, average frond length from base of stipe to tip 8 cm, found on the top surfaces of large boulders ..... *T. bipunctatum*
- Stipe not winged, fronds light green ..... *T. caudatum*

Rhizome not creeping; fronds clustered ..... *T. dentatum*

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The Use of Algae To Indicate Eutrophication in the Streams of Cook's and Opunohu Bays, Moorea, French Polynesia

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ABSTRACT. Eutrophication, the addition of excess nutrients, can have devastating effects on macrobenthic communities through changes in species diversity and composition and the proliferation of algae. To test the effects of eutrophication on the macrobenthic communities in the more developed Cook's Bay and the more pristine Opunohu Bay, Moorea, French Polynesia, I identified and determined the percent cover for all organisms at the mouths of ten streams throughout the two bays. Compared to a control for each stream site, I determined the effects of streams on the species richness, total alga cover, and cover of specific species of algae, including *Padina* sp. In addition, the number of houses, the area of agriculture, and the size of the watershed were determined, along with the nutrient concentrations at all of the streams, to test what factors were responsible for algae growth. Results show that streams do not affect the species richness or total alga cover, in both bays, but were shown to increase the percent cover of *Padina* sp. As the number of houses, the area of agriculture, and nitrate levels increased, so did the percent cover of *Padina* sp. in Cook's Bay. Therefore, *Padina* sp. can be used to indicate levels of eutrophication, and development, in the streams of Cook's Bay, and perhaps more broadly.

Introduction

Worldwide, biological diversity is becoming increasingly threatened. Whether or not biodiversity is valuable simply because it exists, biodiversity has value to humans through goods, services and information (Meffe and Carroll 1997). As the human population continues to grow, so does its demand on the environment.

In Moorea, French Polynesia, the destruction of biodiversity is not due as much to the number of people, but to where the people live. Much of the population lives around the two bays, Cook's Bay and Opunohu Bay, due to accessibility to water and food resources. The increase in the number of houses and agriculture around Cook's Bay and Opunohu Bay affects the bays through the streams and drainage ditches, which run from the hill-tops down to the bays.

Eutrophication is the addition of excess nutrients, such as nitrogen and phosphorus, into water, such as a bay. Excess nutrients come from fertilizers and animal wastes, and are due to grazing, agriculture, and runoff from developed areas (Bell and Elmetri 1995). The island of Moorea has numerous ephemeral streams that can carry large quantities of water down to the bays where the soil and nutrients are deposited. Eutrophication in the coastal ecosystem can have devastating effects. Some of these effects include changes in species diversity and composition, increase in macroalga abundance, as well as food chain effects and indirect effects on humans through illness from accumulating toxins in seafood (Carpenter 1998). In addition, the aesthetic quality of the water can be compromised, as it becomes murky and brown. Because much of the population of Moorea, French Polynesia, relies on the water in its bays for fish to eat or for tourist dollars, eutrophication can have important socioeconomic impacts (McCook 1999).

Macrobenthic communities, including corals, gastropods, echinoderms and algae integrate environmental fluctuations for long periods of time and can be used as measures of the degree of eutrophication caused by the stream which enters into the bay (Adjeroud 2000). Because nutrient concentrations may change at the mouth of the stream day by day due to rainfall patterns, macrobenthic organisms may be used to indicate the long-term effects of eutrophication.

To test how eutrophication affects the composition of macrobenthic communities in

Cook's Bay and Opunohu Bay, I surveyed ten stream mouths with varying degrees of eutrophication. Eutrophication can be especially devastating in bays such as these two, because they have low exchange of water with the open ocean to flush out the nutrients (Gabric and Bell 1993). Both bays have similar shape, size, and orientation but Cook's Bay has significantly more coastal development than Opunohu Bay, so Opunohu Bay offers an ideal natural control site to study the effects of eutrophication on bays.

This study seeks to test four hypotheses: (1) streams running though land with agriculture and development will be more nutrient rich than those running through pristine forest; (2) water richer in nutrients will have more alga growth; (3) species richness and composition will be different between Cook's Bay and Opunohu Bay; (4) some species will be more sensitive to eutrophication than others, and may be used as indicator species. The ultimate goal of this research is to identify species in Cook's Bay and Opunohu Bay that are sensitive to eutrophication, in order to facilitate monitoring of water quality and bay health.

Materials and Methods

Study site

The island of Moorea (S17° 30', W149° 50') is located in the Society Archipelago, in French Polynesia (Figure 1). It is a high volcanic island, surrounded by both fringing and barrier reefs, situated in the South Pacific Ocean. Along the north side of the island are two long bays oriented in the north-south direction, Opunohu Bay and Cook's Bay.

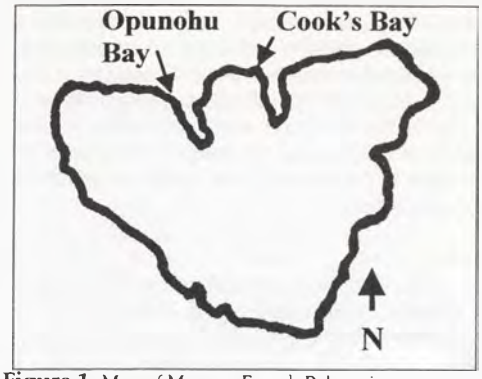

Figure 1. Map of Moorea, French Polynesia.

Data were collected from 2 October to 31 October 2000. A total of ten streams were surveyed, six from Cook's Bay (Figure 2) and four from Opunohu Bay (Figure 3). The stream sites were chosen randomly from all streams along the midsections of each bay. Streams at the baymouth and bayhead were omitted to eliminate factors relating to sedimentation from the large rivers at the bayheads and the open ocean at the baymouths.

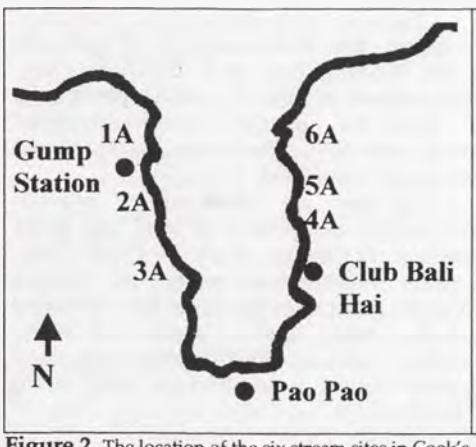

Figure 2. The location of the six stream sites in Cook's Bay, Moorea.

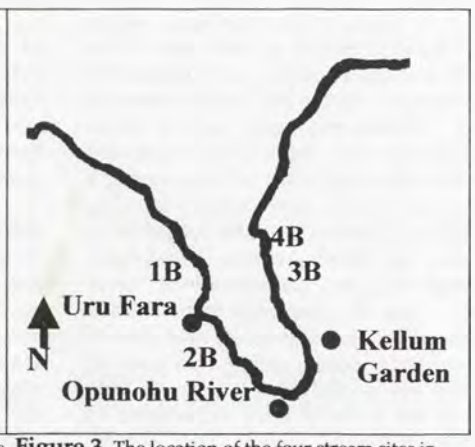

Figure 3. The location of the four stream sites in Opunohu Bay, Moorea.

In each bay, the sites were split between the east and west sides of the bay to control for the normal refraction patterns and currents associated with the reefs and passes. For each of the ten stream sites, a control site was located 50 meters away. A distance of 50 meters was determined after preliminary observations showed effects from streams were localized to within about 50 meters of the stream. In addition, 50-meter distances still had the same substrate and orientation as the streams, in most cases. A coin flip was used to determine if the control was north or south of the stream site.

Sampling

Surveys were conducted to determine the identity, abundance, and distribution of the macrobenthic organisms, including corals, algae, echinoderms and gastropods, at each of the ten stream and control sites. At each site, a ten-meter transect tape was placed at five distances from shore: 2, 4, 6, 8 and 10 meters. Transect tapes were centered on the stream source and were parallel to the shore and each other.

Using a 0.5m by 0.5m quadrat, five samples were selected along each transect using a random number table. In each quadrat, all individuals or colonies visible without removing rocks were identified to genus or species level, and counted, or percent cover was determined for sessile organisms, such as algae.

Along with these biotic measurements, several abiotic factors were determined. First, the surface water temperature was recorded at each site. The stream width and water depth were measured with a meter tape. In the lab, water samples were tested. Before the rainy season, three stream and control sites from Cook's Bay and two from Opunohu Bay were selected using a random number table and water samples gathered. Using chemical test kits supplied by LaMotte, Inc., the nitrate, nitrite, phosphate, and dissolved oxygen concentrations were determined. For the samples from Cook's Bay, a digital pH meter was used, and for the Opunohu Bay sites, litmus paper was used to determine the pH of the water. These tests were done to see if there were differences in water quality between stream and control sites.

After one full day of rain at the start of the rainy season (31 October 2000), we collected water samples from all of the streams within a two-hour period. Water was collected 1 to 3 meters up from the mouth of the stream. The water tests outlined above were repeated on all of the stream water samples.

To characterize the land use above each stream and to locate possible sources of nutrient influx, the number of houses along the stream and in the watershed, the area of agriculture along the stream and in the watershed, and the size of the watershed were determined. These values were estimated by walking along or near the stream as far as possible and collecting data. Alternatively, for the steep-sloped mountains, we collected data by standing near the mouth of the stream and looking up the side of the mountain.

Statistical analyses

Paired comparison t-tests were used to analyze most of the data. The species richness, total alga cover and total number of species were compared between the control sites and stream sites in both Cook's Bay and Opunohu Bay. In addition, the percent cover of the most abundant alga species in each bay were compared between the control and stream sites to test for significant differences. The mean, variance, standard error, and p-value were computed. A significant difference was a p-value of 0.05.

The program JMP was used to compute nested ANOVAs to test whether or not the species richness and alga cover in the stream and control sites were affected differently in the two bays, Cook's Bay and Opunohu Bay.

Pairwise comparison t-tests were used to compare the concentrations of nutrients between Cook's Bay and Opunohu Bay. Concentrations of nitrate, nitrite, phosphate, and dissolved oxygen were compared between both bays. The mean, variance, and p-value were computed.

To test for relationships between abiotic factors and percent of total alga cover or percent *Padina* sp. cover in Cook's Bay, regression analyses were performed. Factors such as the number of houses in the watershed above the stream, area of agriculture in the watershed, nitrate, nitrite, phosphate, and dissolved oxygen concentrations were tested for relationships with alga coverage. The R^2 and p-values were computed for all regressions. For any significant relationships, the equation of the best-fit line was computed.

Finally, multiple regressions testing for interactions between abiotic factors, such as the nitrate concentration with the number

of houses, and the nitrate concentration with the area of agriculture, were computed using the software JUMP, to test for effects on total alga cover and *Padina* sp. cover in Cook's Bay.

Results

Site characterization

The number of houses, area of agriculture, type of agriculture and the size of the watershed at each of the stream sites in Cook's Bay (Table 1) and Opunohu Bay (Table 2) were variable. Overall, there were more houses in Cook's Bay than in Opunohu Bay. In addition, there was more agriculture in Cook's Bay than in Opunohu Bay, with a greater variety of crops grown; bananas, pineapples and coconuts were found growing around Cook's Bay, while only coconut groves were seen at Opunohu Bay.

Table 1. Cook's Bay site information

Site	Coordinates	Location of Control Site	Stream Width (m)	# Houses Along Stream	# Houses In Water-shed	km^2 Agri-culture Along Stream	km^2 Agri-culture In Water-shed	Size of Water-shed (km^2)	Notes
1A	S 17°29'34.9", W 149°49'59.1"	South	3.5	3	10	0	.01 - bananas	.10	Pineapple cannery effluent
2A	S 17°29'29.1", W 149°49'34.9"	South	3.8	0	6	0	0	.25	Gump Station property
3A	S 17°29'38.2", W 149°49'32.4"	North	3.8	1	16	0	.06- pineapples	.75	Near row of houses
4A	S 17°30'04.3", W 149°49'02.2"	South	3.2	4	11	0	0	.20	Near a market and restaurant
5A	S 17°29'49.6", W 149°49'02.1"	North	2	3	7	0	.02- coconuts	.25	Near a market
6A	S 17°29'40.4", W 149°49'05.0"	South	2.1	4	11	.0025- bananas	.005- coconuts	.50	Near a restaurant

Table 2. Opunohu Bay site information

Site	Coordinates	Location of Control Site	Stream Width (m)	# Houses Along Stream	# Houses In Water-shed	km^2 Agri-culture Along Stream	km^2 Agri-culture In Water-shed	Size of Water-shed (km^2)	Notes
1B	S 17°30'24.5", W 149°51'29.8"	South	1	1	1	0	0	.20	Near a market
2B	S 17°30'51.7", W 149°51'18.1"	North	2.2	0	0	0	.04- coconuts	.25	Near fallen coconut
3B	S 17°30'23.0", W 149°51'08.1"	South	1	0	2	0	0	.10	Close to the main road
4B	S 17°30'14.5", W 149°51'10.5"	South	1	2	12	0	.01- coconuts	.12	Near landslide .01 km^2

Species richness

When the species richness, the total number of alga and animal species, was compared between stream and control sites in both Cook's Bay and Opunohu Bay, no significant difference was detected (Table 3).

However, when the species richness was compared between Cook's Bay and Opunohu Bay, the t-test results show there were significantly more species in Cook's Bay than in Opunohu Bay (Cook's: mean=8.92, SE=1.08; Opunohu: mean=3.25, SE=0.456; t-test: df=11, p=0.00112*) (Figure 4).

Table 3. Results of t-tests comparing the total number of species in the stream and control sites in Cook's Bay and Opunohu Bay.

		Mean	Standard Error	df	P-Value
Cook's Bay	streams	9.00	1.69	5	0.477
	controls	8.33	1.51		
Opunohu Bay	streams	2.75	0.750	3	0.247
	controls	3.75	1.25		

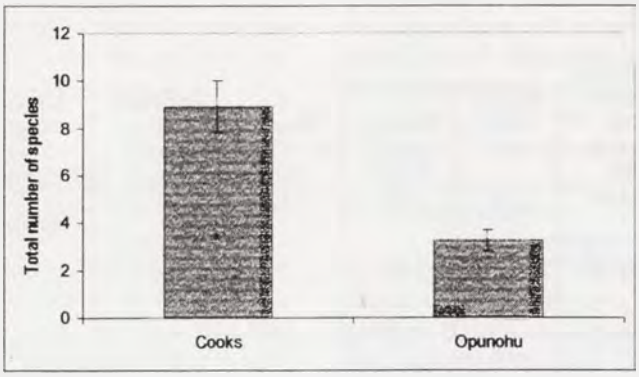

Figure 4. A comparison of the total number of species between Cook's Bay and Opunohu Bay. The error bars represent one standard error.

Alga coverage in streams vs. controls

A t-test pairwise comparison of the total amount of alga coverage showed no significant difference between coverage at the control and stream sites, for both Cook's Bay and Opunohu Bay (Table 4). Although not significant, five out of the six stream sites at Cook's Bay had a higher percent cover of algae than their control sites (Figure 5). Also, three out of the four stream sites at Opunohu Bay had a higher percent cover of algae than their control sites (Figure 6), but differences were not as noticeable as in Cook's Bay for alga coverage.

Table 4. Results of t-tests comparing the total algae coverage in stream and control sites in Cook's Bay and Opunohu Bay.

		Mean	Variance	df	P-Value
Cook's Bay	streams	49.1	1160	5	0.124
	controls	31.3	877		
Opunohu Bay	streams	70.1	253	3	0.263
	controls	65.7	284		

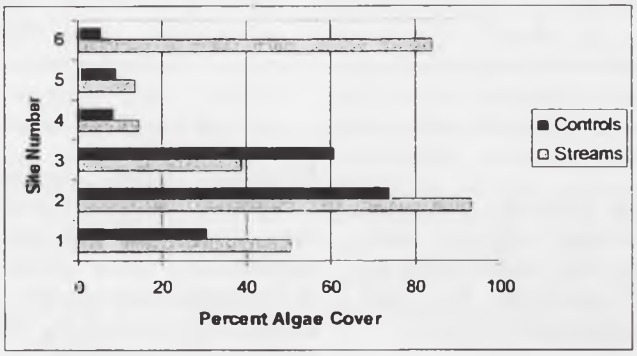

Figure 5. A comparison of the total alga cover in the stream and control sites in Cook's Bay, Moorea.

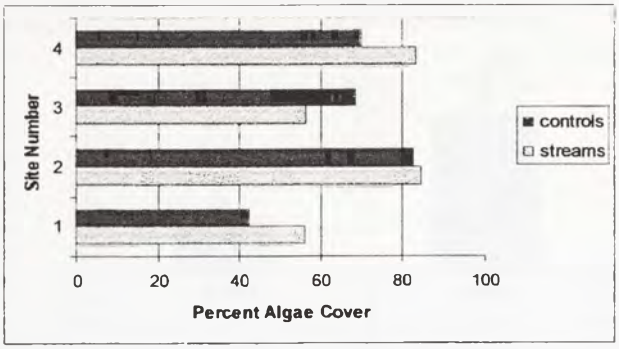

Figure 6. A comparison of the total alga cover in the stream stream and control sites in Opunohu Bay, Moorea.

In addition to comparing the total alga coverage between streams and their controls in Cook's Bay and Opunohu Bay, the most abundant algae in each bay were compared with t-tests (Table 5). In Cook's Bay, *Padina* sp., *Halimeda* sp., and *Ceramium* sp. were all tested for differences between stream and control sites, and only *Padina* sp. showed a significant difference in coverage between the streams and controls (Table 5). This difference is shown in Figure 7, where the mean percent cover of *Padina* sp. is much greater at the streams than the controls. In Opunohu Bay, neither of the most abundant alga species, *Jania* sp. and *Ceramium* sp., showed significant differences in cover between the stream and control sites (Table 5).

Table 5. Results of the t-tests comparing different alga species at stream and control sites.

		Mean	Variance	df	P-Value
Cook's Bay					
Padina sp.	streams	6.38	23.1	5	0.016*
	controls	2.18	2.21		
Halimeda sp.	streams	3.27	22.1	5	0.428
	controls	3.58	10.1		
Ceramium sp.	streams	21.2	1030	5	0.402
	controls	22.4	809		
Opunohu Bay					
Jania sp.	streams	35.0	1770	3	0.162
	controls	30.7	1550		
Ceramium sp.	streams	35.6	1670	3	0.343
	controls	31.6	1350		

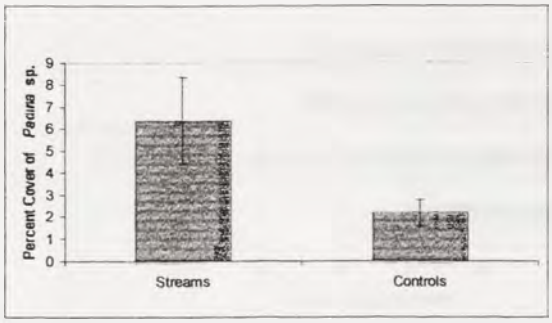

Figure 7. A comparison of the total cover of *Padina* sp. between the stream and control sites in Cook's Bay, Moorea. The error bars represent one standard error. (Streams SE=1.96; Controls SE=0.606)

Different effects by different bays

Although the nutrient differences between Cook's Bay and Opunohu Bay were not significant, and the size and shape of the bays were similar, nested ANOVAs were computed to see if the stream and control sites were affected differently by the two bays. ANOVAs were computed for species richness, *Padina sp.*, and the other most abundant algae. Results show that the stream and control sites are affected differently by the two bays for species richness and *Padina* sp. only, but not for the other abundant algae (Table 6).

Table 6. The results of the nested ANOVAs testing for differences in how stream and control sites are affected by the two bays.

Factor	Source	df	SSQ	Mean-square	F-ratio	P-value
species richness	model	3	156	52.1	4.62	0.016*
	error	16	180	11.3		
total algae	model	3	4680	1560	2.12	0.138
	error	16	11800	736		
Padina sp.	model	3	132	44.1	5.52	0.009*
	error	16	128	7.99		
Ceramium sp.	model	3	568	189	0.166	0.918
	error	16	18300	1140		
Jania sp.	model	3	3550	1180	1.31	0.307
	error	16	14500	906		

Water nutrient tests

Before the rainy season, when there was no running water in the streams, the water in the bay at the mouth of the stream was collected and compared to water at each control site. For all of the factors tested, which included the water temperature, the nitrate, nitrate, phosphate and dissolved oxygen concentrations, and the pH levels, there was virtually no difference between the stream and control sites, in both Cook's Bay and Opunohu Bay (Table 7). The only substantial difference between control and stream sites was for the phosphate concentration for site 1A, the pineapple cannery site.

Table 7. Comparison of the nutrients between stream and control sites in Cook's Bay and Opunohu Bay, Moorea, before the rainy season.

Site	°C	ppm Nitrate (NO_3)	ppm Nitrite (NO_2-N)	ppm Phosphate (PO_4)	O_2	pH
1A- stream	30.5	1.1	0	2	2.6	6.95
1A- control	29.5	1.1	0	1	2.4	7.24
5A- stream	27.5	.88	0	1	2.6	7.40
5A- control	27.5	.88	0	1	2.8	7.51
6A- stream	26.0	1.1	0	1	3.2	7.43
6A- control	26.5	1.1	0	1	3.4	7.54
1B- stream	27.0	1.1	0	<1	9.0	7
1B- control	27.0	1.1	0	<1	9.0	7
2B- stream	27.0	1.1	0	<1	8.6	7
2B- control	27.0	1.1	0	<1	8.5	7

After the first full day of rainfall at the start of the rainy season, water was collected from the streams at both Cook's Bay and Opunohu Bay. Samples were taken from all of the streams, except for stream site 2B, in Opunohu Bay, which had no running water at the time of sampling. Unlike the comparisons of the bay water in Table 6, the nutrient concentrations in the stream water varied greatly (Table 8). For example, the concentration of nitrate ranged from .88 ppm at sites 1A and 2A to 11 ppm at site 3A, just down Cook's Bay. Interestingly, the nutrient concentrations were not correlated with one another; for example, if the nitrate concentration was high, the phosphate and nitrite concentrations may not have been high as well. In addition, I expected the dissolved oxygen levels to decline with increasing nutrient concentrations, which was not apparent in these results.

Table 8. Water test results of the stream sites, after 24 hours of heavy rainfall.

Site	ppm Nitrate (NO_3)	ppm Nitrite (NO_2-N)	ppm Phosphate (PO_4)	ppm Dissolved Oxygen	pH	Water Color
1A	.88	0	2	1.5	7	colorless
2A	.88	0	3	5.2	7	light brown
3A	11	.2	3	6.7	7	golden brown
4A	2.2	.1	3	6.8	7	yellow brown
5A	1.1	0	3	7.6	7	yellow brown
6A	1.1	.2	4	5.0	7	brown
1B	2.2	.1	2	6.6	7	colorless
2B*	-	-	-	-	-	-
3B	.88	0	2	6.8	7	yellow brown
4B	4.4	.2	6	2.8	7	dark brown

*This stream had no flowing water at the time of sampling.

The results of the t-tests comparing the concentrations of nitrate, nitrite, phosphate and dissolved oxygen in the stream water after rainfall between Cook's Bay and Opunohu Bay had very surprising results. For all the nutrients and dissolved oxygen, there was no significant difference in concentration between the streams in Cook's Bay and Opunohu Bay (Table 9), which was unexpected.

Table 9. The results of t-tests comparing the nutrient concentrations between Cook's Bay and Opunohu Bay.

Nutrient	Bay	Mean	Variance	df	P-Value
Nitrate	Cook's	2.86	16.1	5	0.392
	Opunohu	2.49	1.26		
Nitrite	Cook's	0.083	0.010	5	0.305
	Opunohu	0.100	0.004		
Phosphate	Cook's	3.00	0.400	5	0.295
	Opunohu	3.33	2.13		
Dissolved oxygen	Cook's	5.47	4.78	5	0.481
	Opunohu	5.40	2.03		

Abiotic factors influencing alga coverage

Because *Padina* sp. was the only alga species to show a significant coverage difference between the stream and control sites, it was the only species, along with total algae, on which I tested the different abiotic factors, such as land-use and nutrient concentrations (Table 10). The only factors to show a significant correlation with the percent cover of *Padina* sp. were total number of houses in the watershed, total area of agriculture in the watershed, and nitrate concentration in the stream water. None of the factors were correlated with the total alga coverage.

Table 10. Results of regressions comparing abiotic factors on alga cover in the stream sites of Cook's Bay, Moorea.

Variable 1	Variable 2	R^2	P-Value
Total number of houses in the watershed	*Padina* sp. cover	0.888	0.005*
Total area of agriculture in the watershed	*Padina* sp. cover	0.691	0.040*
Nitrate concentration	*Padina* sp. cover	0.743	0.027*
Phosphate concentration	*Padina* sp. cover	0.020	0.780
Nitrite concentration	*Padina* sp. cover	0.394	0.182
Dissolved oxygen level	*Padina* sp. cover	0.005	0.897
Total number of houses in the watershed	Total alga cover	0.049	0.673
Total area of agriculture in the watershed	Total alga cover	0.088	0.569
Nitrate concentration	Total alga cover	0.050	0.671
Phosphate concentration	Total alga cover	0.098	0.545
Nitrite concentration	Total alga cover	0.007	0.872
Dissolved oxygen level	Total alga cover	0.206	0.366

It was surprising how well correlated the percent cover of *Padina* sp. was to the number of houses in the watershed (Figure 8), the area of agriculture in the watershed (Figure 9), and the nitrate concentration (Figure 10). In each case, as the number of houses or agriculture or concentration of nitrate increased, the percent cover of *Padina* sp. increased

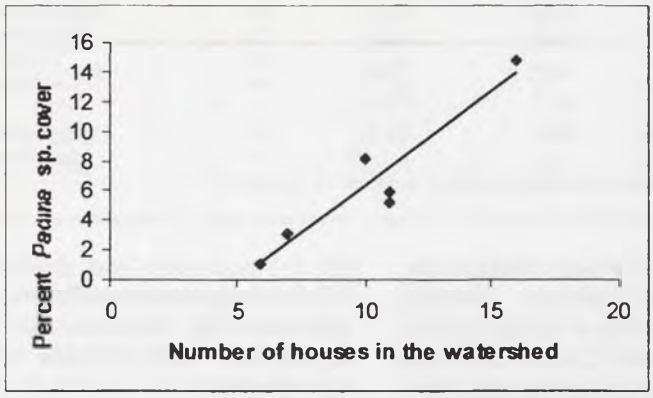

Figure 8. A regression showing the relationship between the percent cover of *Padina* sp. and the total number of houses in the watershed along the streams in Cook's Bay, Moorea. The line represents the best-fit line. (R^2=0.888, y=1.28x-6.17)

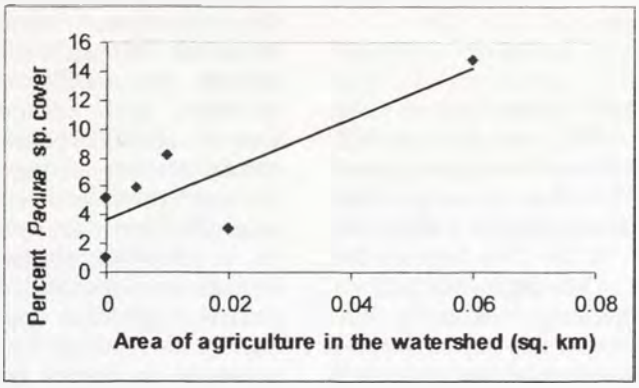

Figure 9. A regression showing the relationship between the percent cover of *Padina* sp. and the total area of agriculture in the watershed along the streams in Cook's Bay, Moorea. The line represents the best-fit line. (R^2=0.691, y=174.53x+3.613)

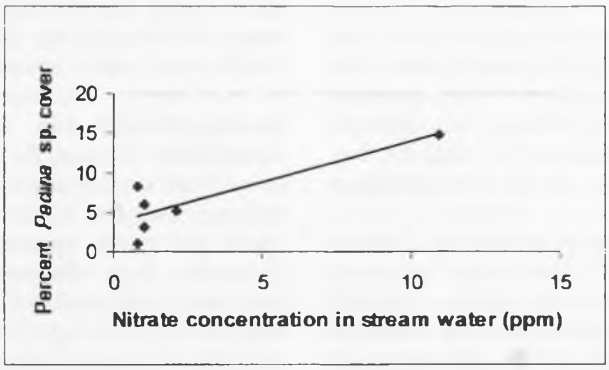

Figure 10. A regression showing the relationship between the percent cover of *Padina* sp. and the nitrate concentration in the stream water of the streams in Cook's Bay, after a day of rainfall. The line represents the best-fit line. (R^2=0.743, y=1.0314x+3.427)

The results of the multiple regressions were unexpected. The simple linear regressions showed significant relationships between *Padina* sp. cover with the amount of agriculture, number of houses, and the concentration of nitrate (Table 10). In addition, they showed no significant relationships with the total alga cover (Table 10). When crossing two abiotic factors in the multiple regression analyses, only when nitrate concentration was crossed with agriculture, did it show an interacting effect on the total alga cover (Table 11).

Table 10. The results of the multiple regressions, testing for interactions between abiotic factors on *Padina* sp. growth and total algae growth.

Interacting variables	Dependent variable	df	SSQ	F-ratio	P-value
nitrate*agriculture	*Padina* sp. cover	1	0.025	0.002	0.970
nitrate*houses	*Padina* sp. cover	1	5.03	1.73	0.319
nitrate*agriculture	total alga cover	1	4770	23.0	0.041*
nitrate*houses	total alga cover	1	2540	1.74	0.318

Discussion

Site characterization

Consistent with observations by London and Tucker (1992) and Rich (1998), there is considerably more development and agriculture in Cook's Bay than Opunohu Bay (Table 1, Table 2). More important than the amount of agriculture in the two bays is the type of agriculture. Pineapple plantations dominate the development of Cook's Bay watershed, while Opunohu Bay watershed is mostly coconuts, with the occasional grove of fruit trees (Galzin 1990; personal observation Table 1 and Table 2).

A substantial amount of nutrients enters the stream water from urban sources, such as construction sites, runoff from lawn fertilizers and pet wastes, and from unsewered houses (Carpenter et al. 1998). A higher density of housing often correlates with more roads which makes the ground impermeable to water, leading to greater runoff. In addition, the clearing of land for the building of houses breaks up the soil, leading to erosion.

The pineapple plantations in Cook's Bay lead to more runoff than other types of agriculture, such as coconut groves which have a lot of undergrowth vegetation, because they use drainage ditches and no groundcover in the fields (London and Tucker 1992). Therefore, the combination of a higher density of housing and pineapple plantations in Cook's Bay, is expected to lead to greater runoff and sedimentation than in Opunohu Bay. The results of the nutrient tests comparing the streams in Cook's Bay and Opunohu Bay (Table 9), however did not detect a difference in the nutrient levels between the two bays. Samples taken at more stream sites around each of the bays may be required to detect a difference.

Species richness

Eutrophication is a major factor in the loss of marine biodiversity (Carpenter et al. 1998). Therefore, it is surprising that there is no significant difference in the species richness between the stream and control sites in both Cook's Bay and Opunohu Bay (Table 3), even though there are more nutrients in the stream water than the bay water. This may be explained by a couple of factors. First, the nutrients may diffuse evenly over the stream and control sites, making no difference in nutrient concentrations over time, as in Table 3, where the nutrient levels are no different in the control and stream sites. Second, the levels of nutrient influx may not be high enough to influence changes in species numbers and composition. According to Gray's (1992) model of the process of eutrophication, changes in species richness and composition occur at higher levels of eutrophication than increases in alga growth.

Results showed dramatically more species of algae and animals in Cook's Bay than in Opunohu Bay. These data may be slightly skewed by the fact that six sites were surveyed in Cook's Bay and only four were surveyed in Opunohu Bay. However, the species richness in Cook's Bay is still considerably higher and would probably still be higher with more samples in Opunohu Bay.

Because Moorea is an isolated island, many of its species are not native. Therefore, many of the species of algae and animals in Cook's Bay and Opunohu Bay are not native to the bays. Because Cook's Bay is more developed and has more boating activity, from boats such as the cruise-liners, there are more vectors for transportation of algae and animals into the bay. This may explain why there are more species in Cook's Bay than Opunohu Bay. Because the nutrients in the two bays are similar (Table 5), the substrate, bathymetry, size, and temperature are similar, and the locations so proximate, little else explains the dramatic difference in species numbers.

Alga coverage in streams vs. controls

One of the most common results of increased nutrients in the water, or eutrophication, is an increased biomass of benthic and epiphytic algae (Gray 1992; Gabric and Bell 1993; Bell and Elmetri 1995; Carpenter et al. 1998; McCook 1999). Although tests for differences in the total alga coverage between the control and stream sites were not statistically significant, I think they are biologically significant. In all streams but one, in Cook's Bay (Figure 5) and in Opunohu Bay (Figure 6), percent cover of algae is higher than the controls. The number of sites surveyed may not have been sufficient to detect the effect of the streams on the total alga cover.

Similar to the total algae results, when individual species of algae were tested for significant differences between percent cover at the streams and controls, most showed no significant difference (Table 5). This could be explained by the fact that increased nutrients

may not affect the growth of these particular species. Alternatively, there may not have been enough samples taken to detect a difference in growth, if the effects of the streams were small.

One species of alga, *Padina* sp, showed a significant difference in percent cover in the stream sites versus the control sites (Table 5). This suggests that the presence of streams dramatically increases the percent cover of *Padina* sp. Therefore, this alga species may be more sensitive to increased nutrients than the other abundant alga species tested. An alternative could be that *Padina* sp. is more tolerant to freshwater than the other species, but a conductivity meter was unavailable, so I could not test the salinity at the sites. Because the effect of the stream was evident in *Padina* sp., and not the other algae, the effect was most likely very small, and the amount of nutrients added minimal, and only *Padina* sp. showed a difference in cover, with the number of samples tested.

One species in the *Padina* genus, *Padina tenius* was tested in a lab by Schaffelke (1999), and was shown to increase its tissue mass by up to 20% with a single pulse of nitrogen and phosphorus. Because this macroalga responds to short-term nutrient pulses, it means that fast uptake rates and nutrient storage, which it can use for longer periods of time in the nutrient poor waters of the tropics, characterize it.

Different effects from different bays

It is important to test if the relationship between control and stream are different in Cook's Bay and Opunohu Bay. That is, if the streams in Cook's Bay are affecting algae and species richness in the same way as in Opunohu Bay. The results indicate that species richness and *Padina* sp. cover are affected differently by the two bays (Table 6). This means that the relationship between the control and the stream is different for the two bays for species richness and *Padina* sp., but not for the other alga species.

I would not expect there to be differences in the relationships between stream and control in the two bays because the nutrient levels were not significantly different in the two bays, and the bathymetry, size, shape and orientation of the bays is the same. These differences may be due to differences in agriculture or density of housing between the two bays.

Water nutrient tests

As was expected, the temperature, nutrients, dissolved oxygen and pH levels were all very similar, or the same, between the water in the stream and control sites before the rainy season (Table 7). Before the rainy season, most of the streams had very little standing water, or no water at all. Therefore, the streams should not be affecting the water in the bay.

Although the results of the stream water tests are variable (Table 8), there are a few interesting patterns. The first is that none of the streams had very low levels of oxygen, except for the pineapple cannery site (1A). The water in this stream has inputs of effluent from the pineapple distillery upstream, which may be making the water more anoxic from the addition of fermenting pineapple juice and alcohol. Because the other sites did not have low levels of dissolved oxygen, it suggests that the eutrophication has not been severe; the ultimate effect of eutrophication is anaerobic conditions (Gray 1992).

One of the most interesting patterns found in the stream water samples (Table 8), is the correlation of nutrient levels, namely nitrate, with housing density and agriculture type. For both Cook's Bay and Opunohu Bay, the highest density of housing corresponded with the highest concentration of nitrate. In Opunohu Bay, the highest density of housing also corresponded with the highest concentration of nitrite and phosphate. The site with the highest nitrate concentration, by far, in Cook's Bay is site 3A. This stream runs through the watershed with a pineapple plantation, which are known to increase runoff and erosion (London and Tucker 1992).

Several authors (London and Tucker 1992; Rich 1998) have described Opunohu Bay as being a "natural control" for Cook's Bay, because the two bays are very similar in all respects except that Opunohu Bay is much less developed than Cook's Bay. However, the results from Table 9 refute this claim. The results of t-tests comparing the nutrient concentrations between Cook's Bay and Opunohu Bay show no significant difference in nutrient concentrations for nitrate, nitrite, phosphate, and dissolved oxygen (Table 9). This means that overall, the streams running into Opunohu Bay may be no more pristine than Cook's Bay.

Abiotic factors influencing alga coverage

The results of the regressions testing for effects of land use and nutrients on alga

coverage suggest that *Padina* sp. may be used as an indicator species for levels of nitrate, the number of houses in the watershed, and the area of agriculture in the watershed (Table 10). As each of these increased, so did the percent cover of *Padina* sp. It was shown before that *Padina* sp. was the only alga to increase in percent cover at the stream sites. Now, these results give possible factors influencing its growth.

The number of houses in the watershed, the area of agriculture in the watershed, and the concentration of nitrate are not all independent of one another; development and agriculture are responsible for the influx of nutrients into the water. So, it makes sense that if one of these factors increases the growth of *Padina* sp., the others will also. The number of houses in the watershed is the factor best correlated with *Padina* sp. cover (Figure 8). The regressions of the area of agriculture (Figure 9) and the concentration of nitrate (Figure 10) had high R^2 values, however they both had one key point that held down the regression line. In both cases, this point is from site 3A, the site with the most houses and the large pineapple plantation above it. More samples, taken from sites with a wider variety of areas and types of agriculture, would add more points along the regression lines, adding confidence that these correlations are biologically significant.

The results of the multiple regression analyses, testing for interactions between abiotic factors were surprising. It would be expected that nitrate concentration would be interacting, or somehow correlated, with the number of houses and the amount of agriculture. However, only the interaction between agriculture and nitrate was significant in the total alga cover (Table 11). Only this regression had a low p-value and a high F-value, which means that the variation in the data is due more to the interacting factors than error by chance.

The interaction between nitrate concentration and the amount of agriculture, and its affect on total alga cover makes sense. With more agriculture, it would be expected that there may be more fertilizers in use, or more erosion, causing a higher influx of nutrients, which in turn, increases alga growth.

Future work

The determination of *Padina* sp. as an indicator species for the effects of development and eutrophication in Cook's Bay will be useful in monitoring the quality of the water and the health of the bay. Because *Padina* sp. is so abundant, and easy to find and identify, it is not difficult to survey its percent cover at the mouth of streams throughout Cook's Bay in order to determine the level of eutrophication in that stream. Changes over time may be determined, as well, if surveys of percent cover are done on a regular basis. Surveying the cover of *Padina* sp. would provide an alternative to testing the nutrient concentrations in the streams, which must be done frequently, and is difficult because the streams are ephemeral and often have no water .

There are several additional pieces of data that could be collected to further this study. First, more streams along the length of each bay should be sampled to increase the sample size, and increase the variability in numbers of houses, and area and type of agriculture. Second, tests should be done on the salinity of the water in the bay at the stream mouths before the rainy season, and again after the rainy season. Third, samples of stream water should be taken over an extended period of time and averaged, to get a better measure of the nutrient concentration in the stream water. Fourth, more accurate characterization of the watersheds should be done by the analysis of aerial photographs of Cook's Bay and Opunohu Bay. Last, lab experiments should be performed, testing different concentrations of nutrients and salinity on *Padina* sp., to determine how each factor affects the growth independently.

Conclusion

Many of the results of this study were as expected, but some were very surprising. The main points drawn from this research are the following. First, streams running through land with development or agriculture, especially pineapple plantations, have higher nitrate concentrations than streams running through pristine forest. Second, there is no difference in the nutrient concentrations of the streams in Cook's Bay and Opunohu Bay, as was expected because Cook's Bay is more developed than Opunohu Bay. Third, streams do not affect species richness or total alga cover, in either Cook's Bay or Opunohu Bay, but they do affect the total cover of *Padina* sp. in Cook's Bay. And last, increases in the number of houses and the total agriculture in the watershed, and the concentration of nitrate in stream water increase the percent cover of *Padina* sp. in Cook's Bay.

Based on the results of this study, *Padina* sp. has been identified as an indicator species of eutrophication and development in Cook's Bay, Moorea. With it, the health of the bay can be more easily monitored and hopefully the effects will then be minimized. This will help to ensure the stability of the macrobenthic communities and the livelihoods of the people that depend on the bay.

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Appendix: Description of Algae from Cook's Bay and Opunohu Bay, Moorea
(Voucher specimens can be found at the Jepson and University Herbaria, UC Berkeley)

Species 1: *Acanthophora spicifera*
Description : This red alga is stiff and brittle with irregular and sparse branching. It grows to 25 cm tall. It ranges from whitish pink to pale brown, green or yellow. Small spinelike branchlets of hairs appear at the tips of stumpy short branches. It is commonly found attached to other organisms, coral rubble or pebbles in the intertidal zone. (Cook's and Opunohu)

Species 2 : *Dictyota* spp. (*D. bartayresiana, D. friabilis, D. hamifera*)
Description : This brown alga is strap shaped with thin, dichotomously branching blades. The color is a light greenish brown, sometimes with a bluish hue. It is often found on hard substrates in moderately shallow waters. (Cook's and Opunohu)

Species 3 : *Halimeda opuntia*
Description : This green alga consists of hard, disklike, calcified segments connected with flexible joints. It grows to form short clumps, about 10 cm tall. It is a major contributor to carbonate sands. It is whitish green to dark green in color. (Cook's and Opunohu)

Species 4 : *Padina boergesenii*
Description : Light brown to yellow-white, this alga is in the only brown algae genus that calcifies. The blades are thin and cup-shaped with rolled margins, and often have concentric rings along them. This species grows on rock and coral rubble. (Cook's and Opunohu)

Species 5 : *Hypnea* sp. (a)
Description : This red alga has a wiry, entangled growth form. The main branches are stiff and cylindrical and have many short branchlets along them. This alga is light brown or yellow in color and often grows with holdfasts on hard substrates. It can grow to about 20-30 cm tall. (Cook's)

Species 6 : *Hypnea* sp. (b)
Description : This red alga has a delicate growth form. The main branches have many tiny hairlike branchlets along them. The alga is light brown or yellow in color. It can grow up to 20 cm tall. (Cook's)

Species 7 : *Galaxaura* sp. (a) (*Galaxaura subverticillata* ?)
Description : This red alga has moderately calcified cylindrical branches that are dark red and ringed by tiny hairlike filaments. The branching is dichotomous, and forms round mounds that are about 4-7 cm high. It is often found in shallow water on coral rubble or rock. (Cook's)

Species 8 : *Galaxaura* sp. (b)
Description : This red alga has moderately calcified flat, wide branches that are dark brownish red in color. The branches are covered in thin hairlike filaments. It is found in shallow waters and grows in clumps up to about 8 cm high.(Opunohu)

Species 9 : *Actinotrichia frigilis*
Description : This red alga is also known commonly as 'spikeweed'. It is pink to orange in color and is made up of calified cylindrical segments. The branches along the segments are about 1 cm long, forming a very dense clump, about 5 to 10 cm across. It can be found growing on hard substrates and sand. (Cook's and Opunohu)

Species 10 : *Neomeris annulata*
Description : This green alga is very distinctive. It grows as solitary individual stalks that are small, hard and cylindrical. The top portion of the stalk is bright green and the lower portion is white, with distinct calcified rings. It tends to grow abundantly in shaded areas on coral fragments, rocks, or sand in the intertidal zone. (Cook's and Opunohu)

Species 11 : *Ceramium* sp.
Description : This red alga is recognized by distinct banding patterns of small cells on the very fine cylindrical filamnets. It is a light brown, low lying, fuzzy feeling alga that grows on rocks and coral rubble, and also grows as an epiphyte. (Cook's and Opunohu)

Species 12 : *Chaetomorpha* sp. (*C. linum* ?)
Description : This green alga forms large, loosely entangled mounds that are yellowish green in color. The filaments of it are somewhat stiff and twisted and the large cells comprising the filaments are joined by dark green bands. Mats are often found in nutrient-rich waters (Littler et al. 1989). (Cook's)

Species 13 : *Rosenvingia intricata*
Description : The filaments of this brown alga a hollow cylinders of a golden to olive brown color. The plants are in entangled mats of clumps. The main branches gradually taper to the ends that have smaller branches along them. (Cook's)

Species 14 : *Jania* sp. (*Jania adherens* ?)
Description : This red alga consists of tiny cylindical filaments made of hard, pink, calcified segments connected with joints. The branching is dichotomous. It is found on hard substrates. (Cook's and Opunohu)

Species 15 : Mystery alga
Description : Either a green or brown alga, this alga is made of thin greenish filaments which form soft paintbrush shaped clumps. These clumps are covered with a brown color. This alga grows in small individual clumps out of the sand. It grows up to 5 cm tall. (Cook's and Opunohu)

Species 16 : *Sargassum sociale*
Description : This golden brown alga consists of leaflike blades that are sparsely scattered on smooth main branches. Short stalks along the main branch have small spherical air bladders on them. Plants grow up to 40 cm tall and are usually found in shallow water with a lot of wave action. (Cook's)

Species 17 : *Turbinaria* sp.
Description : This is a brown alga, golden brown in color, which has tall cylindrical stalks with clusters of firm pyrimid shaped 'leaves' (tapered toward the base and wider at the top). The edges of the 'leaves' are sharply spined and are embedded with an air bladder. A strong holdfast holds the stalk to rocks. (Cook's)

Reproductive allocation in seagrass, *Halophila decipiens* on Moorea, French Polynesia

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ABSTRACT. Reproductive biology, plant plasticity, and reproductive allocation of seagrass, *Halophila decipiens* Ostenfeld, was studied in Moorea, French Polynesia. Four populations of *H. decipiens* were sampled, and quantitative measurements of the vegetative and reproductive structures were taken. Two of the populations lived in substrates composed mostly of silt/clay, and the other two populations grew on substrate of coral sand. The differences in size of the vegetative and reproductive structures were first compared between all four sites and then compared between the two substrate types to see if site or substrate accounted for the size differences. Analysis showed that both site and substrate affected the characters and size of the populations. Populations growing in silt/clay substrate had vegetative structures 4 mm longer and reproductive structures 1 mm longer than those growing in coral sand substrate. Examination of reproductive biology revealed that *H. decipiens* can reproduce asexually and sexually. *H. decipiens* is also protandrous and hydrophilic. Differences in leaf length, internode length, and root length between populations growing on different substrates and under different environmental conditions provide evidence of plant plasticity in *H. decipiens*. A trade-off between allocating resources to reproductive structures instead of vegetative structures was also observed in the Opunohu population. Plants at this site had fewer leaves and shorter internode lengths, but had larger reproductive flowers than plants at the other sites. These results support the hypothesis that plants are able to change to best fit their environment—by changing their morphology and allocation patterns.

Introduction

A plant's ability to change with the environment is crucial to it survival (Bradshaw 1965, Sultan 1992). Since plants are sessile, they gain the evolutionary fitness through the capacity for indeterminate growth (Cox 1988). Plant plasticity allows individual genotypes to express diverse phenotypes that are appropriate for the environment. When a grass, *Polypogon monspeliensis*, was grown under different light and fertility conditions, hundredfold variations occurred (Bradshaw 1965). Plants of the same species have also been known to growth thicker leaves when exposed to direct sunlight, and thinner, broader leaves when grown in the shade. It has been argued that plants enhance their evolutionary fitness by enhancing their plasticity (Bradshaw 1965).

Reproductive plasticity also allows organisms to enhance their evolutionary fitness. A plant can often move from asexual to sexual reproduction, or a plant can become more sex-biased when subjected to different environmental factors (Lloyd 1974, Allen et al 1993, Nishitani et al 1999, Koelewijn et al 2000). Sexual reproduction in terrestrial plants is considered more advantageous in changing or heterogeneous environments, and asexual reproduction is considered more successful in stable, homogenous environments (Philbrick and Les 1996). Asexual reproduction or vegetative reproduction in plants uses numerous methods. Plants use runner, or stolons (long stems that grow along the surface of the soil), rhizomes (underground stems), and roots that produce suckers or sprouts. In some species, the leaves are reproductive and small plantlets can arise from one leaf. In other plants, such as some citrus trees, orchids, and some grasses, apomixes occurs. The seed produced is generally identical to the parent because fertilization is not needed to produce a apomitic seed (Raven et al 1999). Angiosperm reproduction involves pollination, fertilization, seed maturation, and seedling establishment. All these strategies cost the plant energy and the allocation of resources becomes important. There is trade-off between growth and reproduction, and studies have shown that plants are able to change how much and where it allocates resources in different environments. (Pickering 2000, Sato 2000).

Aquatic plants retain many of the terrestrial angiosperm structures, such as petals, stems, leaves, and roots. Aquatic angiosperms can reproduce sexually and asexually, although asexual reproduction is observed more often (Philbrick and Les 1996).

Reproduction in aquatic angiosperms present a major problem their terrestrial counterparts do not—water. To account for the problem of water to terrestrial plant pollen, aquatic plants have adapted numerous methods for pollination. The first method is to keep pollen dry by floating male flowers that transport the pollen to the female. The second method is a pollen raft that flows on the water surface. The third method is underwater pollination (hydrophily), where current carries the pollen underwater. The fourth method is a

combination of surface and submarine pollination (Cox 1993). Reproduction in aquatic seagrasses is usually either hydrophily or epihydrophily.

Geographic range

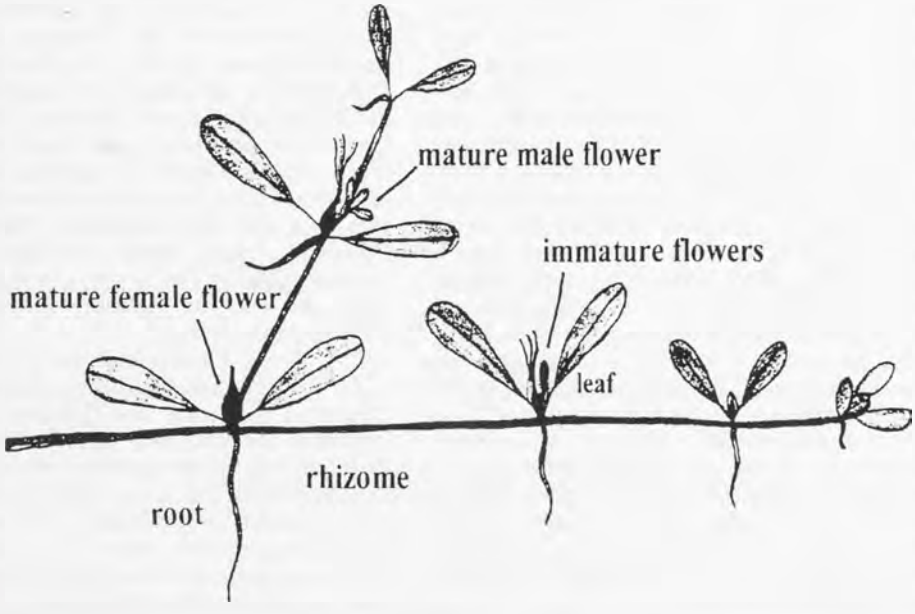
Seagrasses are marine angiosperms that occupy many coastal regions of the world. They are found all around the pan-tropic (den Hartog 1970). They grow in estuarine and near shore continental margins where the water temperature is over 20° C. Seagrasses serve many important functions within their ecosystem. They 1) stabilize sediments, 2) slow the water currents and waves, 3) serve as a refuge for adult and juvenile animals, 4) support high productivity though herbivory and detritus pathways, and 5) keep nutrients in the system by creating an internal nutrient cycle (Wood et al 1969). They grow well in lagoons and bay environments where the reef protects them from rough waves (Larkum and den Hartog 1989). Seagrass can grow in substrate ranging from muddy clay sediments to coarse sand sediments.

In French Polynesia, only one kind of seagrass exists, *Halophila decipiens* Ostenfeld. *Halophila decipiens* is found in many tropical regions of the Atlantic, Pacific and Indian Oceans (den Hartog 1970). In Moorea, Galzin and Pointier found the first documented *Halophila* bed in Opunohu Bay in 1985. Other documented populations occur at the base of Cook's Bay, near the reef pass in front of Cook's Bay (Emmett 1996), and Gump Station (Roderick-Jones 1998).

Halophila decipiens morphology

Halophila decipiens grows along the substrate with horizontal rhizomes that can entangle to create a bed where different plant individuals cannot be recognized (Figure 1). The rhizomes often grow below the substrate, although they also grow with rhizomes exposed. At each node, there may be a root, which can reach over eight centimeters. Nodes are spaced from 10-40 mm, and at the nodes of the primary branch there are a single pair of leaves, 10-25 mm in length, and 3-6 mm in width. The leaf blades are oblong—elliptic, obtuse or rounded, with unicellular hairs. Secondary branching also occurs at the nodes, and sometimes these branches are indistinguishable from the primary branch.

Figure 1. An illustration of *Halophila decipiens* vegetative and reproductive structures. The flowers are found at the tip and develop progressively along the rhizome internode, resulting in the least mature fruits being further from the oldest part of the plant.



Halophila decipiens reproduction

Halophila decipiens is monoecious, and on the reproductive branches at each node, there is one male flower and one female flower enclosed by a transparent spathe. The spathe splits at the top as the fruit ripens. The male females have three tepals that are oblong—elliptical to ovate and obtuse. The anther is approximately 1mm long and transparent, and the pedicel is approximately 3 mm long. After anthesis the complete flower with its pedicel breaks off. The female flower is subsessile, the ovary ovoid and about 1mm. The hypanthium is 1-2 mm long and three, 2.5 mm styles extend from the ovary. The fruit is broadly ellipsoid, 2.5 mm long. The fruit usually contains 30 ovoid seeds that are 0.2 mm in diameter (Hartog 1970).

Halophila decipiens is protandrous. The male flowers mature before the female matures, and ripe fruits are often on branches that have no flowering males (personal observation). Different stages of flower and fruit maturity occur along the branch—the immature flowers are found near the tip of branch and the mature flowers are found the farther the distance from the tip (personal observation). A study conducted in Japan also concluded that the flower and fruit develop progressively along the rhizome internodes, resulting in the least mature fruits being further from the oldest part of the plant (Kuo et al 1995).

Proposed study

Halophila decipiens is a colonizer species, growing quickly in disturbed environments and may have different morphologies in different regions of a clone. Emmett in 1996 found that *Halophila decipiens* puts more energy into growing laterally across the substrate and less into leaf production when growing in the bed margins compared to the interior beds. He found the internode lengths of branches from the *Halophila* bed center to be shorter than internode lengths of branches from the bed margin. Emmett also observed populations of *Halophila* growing in deep lagoon areas had a smaller leaf surface area than populations growing in estuarine areas. Allocation of resources in this case is based on location of the population and the amount of sunlight the population receives. Not much is known about how *Halophila* responds reproductively to different environments, although it has been observed to occur in places differing in substrates and light availability. While there is evidence of morphological plasticity in vegetative parts, no studies have investigated resource allocation for reproductive growth versus vegetative growth.

This study has three goals. The first goal is to study the reproductive structures (i.e. female flowers, male flowers, and seeds) and biology of *Halophila*, since at the time of this study there were few references on reproductive biology of *Halophila*. The second goal is to observe if different populations of *Halophila* allocate resources differently to body parts, such as leaf length, internode length, root length, male flower size, fruit size, seed number, and seed size in response to different environments. The third goal is to see if there is a trade-off in allocation of resources between vegetative and reproductive structures among different populations. I hope to find morphological and structural differences among the populations of *Halophila* and understand their responses to the different environments.

Materials and Methods

Study site

Moorea, French Polynesia lies 25 km Northwest of Tahiti at 17°30'S and 149°50'W. The island formed approximately 1.2 million years ago from volcanic activity, and throughout time the island has eroded to give rise to a highly jagged semi-circular ridge. Mass erosion also formed the two largest bays on the island, Opunohu Bay and Cook's Bay. A barrier reef and fringing reef encircle Moorea with twelve passes intersecting through it. The reef creates a lagoon ranging from 500 to 1500 meters wide. The lagoon is shallow in most areas (0.5m to 3m deep), although the lagoon can reach depths of 10m to 35m in Afareaitu on the eastern side of the island (Galzin and Pointier, 1985).

Four study sites were chosen to study different populations of *Halophila decipiens* from September to November of 2000. The location of the four populations was determined from a study conducted by Emmett (1996), and Roderick-Jones (1998). I collected from one population at Opunohu Bay, one population at the Bridge Site located between Opunohu and Cook's Bay, and two populations at Cook's Bay (Figure 2). The head of Opunohu Bay is a black sand beach, and the bottom slopes from 0.6m to 60m to a silt/clay substrate. Water from the Opunohu stream empties out into the back of Opunohu Bay. The water is murky from the sediment, and the visibility further decreases following rain events.

Table 2 describes the population sizes and environmental conditions. Population 1, the Opunohu Estuarine Site (Emmett 1996) is the largest of the four, and covers an area of 15 ha. Population 2, the Bridge Site, has mainly a sandy substrate, and has good visibility compared to the Opunohu population. The depth of the water ranges from only 0.5 m to 1 m. The population here is about 0.54 ha, and is located near the mouth of a small creek. Population 3, the Gump Station is located in Cook's Bay and the substrate is similar to that of the Bridge—sandy.

Figure 2. Moorea with Cook's Bay and Opunohu Bay. Site 1 was located in Opunohu Bay, site 2 was located between the two bays, site 3 and site 4 were located in Cook's Bay.

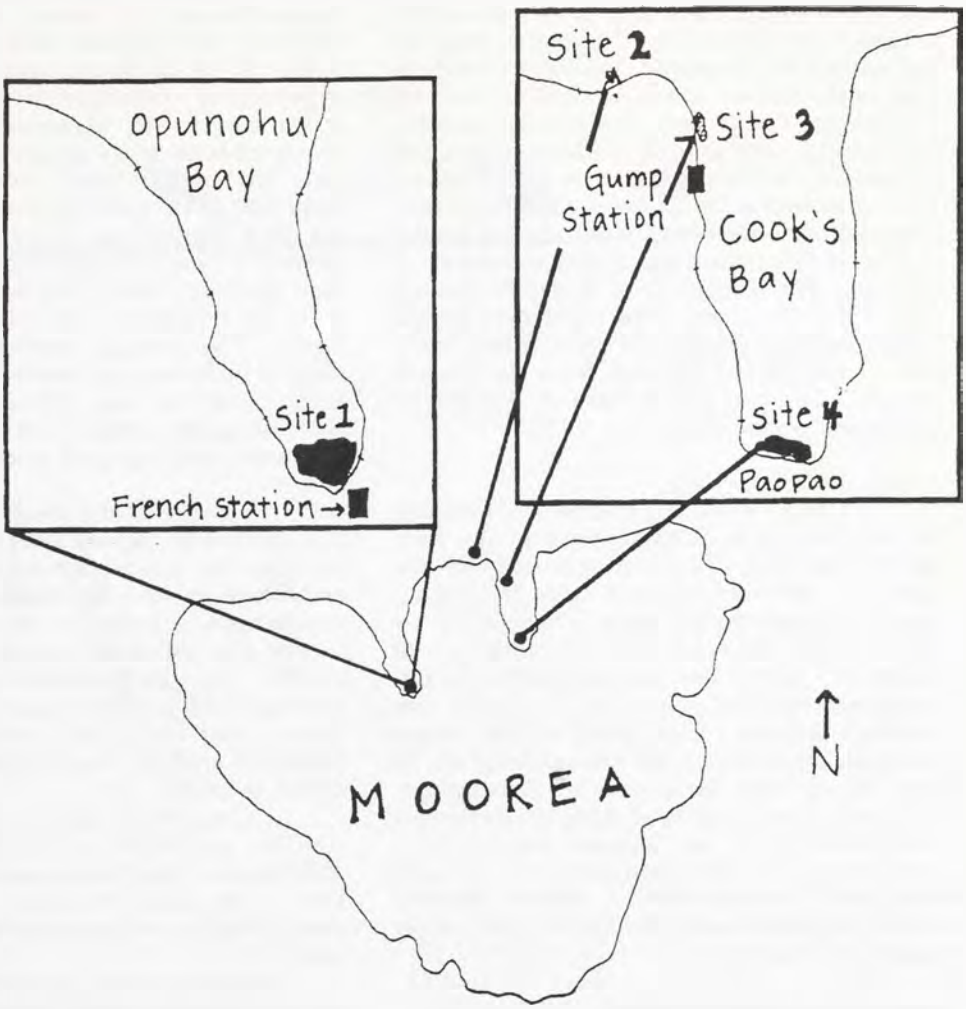


Table 1. Environmental factors affecting four populations of *Halophila decipiens* in Opunohu Bay and Cook's Bay in Moorea

Population	population size	substrate	light available	near possible source of pollution
site 1: Opunohu Estuarine	15 hectares	silt/clay	low	yes
site 2: Bridge	0.54 hectares	coral sand	high	no
site 3: Gump Station	1.2 hectares	corals sand	high	yes
site 4: Paopao Estuarine	6 hectares	silt/clay	low	yes

Unlike the other three populations of seagrass that are too far from shore to be shaded by trees, this population lies in the shade of *Casuarina* sp and *Hibiscus* sp trees, and grows in depths ranging from 0.5m to 1 m. The seagrass beds are located near the Pineapple Distillery—directly in front of the outflow where the distillery releases effluent into Cook's Bay. The population is the third largest, with an area of 1.2 ha. The fourth population, the Paopao Estuarine Site (Emmett, 1996) is located at the base of Cook's Bay where Paopao stream empties. The substrate is similar to that at Opunohu Bay, and is composed of silt/clay. The seagrass grow at depths ranging from 1m to 2m deep. The water here is also highly polluted (Harbaugh unpublished data), due to the effluent flowing from the Paopao stream. The population here is the second largest and is about 6 ha.

Architecture and morphology

Using a dissecting microscope, I studied the morphology of *Halophila* prior to any field work or lab work to familiarize myself with the plant. I collected samples from the Gump Station Site and the Opunohu Estaurine Site by snorkeling in the water and collecting bags of *Halophila*. Using den Hartog (1970), I first confirmed that the plant I was studying was *Halophila decipiens*. I also tried to find female and male flowers from the description given by den Hartog since there were no illustrations. The male flowers and developing female flowers were difficult to see without the aid of a microscope. The developed fruits could be seen without a microscope. After correctly identifying the flowers, I looked at branching patterns of *Halophila*.

Reproductive allocation sampling

At each of the four sites, I collected ten samples of *Halophila decipiens*. The samples collected from the Opunohu Estuarine Site and the Paopao site were collected haphazardly because I could not see where the edges and center of the beds were located. At the Bridge Site and Gump Station Site, I collected five samples from the bed interior and five from the exterior. I collected using a garden shovel, digging 10 cm into the substrate to ensure the roots were attached. Each sample I cut was the size of the garden shove, about 200 cm^2. In the lab, I examined 40, five-centimeter segments of *H. decipiens* from each site, for a total of 160 segments. Using a dissecting microscope and dial caliper, I took quantitative measurements including reproductive status (vegetative or reproductive), leaf length and width, internode length, root length, number of female flowers, length and width of the ovary, stage of the female flower, the number of mature seeds, the diameter of the mature seeds, the number of male flowers, the length and width of the male flowers, and the flower stage (Figure 3).

I dissected the ovary to obtain measurements of female seed count and diameter. Mature seeds were recognized by the presence of a white seed coat (personal observation). Immature seeds were clear and much smaller in diameter. Some ovaries contained both immature and mature seeds, but only the mature seed were recorded and measured. To measure male flower stage, I assigned number one for a developing male (pedicel < 1mm), number two for a developing male (pedicel < 3mm), and the number three for a flowering male (3 tepals present and pedicel > 3mm). The average numbers for the flower stage of both sexes represents how reproductive each population was. All vegetative segments were assigned number one and reproductive segments were assigned number two for data analysis.

I averaged the measurements for each five-centimeter segment within a site and then averaged the quantitative measurements for the entire site to look for difference among the populations. I looked at four combinations of factors that influence morphological changes among the populations—site (location), substrate (silt/clay and sand), site*reproductive status, and substrate*reproductive status. Statistical analysis was performed on the four factors as follows.

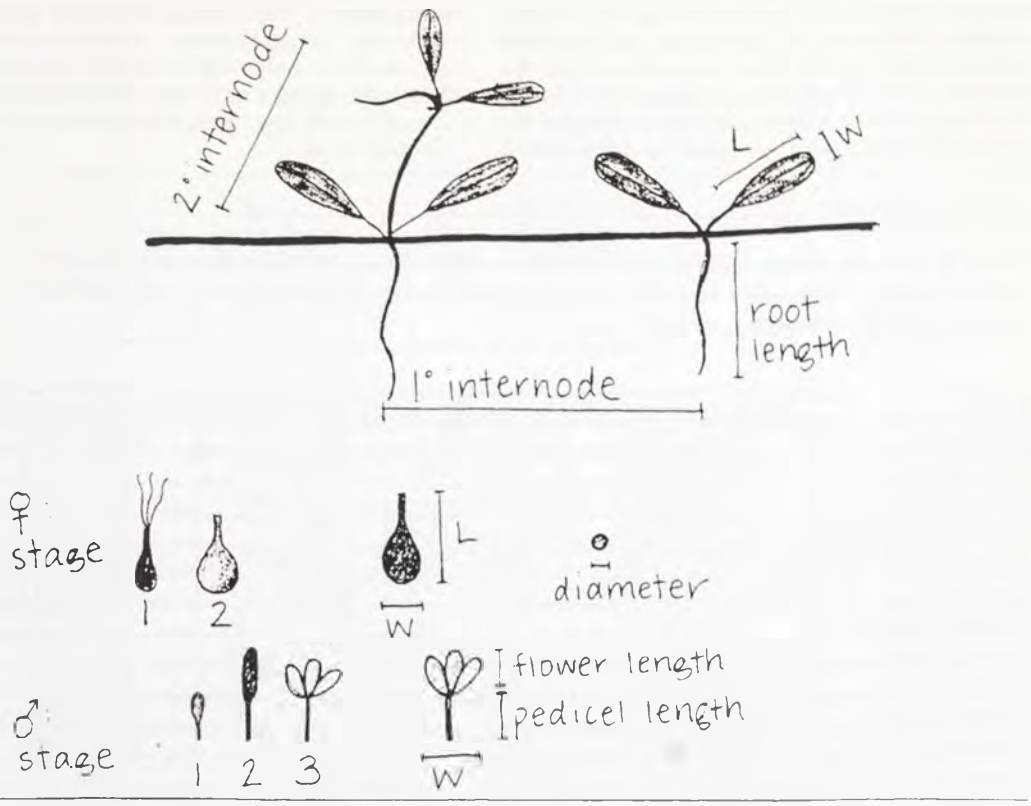
Comparison by site.—I used one-way ANOVA on JMPIN and SYSTAT to look at differences of quantitative measurements among sites. I also used the Tukey Post Hoc test to make pairwise comparisons between the four sites.

Comparison by substrate.—I used one-way ANOVA in JMPIN to look at quantitative measurements between populations growing on different substrates.

*Comparison by site*reproductive status.*—I performed a two-way ANOVA in JMPIN and SYSTAT to look at quantitative measurements taking into consideration the site and the reproductive status as fixed factors. The ANOVA only applied to quantitative measurements that both the vegetative and reproductive plants possessed, such as number of leaves, leaf length, leaf width, internode length, and root length. I also performed a Tukey Post Hoc test.

*Comparison by substrate*reproductive status.*—I used a two way ANOVA in JMPIN to look at whether substrate and reproductive status of the plant as fixed, categorical variables explained variations observed in the following variables: number of leaves per 5 cm segment, leaf length and width, internode length, root length, female flower size, number and size of seeds, and male flower size.

Figure 3. An illustration on how quantitative measurements were taken on *Halophila decipiens*. The measurements included leaf length, 1° and 2° internode lengths, root length, female flower stage, female flower size, seed diameter, male flower stage, and male flower size.



Results

Comparison of plant characteristics showed differences among the four sites ($p<0.5$) (Table 2) and among populations growing in different substrates ($p<0.5$) (Table 3). The four factors contributing to the difference in physical characteristics were 1) site, 2) substrate type, 3) site and reproductive status, and 4) substrate and reproductive status.

Architecture and morphology

Most of the measurements of vegetative and reproductive structures I found were comparable to den Hartog's description (Table 2). Leaf length ranged from 14.79-19.42 mm, leaf width was 4.64-5.73 mm, primary internode length was 16.31-19.87 mm, secondary internode length was 6.57-14.53 mm, male pedicel length was 1.03-3.86 mm, fruit length was 2,.13-3.31 mm, and fruit width was 0.82-1.60 mm. The average number of seeds per ovary for all four populations was less than 30 seeds, which Hartog described previously.

Of the 160 samples I collected, 12 of 140 samples had two male flowers enclosed in the same spathe, and one of 140 samples had two female flowers enclosed in the spathe. The male/male flower samples were collected from the Opunohu Estuarine Site and the Paopao Estuarine Site. The female/female sample was collected from the Bridge Site. Of the total samples, 8.125% had the same sex flower anomaly. In *Halophila decipiens*, normally a spathe contains one male and one female flower.

Reproductive allocation sampling

Comparison by site.—Many plant characteristics differed in size among population (Table 2). The number of leaves ($p<0.0001$) and leaf lengths ($p<0.001$) were different within the sites. The Paoapo Estuarine population had the longest leaf length, the Opunohu Estuarine population had the second longest leaf length, the Gump Station population had the third longest leaf length, and the Bridge population had the shortest leaf length. The Bridge population had the most number of leaves (due to the short internode lengths), the Paopao Estuarine population had the second most number, the Gump Station had the third most, and the Opunohu Estuarine population had the fewest number of leaves. Primary internode

($p<0.0054$) and secondary internode length ($p<0.011$) were significantly different among the four populations. The Gump Station population had the longest primary internode length, the Paopao Estuarine population had the second longest, the Opunohu population had the third longest, and the Bridge population had the shortest. The Gump Station population also had the longest secondary internode length, the Opunohu Estuarine population had the second longest, the Paopao Estuarine population had the third longest, and the Bridge site again had the shortest secondary internode length. Root length ($p<0.0001$) differed between the populations. The Paopao Estuarine population had the longest roots, the Gump Station population had the second longest, the Opunohu Estuarine population had the third longest, and the Bridge population had the shortest roots.

Table 2. Quantitative measurements of plant morphology of *Halophila decipiens* from four populations on Moorea, French Polynesia. All meaurements are in millimeters and represent mean ± one standard deviation. Within each row columns with different letters are statistically at a $p<0.05$.

trait	Opunohu Estuarine (1)	Bridge Site (2)	Gump Station(3)	Paopao Estuarine (4)
# of leaves	7.53±1.77**ab**	9.80±2.87 **a**	7.60±1.75 **b**	8.50±2.55 **ab**
leaf length	18.40±2.57 **a**	14.79±1.29 **b**	15.90±2.01**b**	19.43±1.58 **a**
leaf width	5.25±0.80	4.64±0.51	5.73±7.84	5 43±0.56
1 internode length	17.20±4.72 **ab**	16.31±4.24 **a**	19.87±5.27 **b**	19.74±6.96 **ab**
2 internode length	10.65±1.91 **ab**	6.57±2.10 **a**	14.53±6.89 **a**	10.00±2.53 **ab**
root length	27.72±10.03 **a**	23.67±7.00 **a**	30.76±8.68 **b**	42.48±16.64 **b**
# female flowers	0.51±0.40 **ab**	0.69±0.31 **a**	0.61±0.31 **ab**	0.37±0.39 **b**
ovary length	3.31±1.35 **a**	2.13±0.80 **b**	2.17±1.28 **ab**	2.76±1.56 **ab**
ovary width	1.60±0.82	0.82±0.30	1 41±2.55	1.07±0.60
female fl stage	1.60±0.53 **a**	1.09±0.22 **b**	1.14±0.31 **b**	1.24±0.36 **ab**
number of seeds	16.52±8.99**b**	0.85±2.37 **a**	12.83±1.43**b**	12.88±4.39 **b**
diameter of seeds	0.55±.008 **a**	0.62±0.11 **b**	0.41±0.16**b**	0.63±0.11 **b**
# male flowers	1.39±0.48 **a**	0.59±0.28 **b**	0.53±0.36**b**	0.89±0.64 **b**
male flower length	1.22±0.29	1.01±0.30	1.15±0.31	1.17±0.30
male flower width	0.78±0.19	0.63±0.19	0.68±0.19	0.70±0.23
pedicel length	1.88±1.25	1.25±0.93	1.03±0.61	3.86±8.83
male fl stage	1.90±0.88	1.15±0.54	1.29±0.38	1.57±0.64

The female reproductive parts, such as number of flowers ($p<0.0.018$), ovary length ($p<0.019$), and ovary stage ($p<0.0004$) were significant between the populations. The Opunohu population had the largest and the most mature female flowers at the time of sampling. The Bridge population had the smallest and the least mature female flowers. This population also had the most number of flowers, also due to short internode lengths. The Paopao population had the least number of flowers, due to the long internode lengths, but it had the second highest number of mature and large female flowers. Seed number ($p<0.001$) and seed diameter ($p<0.059$) also differed among the populations. The Opunohu had the most number of seeds, but the seeds had the second smallest diameter. The Bridge population had the least number of mature seeds, but the diameter of the seeds was the second largest. The Paopao Estuarine population had the second highest number of seeds, and the largest seed diameter. The Gump population had the least amount of the smallest seeds.

The number of male flowers ($p<0.001$) and male flower stage ($p<0.059$) were significant. The Opunohu population had the highest number of mature male flowers, and the Paopao population had the second highest number. The Bridge population had the third highest number of male flowers, but the least number of mature flowers. The Gump population had the fewest number of mature males.

The Tukey Post Hoc test made pairwise comparisons between the four sites. The Bridge Site and Gump Station Site were grouped together for five quantitative measurements. These measurements included the number of leaves per segment, leaf length, primary

internode length, secondary internode length, and ovary stage. The Opunohu Estuarine Site and the Paopao Estuarine Site were grouped together for four quantitative measurements. The measurements were leaf length, root length, seed diameter, and the number of male flowers. Both the quantitative measurements and the grouping were statistically significant ($p<0.05$) (Table 2).

Comparison by substrate.—Substrate had an effect on size variation of vegetative and reproductive structures between the four populations (Table 3). The populations growing in a silt/clay substrate had much longer leaf lengths ($p<0.001$) and root lengths ($p<0.0001$) than populations growing in coral sand. The populations in a muddy substrate also had more reproductive structures than the populations growing in coral sand. The muddy substrate populations had longer ovary lengths ($p<0.0032$), more developed ovaries ($p<0.006$), greater seed count ($p<0.001$), more number of males per sample segment ($p<0.001$), male flower length ($p<0.0524$), and more developed male flowers ($p<0.0236$). Since there were only two types of substrate, this comparison did not need a Tukey-Post Hoc test.

Table 3. Quantitative measurements of plant morphology of *Halophila decipiens* from two types of substrates: silt/clay and coral sand, on Moorea. All measurements in millimeter and represent mean ± one deviation. Within each row, measurements that are statistically significant at a $p<0.05$ are denoted with a *.

trait	substrate 1: silt/clay Opunohu and Paopao Estuarine	substrate 2: coral sand Bridge and Gump Station Sites
# leaves per 5 cm	8.0125±0.6894	8.7000±1.5556
leaf length *	18.9109±0.7268	15.3474±0.7845
leaf width	5.3394±0.1213	5.1821±0.7718
1 internode length	18.4693±1.7959	18.0891±2.5121
2 internode length	10.3238±0.4613	10.5464±5.6282
root length *	35.0967±10.4348	27.2166±5.0170
# female flowers *	0.4398±0.0945	0.6498±0.0594
ovary length *	3.0379±0.3899	2.1459±0.0285
ovary width	1.3372±0.3776	1.1104±0.4109
female fl stage *	1.4228±0.2542	1.1101±0.0359
number of seeds *	14.6979±2.5779	6.8436±8.4707
diameter of seeds	0.5901±0.0611	0.5165±0.1525
# male flowers *	1.1425±0.3541	0.5584±0.0409
male fl length *	1.1979±0.0342	1.0770±0.1006
male fl width *	0.7425±0.0567	0.6535±0.0329
pedicel length	2.2679±1.4020	1.1419±0.1545
male fl stage *	1.7368±0.2317	1.3512±0.0926

Comparison by site and reproductive status.—The size of the vegetative structures varied when compared by reproductive status within a site. The plant characteristics that were statistically significant from the interaction of site and reproductive status were: the number of leaves per segment ($p<0.0008$), the leaf length ($p<0.001$), primary internode length ($p<0.036$), and the root length ($p<0.001$). The Tukey Post Hoc Test confirmed that the Bridge and Gump populations were smaller for leaf length and root length than the other two sites, the Opunohu Estuarine and the Gump population had the least number of leaves per segment, and the Opunohu and Paopao populations had the longest leaf lengths (Table 3).

Comparison by substrate and reproductive status.—ANOVA found the leaf length ($p<0.001$) and root length ($p<0.002$) to be statistically significant. Since there were only two types of substrates, I did not need to perform the Tukey Post Hoc Test to do another pairwise comparison.

Discussion

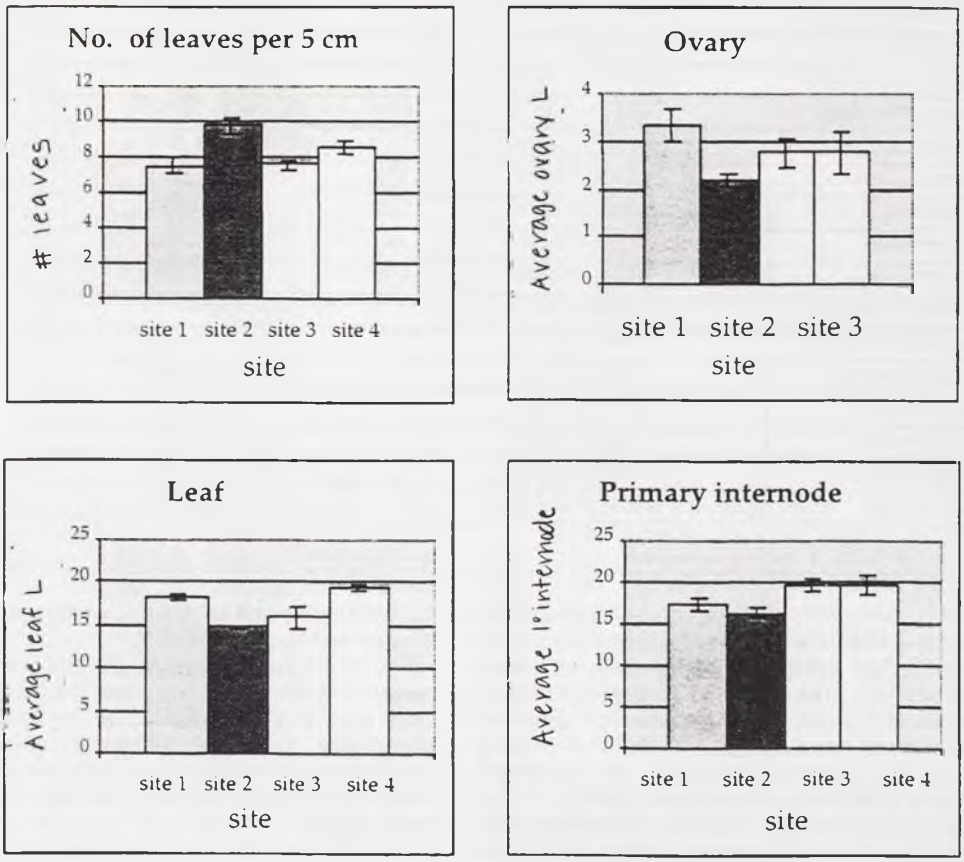
Plant responses to the environment

Plants respond to different environments in many ways. This plasticity is

greater in plants than animals because the indeterminate growth pattern of plants can be modified more easily to produce different expressions of a single genotype (Raven 1999). Some plants that grow in the shade develop thinner and larger leaves than those that grow in the sun. Plants can also change the mode of reproduction, depending on the environment. In Moorea, I observed morphological differences among the four studied populations, and each population showed different responses to the environment.

Most aquatic plants are weightless and growth is limited to the availability of sunlight. Consequently, they tend to invest all their biomass in structures that maximize or enhance photosynthesis (Niklas 1994). The Opunohu and Paopao populations lived in murky, muddy waters, and the light available to these populations is less than the light available for the other two populations. The leaf length of the Opunohu and Paopao populations was longer (Figure 4), increasing the surface area of the leaf, and thereby increasing the ability to capture light in murky waters. The Gump and Bridge populations lived in environments with more light available, but larger leaves may cause shading. Of all the seagrasses, *Halophila decipiens* has the lowest Leaf Area Index (LAI). LAI measures the surface area of photosynthetic tissue to the bottom area. Low LAI reduces self shading (Josselyn et al 1996). The smaller leaf length for the Gump and Bridge populations may be advantageous for optimizing photosynthesis.

Figure 4. Four measurements--number of leaves, leaf length, internode length, and ovary length in *Halophila decipiens* that differed among the four populations.



Halophila decipiens is morphologically and structurally adapted for low light environments. Leaves show different methods the species uses to maximize light availability. *H. decipiens* have thin cell walls, a simple leaf anatomy of two cell layers, densely packed chloroplasts, and reduced biomass allocated to non-photosynthetic material. The ratio of above ground to below ground biomass was 1.06 in a study conducted on *Halophila* in the Virgin Islands (Josselyn et al 1996). My study did not include biomass since there was not a scale sensitive enough on Moorea to measure above ground and below ground biomass. However, leaf length differences among populations show how each population adapted it structures to increase light availability. An increase in four to five centimeters for leaf length in the Opunohu and Paopao populations, in a plant designed to capture light in low light environments, may greatly increase the amount of additional light the plant receives.

Reproductive allocation of asexual and sexual reproduction

Reproductive allocation in plants can also be determined by environmental conditions. In stable environments (more light available), the plant may allocate more resources to asexual reproduction, producing more shoots or buds rather than fruits. In unstable environments (low light available), plants may choose to do the opposite and produce more fruits. In the Opunohu population, there is a trade off between fruit production and number of leaves and internode length. Sexual reproduction increases fruit production, thus the plant puts more energy in fruit production and less energy in growing laterally to colonize new areas. The Opunohu population had the largest fruits and the most number of seeds, but a relative short internode length.

The Gump population has the longest internode length, but some of the smallest fruits and seeds (Fig 4). This suggests that the Grump population may be reproducing more vegetatively, and putting more energy into expanding than producing fruit. Growing in an environment with more sunlight than the Opunohu and Paopao populations makes the environment more heterogeneous for the Gump population. In the silt/clay environments, light availability decreases drastically after a rainfall—due to sedimentation being released from the streams. In the coral sand environments, light availability remains normal after rainfall (personal observation). This makes vegetative reproduction more energy efficient and most likely more successful (Philbrick and Les 1996). As a colonizer species, vegetative reproduction increases establishment rate. Further more, as a opportunistic species, *H. decipiens* have rapid rhizome elongation (Fonesca 1989). Growth is directed toward rapid spreading over the ocean bottom and not at accumulating biomass (Josselyn et al 1996). Studies have shown in some plants that vegetative reproduction is the most advantageous, even if crowding may cause negative density effects (Nishitani et al 1999). The species *Syneilesis palmata*, a summer perennial herb of Japan and Korea had optimal reproduction when the mode of reproduction was purely vegetative. In aquatic plants, asexual reproduction is equally as important for establishment, growth, and maintenance as terrestrial plants, if not more important. In weedy aquatic plants, more emergent species disperse by sexual propogules, and the floating and submerged species disperse vegetatively (Philbrick and Les 1996).

It may be possible that the observed flowering among the populations can be due to timing. One population may flower earlier than another one. If this is true, then we expect to see the populations growing in clearer waters to flower earlier and have larger flowers. Studies have shown that a decrease in light can decrease seagrass growth (Onup 1996, Onup 2000). However, the two populations growing in the murkier waters had more and larger reproductive structures. Thus, although the timing of the samples may affect the observed reproductive status of the four populations, we see the opposite of what we expect. This suggests that perhaps another factor, such as nutrient increase is responsible for the larger plants growing in murky waters.

Seagrass response to nutrients

Increase in nutrients from streams or pollution can increase plant growth rates. Eutrophication can cause noxious weedy plants to multiply. The Paopao and Opunohu streams have historically been known to release excess nutrients into both bays (citation from Moorea class), and the two populations are located at the head of both bays. In other species of seagrass, an increase in nutrients can increase its distribution (Udy et al 1999). The Opunohu and Paopao populations are larger than the Bridge and Gump populations, possibly due to nutrient increase.

A study with the seagrass *Zoster marina* showed that when stressed by salinity, the plants responded negatively to an increase in nutrients, but with relatively low salinity, the plants were stimulated by enrichment (van Katwijk et al 1999). The Opunohu and Paopao populations are located in an environment where fresh water constantly flows in, potentially lowering the salinity level and

increasing the plants' abilities to intake nutrients. I did not take water samples to measure salinity or nutrient levels, but historical practices on the island suggest that nutrients from the valleys may potentially increase seagrass growth.

Sex Ratio

Although the expected sex ratio is 1:1 for *Halophila decipiens*, I found branches with two male flowers per node at two different populations. Possible explanations are unknown, but I recommend further research to study this phenomenon. In some other seagrass species, there is a sex biased ratio favoring more females (Williams 1995, Sato 2000).

Morphological differences were significant for both site and substrate. However, a comparison by site also accounted for substrate differences. However, when comparing differences by site, more factors than substrate type are involved. So while substrate was an important factor in accounting for different morphologies between populations, other factors such as light availability and nutrient increase are also important factors. The results suggest that there are morphological differences between populations and further research is recommended to explain why.

Conclusion

Halophila decipiens showed differences in morphology among four populations on Moorea. When compared by site, the Opunohu population had the biggest vegetative and reproductive structures and the Bridge population had the smallest structures. The different environmental factors from each site, such as light availability, substrate type, nutrient levels, and salinity had an effect on plant structure and its mode of reproduction. When compared by substrate, the populations growing on silt/clay substrate were found to be larger and more reproductive than the populations growing on coral sand. Murkier waters may have influenced these populations to increase leaf length, and thus increase the amount of photosynthetic area. Excess nutrients from fertilizers may also account for the size difference between the four populations. The Opunohu and Paopao populations were likely exposed to excess nutrients being carried by streams from the farmed valleys into the bays.

The differences in plant morphology provided evidence for morphological plasticity. Populations growing in different environments respond by altering their growth to enhance photosynthesis and reproduction. Allocation of resources between vegetative and reproductive structures was also evident. The Opunohu population allocated more resources to sexual reproduction by increasing the size of the flowers and fruits, but resulting in fewer leaves and shorter internode length. Contrary, the Bridge population allocated more resources to leaf production, resulting in many leaves per branch, but the smallest leaves and reproductive structures.

Further research is recommended looking at how substrate type, light availability, excess nutrients, and salinity affects the distribution of *Halophila decipiens*. The male/male flower anomaly should also be studied further to see if possible sex bias occurring among the populations.

Acknowledgements

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Distribution of the epiphytic fern *Asplenium nidus* in the Opunohu Valley of Moorea, French Polynesia

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ABSTRACT. The common epiphytic fern *Asplenium nidus* is found throughout Southeast Asia, through the Pacific and into parts of Africa. Factors controlling its distribution have not been explored. Three 2000m^2 plots at differing elevations in the Opunohu Valley on the island of Moorea were examined to determine factors affecting the distribution of *A. nidus* both overall and along an elevational gradient. Within these plots each *A. nidus* was mapped to within one meter of its true location, and substrate was recorded. Maps generated from these plots were then used to test for clumping of individuals, distance to stream, and preferences in substrate. Additionally a several kilometer long transect was used to record overall frequencies of *A. nidus* in the valley. Results showed that *A. nidus* is clumped in its overall distribution regardless of elevation, and is growing preferentially on basalt boulders and *Hibiscus tiliaceus*. *A. nidus* was also found to be most densely distributed in areas close to streams. This effect became more pronounced as elevation decreased. Although the overall frequency transect did not show differences in densities of *A. nidus* by elevation, supplementary data indicates that their frequency is greater at higher elevations.

Introduction

The epiphytic growth habit is a well-documented and successful adaptation to above ground habitat. There are 83 families of epiphytes containing nearly 29,500 species (Wolf 1993). Epiphytism can be defined as the ability of an organism to live on the living or dead portions of another plant without drawing nutrients or water from its living tissues. This definition may also be

extended to include organisms that live on non-biotic substrates above the forest floor such as large boulders, and do not require access to forest floor soils. However, these definitions may be obscured by indirect uptake of nutrients from a platform plant through shared mychorrizal fungi that live both in the roots of some epiphytes and the living tissues of host plants (Wolf 1993; Lobban & Schefter 1997).

About 10% of the world's vascular flora is composed of epiphytes, about 75% of which are found in the tropics (Heite & Briones 1998). In wet tropical rain forests as much as 35% of the total species and 63% of individual plants are epiphytes (Wolf 1993). Although their distribution has been studied both in temperate and tropical regions (Sugden 1981; Wolf 1993; Gay et al. 1993; Heitz and Briones 1998; Zotz etal. 1999) very little evidence has been presented to explain the mechanisms behind observed distributions. This is due largely to difficulty obtaining climatological and micro-climatological evidence to support explanatory hypothesis describing distribution for any given location or habitat (Wolf 1993). Therefore hypothesis about distribution have been put forth largely based on assumed habitat restrictions including substrate preference, canopy soil characteristics, light intensity, and water and nutrient availability.

Epiphytism has allowed for greater niche filling in areas of high species diversity. Mosses, lichens, liverworts and ferns are common on Moorea, and display a great range of adaptations and morphology (personal communication with Brent Mishler). *A. nidus*, the birds nest fern, is one of the largest epiphytes found on the island. Factors influencing its distribution and abundance demonstrate restrictions on the epiphytic habit, and lend understanding to how such a relatively large species is able to succeed without contact with forest floor soils.

Asplenium nidus is widely distributed throughout Southeastern Asia, the Pacific, and into Africa. It is commonly found growing on trees, boulders, large detritus, and even the forest floor on Moorea. Its upright rhizome gives rise to large simple fronds that grow in a rosette pattern giving the fern the appearance of a large birds nest. These simple fronds, which can be of more than one meter in length, are thin and glossy green with wavy margins and a prominent almost black midrib. They push through the root mass and bed outward and backward to form a crown like arrangement. This creates a platform upon which detritus and litterfall is collected and held in place.

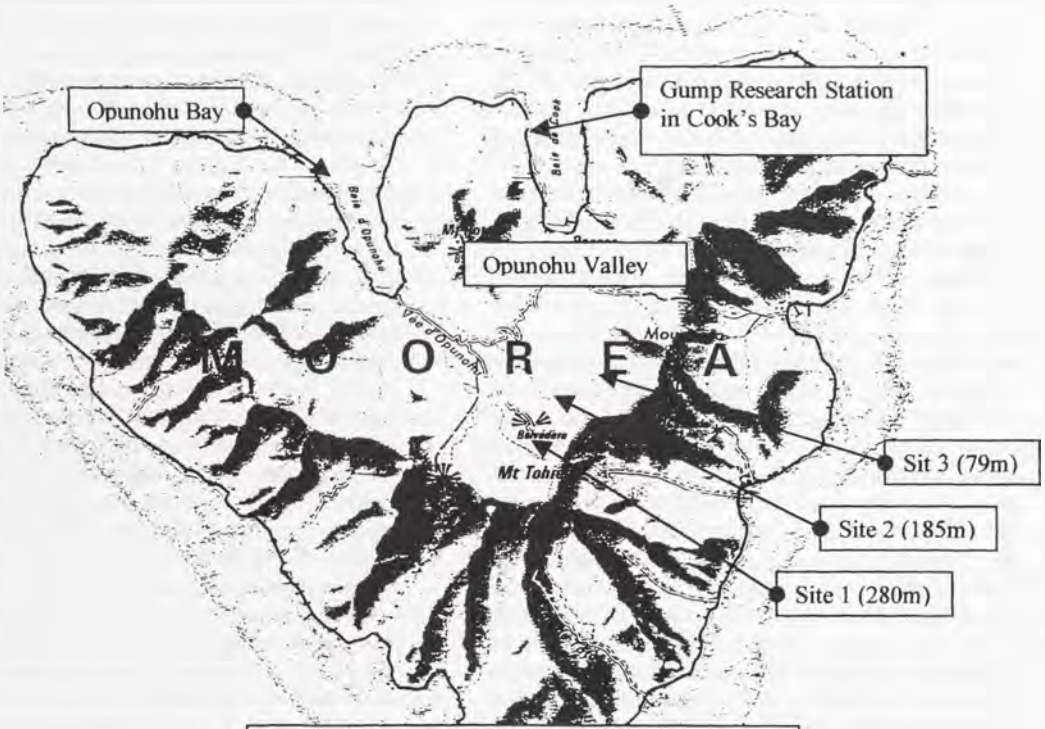

Figure 1: Map of Moorea. Sites indicate 2000m^2 sample plots.

The upright rhizome gives rise to many fine and tightly compact roots covered in small rootlets that form a ball like mass from which the fronds arise. These roots anchor the plant to its substrate, and are key to the epiphytic habit of *A. nidus*. As this material collected in the leaves and crown portion of the plant it decomposes and filters down to the numerous roots growing along the stem that then hold it in place in an absorbent mass (Wee 1978). This then becomes a highly organic self-created and contained soil from which the plant can absorb nutrients.

During rain events this root mass soaks up and retains water. In this way *A. nidus* creates its' own soil and is able to survive without ground water despite its' large size. Specimens observed growing on the forest floor in Moorea seem to be exhibiting the same habits and morphological traits even though they have direct access to better water and nutrient sources.

A. nidus does not reproduce vegetatively, and plantlets arise only from spore (Wee 1992).

A study of their distribution was conducted in the Opunohu Valley on the island of Moorea, French Polynesia (Fig 1). This study focused on elevational gradients of abundance, proximity to streams, and substrate preferences. Due to the difficulties in obtaining and quantifying (micro) climatological data, the hypothesis tested are based on assumed physiological restraints.

Availability of water is an important factor affecting the growth and development of epiphytes (Wolf 1993; Heitz & Briones 1998; Schneider & Carlquist 1999; Zotz et al. 1999). It seems likely then that *A. nidus*, a large epiphyte requiring high humidity, would be found near surface water, and be more abundant at higher elevations were orographic influences result in higher rain fall. The sheer walls of the Mouaputa ridge that runs along the east side of the Opunohu Valley often flow with sheets of water and waterfalls during the many rain events on the island. To determine overall distribution patterns the following hypothesis' were tested :1) *A. nidus* becomes more common as elevation increases 2) Densities of *A. nidus* are greater near streams or rivers than in the forest, 3) *A. nidus* is clumped at all elevations, and 4) That *A. nidus* is exhibiting substrate preference. My objectives were to sample the overall distribution, preferred substrates, radiation tolerance, and distance to nearest neighbor for *A. nidus* in the Opunohu Valley.

Other ecological factors may be important to the distribution of *A. nidus*. As an epiphyte requiring high humidity it is likely that a high percentage of canopy cover is required to prevent desiccation. Therefore the range of canopy cover observed for each specimen in each sample plots was recorded to give a range of tolerance.

Finally substrate may be important for any number of reasons including acquisition of nutrients and water channeling during rain events. The hypothesis that *A. nidus* would be found to grow preferentially on some substrate was tested, but the mechanisms behind that preference were beyond the scope of this study.

Materials and Methods

Preliminary Survey & Environmental Setting

To obtain background information surveys were conducted on all parts of the island of Moorea. The *Asplenium nidus* was present in all valleys surveyed. Three specimens from different locations were collected for examination of overall morphology, and insect and fungal associations. Perspective sites were chosen from relatively undisturbed valleys remaining on the island.

Primary research was conducted in the Opunohu Valley based at the Richard Gump Biological Research Station (Figure 1). This large valley, which dominates the northern interior of the island, offers ample setting for a distribution study the epiphytic fern, *A. nidus* in an undeveloped forest. Established trails in this area allowed access to regions up to 311 meters in elevation. At greater elevations the almost vertical slope of the terrain made sampling too difficult. The coastal regions of the Opunahu Valley are highly developed for housing and agriculture, but a few accessible undeveloped areas still exist at the head of Opunohu Bay.

Two rivers and their tributaries flow through the valley. These are the PaoPao and Opunohu rivers respectively. All sample plots were located along the PaoPao River or its tributaries along the eastern boarder of the valley. All frequency transects were also taken in this area, with the exception of the 0-85 meter transect which was taken at the head of Opunohu Bay. This was necessary because anthropogenic modification of the lowlands near PaoPao River has not left undisturbed forested regions adequate for study.

Overstory vegetation in the area of study was co-dominated by species of *Inocarpus fagiferus* and *Hibiscus tiliaceus*. These two trees are common at all elevations studied as well as in all parts of the island. *Inocarpus* and *Hibiscus* did not share the canopy in overlapping areas, but rather occupied discrete sections of each of the study plots. *Inocarpus* creates a much higher and denser canopy than *Hibiscus*, which appeared to be a primary succession species colonizing light gaps in the forest. Bird's nest ferns were found growing much higher in the canopy

of *Inocarpus* than *Hibiscus*. The understory vegetation was composed primarily of *Myconia*, and several varieties of ferns.

Overall Frequency Transect

To sample the overall frequency of *A. nidus* across the landscape from 0 – 311 meters in elevation a linear transect spanning several kilometers was measured. Elevation and the number of bird's nest ferns was recorded at random intervals along this transect using an altimeter and a 50m transect tape (n=67). This transect was measured in two locations. The first spanned the contour of the east side of the valley along the trail from site 1 to site three from 311 – 79 meters in elevation. The second, which spanned 0 to 85 meters in elevation, was taken at the head of Opunohu Bay on the west side of the valley due to the aforementioned anthropogenic modification of the landscape near the PaoPao river. No bird's nest ferns were found below 50 meters in elevation.

Sample Plots

Three 2000m^2 sample plots at differing elevations were examined to detect potential pattern changes along a gradient. These plots were 40 X 50 meters in size, and located at 280m, 185m, and 79 m respectively (Fig. 1). All three were on the eastern side of the Opunohu Valley in the PaoPao River watershed.

Every *A. nidus* observed within these plots was sampled and mapped to within one meter of its true location. Substrate and percent canopy cover were recorded for each to detect patters in substrate and percent cover preference both between elevations and overall. Descriptions of local conditions including vegetation, and the location and size of light gaps were also recorded.

Elevation readings were taken with an analog metric altimeter. Reported elevations for each site were determined by taking the mean of five readings recorded on separate occasions at each site. There was only a three-meter variation in readings between occasions at each site. Canopy cover prevented GPS readings at all three sites.

Each plot was chosen for the presence of 50 or more bird's nest ferns that were needed for an adequate sample size. At below 100 meters in elevation a smaller sample size (n=35) was used because a greater density of *A. nidus* could not be found at that elevation. Initial observations showed uneven distribution of bird's nest ferns in the valley and samples of adequate size were only found in direct proximity to stream channels, wet or dry. At higher elevations streams channels were far more common, probably due to the steepness of the slope. Therefore, site one was chosen at random from several suitable sites. At lower elevations streams were not as common and sites two and three were chosen for suitable sample size.

In order to map the relative location of individual ferns and to test the hypothesis that their distribution is clumped and concentrated near streams at each site a central transect was laid in the stream bed or within one meter of its edge. This then became the center of the plot. A Silva compass model 426 was used to take perpendicular readings to the central transect creating the 40 by 50 meter grid from which locations of individual ferns were plotted.

Four supplementary sample plots were studied to determine if A. nidus was occurring away from stream channels, and at greater frequencies at higher elevations. These were 10 X 50 meters in size and adjacent to each of my 40 X 50 meter plots (detailed above), with one additional plot at 5m elevation. All supplementary sample plots occurred at distances greater than 20 meters from the streams, and the total number of *A. nidus* was recorded in each.

Results

Preliminary Observations & Incidental Data

During the preliminary study and in the collection of primary data several patterns emerged that are worthy of note. Three samples of *A. nidus* were collected for dissection, two from basalt boulders and one from an *Inocarpus* tree. This was done to examine morphology, invertebrate communities and fungal associations of *A. nidus*. Although ants and other unidentified invertebrates were found on the fronds of samples collected, no invertebrates were found in the root masses themselves.

Fungi were found in the root mass of each sample that appeared to be mychorrizal. Although verification of the mychorrizal nature of the fungi was not possible it would be consistent with current knowledge of tropical epiphytes (Lobbin and Schefter 1997), and be interesting in future studies. It was not possible to experiment with this facet of *A. nidus'* life history with available equipment. Therefore, the focus of the study remained concentrated on the distribution and ecological constraints of *A. nidus*.

In one location it was noted that sporophytes of *A. nidus* were growing on areas of substrate facing away from a large (92m^2) light gap that appeared to have formed before the establishment of most of the young plants. This implies light sensitivity and a need for high canopy cover for the species.

Most specimens at all sites were found growing under dense canopy cover. A hollow tube measuring 4cm in diameter and 11.3cm in length was used to estimate canopy cover for every specimen in all three plots (n=199). Most samples were found to be growing under greater than 95% canopy cover (Figure 2). Samples that occurred under less than 90% canopy cover appeared, for the most part, to be under or near a light gap that formed after the specimen had become established itself.

Overall Frequency Transect

A linear regression was used to examine the relationship between elevation and presence/absence of A. nidus (n=67). The regression did not show significant differences in the occurrence of A. nidus between elevations (p = 0.22).

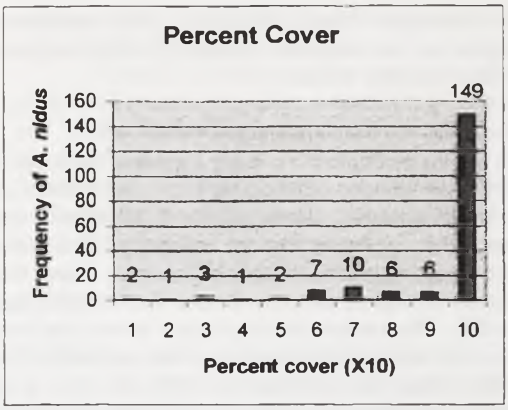

Figure 2: Percent Canopy Cover. Bars indicate the frequency of *A. nidus* by percent canopy cover. 149/199 samples were growing under ≥95% cover.

Substrate Type	Site 1	Site 2	Site 3	Total
Basalt Boulder	50	6	13	69
Hibiscus	3	59	1	63
Inocarpus	2	10	19	31
Detritus	18	2	0	20
Forest Floor	7	2	2	11
Unknown Tree A	5	0	0	5
Total	85	79	35	199

Table 1: Numbers indicate the frequency of *A. nidus* by site, substrate, and in total.

Substrate Preference

A chi-squared test was used to examine the null hypothesis that there is no substrate preference. Results showed that there was a significant deviation from the expected value assuming all substrates were equally likely (p=<0.0001). The assumption was made that substrate was not limited at any given site, and therefore quantitative measures of individual substrates were not recorded in detail.

Numbers of individuals found in each substrate type and in total are shown in Table 1. *A. nidus* was found on basalt boulder samples that ranged in size from 1 to 100 square meters, were found only on mature trees, and grew on detritus of ≥ 9cm in diameter. All those recorded as growing on the forest floor were free from attachment to any other substrate.

Results showed a different preference for substrate at each elevation. Site one showed a preference for basaltic boulders. Site two a preference for *Hibiscus*, and site three showed shared preference for basaltic boulders and *Inocarpus*. Overall preference was shared between basalt boulders and *Hibiscus* trees.

40 X 50 Meter Sample Plots
Distance from Stream

Distance from stream was one of the measures used to determine if *A. nidus* was clumped in its distribution, and to test the hypothesis that proximity to streams becomes more important at lower elevations. *A. nidus* was found to be more clumped near streams at the two lower elevation sites than at the high elevation site one. Distance from stream at that site ranged from 0-21.5 meters, and the frequency of *A. nidus* within that range was more evenly distributed than in sites two or three (Figure 3).

An ANOVA testing the null hypothesis that distance from stream is uncorrected with elevation showed site one to be significantly different than its two counterparts (p = 0.0001). On average samples from site one differed in mean distance from stream

by 4.27 meters from site two and 3.00 meters from site three.

A chi squared analysis testing the null hypothesis that *Asplenium nidus* is equally likely to occur at any distance from stream showed that observed values were significantly different than expected (p=<0.000015). Therefor it can be said with confidence that *A. nidus* is not evenly distributed, and is more densely distributed near streams.

Distance from stream at site two ranged from 0-15 meters, which is very similar to the pattern found in site three. Site two shows a high number of total specimens in the less than or equal too 3.44 meter range compared to the other plots. The overall area of site two is composed of 20.5% stream channels compared 10.5% at site one and 4.2% at site three. Therefore there was a greater chance that any given specimen would be growing closer to a stream channel in site two.

Distance from stream at site three ranged from 0.5-15 meters. A large portion of the samples occurred at less than or equal to 3.2 meters from the stream. This site had a sample size of 35 individuals. This is smaller than the other two sites only because a site with a greater number of individuals was not found in the 70 to 100 meter elevation range.

No bird's nest ferns were found in the 10 X 50 meter supplementary sample plots adjacent to sites two (185m) or three (79m) or at 5m in elevation. However, 18 specimens were found at the 280m elevation site.

Distance to Nearest Neighbor

Distance to nearest neighbor was used as a determinant of clumping independent of distance from stream. For each site it was found that the frequency of A. nidus decreased with increasing distance from it's nearest neighbor (Figures 4 & 5). For site one 72 of 85 samples were within 3.34 meters of their nearest neighbor. At site two 64 of 79 samples were found to be within 1.18 meters of their nearest neighbor, and at site tree 27 of 35 were found to be within 2.6 meters.

A simulation study was conducted to test the null hypothesis that no clumping was occurring. Its purpose was to estimate the distribution of mean nearest neighbor distances, if the same number of plants as was present in each plot had been placed at random. This was compared to the actual mean nearest neighbor distances for each plot. Results of the study, which was run 1000 times, showed that actual mean nearest neighbor distances were significantly less than expected if the same number of plants had been placed at random (p = <0.0001).

An ANOVA was used to test the null hypothesis that distance from stream and distances to nearest neighbor are unrelated. Results showed that distance to nearest neighbor is significantly positively correlated with distance from stream (p = 0.0001).

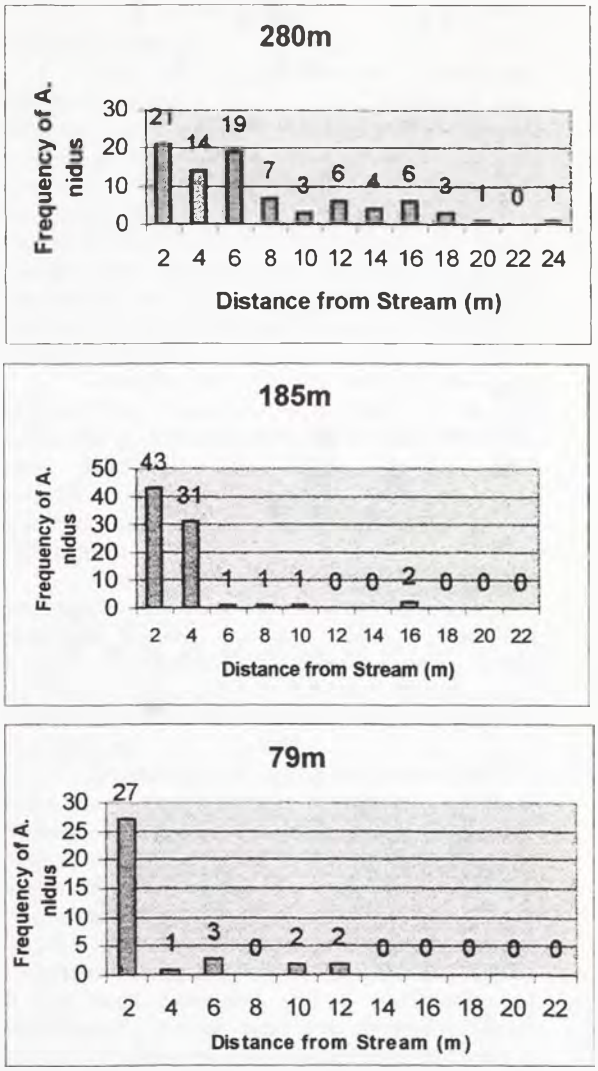

Figure 3: Frequency of *A. nidus* at increasing distance from stream and at differing elevations. Chi squared showed that these values are significantly different than if they were evenly distributed (p=<0.000015), and ANOVA showed that they are significantly different from each other (p=0.0001)

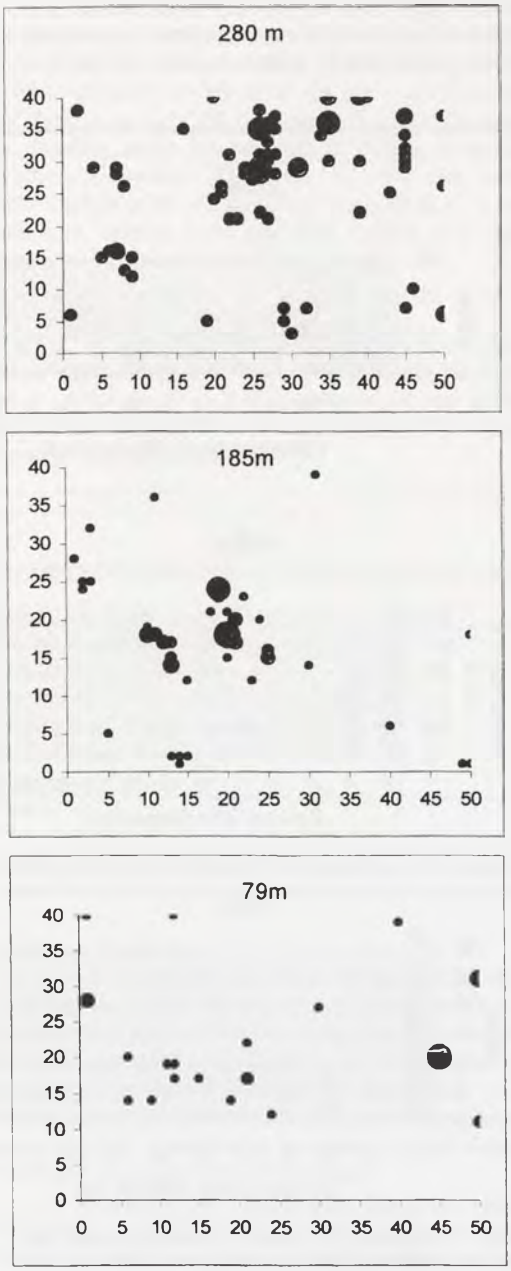

Figure 4: Map of location and densities of A. nidus each 2000m² sample plot. Each dot represents the presence of at least one *A. nidus*. Larger dots indicate proportionately more, up to 10 per dot.

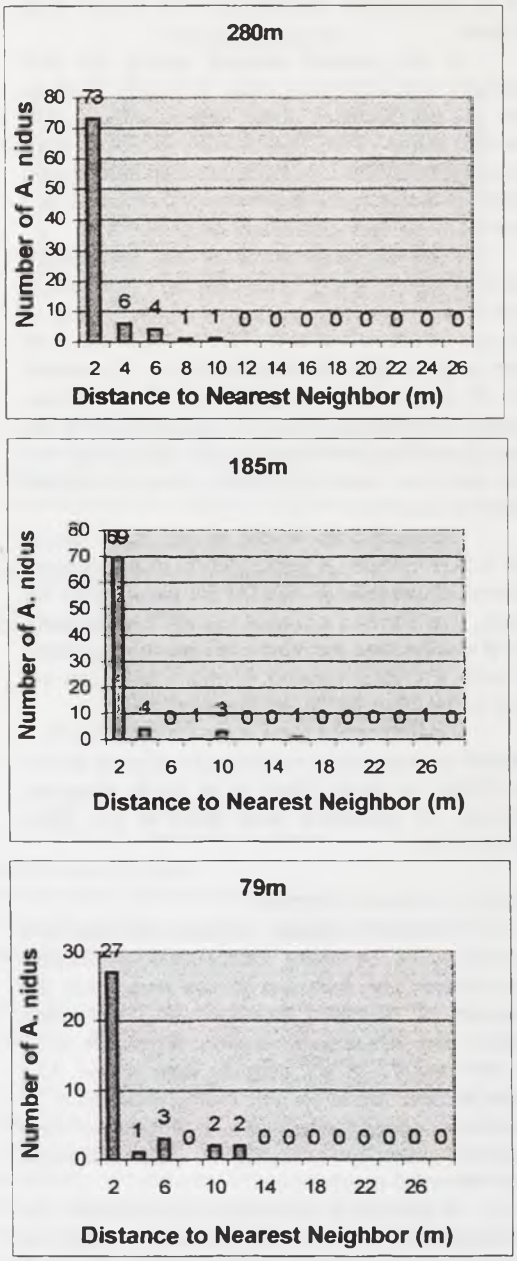

Figure 5: Frequency of *A. nidus* at increasing frequency from nearest neighbor. A simulation study shoed that they are not randomly distributed (p=<0.0001). *A .nidus* appears to be clumped at all elevations.

Discussion

Sampling Problems

Two notable problems with sampling occurred. The first of which was in mapping the location of ferns within sample plots. Boulders, trees, and slope caused slight skewing of transect tapes leading to imperfect measurements of distance from stream. This is most pronounced in areas most distal to streams since the central transect was laid there.

The overall result is that recorded samples may have been closer to streams than records indicate. Therefore conclusions and data that indicate *A. nidus'* growth in proximity to streams are conservative, and may be closer than was reported. Distance top nearest neighbor was confirmed in the field and should not be as effected by this error.

The second problem was with recording specimens of *A. nidus* growing in the canopy. Direct canopy sampling was not possible, and some specimens may not have been recorded. Visual observations were made from ground level with a pair of Bushnell 7 X 35 binoculars, but due to dense canopy cover some samples were undoubtedly missed. Many of those observed growing at heights of > 15 meters in the canopy were just visible through the foliage. It is possible that *A. nidus* is better established in the canopy than this study indicates.

Overall Frequency Transect

The overall frequency transect did not show frequencies of *A. nidus* to be controlled by elevation. However, several other observations contradict this result. Supplementary 10 X 50 meter plots showed that *A. nidus* was occurring at distances greater than 20 meters from the stream only at the site one (280 meters elevation). At that site 18 specimens were counted in the supplementary plot where none were found at the two lower elevation sites (185m & 79m respectively).

Additionally, site one was chosen at random from several sites containing 50 or more *A. nidus* specimens. In contrast site two was chosen because it was the only site available at the prescribed elevation with an adequate sample size. For site three an exception to the minimal sample size had to be made because no greater densities of *A. nidus* were found below 80 meters in elevation.

Seven small, dry streambeds were observed along the frequency transect between 250 and 311 meters in elevation. Six of these were observed to be flowing with water during one rain event early in the rainy season of 2000. This is compared to three total observed between 79 and 250 meters in elevation. At site two the stream was not observed to be flowing at any time and at site three the stream was perennial. Assuming that water availability is a factor controlling the abundance of *A. nidus*, greater abundance should be found at higher elevations where water in more available, particularly during the rainy season.

Direct evidence from the supplementary sample plots and indirect evidence from the number of streams present appear to outweigh the results of the overall frequency transect. *A. nidus* appears to be occurring at greater frequencies as elevation increases, although quantitative evidence as to the relative differences in frequency on an elevational gradient is currently lacking.

Substrate Preference

The data suggests several patterns in substrate preference for *A. nidus*. Preference was different at each elevation, suggesting an elevational gradient. Basalt boulders were preferred at high elevation, *Hibiscus* at middle elevations, and *Inocarpus* and basalt were shared at low elevations. Overall A. nidus shows a shared preference for basalt boulders and *Hibiscus* trees. However, no strong conclusions can be made as to substrate preference between elevations because there was no replication of site by elevation.

Untested environmental variables are likely affecting the proportion of *A. nidus* on various substrates at different sites. These would include water availability from runoff, humidity, light intensity, nutrient availability, canopy soil characteristics, and interspecific competition or interaction.

The need for substrate stability to survive stochastic events may also be important. All sites contained streams that are subject to periodic flooding. It is possible that *A. nidus* survives longer on stable substrates in adverse environmental conditions and is then able to reproduce to colonize that substrate.

Succession may impact the occurrence of *A. nidus* on various substrates at differing locations. Succession of epiphytes is not a clear or predictable process in terms of which species will be pioneers and which will dominate over time. Great variability in species composition of epiphytes has been shown in what are believed to be equivalent habitat types (Wolf 1993, Heitz & Briones 1998, Zortz etal. 1999). It has been postulated that a combination of environment, chance and time determine which species of epiphytes will colonize and dominate a given habitat, with chance being the most important factor (Schuster 1957 as sited by Wolf 1993). Assuming near equivalence of propagules of various epiphytes, the most abundant spores at a given site would colonize and dominate that site.

Asplenium nidus propagates only be spore. Once established on a given substrate, likely by chance, a parent plant would drop most of its spores in the area immediately surrounding itself. Thereby creating a clumping of individuals on a given substrate, and giving the illusion of preference. Proportionally large numbers of immature plants

were observed growing around reproductively mature individuals at all three sites.

Furthermore, *Inocarpus* and *Hibiscus* did not grow in the same area at any of the study sites, except in small margins where stands of the two species interfaced. Spores of *A. nidus* within one stand would have to travel some distance to colonize a new type of tree. Basalt boulders were found under both *Inocarpus* and *Hibiscus* and may have been receiving spores of *A. nidus* from either, but if boulders were colonized first, spores may not have reached suitable platforms in the canopy and would have remained restricted to boulders, detritus or the forest floor.

Distance from Stream

As stated, *A. nidus'* proximity to streams is likely a result of its need for high humidity. Desiccation is a problem for all epiphytes, and a species of this size has a proportionately greater need for water. This is implied by their decreasing frequency with distance from streams. Samples were found at significantly greater distances from streams at site one, 285m elevation, than at the two lower elevation sites. This implies that birds nest ferns are better able to survive at greater distances from streams at higher elevations. No bird's nest ferns were found at distances greater than 50m from a stream at any elevation.

There are a number of mechanisms that may explain these patterns. The number of streams is positively correlated with elevation. These watercourses are often dry, but many flow with water during rain events. As the wet season progresses and the ground water table rises it is possible that these streams will flow more frequently and with greater volumes of water. Very little vegetation was observed growing in these stream channels which lends evidence to greater flow during the wet season.

Streams at greater intervals across the landscape likely result in higher overall humidity levels. Assuming that humidity is one of the factors controlling A. nidus' distribution, it is not surprising that they were found at greater distances from streams as elevation and frequency of surface water increased. It would appear the humidity levels, and not elevation, are influencing the distribution of A. nidus. Provided conditions were favorable it seems possible that distribution patterns observed at high elevations could occur at any elevation. However, no such conditions were observed below 250m in elevation on Moorea

Nearest Neighbor

Asplenium nidus was clumped in its distribution at all sites. In each plot frequency was negatively correlated with distance from nearest neighbor (Fig 4). Clumping is partially explained by proximity to streams. Distance from nearest neighbor is significantly correlated with proximity to streams at all elevations (site one $p=.0005$; site two $p=.0000007$; site three $p=.0004$). Therefore habitat restriction may be forcing individuals to grow in the same area. However, *A. nidus* was clumped in its distribution even in areas distal to streams.

There are other factors that may contribute to the clumped distribution of *A. nidus*. Reproductive strategy may be important. Plantlets of *A. nidus* arise from spore. Most spores fall close to the parent plant, resulting in clumping of individuals, provided environmental conditions were favorable. There was no obvious evidence that young plants were suffering from density dependent mortality as put forth by the Janzen-Connell Hypothesis. Often immature sporophytic fronds were observed growing clustered around actively reproducing mature sporophytes.

Conclusions

There are a several apparent patterns influencing the distribution of *Asplenium nidus*. It was found more commonly at higher elevations where water and rainfall are more abundant, resulting in high humidity levels. *A. nidus'* need for high humidity has resulted in greater densities near surface water, and at higher elevations. At all elevations specimens were found under dense canopy cover, which would help prevent desiccation.

Greater densities near water are correlated with the overall clumped distribution of *A. nidus*, although clumping is not entirely explained by the need to be near water. *A. nidus* was observed to be clumped in its distribution even where it occurred in areas most distal to streams. This is likely a result or reproductive strategy but, interspecies interactions may influence the success of *A. nidus* at locations distal to streams.

At each site *A. nidus* was observed growing in close proximity to microepiphytes that are known to be desiccation tolerant (personal communication with Brent Mishler). Often these microepiphytes, which can absorb 4 - 5 times their dry weight in water (Pocs 1980), were observed growing on or near the root mass of A. nidus. During dry periods the release of that water may maintain humidity levels and support A. nidus indirectly (Wolf 1993). This may provide a mechanism for the survival of large epiphytic species that share niche space with these microepiphytes.

This was a pilot study detailing the distribution patters of *Asplenium nidus* working under assumed habitat constraints related to epiphytes. Future studies could lend more insight into the mechanisms behind its distribution. The effects of (micro) climate changes on epiphyte succession, and the rolls epiphytes play in nutrient cycling are largely unexplored. Work in these areas would deepen the understanding of the ecology of epiphytes, and nutrient cycling in the tropics.

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Observations of Dominance and Feeding Behavior in Human-Fed Freshwater Eels and a Comparison to Naturally Occurring Eels in Moorea, French Polynesia

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ABSTRACT. In easily accessible streams in Moorea, French Polynesia, there are a few select pools where humans feed freshwater eels. Local Residents probably started the trend as soon as they found the eels on the island. Tourist guides throw food into the streams as observers watch *Anguilla marmorata* feed and occasionally fight over the food. Comparisons were made between eels not fed by humans (natural eels) and the human fed eels in the valleys of Cook, Opunohu, and Afareaitu. Eels in the human fed pools were on the average larger, discolored, and not afraid of man. The human fed eels also existed in greater pool densities than the naturally occurring eels. Dominance and feeding behavior studies were performed on the human fed pools by providing a food source in a neutral location in the pool relative to the eels' assumed residence. Time of feeding and any social interactions were recorded with respect to an eels' size during feeding periods usually lasting 30 minutes. A rank order was established based on percent of interactions won and number of exclusions from the food pile. Rank order correlated significantly with eel size. On average, the largest eel was the most dominant. Dominance results were compared with feeding time of each eel size. Total amount of feeding time was expected to increase with eel size, but the relationship between feeding time and rank order was stronger than the relationship between feeding time and size. This study is an introduction to the social behavior of *A. marmorata* and how man has affected the nature of that behavior.

Introduction

Three species of freshwater eel exist on the island of Moorea, French Polynesia. *Anguilla marmorata* is the largest and the most abundant of the three. The two other species of eel, existing in less abundance, are *Anguilla megastoma* and *Anguilla obscura*. *A. marmorata* was the focus of this study because the other two were difficult to distinguish from each other and their presence in this study was relatively small. *A. marmorata* ranges over most of the tropics and even in the temperate north Pacific region (Tesch 1977).

For a long time in Moorea, the local population has been feeding certain eel pools around the island. There are eels residing in these pools that receive food from humans on a consistent basis. Some or most of these eels may depend upon humans for all of their food requirements. The eels have become comfortable with humans and eel feeding has become part of many tourists' daily schedule. The tourists feed the eels canned mackerel and locals feed the eels anything from bread crumbs to fish heads as well as canned mackerel. The human-fed pools will be termed feeder-pools. In the streams further away from the human population, the eels reside in pools with densities less than the feeder-pools. The size of the feeder-pool eels is also unique. The feeder-pool eels are on average longer and heavier than the naturally occurring eels.

Studying dominance in animal societies or groups informs a scientist on the social organization of that group. Survival, and thus fitness, of each individual in that group depends on whether or not an animal conforms to the rules of the group, so to speak. In animals, dominance can be either absolute, linear or partial. Absolute dominance is seen in chickens, and can be described as one animal completely dominating the rest of the group. There is a clear line of rank in the group with each individual holding a position. Partial dominance occurs when there is no clear-cut dominance lineage from the top animal to the bottom animal. Partial dominance is found when the observer has to count up all the attacks and find winners and losers based on the percent of interactions won. A particular animal in a group displaying partial dominance will win some fights and lose some fights. The wins and losses will be about equal in an animal that is positioned in the middle of the dominance hierarchy (Etkin 1967).

Fighting is generally concerned with territory or mating behaviors, making it largely seasonal. For instance, the Indian rhino maintains its territory throughout the year, but the level of aggression fluctuates with the season. Two adults are rarely seen together except when they are fighting or mating (Cloudsley-Thompson 1965).

Figure 1. Map depicting study sites. Shaded regions marked with A or B indicate the natural pools along the stream transect.

There are different ways of becoming dominant. Pitcher explains that "...larger size may need to be coupled with higher levels of aggression before a fish can capture a disproportionate share of the resource. Conversely, early success in social interactions may enhance status, allow an individual to become dominant, and as a consequence, secure more food and grow faster" (1993).

Maybe the best known example of dominance in fish is the Siamese fighting-fish, *Betta splendens,* with aggression developed in both sexes. It is the male, however, that gives the most interesting and colorful displays. Two males may be fighting for whatever reason by means of challenging, biting, locking of jaws, and jockeying for position until one of the fishes surrenders and retreats. The dominant fish will give aggressive displays for some time after a fight, while the loser adopts a subordinate relationship (Cloudsley-Thompson 1965).

In eels, studies on dominance or social structure were not found after substantial searching of the relevant literature. Eels have been known to cluster or group together in large numbers. Thousands of small yellow eels were found between two planks under a weir in Muhlhof (Tesch 1977). The eels could not ascend past the weir and the baby eels had jammed themselves between the planks and actually weakened the structure of the weir because somehow they were able to get food and grow while jammed between the two planks. There have also been cases where multiple eels are found in one burrow. Eel clustering is not limited to dens and burrows however, and yellow eels have been seen in drifting clusters, forming a ball about two meters in diameter. Silver eels have also been seen forming clusters (Tesch 1977).

The main body of behavioral work on eels is done on migrations of the American and European eels. No known work has been done on the social organization and possible dominance hierarchy in the eels of Moorea. Marquet (1986) has studied the abundance and migration of the eels on Moorea. Resh et al. (1999) have done work on the diet of the eels, and found that they feed on freshwater and saltwater prey.

This study compares physical attributes and pool density of eels that occur naturally in the streams with the eels that occur in feeder-pools.

It also addresses the question of whether or not human-induced crowding of individuals has given rise to a dominance hierarchy within the feeder-pool population of eels, and if the ability to dominate other eels has any influence on the feeding behavior of eel size.

The expectations going into this study were that larger eels would hold a higher position in the rank order and this dominance would be expressed in the phenomenon of larger eels spending more time feeding.

Materials and Methods

Site Description

There were three streams where natural pools were studied (Figure 1). The stream A site is located in the Opunohu valley with a total of 24 pools at this site. The transect of pools stretches from site 4 (Opunohu South bridge) to 17° 31' 14.2" South, 149° 50' 47.2" West. Stream B is located in Pao Pao with a total of 6 pools at this site. Pao Pao feeder-pool to 17° 30' 48.2" South, 149° 49' 25.2" West. Stream C (salt stream) is located just South of the main road at PK 12. The family of eels known as Anguillids can exist in both freshwater and saltwater (Tesch 1977). This dry riverbed was tidally influenced and the size of the pool is determined by the amount of saltwater intruding up the dry riverbed. This site contains eels mainly under 12 inches in length. The eels in this pool were classified as natural because they actually avoided mackerel when it was placed in the middle of the stream. The volume of the salt stream was 61 m^3 at the time of the study.

When observing the eels in the natural pools it was difficult to characterize behavior other than the flight response from the observer. For this reason, only the size, species, and location of the observed eels were noted. The size of the pools was estimated by collecting information on the area and depth of the pools. The average volume of the natural pools was 103.5 m^3. The total logged hours observing on the natural pools was 12.48 hours in eight stream walks.

Feeder-pools are defined as any pool containing eels that eat the canned mackerel or any other food supplied by humans. All the pools are visible from a

road, giving easy access for humans. Tourists and/or locals have been seen present at all the feeder-pools. There are a total of 7 feeder-pools in this study. These sites were the only feeder-pools found on the northern side of the island. Study site 1 is located in the village of Papetoai at PK 22 near a side road that runs just west of the post office (17° 29' 33.5" South, 142° 52' 16.6" West). The study area is on the river Vaihana about 35 meters upstream from the mouth of the stream. The area studied has a volume of 22 m^3 and a substrate of rocks, silt, and human litter.

Study site 2 is located in the village of Urufara between PK 19 and 20 on the Western side of the intersection between the main road and the river Urufara. The volume of the pool is 7 m^3, and the substrate is composed mostly of flat rocks.

Site 3 (North Opunohu Bridge) is located in the Opunohu valley immediately west of a small bridge on the road to the Belvedere (17° 31' 26.2" South, 149° 50' 29.8" West). The pool is located just west of the intersection of a stream and a road. It is a man-made pool and can be classified as a feeder-pool. It is assumed that tourists or locals feed the eels at this accessible location. The volume of this pool is 10 m^3, and the substrate is composed of leaves and rocks. Site 4 (Opunohu South bridge) is located in the Opunohu valley further south along the same road as Site 3 (17° 31' 31.5" South, 149° 50' 55.3" West). Tourists have been seen in abundance in the area, but none have been seen feeding the eels. Tourist safari trucks have been observed traversing this stream with a relatively high velocity in the ripple adjacent to this pool. The volume of this pool is 85 m^3 and the substrate is mostly sand and silt with many large rocks on one side of the pool.

Site 5 is located in Pao Pao on a residential road at (17° 30' 56.7" South, 149° 49' 23.6" West). The pool is assumed to be purely resident-fed because no tourists have ever been seen in the area. Residents have been observed feeding the eels at his pool. The volume of this pool is 15 m^3 and the substrate is composed of sand and large rocks.

Sites 6 and 7 are located in the village of Afareaitu. Site 6 is just under the bridge of the main road near the mouth of the river Putoa at PK 9 (17° 33' 02.4" South, 149° 49' 25.2" West). The study area has a volume of 11 m^3, and the substrate is a divided area with one side being a concrete slab under the bridge and the other side being rocks and sand. Site 7 is located at the waterfall of the same river as Site 6. No GPS data are available for this pool because the dense canopy cover inhibits the GPS to receive signals. There is a feeder-pool at the base of the waterfall with many tourists arriving at the pool about every half an hour. The waterfall pool has a volume of 85 m^3 with a substrate of sand and rocks of both large and small size. Both Sites 6 and 7 have relatively limited data on the eels in those pools. Problems exist with gathering data on dominance at Site 6 (near the road) because the eels under the bridge and in the near vicinity do not feed on the mackerel, although people have been observed feeding the eels at this site. It was difficult to collect data from Site 7 (waterfall) due to the frequent presence of the tourists in the pool itself. Eel size and numbers were collected in pools when it was not possible to collect dominance data.

Sampling Methods

Dominance displays were observed by providing a food source for the eels. Canned mackerel was used as the food source because that is what the tourists and residents have been feeding the eels, based on personal observations and investigation. A pile of mackerel was placed in the middle of the stream and a timer was started. The pile of mackerel was placed in a position assumed to be a neutral point in the stream being equidistant to each eel's presumed lair. Polarized glasses were used to reduce glare and improve underwater observations from the streamside. Small amounts of mackerel were thrown in a spray pattern around the pool before each observation to increase the eels' awareness of the presence of the food. Any social interactions or expressions of dominance were noted during and before the feed. Each feed lasted about 25-30 minutes or until the food ran out and all the eels participating in the feed went back to their lairs or previous positions.

Data Collection

During the feed, information on size of an eel, time spent feeding, and social interactions with other eels were recorded from the streamside. Because tagging was not possible, no data on individual eels are available. The results are based on the eels' size alone. The eels' sizes had to be estimated from the streamside in feet and then later converted to meters. This estimation is acceptable because what is important is the size of the eels relative to each other and not necessarily their exact length. The amount of food dropped in the stream and the amount of food left uneaten was also estimated by the naked eye.

Species identification posed a major problem in this study. Some eels were easy to identify as *Anguilla marmorata*, but others were more difficult. *A. marmorata* has a mottled brown and orange pattern along the body. Some eels possessed a coloration that resembled this mottled pattern, but on the dorsal side of the body the pattern was more uniform. This

	Pao Pao	Urufara	Papetoai	Opunohu	Afareaitu
number of pools at site	1	2	1	2	2
number of feeds	7	7	2	4	
number of density counts	3	1	3	6	7
total logged hours feeding/counting	3.01	2.24	0.51	3.52	1.95
maximum number of eels seen	10	8	14	3	3
average size of max eels (m)	0.92	1.06	1.07	0.84	0.41
average number of eels per pool	7.1	5	6.2	1.9	1.42
Average size at pool (m)	0.98	1.09	0.89	0.72	0.87

Table 1. Feeder pool site specification.

uniform/mottled color made it difficult to distinguish if it was just pattern variation within the species *A. marmorata* or a different species such as *A megastoma* or *A. obscura.* Because of the uncertainty of species identification in the field, this study focuses on size alone. Therefore, this study does not focus on species differences or individual differences within a rank order, only size differences.

Method of Data Analysis

The main statistical analysis used was linear regression with Microsoft Excel. Polynomial regression was also used to a lesser degree. Regressions were calculated to correlate size with variables such as feeding time and rank order.

Results

Natural Pools

In the natural pools studied on the stream transects, 19 of the 31 total pools contained eels at any time during the study. Only *A. marmorata* were seen in either Opunohu or Pao Pao. There were eels with the uniform mottling seen at the feeder-pool in Pao Pao, which made identification so difficult at that site. The maximum number of eels seen in a natural pool is 5 in Opunohu with the average in that pool being 0.58 meters in length. The average eel length for all the natural pools was 0.68 meters. The average density of the 19 pools containing eels is 1.77 eels per pool. The total amount of logged observing time in the naturally occurring pools was 12.48 hours.

In the salt stream, the maximum number of eels seen was nine, with an average length of 0.27 meters in length out of those nine. The eels seen there had an average length of 0.27 meters, suggesting that they are juveniles. The species of these eels could not be determined, but all the eels possessed the mottling color characteristics of *A. marmorata.* The average density of the salt stream was 5.6 eels per pool. The total amount of time logged observing at the salt stream was 1.02 hours.

There were four major differences between the natural eels and the feeder-pool eels: size difference, living proximity to nearest eel, color difference, and response to humans. The natural eels were smaller than the feeder-pool eels on average. There were also no observations of any natural eel being over 1.22 meters in length. It was almost common to have two or three eels above 1.22 meters in the feeder-pools. The proximity to the nearest eel was not measured for reasons of difficulty, but the feeder-pool eels lived much closer to each other than the natural eels based on equal hours of observation in both environments. The natural eels had a brown and orange mottling, whereas the feeder-pool eels had a more blue/grayish and black mottling. In terms of behavioral response to presence of humans, the natural eels all fled upon recognition of man. The feeder-pool eels did not flee and some (especially Urufara) would even approach when humans visited the streamside.

Feeder-Pools

The individual pool results for the feeder pools are found in Table 1. Density counts were made when weather did not permit visibility for feeding. The maximum number of eels seen is the most eels spotted during one density count or feed.

In all the feeder-pools, the average length of the eels was 0.91 meters. A total of 20 feeds were performed for a total of 12.25 logged hours. 25 density counts were performed. The average number of eels per pool in the feeder-pools was 4.32.

The maximum number of eels seen in a pool at one time was 14 eels at Papetoai. The average length of those eels was over 1 meter in length. That situation was very unique, and the eels were basically lying on top of one another.

Dominance

Counting the number of aggressive interactions between the eels in the feeder-pools was the main method of determining dominance. Counting the number of exclusions from the pile of food was used as a secondary measure to back up the number of aggressive interactions. Figure 2 shows the dominance matrix made up of the aggressive interactions (style taken from Somers and Nel 1998). Aggressive interactions include biting, nose-to-nose pushing, threat displays, chases, and fleeing upon recognition of another eel.

Subordinate

Dominant	0.53	0.61	0.69	0.84	0.91	0.99	1.14	1.22	1.30	1.45	1.60
0.53											
0.61					2						
0.69											
0.84						1					
0.91		6		1	2						
0.99			1			1					
1.14											
1.22				2	6						
1.30	4			3							
1.45											
1.60			3	2			2				

Figure 2. Dominance Matrix. The column on the left represents the size of the eels that dominated the aggressive interactions that are tallied in the center of the matrix.

Table 2 shows the number of aggressive interactions that occurred during times of observing (style taken from Shaw 1985). It includes all that the dominance matrix includes except for the fleeing upon recognition of another eel. This table is used to rank the eels. The eel size with the least number interactions lost in terms of percent is ranked as the highest in the hierarchy. Typically, the number of interactions won is used, but sizes 1.14 and 0.69 meters never won an interaction, and sizes 1.45 and 0.53 meters never got involved in any interactions. In order to include these sizes in the ranking, the percent-lost measurement must be used. In Figure 3, the percent-lost rank is compared with the exclusion rank. The exclusion rank is calculated by dividing the number of times an eel got excluded from the food pile (B) by the number of times an eel excluded another from the pile (A), as seen in Table 2. The larger two sizes did not get excluded from the pile once, so whatever size excluded the most got the higher ranking. Figure 4 shows a correlation between size and rank based on percent of interactions lost.

Dominance and Feeding Behavior

Figure 5 shows a comparison between the two correlations of size vs. total feeding time and rank vs. total feeding time. There is a more significant p-value in the chart that compares total feeding time and rank ($p=0.07$).

Figure 6 shows a comparison between the two correlations of mean time spent per visit vs. size and mean time spent per visit vs. rank. Mean time spent per visit is the average time an eel size spent feeding at each visit to the food pile. Typically, an eel will visit the food pile a number of times during each feed. Again, the more significant p-value (0.005) belongs to the correlation using rank.

Size Class (ft)	Size Class (m)	# of Interactions	# Lost	% Lost	Rank	# of others excluded (A)	# of others that excluded it (B)	B/A	Rank
1.75	0.53				neutral				
2.00	0.61	8.00	7.00	0.88	7	2.00	8.00	4.00	5.00
2.25	0.69	1.00	1.00	1.00	9				
2.75	0.84	6	5	0.83	6		3		6
3.00	0.91	19	13	0.68	4	6	7	1.17	4
3.25	0.99	4	3	0.75	5				
3.75	1.14	4	4	1.00	8				
4.00	1.22	8	4	0.50	3	1	1	1.00	3
4.25	1.30	3	1	0.33	2	6			1
4.75	1.45				neutral				
5.25	1.60	6	1	0.17	1	4			2

Table 2. Method of ranking different eel sizes.

Figure 7 shows the same size and rank comparison correlating both factors with time feeding alone as fraction of each sizes' total feeding time. A linear regression was performed on the size vs. time alone fraction but the rank data seemed to follow a non-linear trend. A polynomial trend line was then added to the rank correlation with an R-squared value (0.51) that is greater than the size correlation's R-squared value (0.19).

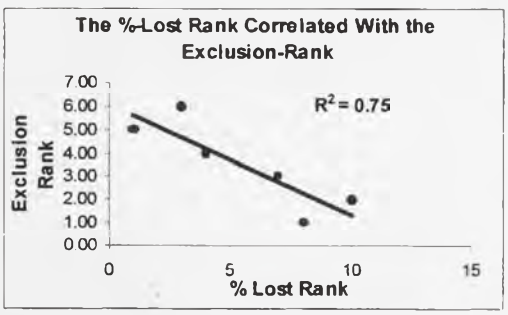


Figure 3. Regression of percent-lost rank with exclusion rank. (p=0.001)

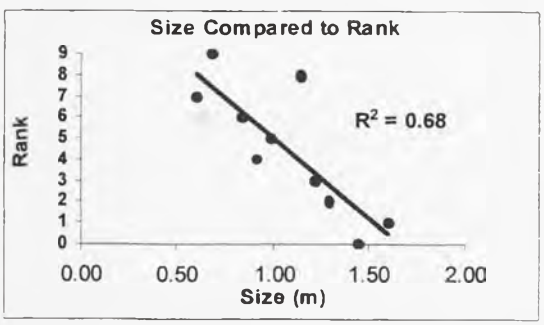
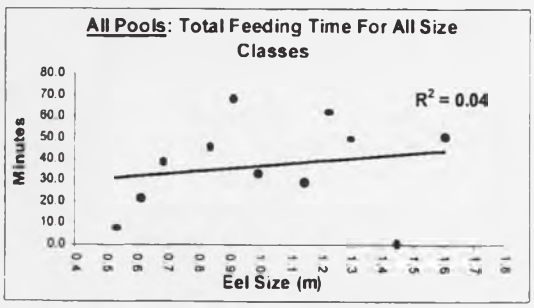


Figure 4. Regression of size and percent-lost rate. (p=0.01)

Figure 8 shows differences in the dynamics of a typical eel feeding alone and a typical feed with multiple eels feeding. One eel feeds off and on for the length of the feed visiting the food pile a number of times. The typical feed with three or more eels shows that a certain eel will feed off and on but is not demonstrating the same behavior of an eel feeding alone.



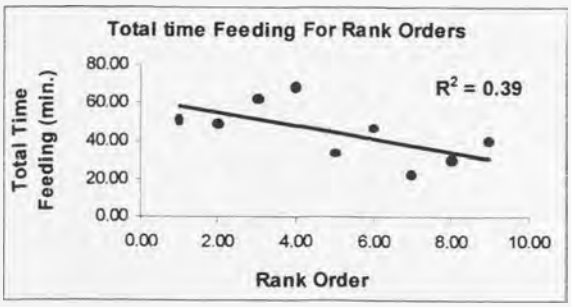
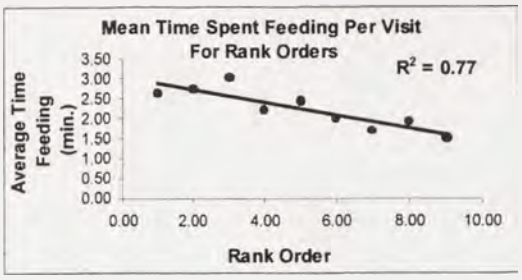


Figure 5. Comparison between the regressions of total feeding time with size and rank order. (Size: p=0.1, Rank: p=0.07)



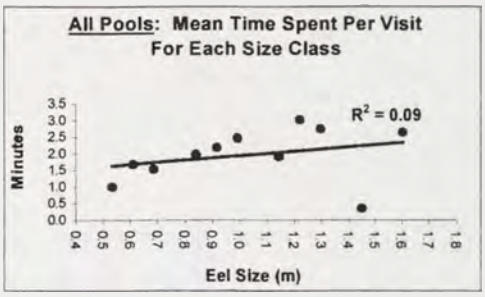
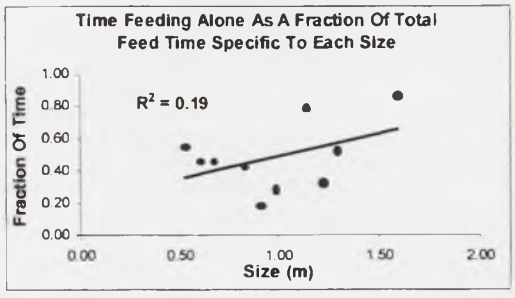


Figure 6. Comparison between the regressions of mean time spent per visit with size and rank order. (Rank: p= 0.005, Size: p= 0.1)



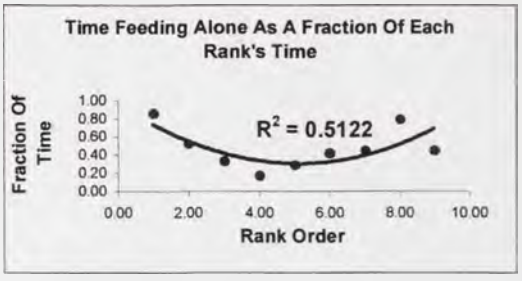
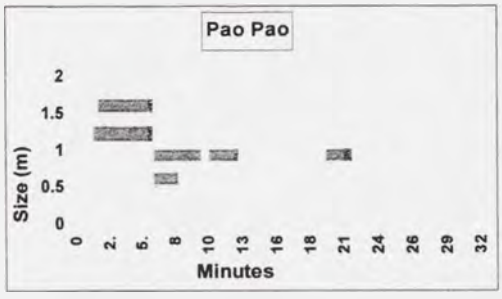


Figure 7. Comparison between the regressions of time feeding alone as a fraction of total feeding time with size and rank order. (Size: p=0.1)



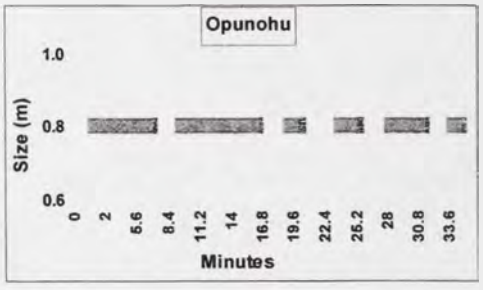


Figure 8. Comparison between the dynamics of an eel feeding alone at Opunohu and 4 eels feeding together at Pao Pao. The shade regions signify the time the eel was present feeding.

Figure 9 shows at what time the aggressive interactions occurred in the feed. The number of aggressive interactions increased from the beginning of the feed to about 10-12 minutes into the feed. These interactions tapered off from 15 minutes into the feed to the end of the feed.

Discussion

Natural Pools and Feeder Pools

In the natural pools, the behavior of avoiding man is expected. It is the approachability of the feeder-pool eels that is interesting. At Urufara, it seems as if the eels will approach anyone with food. Tourists and local children play with the eels to the extent of picking up the animals repeatedly on each visit to the feeder-pool. The animal does not bite, but it is definitely trying to get away from its handler. The eel must be accepting of the human in order to acquire the food. The relationship between humans and the eels is like that of the reluctant pet. However, there is no need for the residents to capture these animals to enjoy them because the animal stays in these pools and

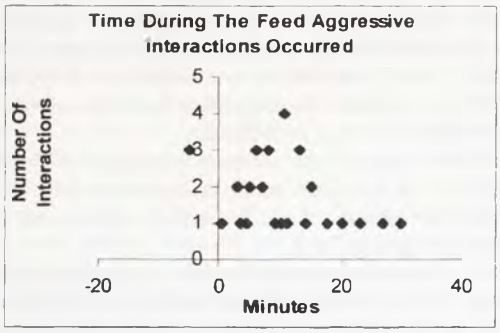


Figure 9. The total number of aggressive interactions taking place during the minutes of the feed. The negative value represents the aggressive interactions during the pre-feed.

waits for food. It may be some early form of symbiosis beginning. The eel needs the human for food, and the human uses the eel for entertainment and amusement.

The difference in color may be attributed to the difference in diet between the natural and feeder-pool eels. The pools where the eels are fed the most mackerel, Urufara and Papetoai, contain eels with the blue/grayish and black pattern. Feeder-pools where the presence of feeders is little to none (Opunohu and the Afareaitu waterfall) contain eels that display the color patterns seen in the natural pools.

The average size of the feeder-pool eels in Opunohu and Afareaitu waterfall is smaller than the average size of the main feeder-pools. The average size data do not accurately reflect the size differences between the two. In the more natural feeder-pools (Opunohu and Afareaitu), eels over 1 meter were not seen. It was common to see eels over 1 meter in the main feeder pools. In Pao Pao, where there may be two or more species are living in a group, it is not uncommon to see an eel over 1 meter there as well. This suggests that either eels are reaching unnatural sizes in the feeder pools, or the larger eels in nature are extremely shy of man and are able to avoid man without detection.

The number of eels per pool is on average greater in the feeder pools than in nature. The pattern of the number of eels per pool is similar to the size pattern previously explained. The number of eels per pool is higher in the main feeder pools than the more natural feeder pools. In Pao Pao, Urufara, and Papetoai, the average number of eels per pool was 7.1, 5, and 6.2. In Opunohu and Afareaitu, the average number of eels per pool was 1.9 and 1.42. In the main feeder-pools there were a lot of large eels living together (larger than 1 meter in length) in relatively close proximity to each other compared to the eels in nature. The naturally occurring eels had an average eel density of 1.77 eels per pool, and the average size of the pool was twice as large as that of the feeder-pool average. The feeder pool eels are on average larger, live in relatively smaller living quarters, are not afraid of man, and possess coloration not seen in the natural pools.

The salt stream data is interesting because it could potentially be some sort of juvenile rookery for *A. marmorata* or other species. No adult eels were seen in this pool. One *A. marmorata* was seen swimming in reaching about 0.6 meters in length. Upon arrival of the larger eel, all the smaller eels sought refuge until about 20 minutes after the larger eel had left the area.

It has been noted that when the living space between animals decreases, the number of aggressive interactions increases. The results, however, are misleading because in a natural setting where the animals have as much space as they want, there are relatively fewer fights (Etkin 1967). What sets the eels on Moorea apart is that the eels are free to leave these feeder pools if they want to, but choose to stay. Do the Eels have to conform to certain rules of a social hierarchy to reap the benefits that man is giving them?

Dominance

In Figure 2, the dominance matrix shows evidence for a dominance hierarchy. Besides the eel sizes that did not get involved into a single interaction, there are only 3 cases out of 36 interactions where a smaller eel was the winner. What is not reflected in the figure is that these cases were usually of an eel that approaches a larger eel from behind and bites the tail of the larger eel. The bitten eel then flees and hides. Usually within a few minutes, the bitten eel returns to the feed. It is unknown whether the bitten eel knows which eel bit him, but in most of the cases of this phenomenon, the larger eel returns to the feed and is aggressive towards the smaller eel that previously bit him.

In Table 2 and Figure 3, it is clear that there is a significant correlation between the rank of eels based on amount of interactions lost and the rank of eels

based on the index of exclusion. It is highly likely that the eels that are winning the battles are also the eels that are causing exclusion of another eel from the food pile. Figure 4 shows that there is a significant correlation between the eel's size and the eel's given rank. The larger the size of an eel, the greater chance that eel will hold a higher rank in its pool. Therefore, it could be said with some confidence that it is the larger eels that are dominating the smaller eels, on average. Of course this cannot be stated with absolute confidence because the dominance hierarchy does not perfectly correlate with rank order. This means there is another factor influencing the dominance hierarchy. A possible factor influencing dominance besides size could be aggression. The fact that size does not perfectly correlate with rank is reflected in the feeding times of different sizes and ranks.

Dominance and Feeding Behavior

In this study, feeding behavior is used as an indirect and incomplete measure of aggression. However, non-aggressive eels will surely not challenge another eel for feeding time at the food pile. Aggressive eels are more likely to challenge another eel for food, leading to more time spent feeding. The eels that spend more time feeding may be more aggressive, more active feeders, or both.

When comparing total feeding time between size and rank, it can be seen that the correlation involving rank has the higher significance value (Figure 5). Not only is the trend line steeper so as to define a relationship more clearly, but the data involving rank fit the line better than the data involving size.

The comparison with mean time spent per visit as the dependent variable (Figure 6) shows a similar trend. The rank data is not only more significant than the size data, but the rank data is significant itself with a p-value of 0.005.

Once an eel arrives at the food pile to feed, it is not necessarily the larger eel that is spending more time at the food pile because he can dominate the smaller eels. It is the higher ranked eel that is spending more time at the food pile.

Time feeding alone can be a measure of dominance. There are many reasons why an eel may be feeding at the food pile alone: others aren't aware of the food, others aren't hungry, and/or others are protecting their territory. Certain precautions were taken to minimize the likelihood that these other factors could be operating.

To increase awareness, a 'pre-spray' of food was issued to all the known eels in the pool before the feed, and food pieces were also thrown in a general spray to all areas of the pool. This is to increase the chance of an eel being aware of a human feeder's presence.

To assess the level of hunger of the eels, the amount of bait left uneaten was estimated for each feed. The amount of bait left uneaten (usually less than 2% of the food placed) was relatively the same for every feed and those results had no correlation with the amount of aggression seen in the pool that day.

Territoriality would not have been easy to identify and that is a complete study in and of itself. It is possible that eels could not be feeding because they are busy guarding their lairs.

Time alone could also be interpreted as time during the feed when eels not feeding are hesitant to challenge the feeding eel. The correlation between eel size and time feeding alone as a fraction of that size's total feeding time is shown in Figure 7. The size data is inconclusive but the rank data shows an unexpected trend. A polynomial trend line has been used instead of the linear analysis previously used because of the pattern of the scatter plot. The decrease in feeding time alone for the middle ranks can be expressed in terms of dominance. One reason why the lower and higher ranked eels are feeding alone could be dominance for the higher ranks, and increased effort in searching for the lower ranks. A higher ranked eel will spend more time at the food pile alone because it has already excluded the rest of the eels and/or the eels aware of the food are too afraid to approach. A lower ranked eel may need to depend on more active searching for food to acquire its resources, and simply finds occasions when there are no higher ranked eels around to compete with. This is not pure speculation because it is based on field observations, but it cannot be used as support for dominance because of the multiple ways to interpret this phenomenon.

In terms of feeding behavior, there are differences between an eel feeding alone and an eel feeding with a group. Figure 9 shows a comparison between typical cases of an eel feeding alone and eels feeding in a group (four eels for this case). An eel feeding alone displays a pattern of feeding for a couple minutes, swimming around the pool for a couple of minutes, and then coming back to feed again. The eel will typically repeat this pattern until all the food is gone. In the case of eels feeding with other eels, a single eel may be hindered by others in the group. When the eels arrive and depart at the feed largely depends on who they are feeding with, and if there are any aggressive interactions between the eels feeding. It is too difficult to say exactly what happened in this particular case, but it is obvious that an eel feeds differently in a group than it does when it is relatively alone and unhindered in the pool.

Suggested Further Studies

(1) More work needs to be done on these feeder-pool eels in tagging studies so more conclusive results

may be attained. (2) Studies of dominance should be carried out in a lab with the facilities to house these animals comfortably and safely. (3) Studies could be done to test for dominance in the natural streams to find out more about the social organization of these creatures in the wild. (4) The concept of territoriality has risen in this paper and needs some attention, because some forms of territoriality have been observed in these streams, with conflicts ending in aggression. (5) The interactions between sexes and different species need further study. (6) Studies done on the social behavior of these animals at night is necessary to discover if the nature of the interactions changes with the hour each day. (7) Studies done on the possible domestication of these eels may be necessary to find out more about the human-induced behavioral changes at work in these feeder-pools.

Conclusions

The results from this study suggest that there is a difference between the naturally occuring eels and the feeder-pool eels. On average, the feeder-pools contained a higher density of eels than the natural pools. The average size of the feeder pool eel was larger than that of the natural eel. Most feeder-pool eels possessed a coloration different than that of the eels found in the natural streams.

There is evidence for partial dominance in the feeder-pool eels with aggression and size both being factors in determining the rank order. The rank of an eel significantly correlated with the amount of feeding time the animal spent at the food pile and with the time spent feeding alone. There is also support for a difference in feeding behavior between an eel feeding alone and several eels feeding within a group. An eel feeding within a group may be hindered by other individuals in the group, and restricted from feeding optimally.

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Temporal Changes in Coral Composition in Moorea During the Holocene

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ABSTRACT The study of coral reef ecosystems is valuable because their health serves as an indicator for both local and global environmental change. Amid recent coral disturbance events in Moorea, the current composition of *Porites* spp., a more tolerant genera of coral, is in question. To test the hypothesis that *Porites* spp. has the potential to dominate the reefs of Moorea and secondly to investigate the responses of *Acropora* spp. and *Pocillopora* spp. to the environmental changes in the Holocene, the percent cover of live and fossil coral was collected at three sites on the island. The data from the live coral reefs was compared to the fossils dated 3,340 to 6,070 years B.P. in order to detect temporal changes in the coral composition of Moorea during the Holocene. Results show that there has been no significant change in the percent cover of *Porites* spp, therefore the null hypothesis cannot be rejected. Data also suggests that the composition of *Acropora* spp. has undergone no significant change in composition in Moorea, whereas the 1983 cyclone may have altered the percent cover of *Pocillopora* spp., favoring an increase in growth. This study suggests that over a long temporal scale, coral communities may be well equipped for disturbance events and climate changes, in light of oscillations they experienced during the Holocene.

Introduction

The study of coral reef ecosystems is valuable because their health serves as an indicator of both local and global environmental change. In French Polynesia, the health of coral reefs is integral to the economic stability of the region's tourism, pearl industry and fisheries. (Hutchings 1994) One of Polynesia's many islands, Moorea, has been the subject of extensive coral reef studies (Montaggiom and Pirazzoli 1984; Galzin and Pointier 1985; Gleason 1993; Galzin et al. 1993, Hoegh-Guldberg 1994; Hoegh-Guldberg and Salvat 1995). Moorea experienced intense cyclones in 1982-83, *Athancaster planci* outbreaks in 1982, 84, and 87, and mass bleaching events in 1983, 1987, 1991 and 1994. Results show that amid these disturbance events, *Porites* spp., a more tolerant genera of coral, have low mortality rates (Hoegh-Guldberg 1994, Gleason 1993; Brown 1997). The mortalities of coral genera: *Acropora* spp. > *Pocillopora* spp. > *Porites* spp., indicate that coral bleaching events could possibly change the composition of coral communities (Hoegh-Guldberg 1995). It was hypothesized that repeated bleaching events have the potential to alter the structure of coral communities toward dominance by more tolerant species such as *Porites* spp. (Gleason 1993). In order to detect changes in coral composition over a long temporal scale, a study of the conglomerate platforms in Moorea was warranted. Therefore, this study was initiated to test the hypothesis that the species composition of coral communities has the potential to be dominated by *Porites* spp., and to also investigate the responses of *Acropora* spp. and *Pocillopora* spp. to environmental changes over time.

The Fossil Record

Coral fossils contain records of reef composition thousands of years before present, and are useful tools in assessing temporal changes. The reef fossil record in Moorea is in the form of conglomerate platforms that have been radiocarbon dated from 3,340 to 6,070 years B.P. providing evidence for their Holocene age (Montaggioni and Pirazzoli 1984). The conglomerate platforms are formed by cementation of coral debris subsequently modified by weathering and erosion (Stoddart 1971). The conglomerate platforms are often one meter thick of poorly sorted, cemented coral rubble deposited by storm deposits and partly overlain by more recent accumulations of sediments (Montaggioni and Pirazzoli 1984). Although much of the marine research in Moorea has focused on live corals, it is important to also look into the Holocene fossil record of coral reefs in order to assess the impact of environmental perturbations on the reef ecosystem (Greenstein and Pandolfi 1997). The recent geological history of living reefs is essential to understanding their long-term community dynamics (Pandolfi and Goldstein 1997). By studying both the conglomerate platform and the live coral

assemblages, this study analyses longer temporal changes of coral cover in Moorea.

Temporal Changes

The geography of Moorea's coral reefs and conglomerate platforms creates an ideal environment for analyzing long-term changes of coral communities. Since a narrow coral reef belt surrounds Moorea, the spatial organization of corals is compressed along environmental gradients (Adjeroud 1997). These gradients create biological subdivisions of the reef into five zones. These zones are defined as the conglomerate, reef flat (lagoon), coral zone (back reef), algal belt, and a frontal zone (fore reef) (Galzin and Pointier 1985) (Figure 1). The goal of this study was to analyze the current percent cover of *Porites* spp. in four of the five zones: the conglomerate, lagoon, back reef, and fore reef of Moorea, to determine if coral reef composition in Moorea has changed since the deposition of the fossils in the conglomerate platform in the mid-Holocene. Therefore, this study tests the hypothesis that *Porites* spp. may be becoming dominant in Moorea, and secondly investigates the responses of *Acropora* spp. and *Pocillopora* spp. to the environmental changes in the Holocene.

Figure 1 Schematic of the coral zonation evident in Moorea (Galzin and Pointier 1985).

Materials and Methods

Site Selection

Fieldwork was conducted on the island of Moorea, in French Polynesia (17°30'S, 149°50'W) during the months of October and November 2000. Three sites were examined on the northern reefs of the island: Temae public beach on the Motu Temae, Aroa Point on the Motu Temae, and the north side of the Motu Tiahura (Figure 2). These sites were chosen because they contain four zones: the conglomerate platform, lagoon, back reef, and fore reef. The presence of these four zones was integral to my study because all three sites had similar geomorphologic characteristics in which to compare data and take samples from similar environments.

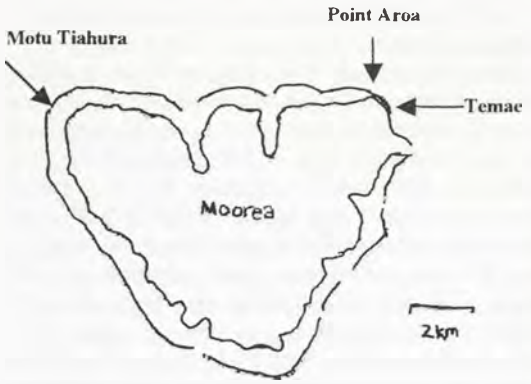


Figure 2. Location of the three research sites of this study.

Site Descriptions

The site at Point Aroa (S 17° 28' 24.2" W 149° 46' 30.8") was approximately 190 meters wide and 140 meters long. The width extended from 20 meters behind the lighthouse, 190 meters to the fore reef. The length spanned 70 meters east (S 17° 28' 24.8" W 149° 46' 28.1") and 70 meters west (S 17° 28' 24.3" W 149° 46' 33.0") of the Point Aroa lighthouse. The length of 140 meters was chosen for two reasons. First, the close proximity of the algal ridge just east of the site created a poor representation of coral zonation. Second, this length was chosen in order to be consistent with the other sites.

The site at Temae Public beach (S 17° 29' 52.5" W 149° 45' 18.8") was approximately 120 meters wide and 140 meters long. The width extended from the landward side of the conglomerate platform 120 meters to the fore reef. The length of the site began at the eastern edge of the conglomerate platform and extended 140 meters west. The length of 140 meters was chosen because the compression of zones just west of the site would result in a poor representation of the reef population. Temae and Point Aroa were sampled as different sites due to their geographical separation by the atrophy of the reef flat for a distance of over one kilometer and replacement of conglomerate by beach rock in the middle of the two areas (Galzin and Pointier 1985).

The site at the Motu Tiahura (S 17° 29' 12.2" W149° 54' 51.4") was approximately 220 meters wide and 140 meters long. The 220-meter width spanned from the landward side of the conglomerate platform 220 meters to the fore reef. The 140 meter length and the location of the site on the eastern edge of the conglomerate was chosen due

to the similarity of zones and geography with the sites at Point Aroa and Temae.

Sampling Design

Four zones were sampled at each site, excluding the algal ridge, in which accurate counts of coral cover is inhibited by thick algal cover of *Turbinaria* sp. and *Sargassum* spp. A pilot study at the Aroa Point site highlighted the necessity for more random plots to get statistically relevant data. Therefore, my sampling design required at least 30 random quadrats in a 50-meter by 50-meter grid, and proportionally fewer data sets in the other smaller grids. The probability level desired was 95% in order to be considered significant, and I employed a two-tailed test in this study. In an attempt to obtain unbiased, precise and representative samples of my population the percent cover of corals, in each zone were sampled by stratified random sampling in three steps:

1. Delinitation of four zones at each research site, conglomerate platform, lagoon, back reef, fore reef, according to biological and geomorphologic gradients including coral cover, depth, and proximity to the algal ridge. (Galzin and Pointier 1985).
2. Random placement of a grid in each zone at each of the three sites using two 50 meter transect tapes. The size of the grid was chosen to be proportional to the size of the zone, so when randomly placed, it would include a representative population of corals in the respective zone (Table 1).
3. Random selection of coordinates in the grid, the number of which in each grid was chosen by proportional allocation. The random coordinates were sampled using a .5 meter^2 quadrat subdivided into 25 squares in order to determine the percent cover of all corals greater than one centimeter in diameter.*

*The sampling of coordinates was modified in the fore reef zone. In these zones a .5 meter^2 quadrat was dropped once as a visual aide, then in the remaining coordinates, percent cover of corals were visually estimated without use of the quadrat, taking into account the increased depth.

	Point Aroa	Temae	Motu Trahura
Conglomerate	18 x 50m	16 x 50m	15 x 50m
Lagoon	50 x 50m	25 x 50m	70 x 50m
Back Reef	30 x 50m	30 x 50m	25 x 50 m
Fore Reef	20 x 50m	20 x 50m	20 x 50m

Table 1. The varying sizes of the grids used in each zone

Data Collection

Percent cover of live coral and fossils was taken following coral codes (Pernetta 1993) (Table 3). Field identifications were checked by comparison with corals referenced at the Centre de Recherches Insulares et Observatorie de Environment, and at the U. C. Berkeley Gump Research Station. Fossil identification was performed by examination with a hand lens, and dead coral cross-section comparison. All corals which could not be identified were labeled as "unknowns." Environmental parameters were also taken at various zones at all sites. Measurements of temperature were taken on site at each sampling session from the top of the water column. Depth was recorded along the y-axis of all marine grids at ten-meter intervals for the larger grids, and in five-meter intervals for the smaller ones. PH was determined at the Gump Research Station for each of the live coral transects using Hydrion Papers.

Code	Description
DCA	Dead Coral with Algae
S	Sediment/Sand
UNK	Unknown/ Other corals
POR	*Porites* spp.
POR RUS	*Porites (Synarea) rus*
ACR	*Acropora* spp.
POC	*Pocillopora* spp.
POC MEAN	*Pocillopora meandrina*
POC VERR	*Pocillopora verracussa*
POC EYDO	*Pocillopora eydoux*
POC DAMI	*Pocillopora damicornis*
HER LIMA	*Herpolitha limax*
FUN	*Fungids* spp.
MON	*Montipora* spp.
PAV	*Pavona* spp.
PAV CACT	*Pavona cactus*
FAV	*Favites* spp.
MIL	*Milliopora* spp.
MOR	*Montestrea* sp.
BR	Beach Rock

Table 2. Coral Codes used in Data Collection

Statistical Analysis

The hypothesis of this study was tested using a one-way ANOVA on JUMP software, with zone as the factor and each site as dependant variables. A Tukey test was then employed if $p<0.05$ to determine significant differences between zones. All means include estimates of standard error, and graphs were prepared on Microsoft Excel.

The data from the four zones at each site was averaged to examine trends in coral composition for the island as a whole, as well as at the individual sites. The three main genera of coral in Moorea: *Porites* spp., *Acropora* spp., and *Pocillopora* spp. were each analyzed separately in order to distinguish temporal changes in coral composition among the three.

Results

Porites spp.

Data from the Point Aroa site was analyzed comparing the percent cover of *Porites* spp. in all four zones. This site showed the lowest percent cover of *Porites* spp. in the fore reef, followed by the lagoon and back reef, and the conglomerate platform. An ANOVA t-test yielded a p-value of 0.59. At the Temae site, the conglomerate had the lowest percent cover followed by the back reef, lagoon, and the fore reef. An ANOVA t-test yielded a p-value of <0.01. According to a Tukey test the conglomerate and the fore reef were significantly different. Data for *Porites* spp. at the Motu Tiahura showed that the conglomerate had the lowest percent cover followed by the back reef, lagoon, and fore reef. ANOVA yielded a p-value of 0.13. The mean percent covers from the four zones at all three sites on Moorea showed that the percent cover of *Porites* spp. is lowest in the conglomerate platform followed by the back reef, lagoon, and fore reef zones. An ANOVA yielded a p-value of 0.40 (Figure 3A).

Acropora spp.

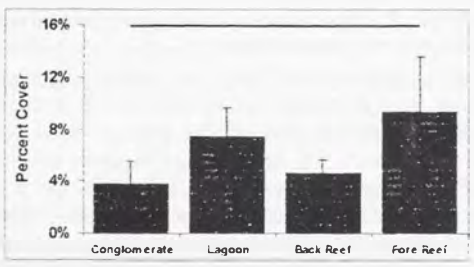
At Point Aroa the lagoon had the lowest percent cover of *Acropora* spp. followed by the back reef, the conglomerate, and the fore reef. ANOVA analysis yielded a p-value of <0.0001. A Tukey Test showed that the conglomerate, lagoon, and back reef are all significantly different than the fore reef. At Temae, the percent cover of *Acropora* spp. was lowest in the lagoon followed by the back reef, conglomerate, and fore reef. ANOVA analysis revealed a p-value of 0.03. A subsequent Tukey showed that no zones were significantly different. Data from the Motu Tiahura showed that the percent cover of *Acropora* spp. was lowest in the lagoon followed by the back reef, conglomerate, and fore reef. ANOVA analysis yielded a p-value of <0.01. The following Tukey test revealed that the lagoon and the fore reef are significantly different. The Mean data for all zones at all three sites showed that *Acropora* spp is lowest in the lagoon, followed by the back reef, conglomerate, and fore reef. ANOVA analysis of *Acropora* spp. data yielded p-value of 0.06 (Figure 3B).

Pocillopora spp.

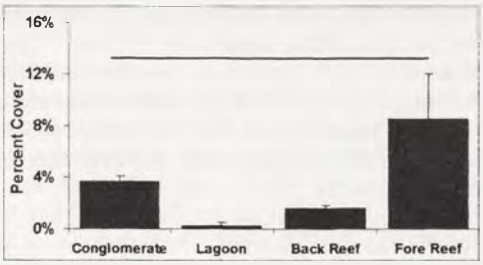
Trends in the percent cover of *Pocillopora* spp. at Point Aroa showed that the lagoon had the lowest percent cover followed by the back reef and the conglomerate, and the fore reef. An ANOVA yielded a p value of <0.001. A Tukey test highlighted a significant difference between the fore reef and all of the other three zones. At Temae, the presence of *Pocillopora* spp. was lowest in the lagoon followed by the conglomerate, back reef, and fore reef. ANOVA analysis yielded a p value of <0.001. Tukey test showed significant difference between the conglomerate, lagoon, back reef and fore reef. At the Motu Tiahura the lagoon had the lowest percent cover of *Pocillopora spp.* followed by the conglomerate, back reef, and fore reef. ANOVA analysis revealed a p-value of <0.0001. A Tukey test yielded significant differences between the conglomerate, lagoon, and back reef with the fore reef. The mean *Pocillopora* spp. data for the four zones at all three sites showed that the lagoon had the lowest percent cover of *Pocillopora* spp., followed by the conglomerate, back reef, and fore reef. ANOVA analysis revealed a p-value of <0.01. A Tukey test revealed a significant difference between the fore reef and each of the other three zones (Figure 3C).

Figure 3. Percent covers of three coral genera in the four zones of all three sites on Moorea. Shown are means and standard errors. Lines connect means that are not statistically different; significant differences indicated by ** ($p < 0.01$)

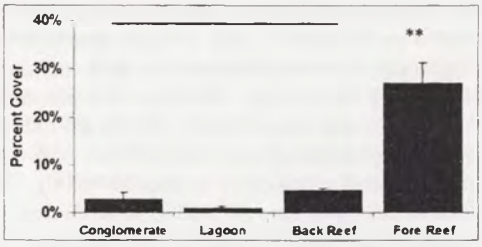
A. *Porites* spp



B. *Acropora* spp.



C. *Pocillopora* spp.



Site	Zone	Porites spp.	Pocillopora spp	Acropora spp.
Point Aroa	Conglomerate	6 +/- 2%	5 +/- 1%	5 +/- 0.1%
	Lagoon	5 +/- 2%	2 +/- 1%	0.1 +/- 1%
	Back Reef	5 +/- 1%	5 +/- 1%	2 +/- 0.1%
	Fore Reef	2 +/- 1%	32 +/- 6%	15 +/- 4%
Motu Temae	Conglomerate	1 +/- 0.2%	2 +/- 0.5%	3 +/- 1%
	Lagoon	6 +/- 2%	1 +/- 0.7%	0 +/- 0
	Back Reef	3 +/- 1%	5 +/- 1%	1 +/- 0.4%
	Fore Reef	9 +/- 4%	31 +/- 4%	4 +/- 2%
Motu Tiahura	Conglomerate	3 +/- 1%	0.6 +/- 0.3%	3 +/- 1%
	Lagoon	12 +/- 3%	0.2 +/- 0.1%	0.7 +/- 0.3%
	Back Reef	6 +/- 2%	4 +/- 1%	2 +/- 1%
	Fore Reef	17 +/- 7%	18 +/- 4%	7 +/- 1%

Table 3. Percent covers for three coral genera at each site. Standard errors are given for each.

Discussion

Porites spp.

The results of this study do not support the hypothesis that *Porites* spp. may becoming dominant in Moorea. Data shows that there has been no statistically significant increase in *Porites* spp. in the coral reefs of Moorea from the time of conglomerate formation in the mid-Holocene to the time of this study. Therefore, the data from the three study sites was not able to reject the null hypothesis that Porites spp. is not becoming dominant.

Although the mean *Porites* spp. data shows no statistically significant change in percent cover since the Holocene, data from the Temae site does show a statistically significant difference between percent cover of *Porites* spp. in the conglomerate and the fore reef (Figure 4). Since the conglomerate had the lowest percent cover and the fore reef the highest, the data suggests that there is possibly more *Porites* spp. now in the living assemblage than was alive during the formation of the conglomerate. However, massive corals such as *Porites* spp. suffer greater degrees of taphonomic alteration than both free-living corals and branching corals, and are therefore more heavily degraded in modern coral death assemblages (Pandolfi and Greenstein 1997). This taphonomic bias for massive coral growth forms to be underrepresented in death assemblages, may be due to their robust skeletons which survive for longer periods of time in the taphonomically active zone and experience dissolution, abrasion, and bioerosion for a longer period of time (Greenstein and Pandolfi 1997; Pandolphi and Minchin 1995). Therefore, the low percent cover of *Porites* spp. at Temae may be attributed to taphonomic bias, and not necessarily an increase in composition especially because the mean data shows no increase in the genus since the mid-Holocene.

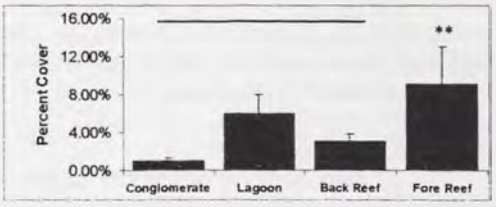


Figure 4. Percent Cover of *Porites* spp. at Temae. Significant differences indicated by ** ($p < 0.01$). Lines connect Zones that are not statistically different.

Acropora spp.

While the mean percent cover of *Acropora* spp. varied between zones, this variability was not statistically significantly different. This suggests that *Acropora* spp., a genera of coral that is more susceptible to disturbance events than *Porites* spp., shows no significant change in composition since the

mid-Holocene However, at the Point Aroa site, the percent cover of *Acropora* spp showed a significant difference between the fore reef and the other three zones (Figure 5). Despite the relatively high percent cover of *Acropora* spp in the fore reef compared to the conglomerate platform, this is off set by the relative low percent cover in the lagoon and back reef. Since research has shown that death assemblages reflect all coral zones due to the array of coral species present (Greenstein and Pandolfi 1997), it may be assumed that since the conglomerate platform was formed from corals in all three marine zones sampled, that its composition of *Acropora* spp could fall somewhere in between the two extremes. Therefore, despite the data from Point Aroa that shows a higher percent cover in the fore reef compared to the conglomerate platform, it does not suggest that the composition of *Acropora* spp has increased since the mid-Holocene

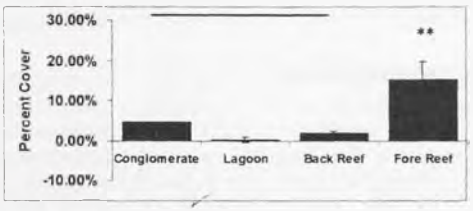


Figure 5. Percent cover of *Acropora* spp. at Point Aroa. Significant differences indicated by ** ($p < 0.01$). Lines connect Zones that are not statistically different.

Pocillopora spp.

The mean percent cover of *Pocillopora* spp for the four zones at all three sites was statistically significantly different and showed a large difference between the fore reef and the three other zones (Figure 3C). The data suggests that there has been an increase in the percent cover of *Pocillopora* spp. since the formation of the conglomerate platform. At every study site, the percent cover of *Pocillopora* spp was statistically significant ($p < 0.001$) and greater in the fore reef compared to the percent cover in the conglomerate platform. There are two possible reasons for the observed increase in *Pocillopora* spp.

First, five cyclones affected Moorea in 1982-1983, three of which caused significant damage (Hutchings, Payri and Gabrie 1994) creating extensive space for settlement by coral larvae (Done and Potts 1992). *Pocillopora* spp. are reproductively mature in half the time of *Acropora* spp (Hoegh-Guldberg and Salvat 1995). Therefore, *Pocillopora* spp. may have increased their population in the fore reef where the most extreme storm damage occurs, between 0-20 meters (Connell 1997). After a cyclone, recovery periods for reefs in the Society Islands are in the order of 50 years (Birkeland 1997). Therefore, the data was collected amid the recovery period for a disturbance event and the increase in *Pocillopora* spp. may be a result. A second possible reason for the difference in percent cover of *Pocillopora* spp is taphomonmic bias. According to fragility tests, *Pocillopora* spp is the least dense and most fragile of the three coral genera, suggesting that it may not be well preserved in the conglomerate platform (Shafler 1995). Therefore, while the data shows a significant increase in *Pocillopora* spp. it is most likely due to cyclonic activity and taphonomic bias instead of a long-term shift toward an increased populations of *Pocillopora* spp.

Coral Composition and The Holocene

The data from this study suggests that there has been no statistically significant change in *Porites* spp. and *Acropora* spp. percent cover from when the fossils were deposited 3,340 to 6,070 years B.P. Over such a long temporal scale, these two genera of coral show no change, partly due to the phenomenon of "time averaging" (Parsons and Brett, 1991). Time averaging is a form of taphonomic bias, in which short-term temporal fluxuations are averaged out of the fossil record during the period of time in which fossils accumulate and lithify. However, what appears to be no change in coral composition, is in fact a dynamic equilibrium of the coral community (O. Granger, personal communication). This equilibrium is reached over a long temporal scale as corals experience various environmental changes including temperature and sea level variation, and more localized events including mass bleaching, *A. planci* outbreaks, and cyclones.

The coral reefs of Moorea have experienced both large-scale and small-scale environmental changes in the Holocene. The data suggests that the composition of coral in Moorea can withstand fluxuations of sea level and temperature. Short-term disturbance events serve as biological experiments regarding the recovery of coral reefs and the possible temporal changes of coral composition. This is evident in the increase in *Pocillopora* spp. in Moorea most likely a result of the 1983 cyclones. Since no temporal changes were found for *Porites* spp. and *Acropora* spp., this study suggests that amid these micro oscillations corals are affected, but when viewed over a long temporal scale, they may eventually return to their dynamic equilibrium.

Caveats and Future Directions of Study

A few caveats: first, due to the large percentage of fossils which were identified as "unknowns," a variety of corals which were in fact present in the fossil record were not reflected in the data. Second, due to the confines of this study data collection was hindered in the fore reef. Percent cover was visually estimated due to depth and high wave action. Therefore, all data in the fore reef was collected using slightly different methods than the other zones, which could possibly affect the proportions of corals observed and tallied. Third, the effects of the 1983 cyclones on Moorea's coral reefs may have altered the temporal scale of this study. Although only *Pocillopora* spp. showed a difference in composition, it may have affected other coral genera although it is not reflected in the data.

The reconstruction of coral composition changes during the Holocene in this study only analyses two points in time, 3,340 to 6,070 years B.P. and today. Further studies are warranted on this topic in order to fill in data on corals during the years in between the fossil corals formation and today. Coral coring is an ideal way to further investigate temporal changes of coral composition.

Conclusion

The hypothesis that this study sought to test was that species composition of coral communities has the potential to be dominated by more tolerant species such as *Porites* spp. There is no evidence to support that the percent of *Porites* spp. is higher now than it was when the conglomerate platform was formed. The data cannot reject the null hypothesis suggesting that *Porites* spp. has not become a more dominant species in coral communities. The data also suggests that the composition of *Acropora* spp. has undergone no significant change in Moorea. However, in the case of *Pocillopora* spp., the 1983 cyclones may have altered its percent cover by favoring increased growth. This data holds important implications for the future of coral reef study.

This study infers that in view of the paleoclimactic history experienced by the corals of Moorea in the mid to late Holocene, predictions regarding the future composition of corals in Moorea may be hypothesized. It is thought that if the projected rates of sea temperature increase are realized, i.e. a 2° C increase in high latudinal coral seas, the upper thermal tolerance limits of many coral may be exceeded (Glynn 1993). However, the data from this study may suggest that *Porites* spp. and *Acropora* spp. may be able to withstand a warming trend in the future, in light of the fluxuations they have survived during the Holocene. Therefore, in the midst of possible global warming, the corals in Moorea may be able to tolerate a future shift in climate. However, how much of a shift they can tolerate is beyond the confines of this study.

Acknowledgements

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Integration of the invasive plant *Miconia calvescens* DC on Moorea, French Polynesia

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ABSTRACT. *Miconia calvescens* DC (Melastomataceae) is a highly invasive and ecologically disturbing plant in the Society Islands of French Polynesia. Its spread is limited in its native range of Central and South America by numerous herbivores and pathogens which are absent in the Society Islands. This two month study examined the interactions of *M. calvescens* with many of the organisms of Moorea, French Polynesia to determine the extent of ecological integration that is occurring as the invasion progresses. The results show that percent defoliation of *M. calvescens* and epiphyll species richness on *M. calvescens* are increasing as the invasion grows whereas epiphyll percent cover on *M. calvescens* and invertebrate species richness on *M. calvescens* are not. Also, insect species collected at night show no habitat preference between *Miconia calvescens, Inocarpus fagiferus,* or *Hibiscus tiliaceus* forests. The results suggest that some degree of integration is occurring with all organisms studied except invertebrates on *M. calvescens*. The speed of the continuing invasion and the robust health of old invasions suggest that little selective pressure is being exerted upon *M. calvescens* in Moorea.

Introduction

Invasive species have long been known to alter ecosystem function, often at the expense of the survival of native species (Elton 1958). Oceanic islands are especially vulnerable to invasions because of their low species richness (Darwin 1859), evolutionary isolation (Carlquist, 1965, 1974), and lack of certain taxonomic groups (Simberloff 1981).

Invading species proceed through a series of stages from arrival and establishment to integration with the native environment and eventual extinction. The following definitions based on Vermeij's (1996) work are representative of the categories seen throughout the literature. An invasion is the geographical expansion of a species to an area previously not occupied by that species. The arrival phase is the dispersal of individuals to the recipient region. Establishment, which is often accompanied by a spread in the population, is the stage at which the new population can sustain itself through local reproduction and recruitment. Integration occurs when the invading organism and the recipient biota respond to each other ecologically and evolutionarily (Vermeij 1996).

The arrival of new immigrants and their integration drives the species life cycle or taxon cycle (Wilson 1961) of island ecosystems. The taxon cycle starts with the geographical expansion of an invading species during which species density is high. This is followed by a slowing expansion and the differentiation of the immigrant into subspecies. The process continues with the ever decreasing geographic range and increasing ecological specialization of populations that become more isolated (Wilson 1961; Ricklefs and Cox 1972). The taxon cycle occurs because of the counteradaptive abilities of the native biota and competition from new immigrants, which at first do not bear the 'counteradaptive load' of the integrating invader (Ricklefs and Cox 1972).

The post-invasion adaptation of immigrant species has a number of causes. The post-invasion adaptations of the insects *Drosophila grimshawi* and *Tyria jacobaea* and the clover species *Trifolium subterraneum* and *Trifolium hirtum* are genetic changes caused by local selection (Barrett and Richardson 1986 and references within). Even a change in only one gene in a plant invader can cause enough morphological change to affect the whole ecosystem (Gottlieb 1984). Conversely, the post invasion morphological changes of many plant species can also be the result of phenotypic plasticity (Willis et al. 2000). Either way, local selective pressures affect the evolution and ecology of invading species.

Miconia calvescens DC (Melastomataceae) (hereafter referred to as *Miconia*) is generally regarded as the most successful and devastating plant invader of the Pacific islands. *Miconia* is native from 20° N in southern Mexico to 20° S in Argentina where its sparse population is limited by many native herbivores and pathogens (Meyer 1997).

Miconia has invaded extensively on the Hawaiian islands and the islands of Tahiti and Moorea in French Polynesia. *Miconia* also has populations in Sri Lanka, Australia, Grenada, New Caledonia, and the Marquesas islands and has been found on the islands of Huahine, Raiatea, Tahaa, and Mehetia in the Society Islands (Meyer 1997).

The spread of *Miconia* on Tahiti and Moorea has been spectacular. Since its introduction in 1937, *Miconia* has invaded 75% of the Tahitian forest and comprises 25% of its canopy (Birnbaum 1991). Up to 100 native plant species, including 35-45 endemic species, are directly threatened by *Miconia* (Meyer and Florence 1996). The invasion on Moorea is younger (plants were first noticed in the early 1970's – Meyer 1997) but no less spectacular as dense stands now dominate most of the higher elevations and are spreading downward.

Very few studies have examined the ecology of *Miconia*, especially in its non-native ranges. Two non-native frugivorous birds, *Zossterops lateralis* and *Pycnonotus cafer*, and the non-native rodent *Rattus exulans* have been observed dispersing *Miconia* seeds in Tahiti (Meyer 1996), and the generalist herbivore beetle *Adoretus sinicus* has been observed to cause up to 50% defoliation of *Miconia* in Hawaii and the Society Islands. *Miconia* has no known pollinators in its new habitats although it is readily self-pollinated (Meyer 1998). Aside from these observations, almost nothing is known about the ecology of this potent invader in the Society Islands.

This study examines the interactions of *Miconia* with some of the organisms on the island of Moorea. Four facets of integration are used to test the hypothesis that *Miconia* is becoming integrated as the invasion progresses. Increases in percent defoliation, epiphyll population parameters, and invertebrate population parameters correlated to increases in invasion parameters are the predicted signs of integration. A lack of insect habitat preference between *Miconia* and two other types of Moorean forests is also an indicator of integration.

Methods

Miconia survey

A cursory survey was performed to qualitatively determine the extent of *Miconia* invasion on Moorea. This survey was used to ascertain changes in invasion levels since the last major survey in 1997 (Bock 1997) and to locate study sites. Twenty of the twenty-four major coastal watersheds (all except Vaiterupe River, Tehiahuri River, Tunahe River, and Vaipahu River valleys) and six additional sites in the island's interior were explored on foot between September 16th and October 10th, 2000. Invasion levels were noted qualitatively based on the following invasion categories assigned by Bock (1997).

Complete invasion-Dense monotypic stands of mature *Miconia* trees at an estimated density exceeding 10 / 10square meters.
Heavy invasion-Most plants are juveniles with up to 10% mature at estimated densities between 5-10 / 10square meters
Moderate invasion-Characterized by either 1) populations of large juvenile and adult trees in estimated densities less than 2 / 10square meters or 2) juvenile and seedling populations with less than 2% mature in densities approaching 5-10 / 10square meters where present.
Preliminary invasion- Mainly seedlings with less than 10% juveniles and no mature plants in sparse densities.
Isolated plants-Mature plants away from any established populations.

Integration study

Eleven sites were chosen to represent the full range of *Miconia* invasion seen on Moorea (Figure 1). Sampling was carried out once at each of the following sites between October 12 and November 7, 2000:

1) October 12, 2000. Located on the east bank of Opunohu Bay at the base of Mt. Rotui on the property of Marimari Kellum at S 17° 30.832', W 149° 50.781'. This site is located at 40-60m elevation. Access from the road is between pk 17 and 18.
2) October 15, 2000. Located near the summit of Mt. Rotui at 850-860m elevation just north of the peak.
3) October 17, 2000. Located in the Vaianae River Valley near the property of Le Ferme Agricole. Located 3km upstream from the ocean and 20m uphill of the river at an elevation of 160-200m.
4) October 20, 2000. Centrally located in Opunohu Valley. The site is .5km along the dirt road that meets road R.T. 23 at the major river crossing bridge at an elevation of 40m.
5) October 22, 2000. Located about .25km after the end of the road along Papeahi River. Access to this road is near pk 5 in the town of Maharepa.
6) October 23, 2000. Located just below a waterfall on a side creek of the Vaioro River in the Niuroa Valley at an elevation of 120m.

Access to this river is near pk 9 in the town of Afareaitu.

7) October 24, 2000. Located .5km upstream along the first side tributary of the major unnamed river in Vaipohe Valley at an elevation of 100-140m. Access is near the town of Vaiare.

8) October 25, 2000. Located near the Belvedere vista point at the end of R.T. 24. The site is .5 km along the trail from Belvedere to 3 coconuts pass at an elevation between 220-260m.

9) October 26, 2000 . Located at the end of the road that follows Vaipapa River. The site is 100m uphill of the river at 160-180m elevation. Access is between pk 18 and 19.

10) October 27, 2000. Located .5km upstream along an unnamed creek that crosses the road .25km south of pk 28 at Le Poissonerie Arimiti. Sampling was done along the creek at 40-60m elevation.

11) November 7, 2000. Located 2km along R.T. 23 when starting from the town of Paopao. It is between 60-80m elevation.

At each study site, a 10m by 10m quadrant was chosen that was centrally located within the observable stand and was reflective of the whole stand (i.e., of average density, plant height, associated forest type). Within each quadrant, the number of *Miconia* plants was counted to determine stand density and 6 plants were randomly selected for sampling. The height and basal diameter at ten centimeters from the ground of each sample plant was recorded. I created an invasion index for each stand that equaled plant density (plants/100m^2) multiplied by average sample plant height multiplied by average sample plant basal diameter. An analysis of variance was performed to determine if invasion levels fall into discrete categories amongst all sites. Invertebrate organisms were collected in 70% ethanol at each sample plant for 15 minutes. Three leaves from the lower half of the foliated section of each sample plant and three leaves from the upper half of the foliated section were selected randomly for epiphyll and defoliation analysis.

Invertebrate analysis

Examination of the invertebrates included a census of total organisms and species richness and an analysis of species diversity using Simpson's diversity index (Simpson 1949) at all sites except #2 (Mt. Rotui) because of time constraints. These data were plotted against the stand's invasion index, density, average plant height, and average basal diameter.

Epiphyll analysis

Analysis of the epiphylls was carried out using a dissecting microscope at 7X to 40X magnification. A 15mm by 20mm quadrant was randomly selected from each of the six sample leaves on all six sample plants. Within each quadrant, the percent cover of each epiphyll species was recorded. The total number of epiphyll species inhabiting the lower half of the foliated plant , the upper half of the foliated plant, and the entire plant was also estimated using the same quadrants. Sampling was divided into these three regimes because of the easily observable differences in epiphyll populations over the height of the plant.

These data allowed the following five measures: epiphyll species richness per plant, epiphyll percent cover per plant, average epiphyll species richness per plant for each stand, average epiphyll percent cover per plant for each stand, and epiphyll diversity for each stand using Simpson's diversity index (Simpson 1949). All five analyses were carried out on each of the three sampling regimes. All percent cover data were subjected to an arcsin transformation to normalize them before further analysis.

The data from the analyses relating to epiphylls on individual plants were plotted against the height and basal diameter of each plant to determine if the dynamics of epiphyll communities are related to plant structure. Average epiphyll values per stand were plotted against the stand's invasion index, density, average plant height, and average basal diameter to determine if epiphyll community dynamics are related to properties of the stand.

Defoliation analysis

Analysis of defoliation was done using a digital camera and the computer program Adobe Photoshop 5.5. The six leaves collected from each of the six sample plants at each site were photographed against a backdrop of known area and this photograph was loaded into Photoshop. The original size of partially defoliated leaves was estimated with drawing tools and the actual size of the leaves and percent defoliation for each plant and each stand were determined using a tool that measures the minimum rectagonal area around objects of similar color (leaves). Finer estimation of leaf area and percent defoliation was done by superimposing the picture over a grid in Photoshop. The percent defoliation for each plant was plotted against plant height and basal diameter to determine if defoliation is

correlated to plant structure. The percent defoliation for each stand was plotted against the stand's invasion index, density, average plant height, and average basal diameter to determine if defoliation is related to properties of the stand.

Principal components analysis

The data from seven independent integration parameters at all sites were subjected to a principal components analysis to identify sites with similar integration patterns. The seven integration parameters were epiphyll diversity, average epiphyll species richness per plant, total epiphyll species richness per stand, average epiphyll percent cover on the lower three sample leaves, total invertebrates, invertebrate species richness, and percent defoliation. Sites were also identified with their habitat type and invasion level to determine if either of those factors affects site grouping.

Insect comparison study

Insect species were collected and compared between forests of monotypic *Inocarpus fagiferus*, *Hibiscus tiliaceus*, and *Miconia calvescens* from October 5, 2000 to November 11, 2000 on the property of Marimari Kellum near study site #1 described earlier. Three collections were made in the *H. tiliaceus* and *Miconia* stands while only two were made in the *I. fagiferus* stand because of time and weather restrictions. The sites were chosen for similar slope, canopy cover, and proximity to streams. Insects were attracted using a portable black light (110V, 11cm fluorescent tube bulb) hung 25cm from a white cloth measuring 3m x 2m at a random location within each stand. The stands were chosen to be as close to each other as possible while preventing radiation from the black light to reach any other type of forest. The light was turned on from 5:30p.m (just before dusk) until 8:30p.m. All insects found at 8:30p.m were collected in 70% ethanol. An analysis of total organisms, species richness, and species diversity using Simpson's diversity index (Simpson, 1949) was performed for each collection. These data were subjected to analyses of variance to determine differences in insect population parameters between stands.

All statistical analyses were performed on the computer programs JMP IN, PC ORD, or Microsoft Excel.

Results

Miconia Survey

Miconia invasion levels have increased in seven of the major areas surveyed by Bock (1997) and there have been no decreases in invasion levels since his survey (Figure 2). Two new heavy invasions of Miconia were found on the eastern half of Moorea. Two heavy and three preliminary invasions were found on the western half of Moorea ,which had never been surveyed. (Figure 1)

Integration study

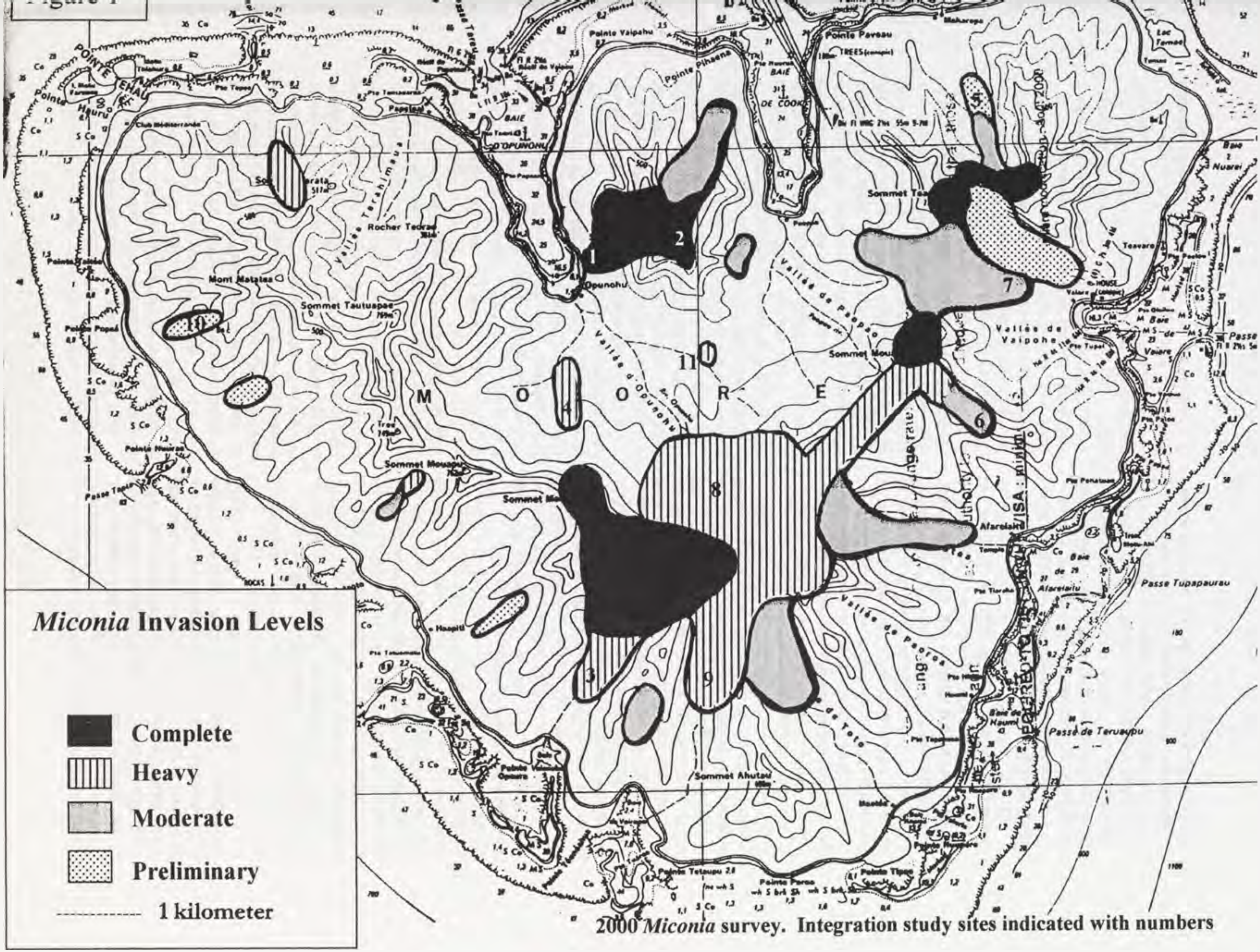
Invasion levels fall into 4 different categories (ANOVA, p=.00004). Sites #1,#2,and #3 make up the highest invaded category with an average invasion index of 3304 +/- 659. Sites #4 and #9 form the second category with an average invasion index of 1740 +/- 50. The third category is comprised of sites #8 and #11 (mean=535 +/- 167) and the fourth contains sites #5, #6, #7, and #10 (mean=30 +/- 11).

Miconia invasions were found in a wide range of habitats. Site #1 was unique because it was the only monotypic stand of *Miconia* in the study. Site #2 was also unique because of its location atop Mt. Rotui, hundreds of meters higher than any other study site. Sites #3, #8, and #9 were located in similar environments of dense *Miconia* within *I. fagiferus* and/or *H. tiliaceus* forests. Sites #4 and #11 shared a similar habitat of patchy *Miconia* within dense forests of *H. tiliaceus* and *Acacia* trees. Finally, sites #5, #6, #7, and #10 all had similar habitats of sparse *Miconia* in dense *H. tiliaceus* forests along streams.

Invertebrate analysis

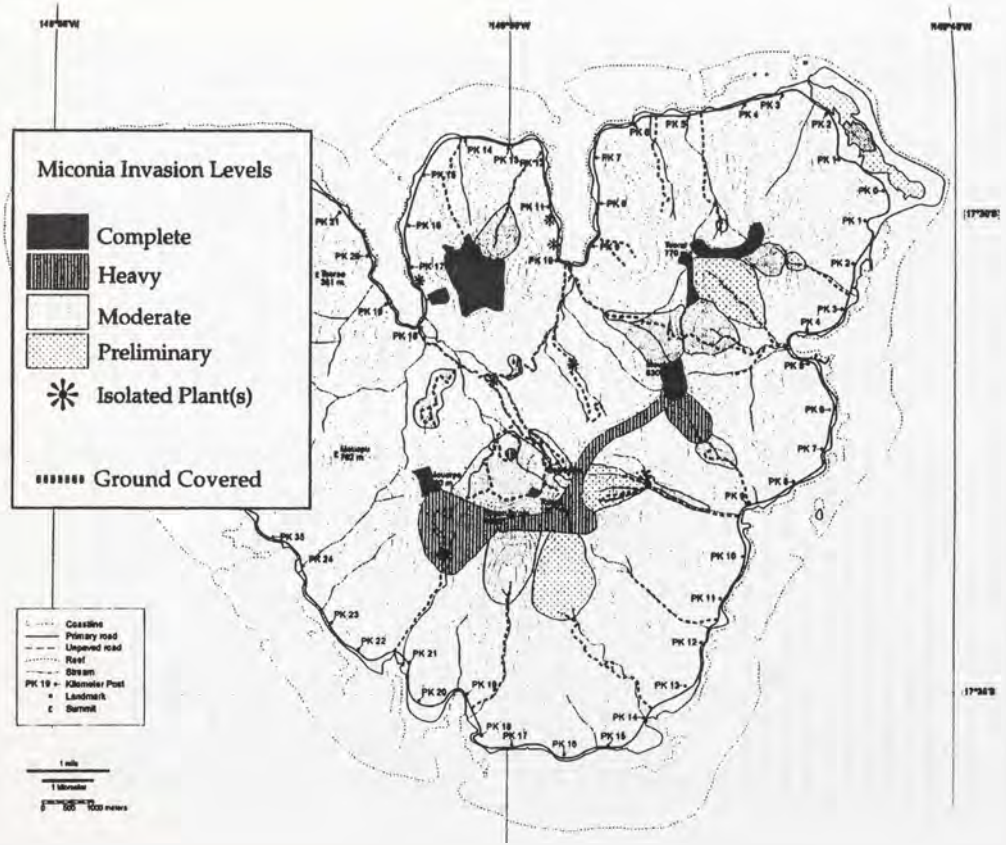
A total of seven invertebrate taxa were found at all sites. Total organisms found ranged between 16 at site #4 and 190 at site #11 (mean = 91.6 +/- 52.0, n=10). Species richness ranged between five at site #1 and 25 at sites #6 and #7 (mean = 16.5 +/- 8.6, n=10). Species diversity ranged between 1.3 at site #11 and 11 at site #9 (mean = 4.9 +/-3.4, n=10) (Table 1). Species composition at sites #1, #3, and #11 was dominated by a snail species in the Endodontiaceae. The invertebrate study revealed two relationships. Invertebrate species richness decreases linearly with an increase in average basal diameter (R^2=.46, p=.03, n=10) (Figure 3) and the total number of invertebrate organisms decreases linearly with an increase in invasion (R^2=.33, p=.08, n=10) (Figure 4). All of the other plots of total invertebrates, invertebrate species richness, and invertebrate diversity versus

Figure 1



2000 *Miconia* survey. Integration study sites indicated with numbers

Figure 2


Miconia Invasion Levels
Complete
Heavy
Moderate
Preliminary
Isolated Plant(s)
Ground Covered
Coastline
Primary road
Unpaved road
Reef
Stream
Kilometer Post
Landmark
Summit

invasion index, stand density, average plant height for the stand, and average basal diameter for the stand show no significant relationships.

Epiphyll analysis

A total of 27 epiphyll species were found at all sites including three plant species, eight crustose lichens, 11 species of fungi, three species of insects, and two species of unknown taxa. Two of the plant species were identified as liverworts, one in the *Leptolejeunea* genus and the other in the *Drepanolejeunea* genus. The other plant was identified as a moss in the *Trentopodia* genus. Epiphyll species richness ranged between 7 at site #1 and 17 at site #3 (mean = 13.5 +/- 2.8, n=11). Average percent cover per leaf ranged between .9% at site #9 and 11% at site #5 (mean = 5.1 +/- 3.2, n=11). Epiphyll diversity ranged between 1.1 at site #5 and 4.3 at site #8 (mean = 2.5 +/- 1.1, n=11) (Table 1). None of the plots of total epiphyll species per plant or epiphyll percent cover per plant against plant height or plant basal diameter revealed significant relationships (n=66).

The plots involving data for the whole stand revealed the following 13 relationships: Total epiphyll species richness on the upper three sample leaves has a 2nd degree polynomial relationship with stand density (R^2=.72, p=.006, n=11) (Figure 5) and invasion level (R^2=.54, p=.05, n=11) (Figure 6). Epiphyll species richness on the lower three sample leaves increases linearly with increasing stand density (R^2=.36, p=.05, n=11) (Figure 7) and has 2nd order polynomial relationships with average plant height (R^2=.45, p=.09, n=11) (Figure 8) and average basal diameter (R^2=.60, p=.03, n=11) (Figure 9). Average epiphyll species richness per plant on the lower three sample leaves has 2nd degree polynomial relationships with average basal diameter (R^2=.76, p=.004, n=11) (Figure 10) and average height (R^2=.65, p=.02, n=11) (Figure 11). Average epiphyll species richness per plant on all sample leaves has 2nd degree polynomial relationships with average plant height (R^2=.62, p=.02, n=11) (Figure 12) and average basal diameter (R^2=.77, p=.003, n=11) (Figure 13). Average epiphyll percent cover per leaf (arcsin transformed) on the lower three sample leaves decreases linearly with invasion level (R^2=.44, p=.03, n=11) (Figure 14) and average height (R^2=.5, p=.05, n=11) (Figure 15) and has a 2nd degree polynomial relationship with average basal diameter (R^2=.43, p=.11, n=11) (Figure 16). Epiphyll diversity on the upper three sample leaves has a 2nd degree polynomial relationship with average basal diameter (R^2=.50, p=.06, n=11) (Figure 17). All other analyses described in the Methods show no relationships with any invasion parameters.

Defoliation analysis

Percent defoliation per plant ranged between 39.7% on a plant at site #2 and 0% on numerous plants (mean = 7.4 +/- 9.5, n=66). Percent defoliation per site ranged between 0.52% at site #7 to 29.6% at site #2 (mean = 7.1 +/- 9.0, n=11) (Table 1). Two relationships were found in the defoliation analysis. Percent defoliation (arcsin transformed) increases linearly with stand density (R^2=.59, p=.006, n=11) (Figure 18) and has a 3rd degree polynomial relationship with invasion level (R^2=.86, p=.003, n=11) (Figure 19). Percent defoliation (arcsin transformed) for the stand is not correlated to average plant height for the stand (R^2=.008, p=.78, n=11) or average basal diameter for the stand (R^2=.11, p=.32, n=11). Percent defoliation (arcsin transformed) per plant is not correlated to plant height (R^2=.12, p=.005, n=66) or basal diameter (R^2=.11, p=.007, n=66).

Principal components analysis

Eighty percent of the variance in the principal components analysis is accounted for in the first three axes. The Eiganvectors of the first axis range between -.4271 and .4568. The Eiganvectors for average epiphyll species richness per plant (-.6256) and total epiphyll species richness per stand (-.6030) are the most correlated to the 2nd axis and invertebrate species richness is most correlated to the 3rd axis (-.7928). (Figures 20 & 21)

Insect comparison study

The total number of insects collected in the *Miconia* forest ranged between 43 and 55 (mean = 49 +/- 3.5, n=3) while the total number of insects collected in the *I. fagiferus* forest ranged from 35 to 56 (mean = 45.5 +/- 10.5, n=2) and the total number of insects collected in the *H. tiliaceus* forest ranged between 45 and 85 (mean = 59.3 +/- 12.9) (Table 2). The total numbers of insects collected in each forest are not significantly different from each other (ANOVA, p=.62) (Figure 22).

Insect species richness in the *Miconia* forest ranged between 21 and 41 (mean = 30.7 +/- 5.8, n=3). Species richness in the *I. fagiferus* forest ranged from 18 to 22 (mean = 20 +/- 2, n=2) and species richness in the *H. tiliaceus* forest ranged between 25 and 46 (mean = 32.7

+/- 6.7, n=3) (Table 2). Species richness does not differ significantly between the 3 forests (ANOVA, p=.4) (Figure 22).

Insect diversity in the *Miconia* forest ranged from 12.8 to 30.8 (mean = 22.0 +/- 5.2, n=3). Insect diversity in the *I. fagiferus* forest ranged from 9.4 to 9.8 (mean = 9.6 +/- .2, n=2) and insect diversity in the *H. tiliaceus* forest ranged from 17.8 to 22.9 (mean = 19.6 +/- 1.7, n=3) (Table 2). The diversity of insects does not differ significantly between the 3 forests (ANOVA, p=.16) (Figure 23).

Discussion

The spread of *Miconia* is progressing rapidly on Moorea. The invasion is advancing downhill in almost all the valleys on the island and it is only a matter of time before the last untouched watersheds are colonized.

Invasion dynamics are affecting the interactions between *Miconia* and some invertebrates of Moorea. The decreases in total invertebrates and species richness observed at higher invasions are driven more by average plant size than by stand density. This may suggest that the ability of insects to adapt to *Miconia* invasions is more dependent on time than stand density as plant size is more correlated to the age of the invasion than is stand density. These relationships would suggest that *Miconia* has not yet integrated with the invertebrate community of Moorea. However, because invertebrates were only collected at the stand level, it is not possible to determine if there are relationships between plant structure and insect community dynamics regardless of invasion level. Future studies should test for this.

Invasion levels have also affected *Miconia* epiphyll communities. There is a polynomial relationship between total epiphylls and invasion and a polynomial relationship between epiphyll species richness and invasion. These relationships, driven more by average plant size than stand density, suggest that stands of intermediate invasion have the most epiphyll species. It is interesting to note that these relationships are made polynomial solely by the site with the largest plants (site #1) and that without this site there would be a strong increasing linear relationship between epiphyll species richness and invasion. Conversely, epiphyll percent cover decreases linearly with increasing invasion. Thus, as the invasion progresses, more epiphyll species are able to colonize *Miconia* leaves (except at sites with the largest plants) but the epiphyll community does not occupy as much leaf area. The decreases in percent cover could be a result of changes in plant structure at different invasion levels. For example, highly invaded sites are usually comprised of large trees with high canopies which lack the lower leaves where high epiphyll percent cover is usually observed. Also, no relationships were noted between epiphyll populations and plant structure across stands. Since the dynamics of epiphyll communities on *Miconia* are not related to plant structure alone, the course of the invasion likely affects the adaptability of epiphylls to this alien invader.

Invasion parameters have the strongest relationship with percent defoliation. While density has an increasing linear relationship with percent defoliation, invasion level has a more complicated 3rd order polynomial relationship with percent defoliation. Such a pattern could reflect a difference in herbivore and pathogen species between sites in different environments. For example, the highest invaded site (#2) has an associated environment that is quite different from any of the other sites because of its location atop Mt. Rotui. This site hosts a very unique biota of endemic species that were not found anywhere else on Moorea. Conversely, the less invaded sites share somewhat similar environments because early invasions typically occur along streams in mixed *Inocarpus/Hibiscus* forests. Future studies should control for the confounding factor of associated environment although this will be difficult given the current structure of the invasion. Also, without knowledge of the cause of defoliation at each site, it is difficult to determine the type and amount of species interactions that have caused the defoliation. Nevertheless, a strong relationship exists between invasion level and percent defoliation. This, in conjunction with the high levels of herbivory seen at some sites, suggests that integration is occuring between *Miconia* and some species on Moorea as the invasion progresses. It is not likely that defoliation has had a significant affect on the growth of *Miconia* in Moorea as the plant is known to tolerate up to 50% defoliation (Medeiros and Loope 1997).

The low percent of variance explained by the first three axes of the principal components analysis and the homogeneity of the Eiganvector values suggest that sites cannot be grouped well based on their integration patterns alone. While the overlay of habitat type produces more consistent non-overlapping groups than the invasion level overlay, neither is an effective

separator of sites. The better grouping ability of the habitat type overlay could be due to its five categories as opposed to four for invasion level. Overall, the principal components analysis suggests that sites with similar invasion levels or environments do not usually share similar integration patterns. Future studies should collect and analyze more environmental data at each site to determine the causes of similar integration patterns between sites.

The insect comparison study suggests that some of the insects of Moorea may have adapted to pure stands of *Miconia*. However, the high variability and low sample size of this study warrant caution in making strong conclusions. It is likely that *I. fagiferus* and possibly *H. tiliaceus* were introduced with arrival of the first Polynesian settlers at least 2000 years ago (Fosberg 1992). Future studies of the distribution and ecology of these three species could shed light on the progression of the taxon cycle in Moorea.

Overall, the results of the epiphyll, defoliation, and insect comparison studies suggest some degree of integration between *Miconia* and the Moorean biota, while the invertebrate study suggests that *Miconia* is still in a stage of expansion. This study attempted to answer a question of evolutionary scale in a short amount of time with ecological data. Thus, there are many different potential confounding factors and environmental variables at each site that could be affecting the measures of integration. However, this study provides an initial survey of the ecology of *Miconia* that can be used to monitor integration all over Moorea in the future. Ideally, the integration process should be examined independently in each type of invaded ecosystem over time.

Conclusion

It is clear that *Miconia* is forming some ecological interactions with the biota of Moorea as it invades. However, the continued speed of the invasion and the robust nature of older stands suggest that this integration process is probably not yet exerting much selective pressure on *Miconia* populations.

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Bioerosion Rates of *Echinothrix diadema* Along the Fore Reef in Moorea, French Polynesia

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ABSTRACT. Coral reef morphology is dependent upon the rate of bioaccretion, the active growth of corals, and the simultaneous erosion of the reef substrate by both physical and biological processes. The rate by which the hard reef substrate is biologically eroded was experimentally determined by exposing pieces of dead coral for one month upon a fringing reef along the western edge of Cook's Bay, Moorea, French Polynesia. The experiment isolated the bioerosion contribution of the sea urchin, *Echinothrix diadema*, from that of large grazing scarid fish. Experimental results show estimated rates of bioerosion to be greatest for grazing fish (4.67 Kg m^{-2} y^{-1}) in comparison to *E. diadema* (4 Kg m^{-2} yr^{-1}). Both rates, when compared to the control (3 Kg m^{-2} y^{-1}), are not significantly different but, instead, suggest that other marine organisms play a role in bioerosion.

Introduction

The high biodiversity of coral reefs have led many to refer to them as the 'rainforests of the sea' and, as with the rainforests, many are becoming increasingly concerned over the long-term viability of coral reefs. Around the world, coral reefs have gained the attention of scientists and the general public alike as wide spread mass mortality is seemingly on the rise. Coral reef health is a dynamic issue dependent on many biotic and abiotic conditions changing over time and space making it difficult to speculate whether the health of a reef is truly in jeopardy or not (Reaka-Kudla *et al.* 1996).

Recent studies, focusing on the roles of oceanic calcium carbonate, have helped scientists to better understand fluctuations in coral reef growth (Bak 1990; Bak 1993; Chazottes *et al.* 1994; Keine and Hutchings 1994; Le Campion-Alsumard *et al* 1993; Peyrot-Clausade *et al.* 1995; Peyrot-Clausade *et al.* 2000; Reaka-Kudla *et al.* 1996). It is generally recognized that these fluctuations are a function of the balance between the active growth, or deposition of calcium carbonate, and the simultaneous erosion of it. The dead reef framework, principally eroded by the mechanics of wave action and biological agents (Hutchings 1986), indirectly redistributes calcium carbonate back into the sea in the form of calcium carbonate powder where it is, in turn, made available for reef building processes, thereby completing the cycle.

The island of Moorea, in French Polynesia, is a high island atoll with a fringing reef, a barrier reef, and a lagoon separating the two. As a result, several unique reef ecosystems surround the island providing for a convenient location to study comparative bioerosion. One such study experimentally showed living coral totally depleted the calcium carbonate content of sea water in the absence of bioeroding organisms, concluding that bioerosive organisms, such as sea urchins, play an important role in restoring calcium carbonate (Le Champion-Alsumard *et al.* 1993). Sea urchins, and other organisms that scrape away coral substrate, known as grazers, have been observed to be the leading cause of bioerosion in many reef studies, both in Moorea and other areas. For example, in a study comparing the rates of bioerosion on a reef in French Polynesia to another in the Indian Ocean, researchers concluded bioerosion was due to two types of grazing bioeroders, namely scarid fish and echinoids, but varied considerably among habitats (Peyrot-Clausade *et al.* 2000). Previous studies in French Polynesia, where urchin densities are comparatively higher than those found in other reef ecosystems showed rates of erosion significantly differed depending upon the species, size and abundance of the grazers (Bak 1990; Bak 1994; Nithart and Salvat 1990; Peyrot-Clausade *et al* 1995). Other studies suggest more complex ecological relationships, such as the spatial and temporal variability of bioeroders (Adjeroud 1997), macrobenthic algae (Steneck 1988), the physical environment and eutrophication (Chazottes *et al.* 1994; Peyrot-Clausade *et al.* 1995) to influence rates of bioerosion.

The aim of this study is to investigate which grazing organisms are most responsible for the bioerosion of the reef framework along a small

stretch of fore reef in Cook's Bay, Moorea (French Polynesia) and to estimate the contribution of each to the calcium carbonate budget.

Materials and Methods

Study Site

Moorea (S17° 30', W149° 50'), a dormant volcanic island surrounded by both a fringing and barrier reef, is located south of the equator in the Society Archipelago, French Polynesia (Figure 1). The island's north side is deeply scored by two long bays, one of which, is Cook's Bay, which runs in a roughly north-south orientation. Along the western side of Cook's Bay, near the mouth, is the Gump Biological Research Station. The fore reef directly in front of the station (Figure 2), commonly thought to be subject to eutrophication and poor oceanic circulation, was chosen for the study.

The reef gently slopes away to 2.5 meters from a sandy sub-tidal shoreline for 80 meters before sharply dropping off to over ten meters. The reef's edge is characterized largely by dead coral reef framework with occasional live coral outcrops.

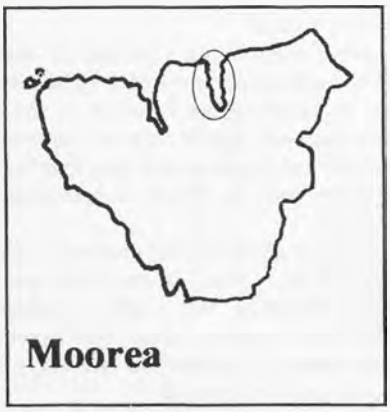

Figure 1. Map of Moorea

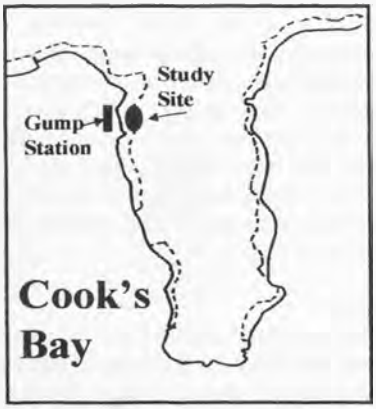

Figure 2. Study site location

Sampling

The reef was surveyed three times at night to evaluate sea urchin species, presence, location and density. Once sea urchin presence and locality were determined, the area was flagged every five meters for 110 meters along the fore reef's edge. Sea urchin density was sampled at night using a $1m^2$ quadrat. These samples were based on 'x' (0-110m) and 'y' (0-1.5m) coordinates randomly assigned from a random number table and taken thirty times (n= 30).

The substrate was sampled 30 times during the day using the same flagging and 'x-y' random coordinate system with a $1m^2$ quadrat to evaluate substrate availability. The substrate was characterized as either dead reef framework, rubble, sand or live coral on a percent cover basis.

A second random sampling survey was done at night, using the same system to correlate urchin presence to a preferred substrate type (reef framework, rubble, sand and live coral). Data was recorded on either the presence or absence of urchins, along with what substrate type they were on.

Experimental Design

Experimental plots were designed having three treatments and a control in each of seven replications along the 110m length the fore reef's edge. Each replication was placed where *Echinothrix diadema* urchin densities were highest in order to best simulate their natural reef substrate habitat. Treatment A, or the 'Enclosure', was designed to test for the exclusive contribution of *E. diadema* to bierosion. Enclosures were constructed of chicken-coup wire mesh completely enclosing the urchin. Treatment B, or the 'exclosure', was designed to quantify bioerosion excluding the sea urchin *E. diadema* but not large scarids such as parrot fish. These 'exclosures' were designed to cover the same amount of substrate but have an open top to allow for large grazing fish to enter. Both treatment A and B measured 60cm on each side. Treatment C, or the 'control' needed no

construction because it was used to test the natural levels of bioerosion. Treatment D tested for algal growth in the absence of both sea urchins and large fish. This exclosure measured 30cm on each side. In each of the seven replicates, each treatment was randomly ordered in it's placement next to the other in order to avoid pseudoreplication.

Bioerosion was measured using data recorded from 28 pieces of dead coral substrate found along the reef's edge. Each piece was removed and measured in the lab for weight and volume displacement. The weight was measured using a digital scale and the volume was quantified by the amount of seawater each piece displaced into a graduated cylinder. Each piece was then placed in the center of each of the cage treatments. The pieces used for the control treatment were placed in the center of a $360cm^2$ square on the reef. After 31 days, the coral pieces were removed from each treatment, their weight and volumes re-measured, their surfaces inspected for signs of grazing and then returned to where they were originally found in the bay. Algal filaments, upon the upward face, were measured in millimeters on all pieces of coral after 31 days.

Data Analysis

The natural density of *Echinothrix diadema* was calculated by dividing the number of urchins found, by the number of times the site was sampled times $1m^2$, or $30m^2$. The experimental density of *E. diadema* was simply $1/.36m^2$ based on enclosure treatment size. Substrate availability was calculated on a percent cover basis. Substrate preference data was analyzed using the Chi-squared equation, to test whether urchins preferred one substrate type to another.

The program Excel was used to analyze volume displacement data for treatments A, B and C using a single-factor ANOVA test to check for variance between treatments. T-tests, assuming equal variance, were also used to analyze variance between treatments A and B, treatments B and C, and treatments C and A. Algal filament length in treatment D was compared to lengths measured in the other 3 treatments and was analyzed using a single-factor ANOVA.

Experimental rates of bioerosion were calculated by converting volume to mass using the average density of algae covered coral ($5.01g\ ml^{-1}$) as used by Peyrot-Clausade *et al.* (1995) multiplied by the experimental density ($1/.36m^2$) and extrapolated to $kg\ m^{-2}\ y^{-1}$.

Results

Site Characterization

Three night surveys showed *E. diadema* densities to be greatest where dead coral substrate was most abundant within 2 meters of the outer edge of the fore reef. Based on these observations, the study site and experimental plot were laid out along a 110m long by 3m wide area along the reef's edge.

Natural *Echinothrix diadema* densities were estimated at $1/.36m^{-2}$, or one urchin per $3m^2$. The density estimate was used to standardize bioerosion rates derived from the experiment because experimental density was artificially set to $3m^{-2}$.

Substrate availability within the study site resulted in 58.5% reef framework, 18.2% rubble, 17.8% sand and 5.5% live coral (Figure 3). The substrate preference data showed 30 of 32 urchins to be found on reef framework and the remaining two to be found on coral rubble (Figure 4). A Chi-Squared test showed significant preference of the urchins to reef framework over other substrate ($p < .001$).

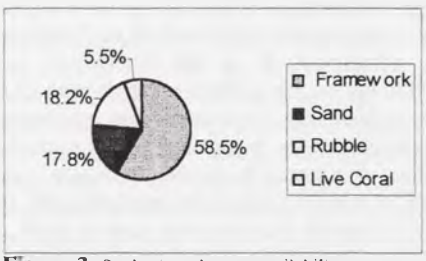

Figure 3. Study site substrate availability

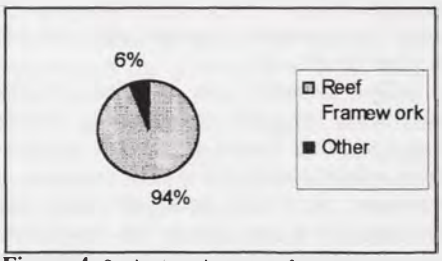

Figure 4. Study site substrate preference

Experimental Bioerosion

Mean volume displacement data recorded from treatments A (23.3ml), B (28.3ml) and C (18.1ml) reflected an increased amount of bioerosion in both treatments A (enclosure) and B (exclosure) when compared to treatment C (the control) (Figure 5) but failed to be statistically significant when analyzed using an ANOVA ($p = .34$). Converted to kilograms per meter squared per year, treatment A equals 4.0 $kg/m^2/y$, treatment B equals 4.67 $kg/m^2/y$ and treatment C equals 3.0 $kg/m^2/y$. The results of treatment A when compared to treatment B were unexpected since it reflects urchin bioerosion less than that of large fish. T-tests used to analyze variance between paired treatments rendered pair AB and pair AC insignificant ($p = .27$ and $p = .20$) and pair BC significant ($p = .05$). When analyzed using an ANOVA, differences in algal growth between treatments was not statistically significant ($p = .07$). The results of the algal length data recorded unexpectedly reflect filament growth to be longest in treatment D (6.6mm), followed by treatment B (5.6mm), A (3.0mm) and then C (2.9mm). This evidence, though not statistically significant, does not support my hypothesis nor does it allow the rejection of my null hypothesis.

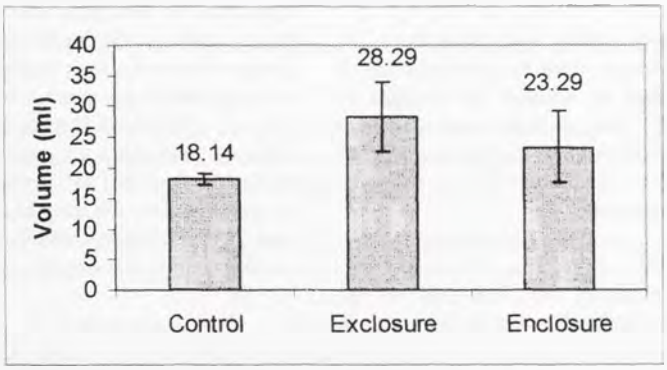

Figure 5. Volume displacement data in each treatment

Discussion

Site Characterization

Densites of *E. diadema* along the fore reef, within the study site, were stable suggesting that home-range and territoriality are small. According to previous research focusing on the density and distribution of urchins, density is a function of the shelter in the reef structure (Nithart and Salvat 1990).

The distribution of substrate within the study site was randomly distributed with observable clumped patterns of live coral and reef framework. Due to the nature of the random sampling method used, it is possible that the data is inaccurate. But for the purposes of this study, which is to estimate the relative contributions to bioerosion, it should suffice. Urchin preference data was very reliable since the data was collected on a non-random basis but rather on observation of all *E. diadema* urchins found within the study site.

Experimental Bioerosion

Bioerosion data collected varied greatly within each replication and between treatments. Over the course of the experimental period several confounding factors were realized that may have significantly contributed to this variation. First, there was noticeable deterioration of urchin health by the 25th day. Urchins in replication #3 and #5 had suffered severe spine damage. By the 29th day, urchins in replication #1, #2, #4 were missing, the urchin in replication # 5 was dead. All missing urchins were replaced that evening to continue the experiment through the 31st day.

In addition to urchin mortality, the experimental cages allowed small grazing fish to take refuge from larger fish, thereby increasing normal fish densities, which in turn, may have influenced bioerosion data indiscriminately throughout the treatments.

A third cage effect possibly affecting the data was algal growth upon the cages themselves. By the 20th day, algae growing upon treatments A,

B and D in replication #4 and #5 had taken on new defenders. The algal type was that which damselfish normally cultivate in their gardens. Damselfish had moved into the treatments and had begun to defend them from presumably fish and urchins alike. In a previous report on damselfish and sea urchins, it was concluded that damselfish-algal mutualism is probably important in preserving and influencing reef framework morphology (Eakin 1988).

A problem, independent of cage effects and probably more important, was the influence of micro- and macroboring organisms on bioerosion. The coral pieces used to calculate bioerosion were probably at various degrees of maturation, which, in turn, would skew density calculations used in bioerosion rates.

Experiments testing bioerosive rates for coral reef environments need to be done over a longer period of time to account for changes in bioeroder density, reef ecology and seasonal change. Rates of erosion cited in this research are estimates for a given time and need to be followed up by subsequent research.

Conclusions

Bioerosion along the fore reef at the Gump Research Station in Moorea is important in determining whether its health is in decline or not. Future bioerosion and accretion rate estimates made for this reef can provide a baseline, for which, it can be applied over time and a better picture of coral health can be understood. Reef ecosystems are inherently heterogeneous, making it difficult to assess whether a net-gain or net-loss of calcium carbonate, at a given time, is an accurate reflection of long-term reef health. But the more research done at specific sites the more we will be able to move toward making smarter decisions in managing the health of other coral reefs ecosystems.

Acknowledgements

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Evidence for symbiosis between species of non-native ants on Moorea, and the coastal tree species *Hibiscus tiliaceus*

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ABSTRACT. The Polynesian island of Moorea has no native species of ants, however 36 species of non-native ants (Hymenoptera: Formicidae) have been identified. Little is known about the effect of the many species of introduced ants upon the local flora and fauna in the habitats they occupy on the island, however ant-plant symbioses are common in tropical environments, and preliminary observations on Moorea suggested that species of ants were feeding upon the extra-floral nectaries of the coastal tree species *Hibiscus tiliaceus* (common name Beach Hibiscus). *H. tiliaceus* is not sited in literature as a true myrmecophyte (ant-plant), however observations suggest potential for a developing symbiosis between ant and plant. The proof of a unique feeding relationship, and the degree to which the feeding pattern and other observed ant behaviors suggested an evolving ant-plant symbiotic association were the focus of this study. Ants were found to favor the leaves of *H. tiliaceus* over those of other tree species, and to focus upon the most distal leaf on a hibiscus branch and the extra-floral gland structures on the underside of each leaf. Indicators of typical ant-plant mutualism other than the provision of food for ant by plant were found to be not statistically significant, if not absent from the current relationship structure. The findings of the study provided insight into potential evolutionary conditions that precede obligate ant-plant symbiosis, including resource competition and limitation, introduction to a foreign environment, and niche vacancy.

Introduction

Symbioses exist on all levels of biological organization and occur between organisms from all kingdoms, including the case of ant-plant symbiosis, a largely tropical phenomena where ant species live in a tight mutualism with a partner plant species. The evolutionary roots and ecological causes of ant-plant relationships are largely speculative (Soepadmo 1978) due to confounding factors such the remote location of many tropical habitats, and a historical perspective in the biological sciences that has tended to ignore symbiosis in general (Amadjian and Paracer 1986). Further, the plant species found in these associations are members of a variety of unrelated families, and the ants are from a number of separate species.

Competition for food sources and suitable colony locations is so pronounced in tropical environments that plants exhibiting some if not all of the diagnostic ant-plant symbiotic traits are often quickly found by ants (Bailey 1924). However the difference between generalized resource exploitation and established ant-plant mutualisms are dramatic. In a typical Ant-plant mutualism the plant provides the ant with a food source and nesting space, while the ants live on the tissue of the plant and aggressively protect it against insect and plant predators, including other species of ants. Extra-floral nectaries, sources of nectar not located in the flower, are a common food source provided by a plant to feed a symbiotic ant colony. In many cases these mutualisms become obligate and both species experience reduced fitness when removed from their symbiont (Soepadmo 1978). The question of how the two organisms develop their tight mutualism can be addressed by examining natural conditions that precede the tight mutualism. Thus examining ant-plant associations in their early stages has the potential to reveal evolutionary pathways towards ant-plant mutualism. Remote islands, such as the island of Moorea, a hot-spot island in French Polynesia in the South Pacific Ocean, provide ideal locations to conduct this research, as many organisms are recent arrivals, in evolutionary time, and are forced to reorganize themselves into new associations for survival in their new environment

The Society Islands have historically relied upon biological invasions for their floral and faunal development. Natural dispersal mechanisms and human commerce have provided constant sources of non-native species to the islands, and the resulting menagerie of species found does not mirror species diversity encountered on comparable mainland habitats. Whole groups of

organisms, such as the family Formicidae (ants), ubiquitous in mainland tropical environments, were absent in the Society Islands until human introduction. The first dates of ant introduction are unknown, however records of ant collections in Polynesia date back to the 1860's (Wilson and Taylor 1967). The first comprehensive collection of known species on the Polynesian islands, including Moorea and the other Society Islands, was completed by Edward O. Wilson and Robert W. Taylor in 1967, in which they described 16 species on the island of Moorea. By 1996, 20 other species had been identified (Morrison 1996), more than double the number of species found in 1967. Descriptive studies have been conducted that detail species composition and interspecific interactions between these ant species, however few studies have focused upon the ants' interaction with their environment, specifically the potential effects the many species may have on more established insect and plant communities. Ants are highly socialized and typically aggressive invaders that have been observed to severely displace established arthropod populations in Hawaii by disrupting feeding networks (Gillespie 1993), thus the study of how introduced ants on Moorea have integrated themselves into the environment has important ecological significance for the local flora and fauna.

Hibiscus tiliaceus (common name Beach Hibiscus) is a tree species (family Malvaceae) that dominates the vegetative structure along the coast of Moorea, especially along the coastlines of Cook's Bay and Opunohu Bay. It is a mangrove associate, presumed to have Indo-Malaysian origins, but the current species range includes the Hawaiian Islands, Polynesia, Melanesia, Thailand, Australia, India and the Indo-Malaysian Peninsula. Beach hibiscus is a pioneer species and readily colonizes newly disturbed areas around the coast and up river valleys (Santiago et al. 2000), however it is found in nearly every forest type on the island of Moorea and at elevations up to at least 250 meters (personal observations). The establishment of the species is so widespread on Moorea that it is considered a native species for all practical purposes, and the approximate date of introduction on Moorea is not documented in literature.

During preliminary observation ants were found feeding on extra-floral nectaries located on the underside of Beach Hibiscus leaves. Ant-*H. tiliaceus* symbiosis is not documented in literature however ant presence on *H. tiliaceus* trees in papua new Guinea has been observed (Dr. Phillip P. Ward, UC Davis, personal communication), thus in this study of potential ant-plant symbiosis, I examine ant distribution on beach hibiscus on different spatial levels to determine the level of specificity the relationship has reached in the context of symbiosis. These spatial scales included landscape, stand, branch, and leaf level organization. Investigating the extent of the relationship between beach hibiscus and the species of ants feeding on it detailed a previously unrecorded symbiosis and revealed the potential for an evolving ant-plant mutualism on Moorea. The study also provided some insight into the evolution of tropical ant-plant mutualisms.

Materials and Methods

Site Selection

I established five primary sites and one supplementary sampling site along Cook's Bay and Opunohu Bay on the North side of Moorea in September of 2000 and sampled the sites during the months of October and November, 2000. Each site was a hibiscus dominant forest patch below 10 meters elevation and selected on the basis of location, tree species composition, plot size, and accessibility. The five primary sites were located along the Moorea coast road at pk (pointe kilometrique) 18, 17.5, 16.5, 12.5, and 11.75 (Figure 1). I attempted to select sites at least 1 kilometer apart, and the two sites located less than a kilometer apart were separated by the mouth of the Opunohu River. Each of the six sites had a human maintained "edge" that delineated a clear division between the *H. tiliaceus* stand and the adjacent vegetation. An "edge" was defined as any continuous stretch of interface between Hibiscus forest and human maintained habitat that displayed previous signs of human manipulation. Chain saw scars and piles of downed Hibiscus debris were used as indications of human management. On three sites (# 1,# 3,#4, and #5), the edge was the strip of grass on the side of the Moorea coast-road, and on two sites located on private property (#2 and #6), the edge was indicated by the transition from *H. tiliaceus* forest to bare mineral soil. Each edge was a continuous 50 to 100 meters of *Hibiscus tiliaceus* forest bordering a stand of *H. tiliaceus* forest over 30 meters deep.

Site 1, located across from the Gump Research Station facility on Cooks Bay, was a *H. tiliaceus-Inocarpus sp.-Cocos nucifera* stand, on a 45 degree slope. Site 2, located on the property of Mari Mari Kellum at Opunohu Bay (pk 17.5), was a *H. tiliaceus-Inocarpus sp.-Miconia calvescens* stand. This site was the only one sampled with an edge

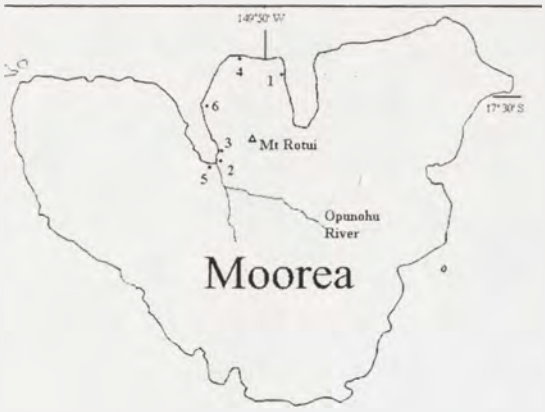

Figure 1. Map of Moorea listing Primary Study Sites (1-5) and one supplementary study site (6).

oriented perpendicular to the Moorea coast road, and situated more than 1 meter off of the road. The site was 100 meters off of the road, on the South–East end of Mari Mari's property, along the mouth of the Opunohu River. Site 3, located at pk 16.5, was also a *H. tiliaceus-Inocarpus fagiferus.-Cocos nucifera* stand, and had a 100 meter edge, and a 30° slope. Site 4, located at pk 14 had the shortest edge, 50 meters, and also had distinct edges on both flanking sides of the plot due to adjacent properties. Site 5, located at pk 18 was a pure hibiscus stand in a mud flat on the South border of the mouth of the Opunohu River. The 100-meter edge had ornamental te plants, *Cordyline fruiticosa,* along the South border. Site 6, at pk 14, was the supplementary site and was located in a private yard. The edge was 100 meters long and the stand was *H. tiliaceus.-Cocos nucifera* dominant with a 30° aspect.

Ant presence on Landscape Level

At 20 random sites, six leaves were hand selected from *H. tiliaceus* and from 2 other dominant plant species (trees were selected over smaller plants when available) and sampled for ant abundance. Sites were selected by the placement of pk markers, and subsequently by the presence of Hibiscus at each marker. Pk markers from 1 to 26 leading North from the Moorea airport, along the Moorea coast-road, were used as random sample points, as hibiscus distribution was unrelated to the location of the pk markers. For a marker to be a useful sample point it had to have Hibiscus and two other plant species present, thus 6 different pk markers were eliminated due to limited vegetation.

Spatial Distribution of Ants within a Hibiscus stand: Branch and Leaf Level

The five primary sites were sampled in order to address questions concerning the spatial distribution of ants across a Hibiscus stand on both stand and branch level of organization. From the "edge" at each site, two 30 meter transects were established at two random points on the "edge" line and run perpendicular to the edge into the patch. One branch within my arm's reach (2.16 meters) was sampled every five meters along the transect line. If no branch intersected the transect line, the closest branch within the 5 meter range between the target number and the next consecutive interval was sampled. On each branch the eight most distal leaves were examined. Ant abundance and position, leaf herbivory, petiole length and gland color were noted for each leaf sampled. Ant abundance per branch was summed for each of the seven ranges of distance on the transect. Canopy cover was recorded for each transect. Novel ant morphs were hand collected during the sampling period in order to compile a complete collection of ant species found on Hibiscus leaves. After performing preliminary identifications in a lab on Moorea, the samples were preserved in 70% ethanol and shipped to U.C. Berkeley for identification.

Site 6 was selected and sampled to determine ant distribution on single *H. tiliaceus* leaves, while not inadvertently duplicating data points from any of the five Primary Sites. 20 branches were sampled along a transect that was aligned identically to those at the Primary Sites. The first 20 individual branches that intersected the transect line, within my arms reach, were sampled as follows. Two leaves from each branch were selected from the 8 most distal leaves on the branch, by assigning each leaf its respective number for its position on the branch (ex: the most distal leaf was #1, the second most distal leaf #2, etc.) and randomly selecting two of the numbers from a random number table. If no ants were present on the leaf, the next leaf towards the tip was selected until a leaf was found with ants present. All samples had at least two different leaves containing ants. 40 leaves were sampled using this method and quantified as follows: Leaves were measured vertically and horizontally to estimate leaf size, gland length and the width of the spread between the two most distant glands were measured to estimate potential feeding area, number of ants in feeding area and number of ants elsewhere on the leaf were tabulated, and % herbivory was estimated. Ants present on the petiole were ignored, as their intended destination on the leaf was unpredictable.

On each leaf, the number of ants and their positions "on or off" of the glands were tallied, and members of different species were collected.

Ant Insect Interaction

At the 5 Primary Sites, time limited sampling was applied to collect data on ant vs. insect abundance across hibiscus branches. Many of the same constraints outlined above applied to this sampling process, such as potential branches were those within my arms length and different species of ants were grouped together, rather than tallied individually. For this component of my study of ant distribution, the sampling period was one hour, and sample branches that were not finished at the end of the period were completed. At each of the five sites sample branches were quantified for total number of ants on a leaf, number of ants on the glands, number of total other insect individuals, types and number of kinds of insects, and percent leaf herbivory.

Data Analysis

All statistical analyses examining patterns of ant abundance within the *H. tiliaceus* landscape were performed using the statistical software Systat© and Jmpin©. The tests used were one-way multi-factor Anova tests with Tukey matrixes and Wilcoxon signed ranks tests. Linear regressions and correlations were also generated for data pattern detection. All graphical figures were created using Microsoft Excel and Systat. Histograms were created to determine how closely data followed the normal distribution, and data that did not follow the normal curve was either transformed appropriately or analyzed using nonparametric data tests (Zar 1999).

Results

General Observations

The hibiscus trees sampled were identified as *Hibiscus tiliaceus* var. *tiliaceus* Linneus., Sp. Pl. 694, 1753 (Rakshit and Kundu 1970). The phenotypic differences between beach hibiscus populations found at different elevations on Moorea has yet to be studied, however visible leaf color differences and differences in mean leaf size suggest there is some phenotypic variation between locations.[1] Six species of invasive ants were identified feeding on the extra-floral nectaries of the hibiscus from the six sites along the North section of the Moorea coast road. Five species, *Pheidole fervens, Pheidole oceanica, Pheidole umbonata, Tetramorium pacificum* and *Tetramorium tonganum* are members of the sub-family Mymrcinae, and the sixth species, identified as *Paratrechina longicornis*, is a member of the sub-family Formicinae (Wilson and Taylor 1967). Voucher specimens for these ant species are deposited at the University of California,Berkeley's Essig Museum of Entomology, and voucher *H. tiliaceus* leaf samples are deposited in the University Herbarium, University of California, Berkeley.

Beach hibiscus leaves of all ages and at all sampling locations, had grooved glands from the base of the petiole radiating out along the primary leaf nerves, approximately 3 to 20 mm towards the edge of the leaf. The glands were furrows of light green or deep pink tissue in young leaves and green tissue with reddish scars in older leaves. Each leaf had on average 3 glands, but gland number ranged from one to seven on some leaves. One individual to many ants occupied spots along these glands and were observed to stick their mouth-parts into the leaf tissue repeatedly. Under the microscope ants were observed to puncture the gland and cause a bubble of fluid to ooze from the puncture. The fluid was then orally injested by the ants. I tasted the liquid and found it to be sweet, however no other tests were performed to determine its chemical composition.

Ant presence on Landscape Level

Of the 21 plant species found in association with *H. tiliaceus* at the 20 sample sites, only five had ants present on their leaves (*Cordyline fruticosa, Barringtonia asiatica, Vigna marina, Leucaena leucophylla, Hibiscus rosa-sinensis).* Ants were absent on the leaves of the following species: *Inocarpus fagiferus, Cocos nucifera, Inga edulis, Hernandia nymphaeifolia, Calophyllum inophyllum, Manihot esculenta, Morinda citrifolia, Eugenia cumini, Spathodea campanulata, Terminalia glabrata/catappa, Dendrocalamus giganteus, Magnifera indica, Ipomea pes-caprae, Phyllanthus urinarius, Colocasia esculenta and Lantana camara* (Fosberg 1996; Petard 1986). A Wilcoxon signed ranks test, pairing hibiscus and the other two plant species found in proximity to it for each of the 20 sample sites tested and rejected the null hypothesis that there was no difference between the number of ants found on each of the three species (p-values 0.002 and <0.001) (Table 1).

[1] For the purpose of this study, differences within coastal populations of *H. Tiliaceus* were assumed to be negligible in terms of effect upon ant behavior.

	A	B	C
A	1.000		
B	0.002	1.000	
C	0.000	0.225	1.000

Table 1. Wilcoxon Signed Ranks Test two-sided probabilities using normal approximation for ant abundance on *Hibiscus tiliaceus* (A) versus species B (B) and species C (C) found in proximity to it.

Spatial Distribution of Ants within a Hibiscus stand

Canopy cover over the 5 Primary sites averaged 75%, and ants were found on hibiscus leaves at all sites. The ant-abundance data was transformed, by taking the cube root of the set, to better approximate a normal distribution, and a one-way Anova showed that ant abundance per branch (one branch was equal to the sum of ants sampled on the eight most distal leaves) when compared to distance from the edge was not significant (R^2=0.130, p-value=0.173). 18.57% of the branches sampled did not have any ants.

Ant presence on Branch and Leaf Levels

A one-way Anova test with eight factors and a Tukey matrix compared per-leaf ant abundance to leaf position on a branch of hibiscus. The analysis calculated significant differences between leaf position and total number of ants on the leaf (R^2=0.092, p-value<0.001)(Table 2). Leaf position versus number of ants on the glands was also significant (R^2=0.108, p-value<0.001). The mean number of ants for each of the 8 most distal leaves on a branch of a hibiscus from the five primary sites were all less than 1, and leaf #1 had significantly more ants than any of the leaves below it on the branch, excluding leaf #2 (figure 2). 72% of the leaves sampled did not have any ants.

	Leaf 1	Leaf 2	Leaf 3	Leaf 4	Leaf 5	Leaf 6	Leaf 7	Leaf 8
Leaf 1	1.000							
Leaf 2	0.767	1.000						
Leaf 3	0.000	0.055	1.000					
Leaf 4	0.000	0.044	1.000	1.000				
Leaf 5	0.000	0.048	1.000	1.000	1.000			
Leaf 6	0.000	0.055	1.000	1.000	1.000	1.000		
Leaf 7	0.000	0.067	1.000	1.000	1.000	1.000	1.000	
Leaf 8	0.005	0.187	1.000	1.000	1.000	1.000	1.000	1.000

Table 2. Tukey matrix displaying p-values for differences in total ant abundance between different leaf positions on a branch. Leaf numbers correspond to positions on a branch relative to the most distal leaf which is considered "Leaf 1". Values lower than 0.05 represent significant differences in ant abundance between two leaf positions.

A Wilcoxon signed ranks test was used to evaluate ant distribution on hibiscus leaf gland area compared to ant distribution on the rest of the leaf surface area. The null hypothesis that the density of ants on the gland area (extra-floral nectaries) was not different from the density of ants elsewhere on the leaf was tested and rejected (p value <0.001). Average ant density was 3.355 ants for the gland area and 0.003 ants for the rest of the leaf surface area.

A correlation between number of ants feeding on glands and number of gland structures present per leaf was not significant (Pearson's correlation matrix p-value 0.174). A line graph, however, displaying the relationship between number of ants and number of glands on a leaf shows the highest abundance of ants were found on leaves with approximately 6 glands (Figure 3).

Ant-Insect Interactions

All ant-insect interaction data was transformed to better approximate the normal distribution by taking its square-root. Average leaf herbivory across the five Primary sites was 7.06% per leaf, and the null hypothesis that herbivory on leaves with ants was equal to herbivory on leaves without ants was tested with an Anova and could not be rejected (p-value= 0.508. R^2=0.001). Linear Regressions comparing ant abundance by location on the leaf to number of insects, number of flying insects, number of sedentary insects and number of insect types on hibiscus leaves generated only two significant relationships (Table 3). The insect groups sampled were separated into informal groups and leaf-hoppers, aphids, mites, grasshoppers, caterpillars and lacewings were all encountered. Linear regression comparing number of ants found not feeding on the extra-floral nectaries to the

number of insects present on a leaf was significant (p-value = 0.030, R^2=0.015), and a line graph displays the non-linear trend in the relationship (Figure 4).

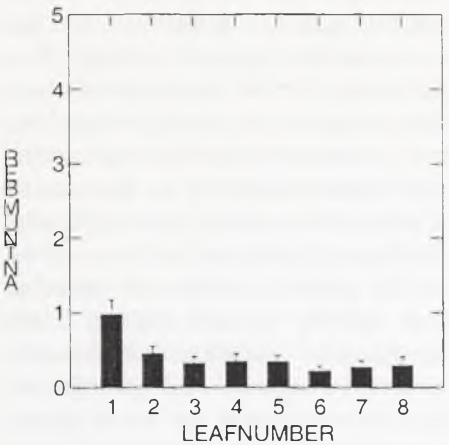

Figure 2. Mean number of ants per leaf position on a branch of *H. tiliaceus* and Standard Error bars for mean ant number. Graph generated in Systat.

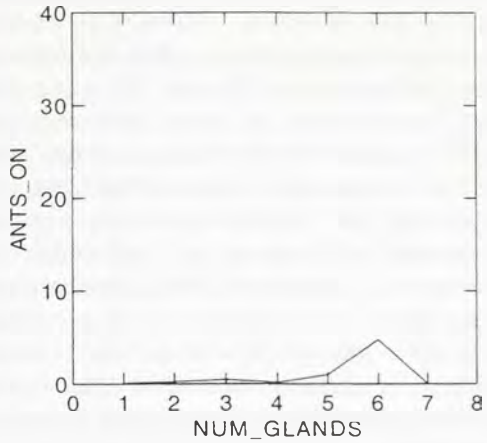

Figure3. Number of ants on the extrafloral nectary glands of a *H. tiliaceus* leaf compared to the number of glands present on a leaf. Graph made in Systat.

	Total Ants On Leaf		Total number ants not on glands	
INSECTS OTHER THAN ANTS ON LEAF:	P-value	R^2	P-value	R^2
Number of Insects	.042	.014	.030	.015
Number of Flying Insects	.169	.006	.169	.006
Number of Sedentary Insects	.077	.010	.077	.010
Number of Types of Insects	.259	.004	.259	.004

Table 3. P-values and R^2 values for regressions between ant position and insect abundance. Ant abundance by position was the independent variable. Significant values are p-values below 0.05 and R^2 values above 0.3.

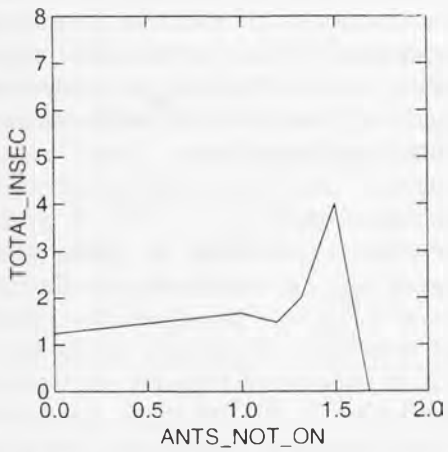

Figure 4. Line graph showing relationship between numbers of ants not on gland structures vs. total nuber of other insects present on the leaf.

On some beach hibiscus trees ant colonies were found under the bark of older, decaying branches that were propped against the living branches or resting against the stem of the tree. Many colonies were physically connected to live hibiscus trees, however no colonies were found established on the living tissue of *H. tiliaceus*. Ant collections revealed that at least six species of ants can be found feeding on beach hibiscus glands along the North shore of Moorea. At a number of sites, more than one species was found feeding on leaves of a single branch, however ant species distribution was not measured in my and I am unable to provide more than qualitative observations on species interactions.

Discussion

General Observations:

The presence of extrafloral nectaries on the underside of *H. tiliaceus* leaves is supported by

observations made from hot-house samples of *Hibiscus sp.* in Germany (Janda 1937). Janda suggests that in many species of hibiscus the glands actually serve to reduce transpiration and/or exude toxins from the plant. There is no mention of potential symbiont partners in this discussion of Hibiscus gland structures, although the nectar from the glands was found to have complex sugars (Janda 1937), suggesting potential as a food source for insects. The internal structure of the Malvaceae clade, including the placement of *H. tiliaceus* with respect to other relatives, is not well understood (Alverson et al. 1999), and DNA investigation could reveal that *H. tiliaceus* is not as closely related to other hibiscus species as was believed. Perhaps it is more closely related to other species of tropical mymecophytes, which would explain its appeal to ants.

Ant presence on Landscape Level

Analysis demonstrated that ant abundance is significant on beach hibiscus when compared to leaves of other plants in its vicinity, supporting the observation that ants are not uniformly across the foliage landscape in Moorea. A single species in the group of associate plants had more ants than the hibiscus at the same location but the anomaly can be explained. The ornamental Te plant, *Cordyline fruiticosa,* had 20 ants per leaf at the one sample location where it occurred, however when I inspected other Te plants along the coast I did not find the same surplus of ants; most of the plants in fact had no ants present. The most likely explanation for the high ant abundance on the sample related to the sample's proximity to a popular picnic area, and the abundance of sweet garbage deposited in the mud-flat area where the sample was found.

Spatial Distribution of Ants within a Hibiscus stand:

The absence of a significant ant distribution pattern across *H. tiliaceus* dominated stands suggests that the ant fauna feeding on *H. tiliaceus* nectaries are not experiencing high levels of competition for the nectaries from other insects, therefore they are not forced to occupy marginal habitats such as human maintained "edges". Results from analysis of ant distribution relative to the edge also revealed the extent of resource availability. The presence of unoccupied branches, approximately 19% of all those sampled, suggests that the level of competition necessary to motivate an ant colony to protect its food source has not developed yet. Abundance of insects with similar nutritional needs is not high enough to exhaust the *H. tiliaceus* resources and necessitate aggressive protection of the resource.

Ant presence on Branch and Leaf Levels

Ants displayed a distinct preference for the newest leaves on a branch (leaves #1 and #2), thus an implied preference for the nectaries on those leaves. Active nectaries are usually found on younger portions of the plant, and nectary "death" (the cessation of nectar secretion) in fact occurs soon after the plant-part is fully mature (Bentley 1977). My findings suggest the presence of an established feeding pattern, as ants use sensitive chemical cues in order to organize workers in the colony, and the choice of leaf #1 and #2 over the other leaves is both coordinated and non-random. On average no ants were found on leaves below leaf #2 on a branch, and as these nectaries were probably dead, previous exploration and establishment of feeding patterns would keep ants off of these lower leaves in the interest of efficiency.

Ants were shown to prefer the gland area of the leaf over the rest of the leaf surface, clearly defining the nature of the ants interest in *H. tiliaceus* leaves as a food provision. Leaves with 6 glands had the greatest number of ants present per leaf. The peak abundance on leaves with 6 glands suggests either that more ants can feed as there are more glands thus more feeding space, or that leaves with 6 glands also produce more nectar, thus attract more ants. The second possibility would need to be further tested as phenotypic differences that have not already been examined could be determining gland numbers on leaves, and nectar production, of phenotypically different *H. tiliaceus* populations. If there is a phenotypic difference between populations, this could lend evidence to the development of a sophisticated ant-plant mutualism as ants are found to favor plants with more glands and through their preferences, could be selecting these phenotypes.

Ant Insect Interactions:

Most of the insects found on beach hibiscus leaves were not in proximity to the gland structures, and few were found directly competing for gland resources. Significant results for total number of ants versus total number of insects on a leaf, and number of ants off of the glands versus number total number of insects on a leaf suggest ants are not aggressively protecting leaves with active glands. Both relationships were positive, and in light of the data two explanations are reasonable. Either the ants are not protective of their food source, and overall insect density per

leaf (ants and other insects) depends upon the carrying capacity of the leaf, which would explain the steep fall off in the graph (figure 4), or perhaps more insects on the leaf encourage more ants to accumulate and protect their feeding rights. Both total number of ants and number of ants not on glands are greater when total number of insects is greater, suggesting that total ant number per leaf increases with the increase in other insects. Causation in the relationship however is still speculative. It is appears that ants are not fighting off any of the groups of insects that I sampled on hibiscus leaves, yet Bentley (1976) suggests that even in the absence of an obligate ant-plant mutualism, herbivore damage can be reduced simply by the presence of ants visiting the nectaries. I was unable to test this hypothesis as I found no hibiscus sites that lacked ants to use as controls for herbivory rates. However, were this to be the case in these coastal hibiscus patches, there is future potential for the ant-plant symbiosis to become more obligate, as selective pressure would favor plants with less herbivory, thus plants with ants. As plants with ants would be those with productive nectaries, hibiscus plants with preferable nectaries would be selected for.

Studies of interspecific ant interactions on Moorea suggest that two of the ant species identified feeding on *H. tiliaceus* leaves, *Pheidole fervens* F. Smith and *P. oceanica* Mayr are behaviorally and numerically dominant in the communities where they occur and maintain mutually exclusive distributions (Morrison 1997). The increase in ant numbers on leaves with more insects could be accounted for by the dominance, atleast within different species of ants, of two species found feeding on beach hibiscus nectaries. The dense structure of *H. tiliaceus* stands provides a negative incentive for ants to colonize a single beach hibiscus tree, as the food resources they desire are abundant across the landscape. If *H. tiliaceus* stands rather than individual trees are considered as individual hosts, respective to ant feeding, it could be argued that ant colonies are already living on their host, and have satisfied a significant requirement for ant-plant mutualism.

Conclusions

It is possible for a plant that already possesses extra-floral nectaries to develop into a myrmecophyte, or ant-plant, under conditions in which the plant has increased fitness due to ant protection. From the research conducted and described, the existence of a feeding symbiosis between non-native species of ants and the *Hibiscus tiliaceus* tree on Moorea is apparent. Further, due to the recent arrival of these ant species (Morrison 1995), the relationship, if maintained, has the potential to develop into an ant-plant mutualism granted a number of biotic conditions are met. 1.) An overall increase in the insect population density on Moorea, and specifically in *H. tiliaceus* patches, is necessary to promote competition for the food resource 2.) Direct competition for gland nectar from other insects is necessary in order to force ants to eliminate potential herbivore predators. 3.) Niche vacancy must become less pronounced on Moorea and force ants to establish their colonies closer to their food source. *Hibiscus tiliaceus* survived and flourished on the island of Moorea long before the introduction of ants, and perhaps in the absence of resource competition, the *H. tiliaceus* tree ecosystem currently provides little incentive for the ants to abandon their generalist lifestyle.

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Intertidal and Supra-Littoral Microbial Mats of the Temae Mudflat on Moorea, French Polynesia

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ABSTRACT. Microbial mats covering a partially inundated mudflat on the island of Moorea, French Polynesia were studied during October and November 2000. Initial surveys indicated the presence of seven phenotypes. In order to test this classification and to characterize and describe the mats, 33 mats encompassing all seven types were examined using light microscopy. The mats were dominated by cyanobacteria belonging to the genera *Lyngbya* and *Microcoleus*. Other cyanobacteria genera including *Oscillatoria*, *Phormidium*, *Xenococcus*, and *Chroococcus* as well as unidentified cyanobacteria and diatoms were also present. Species dominance and frequencies differed between the pre-defined types. All mats were vertically stratified, although to different degrees, and thickness ranged from 1mm to 30 mm. Although the number of laminations differed, most mats had an upper green gelatinous layer and a lower reddish layer. A PCA analysis comparing the environmental and compositional data for 27 mats challenged the pre-defined categories, suggesting that the mats are better grouped into four categories. These results along with a cluster analysis and findings in the literature indicate the presence of two primary types: a Smooth/Reticulate mat and a Blister mat, as well as two types along the periphery of these in various stages of lithification. Extensive extracellular polymer production was present in all mat types, and allowed the mats to bind sediment. Grazing and bioturbation were present in most mat types, and consisted primarily of nematodes and amphipods. Average salinity of the groundwater was 6.8% and temperatures ranged from 27.5-48 °C, indicating an extreme hypersaline environment similar to those characterized for microbial mats in other tropical regions.

Introduction

Fossil records indicate that cyanobacteria were one of the first forms of life to exist successfully on earth. As a group, they are thought to have introduced oxygenic photosynthesis, changing earth's early atmosphere and the production of organic matter (Golubic 1994). Their unusual success in the primordial environment in which few other organisms could develop or survive allowed cyanobacteria to diversify in form and function. Much of this rich record is preserved in organosedimentary structures known as stromatolites. Thought to have dominated intertidal and subtidal environments through much of Earth's history, stromatolites are vertical structures consisting of many horizontal laminations. Each layer marks a historical border between the surface of an ancient microbial mat and water (Golubic 1994, Walter et al 1992).

Some ancient forms of cyanobacteria have continued to succeed and can be found in modern environments (Golubic 1994). Modern microbial mats, for example, are coherent organic layers formed by microorganisms at the surface of sediment, marine sediment, or rock (Des Marais 1990). These communities are often laminated and usually stabilize or bind sediment. Microbial mats are found in a wide variety of environments, including ice-covered Antarctic lakes, thermal springs, and on rocks with high dessication. These structures are most widely distributed and studied, however, in hot springs and marine intertidal and hypersaline environments (Bauld et al 1992). Studies indicate that the distribution of cyanobacteria "on protected and subtidal flats in marine coastal environments is cosmopolitan, especially in tropical and subtropical regions (Hussein and Khoja 1993)."

The habitats of these microbial communities are often extreme, characterized by hypersalinity, high temperatures, insolation, and other features (Golubic 1994, Bauld 1992). Cyanobacteria are also capable of tolerating extremes in the availability of "energy, nutrients, and water (Des Marais 1990)." The ability to survive in harsh conditions was likely influential in their development and success on the early earth and an important factor in their modern widespread distribution. In these conditions, cyanobacteria are usually unchallenged as the predominant compositional component (Golubic 1994).

Since microbial mats are thought to have been one of the oldest biological communities on earth, the study of their structure, composition, growth, and function offers insight into early life on earth. In addition, understanding the development of diversity and its preservation in stromatolites is important to searches for evidence of life in extraterrestrial environments. Mat communities have been found and studied in many regions of the world, particularly in Shark's Bay, Australia, home to a wide diversity of mats (Golubic

1985). Until now, no large cyanobacterial mat populations have been described from the Society Islands. Visual surveys of the mudflats along a marine channel on the island of Moorea, French Polynesia, during the month of September 2000 revealed a developed community of microbial mats on the mudflat surface in the intertidal to supra-littoral zones.

A wide variety in morphology was immediately evident across the community. Growth patterns, color, texture, size, and thickness all changed with increasing distance from the incoming tide and appeared to divide the mats into seven phenotypes. Research on these mats was carried out during October and November 2000 to test the following hypotheses:

1) The microbial mats live in an extreme environment characterized by hypersalinity and high temperatures.

2) The mat communities are dominated by cyanobacteria.

3) Differences in environmental characteristics and composition support the validity of the seven types indicated by the variation in phenotype.

The purpose of the study was to describe and categorize the mats on Moorea according to composition, the environmental parameters defining their habitat, structure, and phenotype. In addition, information on the structural characteristics of the mats, their interaction with the surrounding environment in the form of bioturbation or grazing, and their ability to bind sediment was collected. A more thorough understanding of the mat communities on Moorea would allow for additional insight into the form and functions of these cyanobacterial structures as well as for comparison with similar communities around the world.

Materials and Methods

Study site

The island of Moorea, one of the Society Islands of French Polynesia, is located at S 17°28" and W 149°46". At the northeast corner of the island lies the motu Temae (Fig 1). Although motus usually lie off the coast of an island, Temae is unusual in that it is directly connected to the main landmass of Moorea. However, this connection occurs only at its southernmost tip, and the remainder of the motu is separated by a lake and channel, which are filled with water from the Pacific Ocean. The water in this system is flushed daily as tides rise and fall, low tide occurring at approximately 6 am and high tide at approximately 12 pm. This pattern of flux has created an intertidal environment along the mudflats that border the channel between Lac Temae and its outlet to the lagoon, and the difference between the high and low water lines may be as great as 130m, depending on the lunar cycle.

Mudflats occur sporadically on both the east and west sides of the channel leading from Lac Temae to the Pacific. A number of accessible sites were surveyed for the presence of visually significant microbial mat communities. After a general survey of sites on either side, the mudflat bordering the east side of the channel just north of Lac Temae and Moorea International Airport was chosen as the research site, since, of the sites observed, its mat community appeared to be most developed and accessible. This site was named the Temae mudflat.

The Temae mudflat is approximately 210m by 300m, as measured at intermediate tide and described by its largest dimensions. It is partially covered by salt water daily at high tide, although the majority of the surface remained dry continually during the period of the study. Water enters from all borders along the channel. However, the most significant source of water for this intertidal area is found at the northeast corner, where tidal flux is extremely high and the largest colony of microbial mats is found. The eastern border of the mudflat is lined by vegetation dominated by Coconut and Hibiscus trees and *Cassytha*. A thick reed marsh begins 180 meters from the southeast end of the mudflat, and lines the boundary between the mudflat and other vegetation.

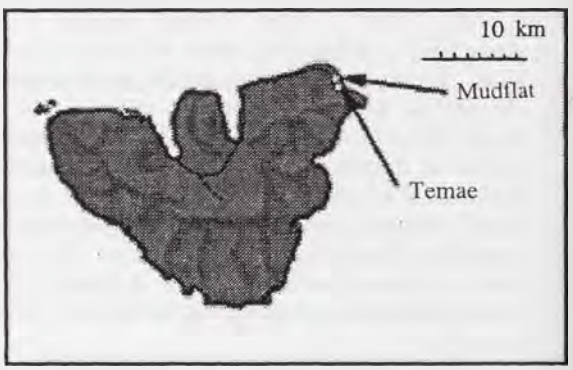

Figure 1. Map of Moorea showing Temae at the northeastern corner, and the location of the mudflat.

Mapping

An initial visual survey of the Temae mudflat indicated the presence of up to 8 phenotypically different types of mat. Types were defined as separate according to color, thickness, texture, and morphology. In order to map the distribution it was necessary to map the mudflat itself. The survey determined that the

necessary dimensions to include all eight types were approximately 300 by 210 meters. Choosing a point approximately in the middle of the mudflat, a baseline was laid from southeast end to the northeast corner, using a 50 m transect tape. Blue flags were placed every 10 meters and pink flags every 50 meters, and were connected with string, so that the line was clearly visible from any point on the mudflat. The baseline ran from S 17°28.896', W 149°46.150' to S 17°28.778', W 149°46.220'. The transect tape was then taken out perpendicularly to the baseline to measure the distance to each peak and valley in the vegetation on the east side, and to the water on the other side. These values were plotted on graphing paper using the baseline as the x-axis. Points were later connected to create a map of the area.

The transect tape was then run out to the perimeters of the mudflat on each side of the baseline at 25 meter intervals. The points at which the different types of mats started and stopped along the tape and across the last 25 meters were recorded. This data was then translated to a map indicating the limits, but not density, of each type.

Environmental Data and Mat Collection

Of the eight types originally observed, seven were chosen for analysis. The eighth type was not included due to time restraints and its removed location. The mat types were separated into two categories: those with larger and smaller areas of distribution. These terms were defined relative to each other. Five types fell in the first category, two in the second. Based on a stratified random sampling strategy with proportional allocation, a random number table was used to choose coordinates for five points within the habitat of each of the mats in the first category, and four points for each type in the second category.

In the field, the mat closest to the random point was chosen for collection. Environmental data was taken at each point. Temperature was measured with a 76 mm Immersion Thermometer, which was inserted into the top layer of the map for two minutes. Using a hand trowel, a hole was dug until hitting the water table, and the depth was measured. Water was then allowed to fill the cavity and was collected using plastic bottles. The pH of the water was immediately tested with litmus paper. The remainder was held for dissolved oxygen tests. Two tests were done for each mat type using a LaMotte Dissolved Oxygen Model 7414 test kit.

The approximate dimensions of the mat were measured with a tape measure and its general morphology was noted. A piece of the mat approximately 15 cm by 10 cm was then cut and removed, and stored in a labeled zip lock bag. Additional 3 cm by 3 cm cubes were also removed to check for deeper layers since larger pieces were not stable and had a tendency to crumble.

Mat Analysis

Structure- The mats were analyzed in the lab, using a number of characteristics as a guide for description. To begin with, the general morphology of each mat was recorded, including color, size, and growth pattern, and the general appearance was sketched. The surface texture was then analyzed using an Olympus Tokyo dissecting microscope with G10X 22 lenses and a magnification of 2. Differences corresponding to color changes on the surface of single mats were noted. Each mat was then cut in half and the cross section examined using the dissecting scope. The number, color, texture, and height of possible layers in each mat were recorded. Height was measured using a Vernier Type 6914 caliper. A perpendicular cut was then made in the mats and the opposing cross section examined for any differences in layering, in order to insure that no growth patterns were overlooked.

Examination of the layers also involved study of the sediment found in the mats. All layers were closely studied in order to determine whether they bound sediment, and to what degree. This also involved determining whether any pattern existed in sediment orientation. In addition, the layers were examined for information on mat fabric, in particular to determine whether organization was patterned or random.

Composition- After the preliminary structural analysis of the mats, a compositional analysis was performed. In each type, a random mat was chosen for a detailed layer approach. This consisted of analyzing two slides from each layer observed in the earlier analysis. The slides were prepared by slicing 3mm by 3mm squares from each layer, and placing each piece on a frosted micro-slide. The slices were then compressed with dissection tools. After adding two drops of water and a cover slide, the slides were analyzed with an Alphaphot-2 YS2-H Nikon compound microscope. Using the 40X objective, the organisms at the center of the view were recorded at 25 points on the slide. The slide was moved approximately 0.5 mm with each turn, and the general composition of the view recorded with points. If multiple species were seen under a single view, points were recorded proportionally. Unknown species were sketched or photographed.

For the remaining mats, a general compositional study was performed. Two mm by two mm slices were

taken from the top layers, defined as the topmost layers not yet separated by layers of sediment over 0.5 cm, and slides made in the same fashion as above. The slides were analyzed using the same method, but using 50 points. Initial pilot slides indicated that although representative proportions were clear at 25 points for a single layer, a higher number of points were necessary for combined layers due to the increase in organic material. Using a 50 point and 100 point species area comparison, 50 points were shown to be sufficient to give the correct compositional proportions. Five slides were prepared for the top layers of each mat. Additional slides were made if deeper layers were found within the mat. In addition, evidence of bioturbation and grazing was noted.

Physical Parameters

A final segment of the experiment examined the physical parameters of the environment in which the mats grow. During a 7-8 hour monitoring of the Temac mudflat, temperatures of each mat type were taken at sunrise, mid-morning, and at mid-day in order to determine the daily temperature range to which the mats are subject. In addition the levels of tidal rise and fall were observed and recorded. General observations of the mats were taken before and after rainfall. Samples of water collected from mats of different types during earlier data collection were combined in order to determine the average salinity of the environment. 250 mL (151grams) of water in a glass beaker were placed in a drying oven for approximately 7 days at 60°C. After all water had evaporated, the remaining salt was weighed and used to determine percent salinity.

Data Analysis

Data manipulation- For each of the 27 mats for which the 5-slide compositional analysis was carried out, the frequencies of all organisms over the 50 points were calculated. These were then averaged between the five slides. This produced average frequency data for all components within each mat. All percent data was transformed in Excel so that final data used in analysis was normal.

Tests-In order to determine whether the assigned types were actually valid categories, a Principle Components Analysis (PCA) and a Cluster Analysis were run using both environmental and compositional data. Both programs use chosen variables to determine the degrees of relatedness between all sites studied. PCA, run in Systat, compares the values for all variables between sites, which are entered under code names. Using this data, it determines which variables most strongly explain the variance within the data, and uses this to plot all sites on an ordinate diagram in which the two principal components, or variables, that explain the highest variation form the coordinate axes. Spatial distribution indicates the degree to which the sites relate to each other, with the most closely related sites being found in closest proximity, and takes all variables into account. Thus, pre-defined groupings are compared to the clusters resulting from statistical similarity.

The Cluster Analysis, also performed in Systat, similarly examines the relationship between all sampling sites. The program enters the number of common species between mats into a formula for Jaccard's Coefficient of similarity (J), which measures similarity according to the presence or absence of each species. The highest coefficient indicates the closest relationship between two samples. Once this is determined, new coefficients are calculated in order to determine the next closest relationship. This process is repeated until the degree of similarity between all samples is resolved. As a Q-mode comparison, the cluster analysis compares simply the absence or presence of species between samples rather than the relationship between the individual species within a sample. Thus, the analysis gives a rough index of similarity and does not compare differences in species frequency. The coefficients of comparison are used to draw a cluster tree, in which closely related sites are grouped together.

Both tests used the same variables. The environmental variables were temperature, water table depth, and pH. Compositional variables included the ten most common species. These were red alga, green bundles, green mass, thin green filaments, clear filaments, red mass, red dot, green cells, blue-black cells, and green bulbous cells (preliminary names rather than final identifications were used as labels).

Spearman test- a Spearman test was run in Systat in order to determine correlations between the various environmental and compositional variables. This analysis gives correlations between all entered variables as well as p-values stating whether they are statistically significant. In addition, a Regression test and Jmpin Spearman Correlation test were performed using data on the thickness of the mats and their distance from the water in order to test for a relationship between the two variables.

Results

Mapping, Identification, and Distribution

Seven types were initially identified according to phenotype, including thickness, color, morphology,

and texture. Five of these types occurred along a north to south gradient with increasing distance from the incoming tide.

The type closest to the tide was called "Thick Mat." This type was frequently covered with water by the tide. It was almost continuous in cover and had a firm consistency. The coloration of the mat was primarily green, dark green, and black, with occasional pink spots, and the surface was covered with an extensive network of small ridges interrupted by smooth green spots.

The next type was called the "Black/Red Mat," because of frequent reddish or pink spongy bumps on its surface. This type was rarely covered with water and was less continuous than the Thick Mat. Its surface was smooth and occasionally white because of dessication.

The "Furled Mat" type occurred adjacent to the Black/Red Type and had a unique appearance. Although much of the mat was smooth and black, its edges were curled up and unattached from the surface in large clumps. The area around this mat was often moist, but wasn't covered by the tide.

To the south of the Furled Mat, another type called the "Black Mat" was found. This type was gray to black in color. Its surface was flat and smooth and covered with sediment in some areas. The mat was less continuous than the Black/Red Mat and was irregular in shape. The Black mats were never observed to be covered by the tide.

A fifth type, the "Gray Mat" was heavily covered by sediment. Its surface was gray and smooth. The mat was dry and cracked, occurring in irregular shapes and was not continuous over the surface of the mudflat. This type occurred farthest from the incoming tide.

In addition, two types were found along the eastern edge of the mudflat. The "Raised/Flat" type was black to white in color and characterized by a combination of raised and flat topography. These mats were dry and brittle and clumps of mat broke off easily. The tide was never seen to cover this type.

The final type, the "Blister Mat" occurred in a small marshy region along the border of the mudflat. This surface of the mat was raised in small hemispherical and often hollow cauliflower-like bumps, which were often flipped or broken. The surface was dark green and flexible. This type was never observed beneath the tide.

Using these descriptive categories as guidelines, the distribution of the seven types was mapped on the mudflat (Fig 2).

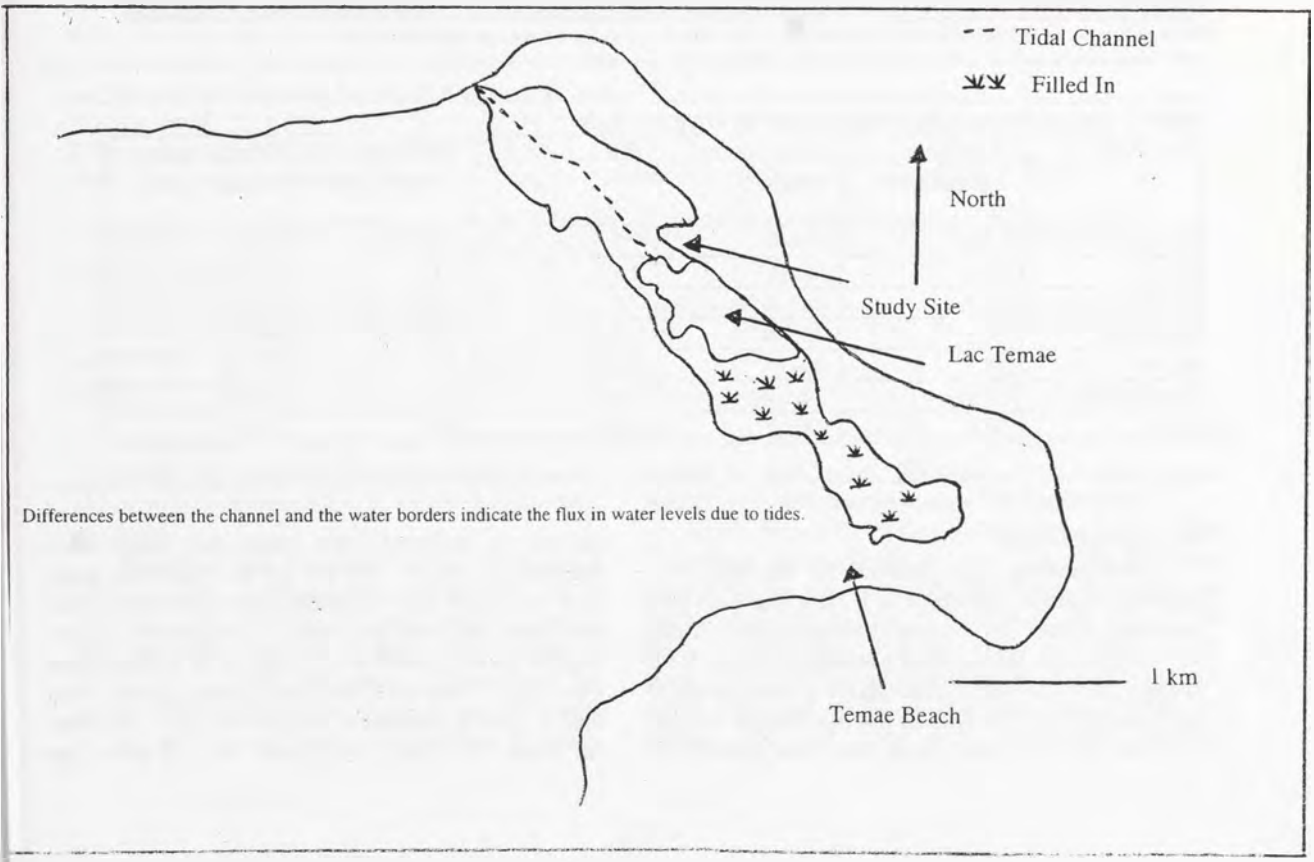

Figure 1b. Closer map of Motu Temae, showing the location of the study site with respect to Temae Beach and Lac Temae.

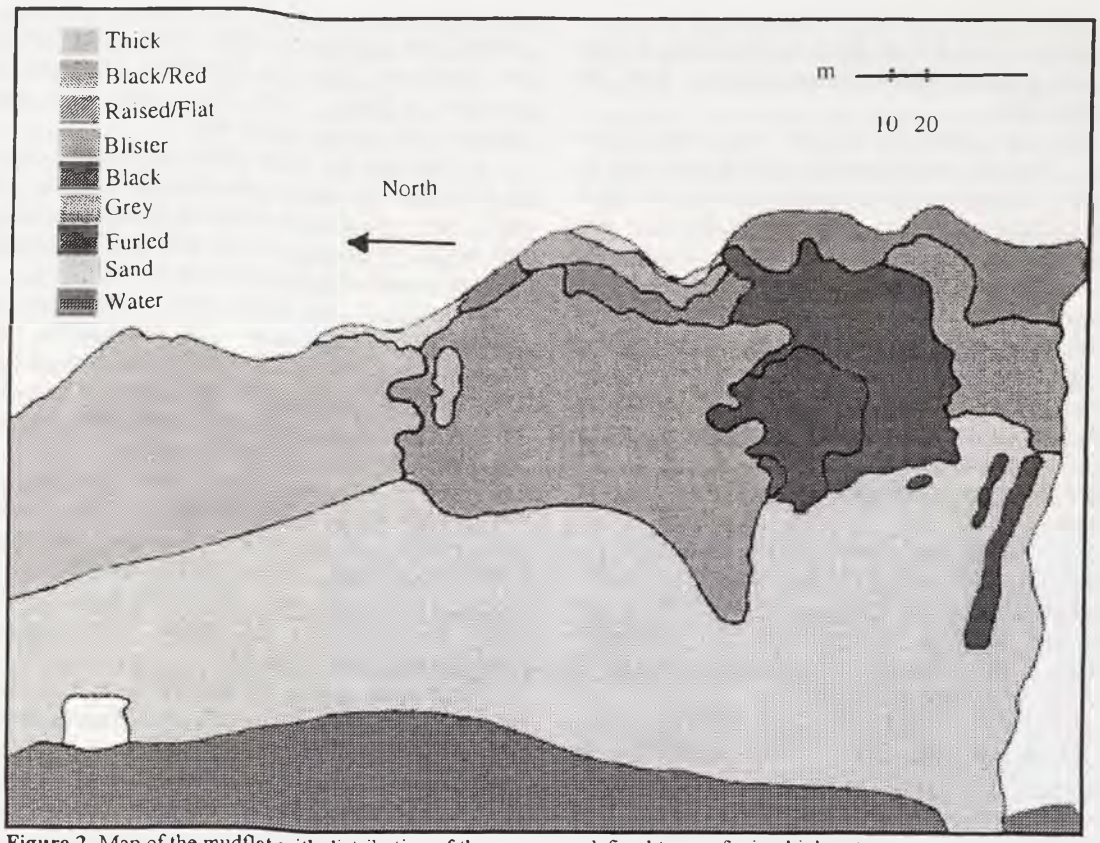

Figure 2. Map of the mudflat with distribution of the seven pre-defined types of microbial mat

Table 1. Average environmental parameters for the seven mat types.

Mat Type	Average Temperature °C	Temperature Range °C	PH	Dissolved Oxygen (ppm)	Water Table Depth (cm)
Furled	37.6	29-44	7.2	1.43	18.2
Gray	39.4	28-48	7.375	1.4	25.025
Black	40.8	28.5-47	7.4	1.35	20.3
Black/Red	36.3	28.5-47	7.22	0.2	19.1
Blister	34.3	29-39	7.875	1.2	22.85
Raised/Flat	33.2	27.5-42	7.68	2	27.86
Thick	36.6	28-42	7.08	0.7	17.6

Physical Parameters

Environmental data gathered for the mats was extremely variable. However, it is clear that each type possessed distinct environmental characteristics (Table 1). Temperature did not show a standard gradient with distance from the water, although the greatest range in daily temperature was seen in the type located furthest from the incoming tide. Types that were periodically covered or saturated with water had much lower temperature ranges. The pH for all types was basic, with the blister and raised/flat types showing a much more basic pH than the others. This may be due to rainfall the day previous to collection of data for those two types. Water with dissolved oxygen content lower than 2 ppm is unsuitable for marine life. Therefore, the dissolved oxygen tests show that all seven mat

types grow in anoxic conditions. Dissolved oxygen values are for groundwater since most types were never covered by surface water. The average salinity of the water in the water table was 6.8%.

Surface Texture

Gray Mat- The surface of the Gray mats was characteristically caked with small sandy sediment and fairly smooth, with occasional larger embedded grains. Clear filaments were visible on the surface of the sand, although no pattern was seen in their organization. Occasional dark green filaments also occurred on the surface. Some mats had rare darker greenish/yellowish surface spots, which appear to be covered with a gelatinous yellow algae. The dominant form on the surfaces, however, was the sandy cover with translucent algae.

Black Mat- Similarly to the Gray mats, the surface of the Black mats was characterized by a porous sandy cover. However, the Black mats had more frequent dark splotches, which appeared almost mossy. These were covered with thick mats of thin dark green filaments, more visible around the edges of the dark spots. They appeared to be arranged randomly. The yellowish gelatinous algae seen on the Gray mats was also seen occasionally on raised portions of the Black mats. In addition, one of the Black mats contained a raised reddish/gray spot, which had a spongy texture when viewed under the dissecting scope.

Furled Mat- The Furled mats were generally gray and flat in the center and became black/green in color toward the edges. The central parts of the mats were attached to the sediment beneath, while the edges were raised, bumpy and lifted up easily from the substrate. The gray center was generally covered in sand and occasional dark, green filaments and appeared wet and spongy when magnified. The edges were irregular, with many protrusions, and appeared mossy. The filaments were far more numerous on the surface of the darker areas, and when scraped off and examined with a compound microscope, appeared to be dominated by red alga, with occasional green bundles.

Raised/Flat Mat-The surface of this type was rigid and encrusted, usually covered with alternating flat segments and ridges and mounds. There was heavy pebbly sediment on the surface. This was covered by a layer of green gelatin of alternating thickness, in which thin light green filaments could be seen. Most of the surface was covered by small black and brown flecks. The raised mounds were usually darker, almost black in color, and appeared moist and mossy. The gelatinous base was found beneath this area as well, and was covered with dark filaments, forming the mossy texture.

Blister Mat- These mats appeared to be loosely bound to the substrate beneath. They were extremely knobby and occurred in alternating bumps. The entire surface was dark green/black in color and was covered in small sediment. The surface was mossy in appearance, extremely moist, and had a thick gooey algal cover. Occasional filaments and black dots were visible. A number of clear cylindrical filaments were visible, and appeared to be the feces of a grazer of the mats.

Table 2. Thickness of all seven pre-defined mat types

Mat Type	Furled	Black	Grey	Raised/Flat	Blister	Black/Red	Thick
Thickness Range (all layers) (mm)	4.7-22.4	2.9-34.2	1-2.3	2.45-14.4	3.8-6.3	12.1-30.35	5.4-20.65
Thickness of top layers (mm)	1.7-4.9	1.35-6.6	1-2.3	1.1-5.9	3.8-6.3	3-5.35	2.8-9.7

Black/Red Mat- The surface was covered with a gelatinous base, ranging from clear/pink to bright green in color. Within the green portions, small, light green filaments were visible. Grey portions of the mat had a sandy sedimentary covering over the gelatin. Darker black/green sections were also found on the surface and were characterized by thick masses of dark green filaments. Red knobs on the surface were soft, spongy masses when magnified.

Thick Mat- The surface topography of these mats was unique and extremely variable. The most noticeable feature was the presence of spidery ridges accentuated by the accumulation of lines of sand along the edges. The surface ranged from a lighter green to a dark, almost black green, and had occasional red spots similar to those in the black/red mats. A gelatinous base covered the surface, and was filled with small green bundles.

However, in the darker area, dark filaments were also visible and formed a thick cover over the mat, arranged in spreading, hair-like clumps across the surface. The mat was thick and firm, and felt almost hairy to the touch. However, occasional spots on the dark green portions were completely smooth and homogenous in color and texture.

Structure

Thickness- Mat thickness ranged from 1-34.2 mm. The wide variation was due to the fact that some mats had deep dark organic layers beneath thick layers of sediment, while other mats consisted only of the top brightly pigmented organic layers. When taking only the top actively growing layers into account, the thickest mats were most consistently found closest to the water in the Thick and Black/Red types (Table 2). The upper layers of all mats were cohesive, although larger pieces of Blister and Raised/Flat mats tended to fall apart. Lower organic layers and thick sediment layers were most common in Black and Furled mats, whereas the other mat types consisted primarily of the top layers.

Regression and correlation tests comparing thickness of the top organic layers and distance from water within the Thick, Black/Red, Furled, Black, and Gray types indicated a statistically significant relationship between the two variables (Table 3). Thickness and distance from tide are negatively correlated with a Spearman Rho value of -0.6990 and P-value of 0.0001. Thus, in general, thickness of the mats decreased as the mats occurred farther from the incoming tide. The regression test gave a R-square value of 0.468789 with P-value 6.01E-09, indicating a causal relationship between the two variables. Only the top layers were used since composition slides indicated that most living microorganisms occurred in these strata.

Table 3. Regression and Correlation Data for Distance from water v. Thickness of mats

Independent Variable	Dependent Variable	Spearman Rho (Correlation)	Correlation P-value	R-square value (Regression)	Regression P-value
Distance from water	Thickness	-0.6990	0.0001	0.0001	6.01E-09

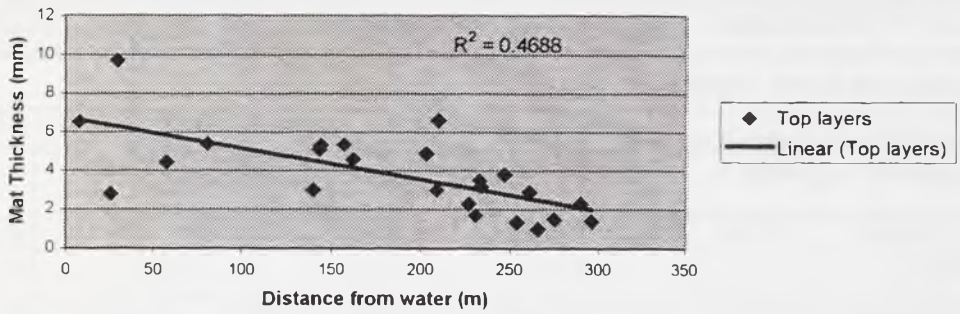

Figure 3. Regression graph for the relationship between the distance from water and mat thickness.

Laminations- All mats had some degree of lamination. Laminations were defined in this study as distinct horizontal layers of organic or inorganic material arranged in vertical rows in a mat. Thick and Black/Red mats were well developed. All mats in these types showed vertical laminations of thick organic layers. Furled and Black mats were also well laminated, but had thinner organic layers interspersed with multiple layers of sediment. Blister mats showed poor lamination; although this type had pigmented layers, they were not as cohesive as those seen in well-laminated mats. Laminations in Raised/Flat mats were interrupted by a cemented sediment layer/organic precipitation, while those in Gray mats were difficult to discern because of heavy sediment accumulation.

Pigmentation- Laminations were accentuated by the presence of pigmented layers. Although the number and color of layers differed between mats, the most common sequence involved the following structure: one to two upper green layers followed by a salmon brown layer. These were occasionally

interrupted by or repeated underneath additional layers of sediment.

Sediment

All mat types and all layers examined bound sediment. A number of differences were seen between the various types, however. For example, the Gray type was characterized by heavy sediment interspersed with thin algal filaments. This sediment consisted of grains approximately 1 mm in diameter, situated almost directly below the surface of the mat.

The Raised/Flat type also possessed an unusual sediment-binding strategy. This mat type was brittle and hard to the touch. The cross-section analysis indicated that this was due to the presence of a cemented layer of sediment or precipitate, approximately 0.8 mm thick, lying beneath the first layer of the mat. The sediment was white in color, continuous, and easily broken with pressure.

All other types were far more malleable. All bound sediment primarily between algal layers. Although grains were embedded within the algal layers, the greatest concentration of sediment in the remaining 5 types was found in thin strata between the very top layer and lower algal layers or beneath the combined top layers.

In addition, all types showed the accumulation of some sediment on the surface of the mat. This was most obvious in the Gray type, in which the entire top layer was coated with sand. Other types were more variable, possessing small areas that were covered with sand, and other areas with larger grains embedded into the surface.

Mat Fabric

The magnification of the tools available was not sufficient to discern any mat fabric. However, each of the types examined showed the presence of horizontal laminations parallel to the surface of the mat and following its topography.

Type Analysis

The Principal Components Analysis confirmed three of the predefined mat types, but challenged the grouping of the remaining four types. The Blister mat, the Raised/Flat mat, and the Gray mat were all plotted in distinct groups, indicating their validity as separate types based on compositional and environmental data (Figure 4). The Black/Red and Stratified mats were also plotted in clusters, although they were not nearly as removed as the three types listed above. Plots for the wet and black mats were interspersed, suggesting that the two compose a single type. These plots were also extremely close to those for the black/red and stratified mats. This indicates that all four types may actually be slight variants within a single encompassing type, and that they are more closely related to each other than to the other three types. The mats in these four types appear to be part of a single larger category occurring along a continuum defined by the changing frequency of the red alga, green cells, and temperature. Thus, the PCA data yields four final types, rather than the initial seven.

The PCA graph also included Eigenvectors, vectors representing each of the variables and explaining a percent of the variance within the data (Table 4). These vectors also explain the groupings described above, since the principal components explaining the most variance within the data were placed on the axis. For example, the frequency of green cells explained most of the variation along the x-axis. Temperature, on the other hand, decreased along this axis, but was not as influential. The frequency of green mass most explained location along the y-axis. This correlated negatively with the frequency of red alga. Thus, variation was most explained by the frequencies with which green masses and green cells were found in the mats.

	PC1	PC2	PC3
Eigenvalue	4.97	2.98	0.884
Percent	38.27	22.92	11.43
Cum Percent	38.27	61.19	72.63
Eigenvectors			
Temperature	-0.369	0.014	0.139
Water Table Depth	0.165	0.295	-0.176
PH	0.283	0.083	0.457
Red Alga	0.057	-0.542	0.149
Green Bundles	-0.378	0.162	0.014
Green Mass	0.004	0.525	-0.068
Blue/Black cells	0.348	0.143	0.133
Thin Green Filaments	0.289	-0.136	-0.438
Green Bulbous cells	0.370	0.058	0.264
Clear Filaments	0.198	0.299	-0.299
Green cells	0.400	0.027	0.296
Red Dot	-0.228	0.129	0.489
Red Mass	-0.126	0.402	0.1313

Table 4. Principal Component Variables for the 13 environmental and compositional variables of each plot. Eigenvalue gives the variance explained, Percent the percent of the variance explained.

The Cluster Analysis results further challenge the predefined types (Fig 5). The cluster tree produced with the data indicates that the Wet, Black, Black/Red,

Nonparametric Measures of Association

Variable	by Variable	Spearman Rho	Prob>|Rho|
WaterDepth	Temperature	-0.2416	0.2247
pH	Temperature	-0.3319	0.0908
pH	WaterDepth	0.1826	0.3621
Red Algae	Temperature	0.0575	0.7758
Red Algae	WaterDepth	-0.1267	0.5289
Red Algae	pH	0.0946	0.6387
Green Bundles	Temperature	0.7223	<.0001
Green Bundles	WaterDepth	-0.1593	0.4273
Green Bundles	pH	-0.3404	0.0823
Green Bundles	Red Algae	-0.1849	0.3557
Green Mass	Temperature	-0.0069	0.9729
Green Mass	WaterDepth	0.2912	0.1405
Green Mass	pH	0.1077	0.5928
Green Mass	Red Algae	-0.7024	<.0001
Green Mass	Green Bundles	0.1274	0.5267
BlueBlack	Temperature	-0.6597	0.0002
BlueBlack	WaterDepth	0.2245	0.2604
BlueBlack	pH	0.5611	0.0023
BlueBlack	Red Algae	-0.2830	0.1527
BlueBlack	Green Bundles	-0.4946	0.0087
BlueBlack	Green Mass	0.3301	0.0927
TGR	Temperature	-0.6044	0.0008
TGR	WaterDepth	0.0651	0.7471
TGR	pH	0.0593	0.7690
TGR	Red Algae	0.0311	0.8775
TGR	Green Bundles	-0.7970	<.0001
TGR	Green Mass	-0.1406	0.4842
TGR	BlueBlack	0.2694	0.1742
GreenBulbous	Temperature	-0.5457	0.0032
GreenBulbous	WaterDepth	0.4160	0.0309
GreenBulbous	pH	0.6276	0.0005
GreenBulbous	Red Algae	-0.0180	0.9289
GreenBulbous	Green Bundles	-0.6027	0.0009
GreenBulbous	Green Mass	0.1434	0.4756
GreenBulbous	BlueBlack	0.5781	0.0016
GreenBulbous	TGR	0.2776	0.1609
CIR	Temperature	-0.4032	0.0370
CIR	WaterDepth	0.0681	0.7357
CIR	pH	0.3194	0.1044
CIR	Red Algae	-0.4828	0.0107
CIR	Green Bundles	-0.2980	0.1312
CIR	Green Mass	0.2881	0.1451
CIR	BlueBlack	0.5427	0.0034
CIR	TGR	0.2078	0.2984
CIR	GreenBulbous	0.2301	0.2482
GreencELLS	Temperature	-0.6514	0.0002
GreencELLS	WaterDepth	0.3122	0.1129
GreencELLS	pH	0.7360	<.0001
GreencELLS	Red Algae	0.1275	0.5261
GreencELLS	Green Bundles	-0.6803	<.0001
GreencELLS	Green Mass	0.0149	0.9413
GreencELLS	BlueBlack	0.6836	<.0001
GreencELLS	TGR	0.2888	0.1439
GreencELLS	GreenBulbous	0.8952	<.0001
GreencELLS	CIR	0.2868	0.1469
Red Dot	Temperature	0.4182	0.0299
Red Dot	WaterDepth	-0.2979	0.1312
Red Dot	pH	-0.0685	0.7343
Red Dot	Red Algae	-0.2678	0.1768
Red Dot	Green Bundles	0.5837	0.0014
Red Dot	Green Mass	0.1376	0.4937
Red Dot	BlueBlack	-0.2507	0.2072
Red Dot	TGR	-0.5742	0.0017
Red Dot	GreenBulbous	-0.2507	0.2072
Red Dot	CIR	-0.2140	0.2838
Red Dot	GreencELLS	-0.2801	0.1570
Red Mass	Temperature	0.3612	0.0642
Red Mass	WaterDepth	0.1315	0.5132
Red Mass	pH	-0.0168	0.9339
Red Mass	Red Algae	-0.5422	0.0035
Red Mass	Green Bundles	0.3887	0.0451
Red Mass	Green Mass	0.5182	0.0056
Red Mass	BlueBlack	-0.1671	0.4049
Red Mass	TGR	-0.2473	0.2136
Red Mass	GreenBulbous	-0.1671	0.4049
Red Mass	CIR	0.1365	0.4973
Red Mass	GreencELLS	-0.1867	0.3511
Red Mass	Red Dot	0.3394	0.0833

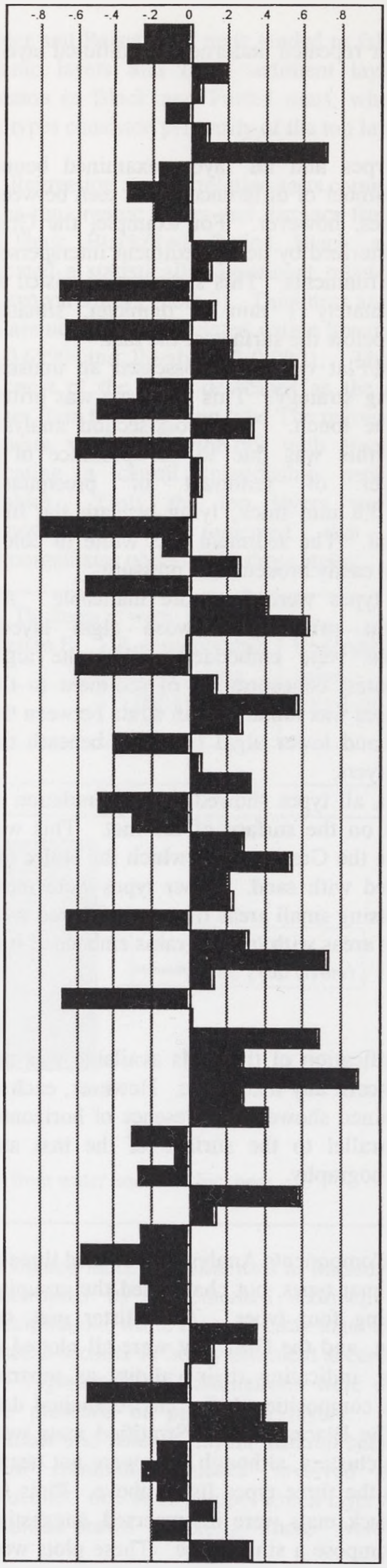

Table 5. Spearman correlations and p-values for environmental and compositional variables.

and Stratified types are all closely related and cannot be clearly split into smaller categories based on presence/absence data. It also indicates a possible relationship between the gray mats and the raised/flat mats. Some degree of grouping is also observed for blister mats: two of the three sites included are grouped together. In addition, the raised/flat mats appear to be more closely related to the blister mats than to the larger inclusive group of mats

Spearman Results

Many of the environmental and compositional variables showed statistically significant correlations with each other (Table 5). Green bundles and temperature; blue/black cells and pH; green bulbous cells and water depth, pH, and blue/black cells; clear filaments and blue/black cells; green cells and pH, blue/black cells, and green bulbous cells; red dots and temperature and green bundles; red masses with green bundles and green masses showed positive correlations. Negative correlations were seen between green masses and red alga; blue/black cells and temperature and green bundles; thin green filaments and temperature and green bundles; green bulbous cells and temperature and green bundles; clear filaments and temperature and red alga; green cells and temperature and green bundles; red dots and thin green filaments; and red masses and red alga.

Composition

During compositional analysis 38 different types of cyanobacteria were found in the mats. Since no identification resources were available in Moorea, all types were sketched and described for later identification. Original descriptions centered around the 12 most common types: red alga, green bundles, isolated filaments, clear filaments, green mass, red mass, red dot, blue-black cells, green bulbous cells, small green cells, thin green filaments, and red organic material. Additional types were also recorded. Cyanophyte taxonomy and identification is filled with controversy and difficulty, so positive identification of the different species was extremely difficult (Hussain and Khoja 1993). However, the dominant types of cyanobacteria were identified with the help of Dick Moe at the Jepson Herbarium and according to descriptions and pictures in Hoffman (1999), Tseng (1983), and Desikachary (1959). These were classified primarily by genera.

The cyanobacteria described as red alga was identified as the filamentous cyanobacteria *Lyngbya aestuarii*. This gliding filamentous cyanobacterium was characterized by a lamellated, dark brown pigmented, tubular sheath surrounding a single green trichome composed of stacked cells (Bauld et al 1992).

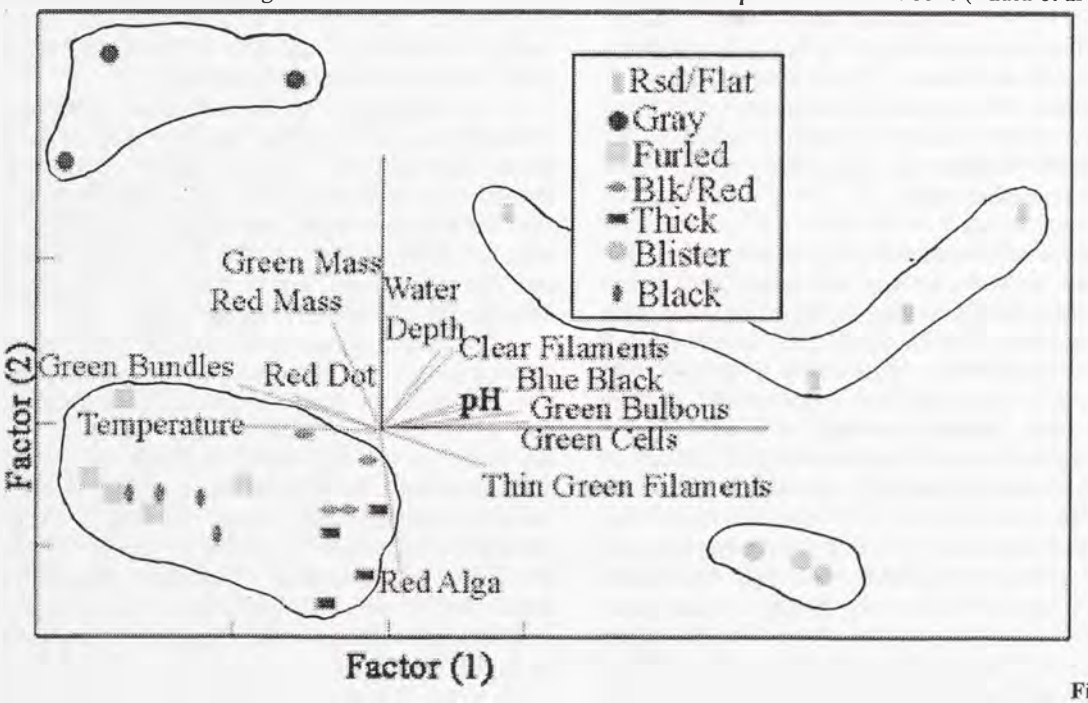

Figure 4.
PCA ordinate diagram showing relatedness of the different mats with spatial groupings. Principal components, green cells and green masses, form the axis.

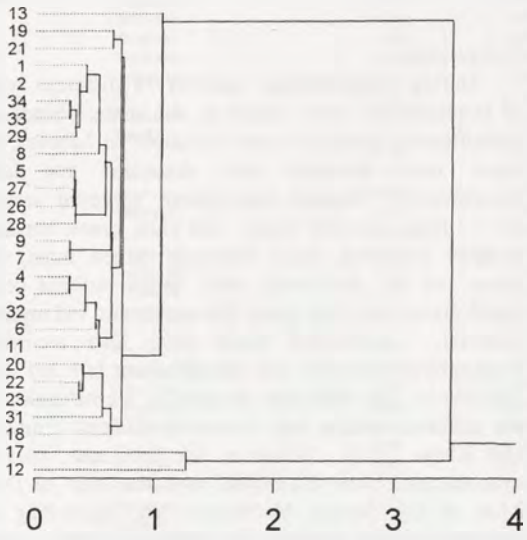

Figure 5. Cluster tree grouping related mats together based on the presence or absence of environmental compositional variables. 1-5: Furled mats; 6-9: Black mats; 11-13: Gray mats; 16-19: Raised/Flat mats; 21-23: Blister mats; 26-29: Black/Red mats; 31-34: Thick mats

Isolated green trichomes with similar dimensions may have been filaments of *Phormidium* or unsheathed *Lyngbya*. The term green bundles referred to *Microcoleus chthonoplastes* and occasional filaments of *Oscillatoria*. *Microcoleus chthonoplastes*, a gliding filamentous cyanobacterium, was typically found as a bundle of trichomes enclosed in a thin hyaline sheath (Bauld et al 1992). In addition to the bundles, isolated green filaments with similar proportions exhibited considerable movement. These were identified as *Oscillatoria*. Thin green filaments were most likely *Phormidium*, with some *Oscillatoria*. The red organic material was identified as extracellular polymer. Other types are identified below.

The two dominant genera of cyanobacteria within all seven types of mats appeared to be *Lyngbya* and *Microcoleus chthonoplastes*, although different types consisted more of one than the other. Other common genera included *Phormidium* and *Oscillatoria*. In addition, a species of *Chroococcus*, possibly *Chroococcus turgidus*, described as a large, green, bulbous group of cells, was found in the Raised/Flat and Blister mats. Similarly, small bright green cells resembling a *Chroococcus*, *Anacystis*, or *Aphanocapsa* genus were found only in the Raised/Flat and Blister mats. Clumps of blue/black cells were also found uniquely within these two types. These were tentatively identified as *Xenococcus chaetomorphae* but may also be *Entophysalis major*. The red dots remained unidentified. The green and red masses, which appeared to consist of smaller cells were also unidentified. However, it is also possible that these are clusters of anoxygenic bacteria, common in other microbial mats described from tropical areas. A number of other cyanophyte species were seen more rarely. For example, *Calothrix confervicola* was found in one mat. Long, brightly colored filaments were observed in a number of mats. These were bright pink, blue, or purple in color, and appeared to be wide and long but flat. In addition, a cluster of purple sulfur bacteria resembling images in Fenchel and Kuhl (2000) was seen in a slide of a thick mat.

The dominant species types were found in all mats although proportions differed between types. Rarer species were more commonly type-specific. Occasional diatoms were also observed in many of the types but were not common. Many less common cyanobacteria were not observed but consisted of both filamentous and coccoid cyanobacteria. Mats were generally dominated by filamentous cyanobacteria.

In addition, composition changed with depth within a mat (Fig 6). Although a certain cyanobacteria may have been the dominant genus within a mat, it was often found primarily within the first few millimeters of the mat, while the lower layers were often dominated by organic material, unidentified coccoid bacteria, and empty sheaths. Thus composition changed significantly along vertical gradients even within mats. In addition, changes in surface texture within a small area of the surface of mats indicated that composition frequencies were most likely not fixed for the mat types and varied within small areas. However, dominance by certain genera was consistent.

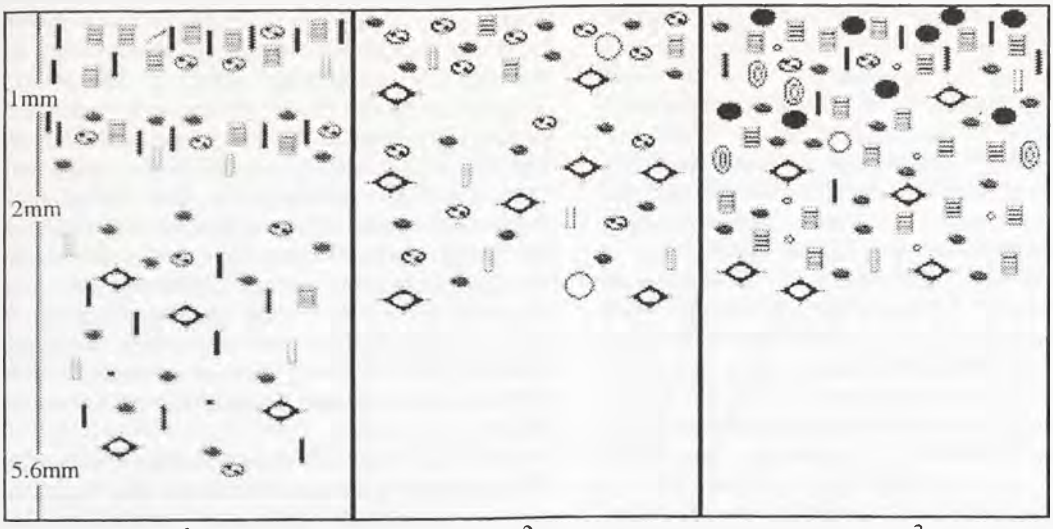

Figure 6. Schematic representation of changes in composition with depth in a mat. Mats are, from left to right, a Thick (1), Black Red (2), and Blister mat (3). Black lines are green filaments. Segmented lines are *Lyngbya*, circles with dots are *Microcoleus*, black smudges are organic material, small black dots coccoid cyanobacteria, triangular figures possibly *Phormidium*, clear lines clear filaments, large open circles green masses, concentric open circles *Chroococcus*, small open circles green cells, large black circles blue/black cells.

Although both *Lyngbya* and *Microcoleus* dominated the mats forming the Smooth/Reticulate type, one was usually more common than the other. *Lyngbya* was the dominant alga in the Thick Mat, whereas *Microcoleus* was most common in the other three types. Similarly, *Microcoleus* was the dominant alga in the Gray mats, which had almost no *Lyngbya*, and also had large numbers of clear filaments. Both *Lyngbya* and *Microcoleus* were common in the Raised/Flat and Blister mats, although these two types also had high frequencies of various coccoid cyanobacteria.

Bioturbation and Grazing

The most common grazers found in the mats were nematodes. These were particularly common in the raised mat type. Large numbers of amphipods were also found in this type. Other grazers seen included an unidentified clear worm, segmented yellow burrowing insects, and black flies. Many neritid, Ceritid, and other snail shells were observed in the sediment layers. No living megafauna grazers were observed in any of the mat types.

Discussion

The environmental parameters of the mats at Temae are similar to those gathered for microbial mats in other tropical regions. Salinities in the literature range from 3.5-11%, so the measured value of 6.8% is approximately average in comparison to other studies (Lopez-Cortes 1990). This salinity includes the mats among the ranks of hypersaline microbial communities. The anoxia observed in the groundwater collected is a common phenomenon in the lower layers of microbial environments, which usually consist of a combination of aerobic and anaerobic microorganisms (Des Marais 1990). The pH for all sites was basic, between 7 and 8, with the most basic values gathered from the Blister and Raised/Flat mats. This agrees with data collected for most intertidal mats, which usually have an even more basic environment, ranging from 7.9-8.4 (Mir et al 1991). Similar mats are usually found in environments with high temperatures. The temperatures recorded at Temae are almost 10°C hotter than those seen in most other study sites. Given the data and comparisons, the mudflat at Temae can be accurately termed an extreme environment.

All mat types were laminated, although to different degrees. Lamination is most developed in mats dominated by filamentous cyanobacteria (Bauld 1992). The filamentous morphology is better suited to

trapping and binding both organic and inorganic material. The cell morphology of coccoid cyanobacteria is poorly suited for the construction of laminations (Bauld et al 1992). This explains the poor lamination observed in the Blister mats, composed largely of coccoid cyanobacteria, and the well-developed laminations in the Smooth/Reticulate mats, predominantly composed of filamentous microorganisms. Laminations were accentuated by differences in pigmentation between the different layers. Top layers were usually various shades of green, while lower layers were often salmon brown or yellowish in color. Differences in pigmentation are likely a result of changes in composition, which changed with depth in a mat (Des Marais et. al 1992). Different microbial components have been recorded to correlate with specific chlorophylls (Mir et al 1991).

In addition, all mats were composed primarily of an organic matrix of polysaccharide and empty sheaths. The organic material produced by cyanobacteria and the accumulation of sheaths are the major cause of the growth of cyanobacterial mats (Fenchel and Kuhl 2000). The extracellular polymers in addition to small filamentous cyanobacteria such as *Phormidium* also result in the cohesiveness observed in the mats (Bauld et al 1992). Furthermore, this organic matrix is influential in trapping and binding sediment, and is thus important to the stratigraphy that results in formations such as stromatolites (Mir et al 1991).

The accumulation of sediment may have an additional purpose. Sediment cover was most extreme on the Black and Gray mats. These are also located farthest from the incoming tide and are subject to the highest level of insolation and dessication. Studies of microbial mats in Saudi Arabia suggest that the accumulation of soil over mats provides additional water-holding capacity, a method of reducing temperature, and a shield from grazing invertebrates (Hussain and Khoja 1993). This is particularly important at the edges of the mudflat where grazers are not inhibited by the inundation present closer to the water.

The grazers observed in the Temae microbial mats were similar to those described in mats from similar environments. Meiofauna such as nematodes and amphipods were most common, particularly in the Blister mat type. No megafauna grazers were observed. This is most likely a result of the high temperatures and hypersalinity of the environment, which most large grazers are incapable of withstanding (Farmer 1992).

The cyanobacteria *Lyngbya aestuarii* and *Microcoleus chthonoplastes* are the predominant components of the mat communities. A number of other cyanobacterial genera are also present, including *Phormidium*, *Oscillatoria*, and *Chroococcus*. Occasional diatoms were observed, but the mats were formed primarily by cyanobacteria. Golubic (1994) states that in extreme environments cyanobacteria are often the dominant primary producers. This is most likely due to the fact that cyanobacteria are capable of surviving in conditions that restrict the distribution of most other organisms, rather than that cyanobacteria themselves are restricted to these conditions. Successional studies in more moderate environments indicate that cyanobacterial mats in these environments are often overgrown by other organisms, which are incapable of surviving in the extreme conditions in which microbial mats are most well developed (Golubic 1994). However, some environments may be more favorable to certain cyanobacterial species than to others.

The Spearman correlation tests showed a number of statistically significant correlations that suggested that environmental parameters influence the prevalence of different cyanobacteria. For example, the blue/black cells identified as *Entophysalis major* decrease in frequency as temperature increases, but increase as the pH becomes more basic. The same trends occur with the frequency of the small green cells. Similarly, the frequency of *Chroococcus* increases with distance to the water table and pH, but decreases with increasing temperature. Although this does not imply causation between the variables above, it offers future regression studies as possibilities for explaining the distribution of the various cyanobacterial genera.

Results from the PCA and Cluster Analysis challenged the validity of the original seven pre-defined mat types. The Thick, Black/Red, Furled, and Black mats were all placed in a single type. This type, labeled Smooth/Reticulate mat, is not homogeneous, but rather shows variation that appears to be arranged along a continuum suggesting a gradient with distance from the water. The Thick mats were characterized by a reticulate network of small ridges on the surface. The remaining three types, all located farther from the incoming tide, showed fewer ridges and an increasingly smooth surface as distance from the water increased. This pattern in surface morphology may be a result of changes in composition. The Thick mats are dominated by *Lyngbya aestuarii*, which is known to interconnect to form thin ridges (Golubic 1985). As the filamentous cyanobacteria glide in phototaxis, they become entangled, resulting in the reticulate surface of these mats (Bauld et al 1992). On the other hand, the other three types are dominated by *Microcoleus chthonoplastes*. This filamentous cyanobacterium is

known to construct smooth mats with flat surfaces (Bauld et al 1992). Thus, the gradient in morphology is likely a result of the gradient in the frequencies of dominant cyanobacterial genera.

The morphologies and composition of these mats are similar to those described in the literature, particularly from Shark Bay, Western Australia and San Carlos, Mexico. The Smooth mats closely resemble Smooth/Stratiform mats described by Bauld et. al. (1992): they are marginal marine mats with flat surfaces and dessication cracks, are dominated by *Microcoleus chthonoplastes* and other filamentous cyanobacteria, have fine lamination, and trap sediment forming frequent sediment layers. Similarly, the Reticulate mats resemble Tufted/Reticulate mats described in the same article. These are marginal marine mats, often located near or on smooth mats, with interconnecting ridges along the surface, dominated by *Lyngbya aestuarii* and other filamentous cyanobacteria, finely laminated, capable of binding sediment but with lower sediment influx.

The similarities in structure and composition between the mats in these four pre-defined groups suggest that these two documented types are found at Temae, but are closely related and occur along a continuum. This may be supported by research that suggests that *Lyngbya* filaments invade areas colonized by the smooth mat and are usually dominant in younger mats because of cyanobacteria's ability to fix nitrogen (Golubic 1985, Mir et al 1991). In addition, *Microcoleus*, which occurs in the greatest range of habitats in the intertidal zone, is thus thought to be capable of tolerating the greatest range of environmental conditions (Golubic 1985). This is supported by the Spearman correlation data, which showed that the occurrence of *Microcoleus* was significantly positively correlated with temperature, suggesting that it is dominant in more restrictive environments. It often dominates both new and well-established mats. Thus, the gradient seen in the Smooth/Reticulate type may be evidence of a progressive succession by *Lyngbya*, or of an established equilibrium in which *Microcoleus* is dominant in the harsher environments facing the mats rarely covered by water. The presence of both species in across all pre-defined types suggest that the latter is the more likely explanation, and that in either case, the mats are closely related.

The second type indicated by PCA is the Gray type. This mat does not resemble other mats described in the literature. However, the high temperature range, the degree of dessication, and the heavy sediment observed within this type suggest that it might be a marginal form of another mat type in some stage of lithification, colonization, or degeneration. This is supported by the high frequency of clear filaments within the organic layers of the mat. Compositional analysis of this type showed almost no filaments of *Lyngbya* but a relatively high frequency of *Microcoleus*. This suggests that the Gray type is related to the Smooth/Reticulate type described above. The prohibitive extremity of its environment may restrict the presence of *Lyngbya* and be the causal factor for the differences that classify it as a separate type.

PCA results grouped all mats from the Blister type in a distinct cluster, supporting its validity as a mat type. When compared to mats described in the literature, two possibilities for its identification arose. The first option involves blistering of the Smooth/Reticulate type. Blistering can occur in any mat type and involves convolution of the mat, creating a series of curvatures with hollow interiors. It is common among mats in the uppermost intertidal areas, particularly in smooth mats (Golubic 1985). The Blister mats may also be a form of Mamillate or Pustular mat. This mat usually occurs in the upper intertidal areas and undergoes extensive subaerial exposure. Its surface is covered with small rounded protrusions that are warty in appearance. It is dominated by *Entophysalis major*, a coccoid cyanobacterium, is poorly laminated, and is capable of binding sediment (Bauld et al 1992). The prevalence of coccoid cyanobacteria, the poorly formed laminations, and the characteristic appearance of the Blister mats suggest that these may be related to Pustular mats found in other areas. However, positive identification of the cyanobacteria tentatively identified in the *Entophysalis* genus is necessary in order to confirm this relationship. However, the spatial separation of these mats on the PCA graph, determined primarily by compositional differences in coccoid cyanobacteria, suggest that it is not simply another form of the smooth mat.

Raised/Flat mats were also grouped as a distinct type by the Principle Components Analysis. Spatial organization on the ordinate diagram suggests that this type is most closely related to the Blister mats. The two share communities with high frequencies of coccoid cyanobacteria and are separated by differences in the prevalence of unidentified green masses and *Lyngbya*, which is rare in the Raised/Flat mats. This type does not resemble any of the mat types found in the literature. However, its compositional similarity to the Blister mats in addition to the presence of a distinct layer of mineral precipitate suggest that this type may actually consist of Blister mats in a stage of lithification. Carbonate precipitation occurs in all mat

types and promotes stromatolite formation (Golubic 1985). The presence of both flat surfaces and raised protuberances further supports this possibility. On the other hand, the Raised/Flat mats may constitute a microbial mat not yet described in intertidal environments.

Thus, PCA results indicate that the microbial community at Temae consists of four mat types: a Smooth/Reticulate type occurring on a gradient with increasing distance from water, a Blister type, and two types in the process of lithification or degeneration. Results from the cluster analysis support the validity of these four types.

Although the general morphology and composition of the mats at Temae bear similarities to those described in other intertidal mats, the microbial communities in this study also show unique differences. For example, most mats described from similar environments were covered by a gelatinous layer dominated by diatoms. All diatoms recorded in the Temae mats occurred within the filamentous layers rather than at the surface. This phenomenon was observed in an incubated artificial mat but is unusual in nature (Fenchel and Kuhl 2000).

In addition, the mats were unusual in that few bacteria were observed. Only one anoxygenic bacterial colony was recorded from the Thick mat type. This difference may have a number of explanations. To begin with, the resolution and magnification available in the light microscopy used may not have been sufficient to distinguish the bacteria. On the other hand, these bacteria may actually not be present in the mats. Although purely cyanobacterial mats have been described, mats with lower anoxygenic bacterial zones are more common in hypersaline intertidal environments (Pierson 1992, Des Marais 1990). This environment is unusual in that during the study period, it consisted almost entirely of a sediment-air interface rather than a sediment-water interface. Oxygen diffusion is greater in air than in water. Thus, oxygen can penetrate more deeply in mats that are dessicated. Since these bacteria are very sensitive to oxygen and may not be able to establish in fluctuating conditions, the air-sediment interface may prevent the development of anoxygenic communities (Mir et al 1991). However, dissolved oxygen levels indicate that some level of anoxia exists beneath the mudflat surface. Further research is necessary to determine the reason for this difference.

Many opportunities for further research exist on the Temae mudflat. At the end of the research period, an additional mat community was identified in the subtidal zone. This mat is much thicker than any of the mats described in this study and is covered by large tufts. Initial observations suggest that it may be a more developed *Lyngbya* mat or may also be an example of an intermediate in the cyanobacterial mat-macroalgal community succession observed in other areas (Golubic 1985, 1994).

Modern microbial mat studies increasingly focus on physiological relationships within mats in addition to structural, morphological, and compositional characteristics (Pierson 1992). Using extremely sensitive instruments, these studies detect and record important changes that occur in the complex microenvironments within these mats (Jorgensen et al 1992). The diverse and developed mats at Temae are an excellent model for further physiological and pigment tests. In addition, TEM and SEM would better describe the microscopic structure, commun[illegible] interactions, and sediment-binding mechanisms wit[illegible] these mats.

The study of microbial mats is important to understanding the development of early life on earth, particularly in the form of stromatolites. The mat types identified at Temae are analogous to a number of Proterozoic structures, including the Transvaal Dolomite and Stoer Group (Bauld et al 1992). Understanding the mechanisms at work in these microbial mats through the techniques described above as well as through molecular and carbon testing is extremely important to the study of these fossil structures (Pierson 1992). The microbial mats at Temae, which include a variety of types as well as mats possibly in the process of penecontemporaneous lithification are important models for microbial, archaeological, and astrobiological study.

Conclusion

The mats studied at Temae show features characteristic of most microbial mats found in tropical intertidal environments. All mats were laminated, although to differing degrees, and showed changes in pigmentation and composition along a depth gradient. The microbial communities were composed predominantly by filamentous cyanobacteria such as *Lyngbya* and *Microcoleus*. The effects of changing frequencies of these species yielded excellent examples of the close relationship between composition and structure in microbial mats.

The initial seven types identified on the mudflat by phenotype were modified to form four mat types upon analysis of environmental and compositional data. Two of these types resemble microbial mat communities described in Shark Bay, Australia, as well as in other studies. The remaining types may be new communities or marginal samples of the previous types in various stages of lithification or degeneration.

The hypersaline intertidal environment at Temae is similar to the habitats characterized in many other microbial mat studies. However, the mats described from Temae were composed almost entirely of cyanobacteria. On one hand, this difference warrants further research to ensure that this was not simply a result of insufficient magnification to see other microorganisms. At the same time, however, the dominance by cyanobacteria clearly demonstrates the high tolerance these orgamsms have for environments that restrict the success of other organisms. This property of microbial mats may make them important players in interacting with polluted environments in the future. The well-developed mats and diversity of form present in this community create an important resource for further cyanobacterial mat research as well as for a deeper understanding of the development of ancient communities.

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Locomotion and Distribution of the Cushion Star *Culcita novaeguineae* in Cook's Bay, Moorea, French Polynesia

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ABSTRACT. The cushion star *Culcita novaeguineae* is a little studied sea star in the family Orestridae. It plays an important role in the reef ecosystem due to its high numbers and predation on the organisms of coral reef assemblages. Previous studies on *Culcita novaeguineae* found the sea star to selectively predate coral (Glynn 1986). It was noted during this study that *C. novaeguineae* was not documented eating coral in the study area on Moorea. *C. novaegineae*'s diet consists mainly of sponges, algal felt and epibenthic film. The sea star did show a habitat preference; it was mainly found on sand and coral rubble substrate. In addition it was also within 1m of *Porites spp* coral thirty five percent of the time it was observed during the distribution study. The locomotion capabilities were also a major consideration of the study. The locomotion was studied in both diurnal and nocturnal settings with the most activity being noted during the nocturnal measurements. The cushion star was found to move as much as 9.74m/h and had an average nocturnal movement of 1.78m/h. *C. novaeguineae* is the second most important sea star in coral reef ecosystems due to its selective predation of *Poccilopora* spp. and *Acropora spp.* coral. The selective feeding of *C. novaguineae* has an impact on the reef assemblage. This impact is substantial considering that these types of coral are important builders of the reef structure (Goreau et al. 1972). A comparison of the behaviors, locomotion, and rates of coral consumption of *C. novaeguineae* in relation to another sea star, *Acanthaster planci,* will be discussed to further highlight the importance of *C. novaeguineae* in the ecology of the reef. *A. Planci* was chosen for comparison due to its similar distribution to the *C. novaeguineae* and its role as a key predator in the coral reef ecosystem.

Introduction

A coral reef is one of the most biodiverse ecosystems in the world. The ecology of coral reef communities is challenging to study, due to the complicated interactions of the organisms of coral reef assemblages. *C. novaeguineae* is an important organism in the reef ecosystem, because it is a predator. *C novaeguineae,* like many other sea stars, is a generalist in diet. Yet it also has been proven to have food preferences (Glynn 1986), which makes its role in the ecosystem more important, because it selectively has an influence on what organisms live and die in the coral reef ecosystem.

Little is known of the habits, behavior, and locomotion or habitat preference of the cushion star. These are important factors in understanding the role of this large predator in the reef ecosystem. Researchers have found the cushion star in many localities and island chains in and around the West Indies and Hawaii (Glynn and Krupp 1986). The range of the cushion star is large; it is one of the most widespread asteroid species in the Indo-Pacific region (Yamaguchi 1977). Cushion stars are found from Okinawa to the southern end of the Great Barrier Reef (Livingston 1932).

Many previous studies have stated that *C. novaeguineae* eats corals (Goreau et al. 1972; Yamaguchi 1977; Krupp1986; Clark 1946; Livingston 1932). This fact alone makes the study of *C. novaeguineae* an important one. This diet of coral is important because of the effects that selective predation has on reef assemblages. As an example of this, one can look at the effects that the crown-of-thorns sea star *Acanthaster planci* on reef ecosystems. *A. placi* is also a corallivore and has been noted as one of the main factors in controlling the amount and type of coral present in certain

island localities (Goreau et al. 1977). The distribution, movement, diet and life history of the *A. planci* is closely related to that of *C. novaguineae*. These similarities and the amount of information available on *A. planci*, make it a good comparison to *C. novaeguineae*.

C. novaeguineae has been documented to be more abundant on coral reefs than A. *planci* in Saipan and the Marianna Islands (Goreau et al 1972). Yamaguchi (1975) also documented *C. novaegineae* as being more prevalent in Guam and the Marianna Islands. In Hawaii, on the island of Oahu, Glynn (1986) noted a higher presence of *C. novaeguineae* than *A. planci*. Evidence has shown that *C. novaeguineae* does prey selectively on certain species of coral and is therefore an important factor in the structure and relative abundance of corals in reef communities (Glynn 1986). Sea stars like the *A. planci* have been documented eating and traveling in aggregations. The *A. planci* also congregate when not eating (Birkeland 1990). Part of the locomotion study of *C. novaeguineae* on Moorea noted the movement of the cushion stars and also looked at the data to see if there is a relationship of sea stars movement to one another. Looking to see if they were moving in a set direction like the *A. planci* or if their movement had any correlation to one another. The locomotion capabilities of *A. planci* have been a part of previous studies (Birkeland 1990, Endean 1976). Since locomotion is so important in controlling how much coral or benthic organisms that can be predated upon, it is an important factor in the assemblages of reef ecosystems. Hence, the locomotion of the cushion star was one of the main focal points during the study. Goreau et al. (1972) noted during a study in Saipan that the cushion star is diurnally active. During that same study in Saipan Goreau also noted that *C. novaeguineae* was not actively eating on a large percentage of their study dives.

The determination of habitat preference for the sea star was a goal in my research. It was noted in previous papers that *C. novaeguineae* preferred areas with low topographical relief. Some studies stated that *C. novaeguineae* was not found in areas that have high surge or low salinity, like near shore environments or close to stream mouths. After doing some preliminary surveys of the cushion stars in the study area I noticed some trends. While conducting snorkeling surveys I noticed the cushion stars were often near *Porites spp.* Coral, on sand, and on coral rubble substrate. This is my first hypothesis: that *C. novaeguineae* prefers sand and coral rubble substrate near heads of *Porites spp.* coral. I hypothesize that in this particular area, the cushion star does not live on or actively eat live coral. The cushion star is more active nocturnally in both eating and locomotion respects. In locomotion, the star moves almost twice as much at night as in the day. I also noticed in my preliminary surveys that larger sea stars seemed to move quicker than smaller ones. This was the basis for my second hypothesis: there is a correlation between movement capabilities, or average movement, and the size of the starfish. From reading papers about *A. planci*, I learned that these stars often travel and eat in aggregations, which contributes to their outbreaks (Birkeland 1990). In an *A. Planci* outbreak the large aggregations of sea stars are more likely to completely kill large heads of coral. As opposed to a few sea stars eating sections of larger heads of coral. Coral recovers quicker when some sections of the heads are still alive. During the preliminary surveys of the *C. novaeguineae* in the study area, I did not notice any relationship between the movements of the cushion stars. In fact, they seem to be a solitary animal that does not live in congregations. This observation led me to look at the *C. novaeguineae*'s movement in relation to one another. My hypothesis is that the direction of movement is haphazard, with little to no movement in relation to the direction or movement of other sea stars in the surrounding area.

Materials and Methods

The main study site was located off the Gump Research Station located on the south side of Cook's Bay in water that varies in depth from a half meter to six meters (Figure 1). Specimens were collected from the outer reef of Cook's Bay, forty meters south of the green buoy along the outer barrier shelf in

water that was between five and ten meters in depth. Specimens were collected in Cook's Bay, south of the station along the coral ridge approximately fifteen meters from shore in water that was three to five meters in depth. After preliminary surveys in various locales around Moorea, the study sight was chosen due to the relatively high percentage of cushion stars in the area.

The distribution of the star was the first segment of the study. Variables studied in this stage included: location using a map and underwater grid, depth, substrate, color, size and behavioral observations, such as proximity to known food items and coral or large rocks. I studied their distribution during both night and day to measure the differences.

The distribution studies were conducted at various times in the day and night for better representation of distribution on a twenty-four hour cycle. Samples were done with a grid map on the ground in the area and an underwater compass (Figure 1). The grid was constructed using a transect tape to measure ten meter sections south to north along the shore in front of the Gump Research Station. With these sections marked I then used the transect tape to measure ten meter sections into the bay, which were triangulated with known markers to form ten meter square blocks. The grid went west to east from the shore into the bay until the coral shelf drops below 10 meters. The area was marked with underwater flags labeled A through K, going from shore out, or west to east, then 1 through 9 going from the Gump station boat dock towards the mouth of the bay or south to north. Using the markers for location recording purposes and frames of reference, an underwater compass and kick cycles (certain number of fin kicks equals a meter, previously measured using a transect tape) were utilized to record area and direction. I used the markers to record where certain stars were located. The grid also gave a frame of reference during the night samples. I utilized various methods to make sure I missed as few sea stars in the area as possible while doing my nocturnal and diurnal samples. By taking a compass reading on nonmobile features on the shore, I was able to set up a grid for snorkeling. The snorkel corridor was set up such that I snorkeled in the middle of a two-meter wide area (this is an area that can be visually inspected for cushion stars with a low percentage of error). The aforementioned technique combined with counting kick cycles, enabled me to be confident that I surveyed the area with a low percentage of error.

While conducting these counts I recorded the depth of the star with a rigid pole that was previously measured and labeled in meters and centimeters. I used the pole to measure the depth of the *C. novaeguineae*. I also noted their color. In addition, I would examine the *C. novaeguineae* for signs of health and to see any trace of predation present, such as scrapes, flaws, or discoloration. The substrate where I found the sea star was recorded. When picking up the star I would look to see if the stomach was extruded, suggesting it was eating (Glynn 1986; Yamaguchi 1977). I also measured if the star was immobile with no tube feet extended; this could be extrapolated by how hard the star was to pick up. I also noted the proximity of the *C. novaeguineae* to corals and large rocks. Proximity to known food sources such as sponges, tunicates and other invertebrates was also noted.

To establish an idea of overall habitat availability, I took fifty random quadrat samples in the study area (Figure 2). I randomly chose two sets of points; one set was along the shore in front of the Gump Research Station going north to south. The next set of points went east to west or from the shore out into the bay. I utilized a compass to make a straight line from the point along the shore, into the bay. The compass helped me to keep the line straight while snorkeling from the shore out into the bay. I measured kick cycles (as previously described) to get an idea of how far I was into the bay to complete the triangulation that was where I took the measurement.

Before tagging and releasing the stars to do the locomotion study, I took various measurements to record the size of the stars. I first measured the distance from the mouth region to the ray tip. The next measurement was the distance between the ends of the rays

on the side of the cushion star. I then measured the overall length of the cushion star. For volume measurements, used water displacement. I measured the amount of water (in ml) that it would take to fill the container and cushion star to a known level. This method of measuring volume was used to make the measurements. For the partial dry weight measurement I left the sea star out of the water for ten minutes before taking the measurement using a small digital scale. I felt it necessary to use two methods to measure the weight due to the challenge of getting accurate measurements on an organism that can fill itself with water and change its shape and weight.

The locomotion of the star was measured using various methods that were changed for better recovery and ease of study throughout the project. First, I marked the location of the star with a flag, then returned in three hours to find the flag and the star to record distance moved by using a transect tape along the substrate. When the star was located, I would then measure the distance the star moved. I attached a seven pound weight to the end of the transect tape which I would drop by the flag. I then swam to the star dragging the tape behind me. Next, I swam the length of the transect tape back to the marker flag to make sure that the line was straight and not going over large rocks or heads of coral that would throw off the accuracy of the measurement. After that I swam back to the sea star to make the measurement.

The previous method was not exact enough to find the same sea star and the recovery rate was low in trying to find the specimens. The natural markings on the stars were not conclusive enough to positively identify one from another. The flagging method proved time consuming due to the challenge of finding not only the sea star but also the flag. Therefore I began marking the sea stars with fishing line and tape for accuracy in finding and identifying the individual.

With the tagging complete, I then created floats out of styrofoam tied to fishing line. I then tied the fishing line to a rock or piece of coral rubble, which was placed next to the stars on the substrate. These floats allowed me to easily locate the marker on the surface and swim to it. I located the stars using a concentric circle search from the marker out a half-meter at a time until the sea star was found. Upon recovering the sea stars, I measured not only how far they had moved but also the direction of travel taking a compass reading on the direction that the sea star moved. I did this by measuring the direction of the sea star from the flag.

Results

For the habitat preference aspect of the study I found that *C novaeguineae* did show a habitat preference. When I took the fifty random quadrats of the bay I found that sand and coral rubble were the two predominant substrate types available in the study area. The results of the samples of the bay are shown in Figure 2.

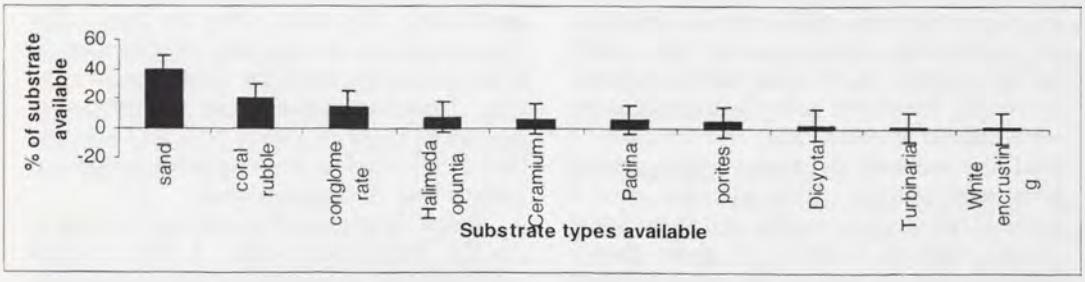

Figure 3. This table represents the percent of different substrates in the study area

Sand and coral rubble were the most prevalent substrate types available. *Porites spp.* coral occurs only 3.75 percent of the time. Yet when I did the distribution surveys the sea stars were within one meter of *Porites spp* coral 35 percent of the time. While doing the distribution study we found the sea stars the most on sand and coral rubble substrate. The interesting and significant part is that the stars were found on both coral rubble and sand substrate 40 percent of the time. This is interesting because coral rubble substrate accounts for 20 percent of substrate available in the study area.

Figure 4 is the percent of the time that the cushion stars were recorded on different substrates during the nocturnal and diurnal distribution samples. *C. novaeguineae* preferred sand and coral rubble substrates and the stars were found on them 40 percent of the time. The *C. novaeguineae* were found on conglomerate less than it was present in the random samples.

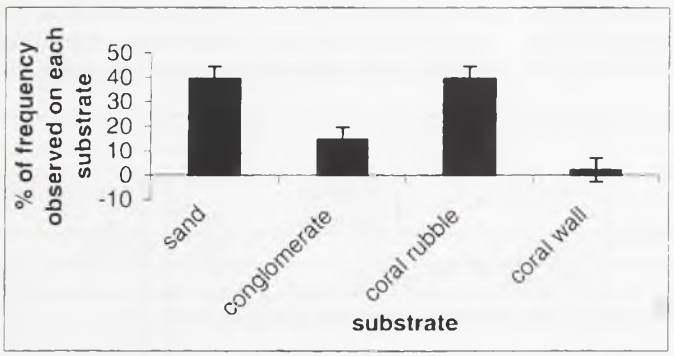

Figure 4. Shows the percent of the time the tagged sea stars were found on different substrates.

When I statistically analyzed the results of the distribution and random quadrat samples I used a Chi-squared analysis to compare the frequencies of the two samples. The results of the analysis were statistically significant. I found a chi- squared value of 36.446 (df=5).I also made a qualitative observation that the *C. novaeguineae* were within one meter of *Porites spp.* coral 35 percent of the time they were observed.

My null hypothesis, that cushion stars show the same amount of activity in both the nocturnal and diurnal periods was not proved; the cushion star is more active at night. The average rate of movement for all the cushion stars was diurnally 19.447 cm and nocturnally 178.456cm. Figure 5 shows the average amount of movement both nocturnally and diurnally for the tagged *C. novaeguineae.*

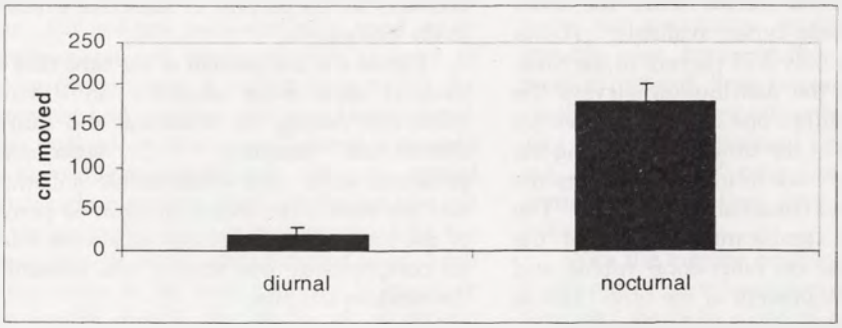

Figure 5. The average movement of tagged sea stars for both nocturnal and diurnal periods.

I used a t-test to compare the difference between nocturnal and diurnal samples. Note the 1.27E-06 value that was found from the one tail t- test this value means that a low percentage of this information is random and significantly different between day and night.

	Mean	Variance	Degrees Freedom	P(T<=t) one-tail
Diurnal	19.447	1736.51	19	1.27E-06
Nocturnal	178.4657	9492.964		

Table 1. This table shows the results of the one tail t test comparing nocturnal and diurnal samples tested.

During diurnal samples the stars moved an average of 19.4 cm on average for all the samples. In the nocturnal cycles the tagged sea stars moved an average of 178.5 cm for all samples taken. These findings lead me to believe the hypothesis that *C. novaeguineae* are more active nocturnally. Over the course of three nights and early mornings, I made the hypothesis based on observations that 80 percent of the cushion stars being measured found a hole or crack in *Porites spp.* coral and hid there at between four and five a.m. *C. novaeguineae* then stayed in that place until an hour after sunset. The sunsets in Moorea occurred between 18:00 and 18:30p.m. The sea stars became active in locomotion respects between 18:30 and 19:00. I observed this cycle for three nights two of which were consecutive. The direction of movement was consistent for the time hypothesized, about two and a half hours on average. When the stars did change direction they held that course for at least an hour.

To analyze the correlation between the ratios of size of the sea star to movement I used a linear regression analysis. When comparing the size measurements to the amount of average movement of the tagged sea star, the results were biologically significant ($p = 0.094$). The values for the other measurements did not show results that were as significant as the volume and nocturnal movement regressions.

Volume	Stomach to tip of Ray	Distance between rays	Overall length	Wet/dry weight
R square .147953	0.0399	.0399	.097395	.019182
P value 0.094018	.398475	.398475	.180392	.560351

Table 2. This table shows the R squared and P values from the linear regressions of size to movement.

The largest sea star moved the furthest and the smallest moved the least, but was not shown to be significantly correlated using a linear regression analysis. Analyzing the size of the sea stars by volume made tagged sea star ten the largest. This *C. novaeguineae* moved on average 7.42 cm diurnally and 373.57cm nocturnally over 25 observations. The smallest sea star by volume was tagged sea star 3. This star moved 1.77cm diurnally and 108.67cm nocturnally over 21 observations. The fact that the largest moved the furthest and the smallest moved the shortest distance helps to validate the hypothesis that the size of the sea star does play a factor in the amount of locomotion possible figure 6.

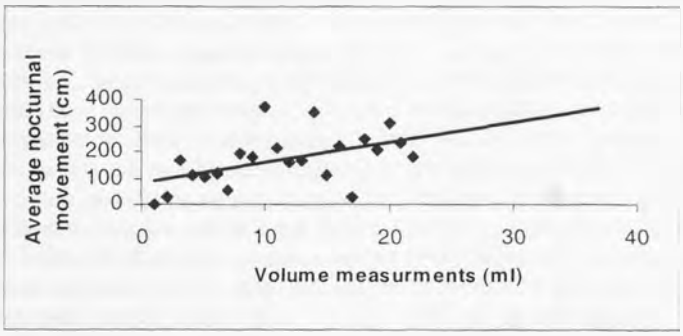

Figure 6. The following scatter plot shows the linear regression of volume to nocturnal locomotion of the sea stars sampled.

By looking at figure 6, one can see a trend in the grouping of the dots representing the movement and volume measurements. If we had a larger sample size the grouping around the line would be tighter, the results possibly would have been more statistically significant.

The movement of each cushion star did not relate to the proximity of one star to another in the study area. To analyze this, I noted the direction of movement for each sea star and compared them by drawing out the trajectories and distances moved over each nocturnal sample. The sea stars did not show any correlation between proximity of one to another in the study area Figure 6.

Discussion

The hypothesis that *C. novaeguineae* is more active during the nocturnal period was statistically significant ($p=1.27x10-6$). The results and data sections clearly show the nocturnal locomotion preference. A reason for nocturnal activity could be competition for habitat space with other benthic reef organisms such as crabs and other echinoderms. Qualitative observations in the lab and during the distribution and locomotion studies indicated that *C. novaeguineae* is photosensitive, this could also be a factor in the stars preference towards nocturnal activity.

The cushion star is challenging to get accurate size measurements on. The semi dry weight test was not accurate enough, due to the cushion stars propensity to suck in water and expand to a point of being rigid when threatened. Nocturnally the cushion star flattened itself out, as opposed to diurnally when it formed a round shape. The overall length measurement was not accurate enough due to the various shapes that the cushion star can employ over a short period of time. Some stars were round in shape with their arms sucked into their body and the center of them pushed up. Others had a flatter shape after sitting on the lab table for a minute. These different shapes assumed by the *C. novaeguineae*, confounded the overall length measurement. The volume measurement with the ray length and distance between measurements were fitting to the cushion stars shape. Hence, the regressions of these measurements to the nocturnal locomotion showed more significant results.

The small amount of research done on *C. novaeguineae* makes it necessary to compare this study to other studies done on other species of sea stars. When comparing different sea stars I chose sea stars that had similar distributions, diets and habitat preferences, such as *A. planci*. The locomotion of *C. novaeguineae* is comparable to the faster moving *A. planci*. The challenge in this comparison comes from the large discrepancy in the amount of movement of the *A. planci* in different studies and in various contexts. *A. planci* has been documented moving at 20m/h on sandy substrate (Endean 1969). This rate is faster than the fastest moving *C. novaeguineae* observed in my study, which moved 9.74m in one hour. On an overall the average amount of nocturnal movement for all sea stars in this study was 1.78 cm/h. This value is substantially lower than the average *A. planci* adult movement of five to ten meters per hour (Endean 1969). The cushion star average is lower due to the fact that juveniles less than 19cm in diameter were factored into the locomotion averages in this study. For instance, *A. planci* in Hawaii moved 0 to 18 m/day (Birkeland 1990). This factor, compared to the most active sea star in this study, which moved 50.60m/day, indicates that in some areas the sea stars movement can be comparable.

A. planci has been documented to aggregate in large groups, ranging from five to hundreds of individuals (Birkeland 1990). Yamaguchi (1975) found that *A. planci* would aggregate when there are high population densities in a given area. The size of the area is hard to estimate with the data available and more work needs to be done on aggregation behavior in *A. planci*. There is a difference in the aggregation behavior of *C. novaeguineae* and *A. planci*. *C. novaeguineae* does not move in relation to one another. This trend held true even when the *C. novaeguineae* were released within 0.5 meters of one another. *A. planci* has been observed aggregating in numbers of 30 or more, which move in one large group for up to three months (Birkland 1990). This is one of the main factors in *A. planci* being as devastating to reefs as it is. The devastation that *A. planci* causes to coral reef assemblages make it more important in the reef ecosystem than *C. novaeguineae*.

There are many possible reasons why *C. novaeguineae* does not congregate like *A. planci*. It would make sense to aggregate to defend against predation and to have others also take some of the nematocyst damage from eating coral. It has been noted in past studies that if the *A. planci* populations are low in a given area, then they do not aggregate (Yamaguchi 1975). Perhaps there is a similar response to low population densities for *C. novaeguineae*.

The amount of coral consumed by *C. novaeguineae* has a basis for being compared to *A planci* because it is considered the second most important corallivore of the Asteroid family (Glynn and Krupp 1986). *C. novaeguineae* is able to kill some coral colonies (3-8 cm in diameter). Yet they are unable to kill larger colonies (Krupp and Glynn 1986). Hence, cushion stars do not play as large a role in the reef ecosystem as does *A. planci*. *C. novaeguineae* can affect the community structure of the reef ecosystem by slowing the recruitment of prey species like *Pocciloporo spp. C novaeguineae* has been documented eating coral at a rate of 0.9 to 1.0 m^2/year (Birkeland 1990). Pearson and Endean (1988) observed *A. planci* eating coral at rates of

116cm^2/day. (Krupp 1986) found the mean feeding rate of *C. novaeguineae* to be 27.8cm^2 /day. The *A. planci* rate of movement is higher, but there is a basis for comparison based on the numbers.

Nocturnal observations provided the most locomotion and activity, including eating. I noted eating by turning the starfish over to see if the stomach was extended. *C. novaeguineae* was mostly eating nocturnally. Goreau et al. (1972) noted that the *C. novaeguineae* was diurnally active and during that study those researchers found the cushion star to be eating a small percentage of the time. I propose that had Goreau done nocturnal as well as diurnal samples, Goreau would have noted more of the cushion star eating.

By this same logic, I feel many researchers that have done *A. planci* studies may be overestimating the amount of coral predation the *A. planci* is capable of. Endean and Cameron (1969), Yamaguchi (1977), Krupp (1986), Livingstone (1932) and others have noted that the cushion star leaves a white scar on coral after it has extended its stomach and eaten some of the polyps. *A. planci* leaves a similar scar on coral after eating. The white scars left behind when a corallivore eats, could come from either species *C. novaeguineae* or *A. planci*. One can see how the credit of eating coral polyps would go to the much studied and written about *A. planci* as opposed to the little studied or understood *C. novaeguineae*. Hence the cushion star needs to be observed more closely due to its importance in eating certain species of coral that contribute to shaping the assemblages of the reef ecosystem.

During my study in Moorea I never once noted the cushion star eating or even traveling over live coral in my study site, or elsewhere around the island. Yet, there are numerous studies that have shown the *C. novaeguineae* to be a corallivore. One such study shows the cushion star has a preference for certain kinds of *Pocillopora* spp. (Glynn and Krupp 1986). This eating of corals has been recorded by other studies (Endean 1969), (Goreau et al. 1972). The fact that the cushion star chooses *Pocillopora damicornis* and *Pocillopora meandrina* over *Porites compressa* (Glynn1986) means that the cushion star could have a definite effect on reef assemblages.

Possible reasons why no observations of *C. novaeguineae* eating coral were noted in this study are the small percentage of *Acropora* spp. and *Poccilopora* spp. in the study site. *Porites* spp. is perhaps not eaten in the study site, due to the large amount of nematocysts in *Porites* spp. as opposed to *Poccillopora damicornis* (Glynn and Krupp 1986). *Poccillopora damicornis* was the preferred coral food choice in previous studies on *C. novaeguineae* (Glynn and Krupp 1986). The majority of *Porites* spp. in the Moorean study site were mushroom shaped making it challenging for the cushion star to feed on them, due to its lack of climbing ability (Birkeland 1990). This climbing limitation was observed in other studies on *C novaeguineae* as well. Glynn (1986) and Goreau et al. (1972) noted *C novaeguineae* 's inability to climb vertically over large heads of coral. Although this would not keep *C. novaeguineae* off all the *Porites* spp. present in the study area, some *Porites* spp. heads were broken or low to the ground. Yet no feeding scars or visual observations were made of *C. novaeguineae* on live *Porites* spp. during the diurnal or nocturnal samples.

There are multiple reasons that could explain why the cushion star in Moorea does not eat coral. One such reason is the presence of crustacean guards has forced a change in the diet of the cushion star. Glynn (1988) noted that crab and shrimp live on certain kinds of coral and will defend their home coral heads from corallivores such as the cushion star. Glynn (1988) also noted that the protective behavior of the crustacean guards was the most aggressive towards *A. planci* because the species is known to kill coral. Aggression against other corallivores that do not kill the coral are less intense. Crustacean guards are present in reef building families of coral, such as *Pocciloporid* spp. and *Acroporidae* spp. varieties. These *Pocciloporad* spp. and *Acroporad* spp. corals are the corals of dietary preference for *C. novaeguineae* (Glynn and Krupp 1986). Perhaps this is one of the reasons why no cushion stars were observed predating or traveling on coral in this study.

The prevalence of *Porites* spp. coral as compared to the preferred dietary *Pocciloporid* spp. coral species may also be a factor in keeping *C. novaeguineae* from being a corallivore in the study site on Moorea.

Another possible explanation for *C. novaeguineae* not eating coral in Moorea could have to do with a biogeographic break in the species. In a study on the starfish *Linckia laevigata* Williams (1996) noted there was a difference in the species due to upwelling and ocean currents changing the larval dispersal available. The same situation could be true for *C. novaeguineae* in Moorea. Moorea is biogeographically separate from many other island chains and mainland Asia and Australia. As noted by Williams (1996) there are more options for genetic variability in the Pacific Ocean as opposed to the Indian Ocean due to the number of islands and atolls in the Pacific. Perhaps *C. novaeguineae* in Moorea have adapted to not eat *Pocciloporid* spp. coral species that are protected by crustacean guards. Instead they favor easier prey items consisting of algae sponges and epibenthic film. It is commonly excepted that sea stars are opportunistic eaters this could be an adaptation to continue this trend.

Conclusions

I found that the cushion stars in my study area, were more nocturnally active. The movement of the cushion stars had no relationship to the other cushion stars in the study area. I also found that the cushion stars did show a preference for sand and coral rubble substrate. The fastest moving tagged cushion star moved 50.60 meters in a 24-hour period. During my samples of cushion stars on Moorea never observed a cushion star eating or traveling over live coral. For future study I would recommend conducting feeding tests on Moorean cushion stars to see if they show a trend similar to the cushion stars mentioned in Glynn and Krupp's (1986) study. If the cushion stars did prefer certain corals to other food items, this could show a different dietary preference in the lab than in the field.

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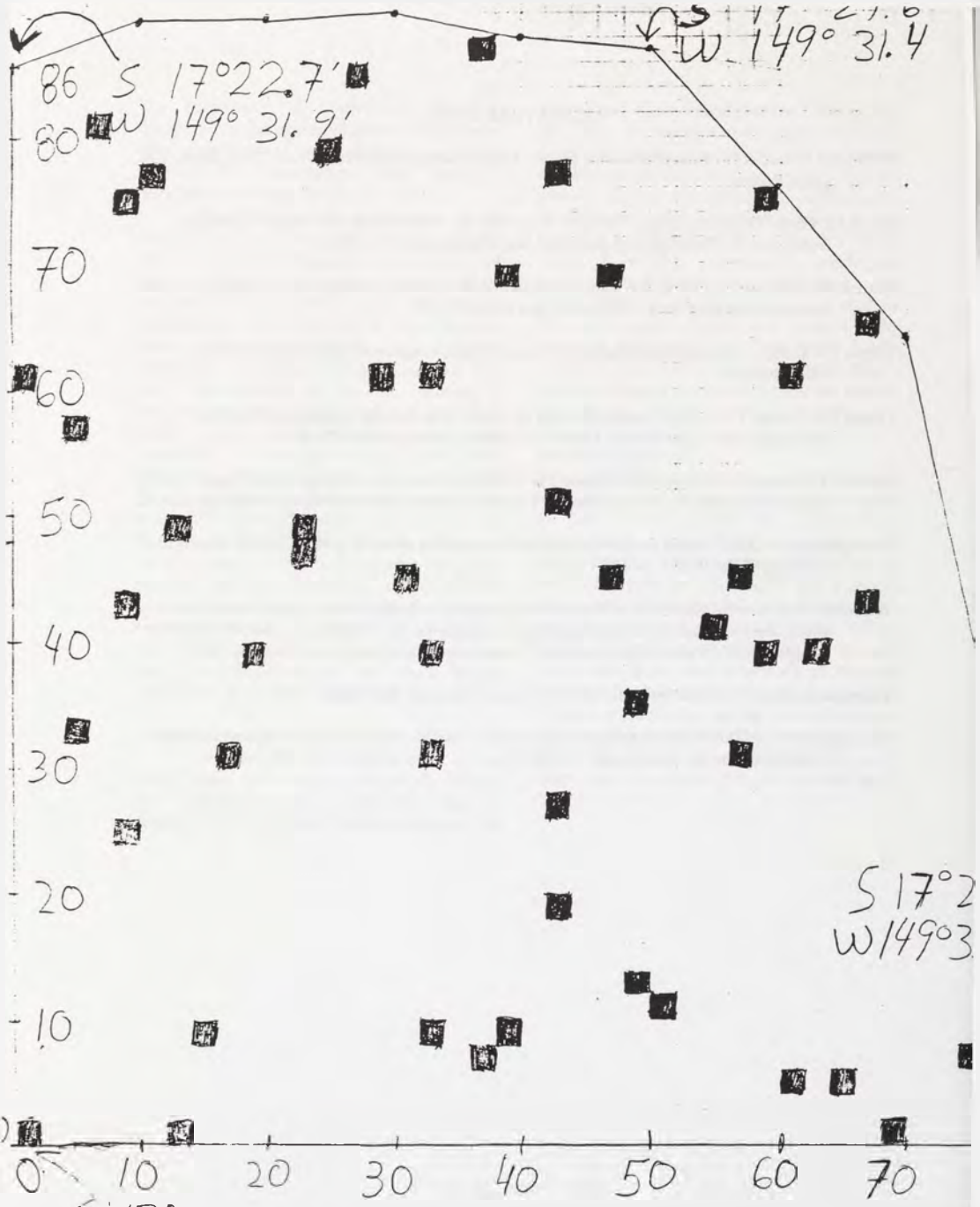
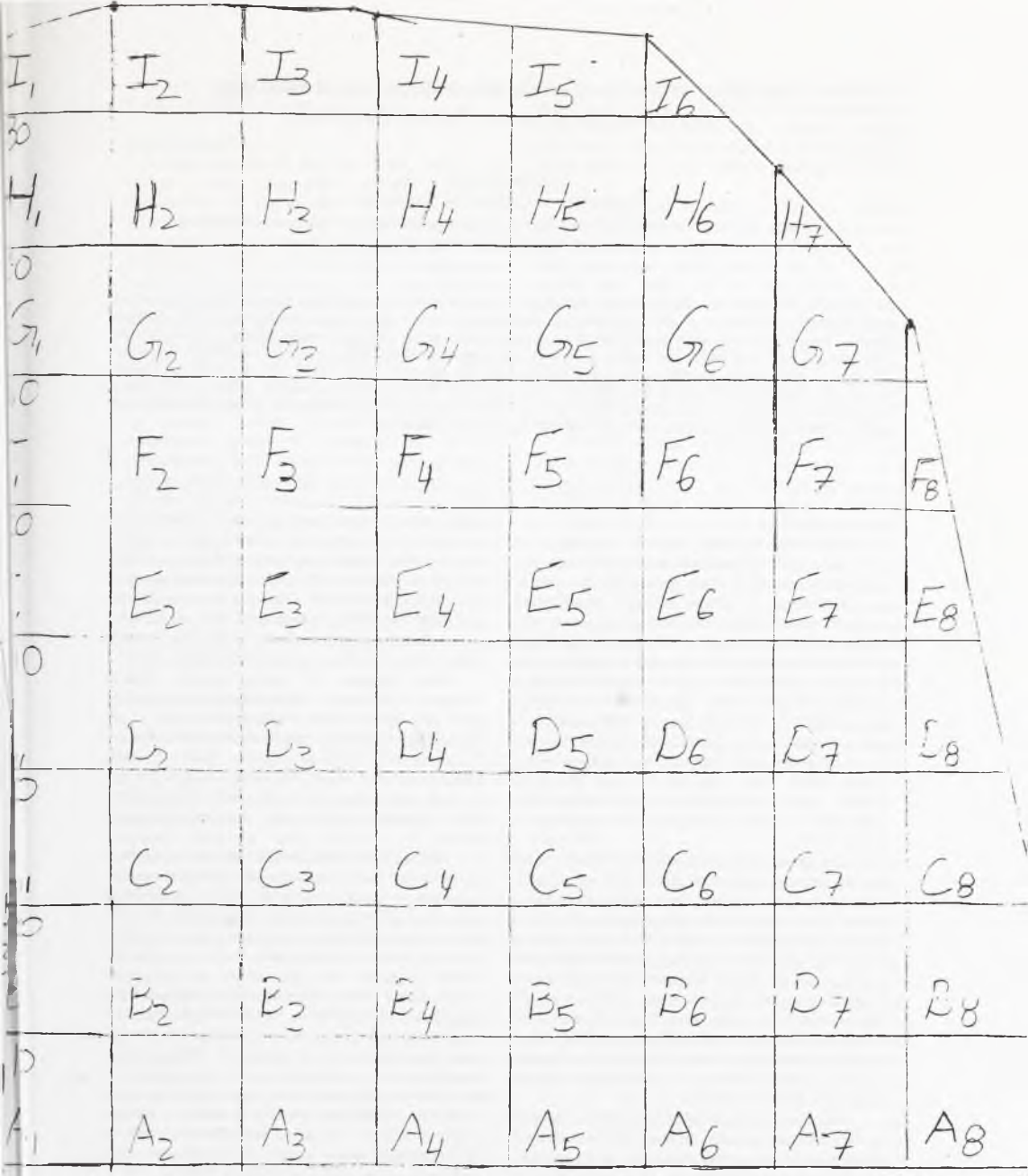


Figure 1



Grid map of study area.

The effect of livestock on diatoms, shrimp, and snail eggs in streams on Moorea, French Polynesia

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ABSTRACT. As tourism is central to Moorea's economy, it is in the island's best interest to insure that farming practices are not polluting the island or its bays. The principle objective of this study was to investigate the effects of livestock on streams. By using chemical and biological indicators of water quality, stream subsites upstream, adjacent, and downstream from livestock were compared to one another. Biological indicators used include shrimp, snail eggs and diatoms. Chemical indicators of water quality investigated were pH, nitrate levels, and dissolved oxygen levels. The data from the streams showed that diatom diversity as well as distribution of snail eggs differed between the three subsites. Additionally, for many of the diatom types there were differences in distribution between subsites. Livestock practices on Moorea affect the water quality of the streams as detected by the diatom and snail egg biological indicators.

Introduction

Biodiversity can be used as a monitor of ecosystem health. A crucial element of sustainability, biodiversity maintains stable gene pools and aids in the preservation of natural systems. It assists in recovery from disturbances and is important for natural succession. One of the most bio-diverse habitats, coral reefs are threatened by nutrient and sediment runoff from stream mouths carrying effluents from livestock, agriculture, and sewage (Nebel 1998). Although loss of biodiversity is a global phenomenon, most extinctions within the last few 100 years have occurred on oceanic islands (Nebel 1998). This is because the small size of the islands limits population size and makes them vulnerable to human alterations to the environment (Nebel 1998).

Most agricultural and livestock cultivation of the Society Islands occurs on the Windward Islands. Situated in the mid Pacific Ocean, Moorea's isolation forces it to supplement costly imports with local livestock. Presently one third of the land in Polynesia is used for agricultural purposes, and this use of land provides enough food to feed three fourths of today's Polynesian population (Merceron, 1996). As the Polynesians modernize, they have increased the amount of meat in their diet (Merceron 1996). Much of the beef and lamb is imported from New Zealand because local production is insufficient to meet demands (Merceron 1996).

With the growing demand for meat accompanied by the increasing population, there will be a strain to develop land for livestock cultivation as has occurred in the Marquesas (Merceron 1996). As of 1996 Polynesia had an adequate local supply of pork (Merceron 1996). A switch from beef to pork in the diet would enable Polynesian supply to meet demand, thus reduce importation (Merceron 1996). As with many underdeveloped nations, much of the food produced in Polynesia is wasted due to poor transportation methods and storage (Merceron 1996). In 1960 Polynesia was 85% self sufficient in terms of food supply, yet in 2000, Polynesia was only able to produce 25% of what it consumed with much of its importation coming from France and New Zealand (John Claude Gaulthier, personal interview).

After decades of French control, French Polynesia will become independent in the coming years. By 2015, French Polynesia is expected to be financially independent, which means France will no longer subsidize expensive imports. At a time when Moorea and all of French Polynesia is preparing to go through many changes, it is important to look into the local livestock practices and their environmental effects.

Immediate economic benefits of food production on the island include reducing the demand for costly importation. Another effect of food production that must be taken into account is damage to the environment. Environmental damage can be caused by the effluent of livestock farms in the form of point source (piggaries and aquiculture) and non-point source (cattle farms) pollution. Often effluent from piggaries and aquiculture is discharged into the nearest stream (personal observation). Although there are laws for the treatment of effluent, many smaller farming operations have too few animals to be covered under the laws or do not abide by these laws because they are not strictly enforced and are costly to follow. Piggaries are required by law to have holding tanks where microorganisms and pathogens can be reduced (Hutchings 1994).

Indicators

Stream ecosystems are complex environments subject to pollution from their watersheds. In order to

investigate stream health, physical, chemical, and biological indicators were used.

Physical indicators

Stream parameters such as width, depth, and current were measured because physical characteristics of streams show differences between grazed and ungrazed areas. Ungrazed areas tend to have greater canopy shading, stream depths, bank height, and smaller widths than grazed riparian areas (Knapp 1996). Cattle grazing at the stream bank may diminish stream depth and increase stream width. Another water quality constituent investigated, current, transports nutrients, organics, plankton, and benthos, all of which provide food to higher organisms and transport silt and sediment (Mackenthun 1969). Temperature has many effects on streams. With livestock effluent stream temperature would be expected to increase (Mackenthun 1969). Increased water temperature can lead to stratification, alter reproductive cycles, and decrease primary productivity (Mackenthun 1969). Temperature is inversely related to the solubility of gases in water, thus increased temperature will lead to decreased DO levels (Mackenthun 1969). Rises in temperature speed up aquatic organisms' metabolisms as well as bacterial breakdown processes, which further reduce DO levels (Mackenthun 1969). Current also oxygenates streams (Mackenthun 1969).

Chemical indicators

PH is important in its effects on stream organisms' biological pathways (Mackenthun 1969), Nitrate levels often increase with the presence of livestock. Dissolved oxygen (DO), which is introduced from the atmosphere or by plant photosynthesis and removed by respiration, provides stream organisms with oxygen for life and reproduction (Mackenthun 1969). At low DO levels, many organisms undergo stress and fail to compete with other species (Mackenthun 1969). DO can be increased by riffles, which increase the concentration of atmospheric oxygen in the water (Mackenthun 1969).

Biological indicators

Good biological indicators of livestock eutrophication are organisms highly sensitive to changes in water chemistry, abundant in stream environments, widely distributed, with well-studied taxonomy and ecology. Past ecological studies have involved the use of diatoms, shrimp, and snails as biological indicators. Shrimp have been used to examine the health of Florida Bay (Ehrhardt 1999), the pesticide levels in southeastern Queensland intertidal marsh pools (Brown 1996), the San Francisco Bay (Vendlinski 1995), and the environmental impact of a fertilizer manufacturing plant (Clarke 1993). Snails have also been widely used as bioindicators to compare metal concentrations at different sites (Gupta 1998), to study the success of east-southeast Madagarscar's natural reserves (Emberton, 1996), and to examine the levels of TBT contamination in Cork Harbour, Ireland (Minchin 1996).

As biological indicators, diatoms provide valuable baseline data due to the preservation of their valves in sediments (Reid 1995). Diatoms, due to their autotrophic nature, often form the base of aquatic food chains and are thus crucial to the productivity of aquatic ecosystems (Cox 1996). Due to their ecological specificity, ecological importance, and ease of collection, diatoms are useful biological indicators (Cox 1996). Of all biological indicators, diatoms have one of the shortest generation spans and thus respond quickly to environmental change and provide early warning of pollution (Stoermer 1999).

Previous research on anthropogenic disturbances and their effects on streams has been carried out on Moorea (Shiley 1992). A 1992 study investigating the effects of canopy removal, dams, and pineapple plantations on adjacent streams as indicated by algal communities found significant variation among control and treatment sites at both the canopy removal and dam sites. The study found no significant variation between control and treatment sites for pineapple plantations (Shiley 1992). A problem with this study is that it had only one to two site replicates per type of disturbance investigated. My study looked at 7 sites affected by livestock (all of the sites on the island that met the site requirements of having livestock adjacent to a year-round stream). Additionally, my study incorporated the use of a broader range of biological indicators.

Materials and Methods

Study Sites

On the island of Moorea in the South Pacific, located at 17° 30' South latitude and 149° 50' West longitude, seven study sites were selected in order to investigate eutrophication of streams by livestock (Figure 1). Three of the sites were adjacent to cattle farms, three to piggaries, and one to a shrimp farm. Each study site consisted of three sub-sites: one 50m upstream from the farm (subsite 1), one at the base of the farm (subsite 2), and one 50m downstream of the farm (subsite 3) (Figure 2). Qualifications for sites required that they be year-round streams with sunlit riffles bordered by riparian fringe and adjacent to livestock. One variable that differed between the sites is that some of the farms created point source pollution while others created non-point source pollution due to how the animals were confined. Two control sites (sites G and H) insured that differences found among treatment subsites were due to the adjacent livestock as opposed to some other confounding factor. In further efforts to eliminate confounding factors, upstream stream sites and

watersheds were explored and possible pollution sources were noted (Table 1). The sites ranged in the type of livestock, number and density of livestock, as well as in degree and type of upstream disturbance (Table 1).

Field Materials and Methods

To test for the eutrophication of streams I collected data for pH, nitrate, dissolved oxygen (DO), shrimp, snail eggs, diatoms, depth, width, current, and temperature. Samples were collected from October 2, 2000 through November 3, 2000. Samples for sites A-H (Figure 2) were collected before the rainy season began and sample I was taken after a daylong rainstorm. Stream width and depth were measured with a meter transect tape. The transect tape was also used in conjunction with a floating bottle top and stopwatch to determine the stream current. This was done by releasing the bottle top and measuring the distance per unit time. A thermometer was used to measure the stream temperature in the center of the stream as well as the temperature approximately .3 meters from each bank.

Biological indicators used were shrimp, snails, and diatoms. Each indicator included multiple species. The common snail species found in streams on Moorea are primarily *Neritina canalis*, although some *Neritina turrita*, *Septaria porcellana*, and *Nerita spinosa* are also found (Resh, personal communication). Three genera of shrimp in the streams are *Macrobrachium*, *Caridina*, and *Atya* (Resh, personal communication). Sixty-four diatom species were documented.

Shrimp counts were recorded as the number of shrimp that could be found in the pool above the subsite riffle within five minutes. Three species of shrimp were observed. The shrimp were not distinguished from one another or classified due to difficulties in catching them. Snail egg counts were made by starting at the base of each subsite and examining the first fifteen rocks (the size of my fist being the minimal acceptable size) as I made my way upstream. The number of eggs on each rock was recorded. As with the shrimp, the snail eggs were not broken down by species due to lack of scientific descriptions of the different eggs. Water samples were collected by submerging sample bottles into the stream below the most extreme part of the riffle. Rock biofilm samples were collected by selecting two rocks (again with the minimum size being that of my fist) from the base of each subsite and using a scalpel to scrape a $2cm^2$ section off each rock surface. The scrapings were then transferred to a sample vial containing approximately 1mL of stream water. Samples were refrigerated and analyzed for diatoms and chemical constituents within 48 hours after collection.

Laboratory Techniques

Slides of the water samples were prepared in the laboratory for diatom identification. Rock scraping samples were homogenized by suspension. Heavy particles were allowed to settle to the bottom and expelled. Two drops of the remaining sample were placed onto a slide. These slides were analyzed under a light microscope at 400X magnification for the presence of diatoms. Slides were analyzed left to right, and top to bottom. Diatoms were classified into morphologically distinct types according to appearance under 400X magnification (Figure 3). In future studies it is recommended that the slides be sent to a diatom expert for precise species identification. The identification of diatoms differs from other algal groups in that their classification is based primarily on cell wall morphology. Frustule morphology is characterized by symmetry, shape, and valvar markings deciphered under light microscope.

Water samples were analyzed in the laboratory to determine pH, nitrate levels, and dissolved oxygen content. Litmus paper was used to determine the pH, and test kits were used to determine nitrate and dissolved oxygen levels.

Data Analysis

Simpson's index was computed for the diatoms at each subsite to get an index for diatom diversity.

Simpson's Index: $D = 1/\sum p_i^2$

D = the Simpson's diversity index

p_i = the proportion of species i

Raw data for the dissolved oxygen levels was fairly close to the normal curve, but data for shrimp, snail eggs, and diatom diversity deviated from the normal curve. To statistically analyze the data with a one-way Analysis of Variance (ANOVA) it first had to be transformed so it would more closely approximate the normal curve because an ANOVA is a parametric test. The best transformation for each data set was selected by analyzing the probability graphs and histograms of different transformations to see which best followed the normal curve (Table 2). The snail egg data was transformed to be as close to the normal curve as possible, taking into account the number of data points that were zero. A one-way ANOVA was preformed on the transformed data to compare the different levels of organisms and dissolved oxygen at subsites 1, 2, and 3. The independent variables were site, subsite, and livestock type. The dependent variables were analyzed one at a time and were the different indicators of stream water quality.

Figure 1. Map of site locations on Moorea

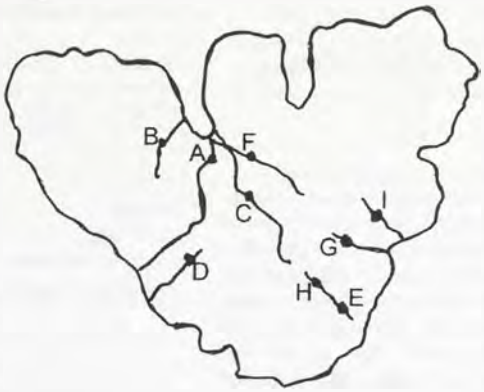


Figure 2. Sites A-I.

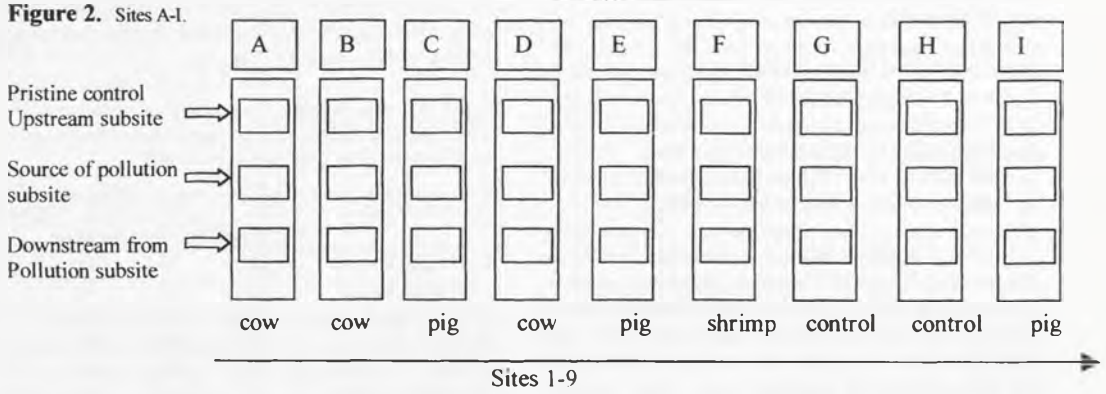


Table 1. Site descriptions

Site	Location	Livestock Type	Livestock upstream	Agriculture upstream	Other possible upstream pollution
A	Opunohu Valley	Cattle, 40 head	2 horses 100m upstream	Old papaya orchard 100m up	Road between at and downstream subsites Bridge construction
B	Opunohu Valley	Cattle, 10 head	Small agricultural plot 20m off stream	No	One house
C	Opunohu Valley, Agricultural School	Pig, 22 large (6') 50 small (3') 16 piglets (1')	No	No	No
D	Opunohu Valley	Cattle, 10 head	One horse 100m upstream	No	One house
E	Haipiti Valley	Pig 54 head	2 horses 100m upstream	No	7 houses
F	Opunohu Valley, base	Shrimp aquiculture	Yes 100m upstream	No	No
G	Afareaitu, Waterfall and stream	No (Control site)	No	No	Tourists throw mackeral into waterfall pool
H	Vaihau Village exit	No (Control site)	No	No	No
I	Afareaitu	8 head captive 30 head free-range	No	No	10 houses, garbage in stream, sample taken after rainfall

Table 2. Transformations performed to adjust data to conform more closely to the normal curve.

Indicator	Transformation
Shrimp	$X^{1/2}$
Snails	$X^{1/2}$
Diatom (Simpson's index)	$X^{1/3}$
Dissolved Oxygen	X

Although the transformed data gave a closer approximation to the normal curve than the raw data, it still failed to conform ideally to the normal curve. Due to this, the Mann-Whitney U-test, a non-parametric test was preformed because it is non-parametric. With the Mann-Whitney test, the raw data was used as opposed to the transformed data of the ANOVA test.

Equations for the Mann-Whitney U-test.
$U = n_1n_2 + (1/2)\, n_1 (n_1 + 1) - R_1$
$U' = n_1n_2 - U$
n_1 = the number of samples in column one
n_2 = the number of samples in column two
R_1 = the sum of the ranks for column one

With the Mann-Whitney U-test, if U or U' is greater than U critical (which is based on n_1 and n_2 and found in a table) then the null hypothesis can be rejected. In all the Mann-Whitney tests, the null hypotheses were that the water quality indicators had the same values at all subsites.

Chi-Square Test

Distribution of Diatom types were analyzed with the chi-square statistical test. Of the sixty-four diatom species found, those occurring only at one site were eliminated as were diatoms represented by fewer than five specimens. Additionally, diatoms that would have an expected frequency for the chi-square statistic of less than five were eliminated (Freedman 1998). The remaining diatom species were analyzed with the chi-square statistic to see if species occurred in different abundances at different subsites (Table 6).

Results

ANOVA

Diatom diversity, investigated with Simpson's Index, ranged from the least diverse, D=0 to the most diverse D=10.1083. The use of Simpson's diversity index data of diatoms in the ANOVA allows one to see if diatom diversity changes between subsites (Table 3). The p-values from the ANOVA were of statistical significance for diatom diversity and snail egg counts. The inclusion of site as an independent variable allows the test to block by site, which corrects for the fact that the sites were not replicates of one another. It is important to have this blocking factor because sites differed by type of livestock as well as in location on the island.

Table 3. P-values for one-way Analysis of Variance with site, livestock type, and subsite as independent variables. Dependent variables, taken one at a time were shrimp, snail eggs, diatom diversity, and dissolved oxygen level.

Indicator	p-value
Shrimp	0.455
Snail eggs	0.002*
Diatom (Simpson's index)	0.007*
Dissolved Oxygen	0.194

Mann-Whitney U-Test

In all of the Mann-Whitney tests preformed on the non-transformed data, no null hypotheses could be rejected because the U critical value exceeded all of the U and U' values (Table 4).

Table 4. Mann-Whitney U-test results. Subsites 1, 2, and 3 refer to upstream from the livestock, at the source of the livestock, and downstream from the livestock, respectively.

Paired subsites	U value	U' value	U critical value
Snail eggs 1 vs. 2	19	35	41
Snail eggs 1 vs. 3	23	26	41
Shrimp 1 vs. 2	28.5	20.5	41
Shrimp 1 vs. 3	20.5	28.5	41
Diatom 1 vs. 2 (Simpson's index)	32	17	41
Diatom 1 vs. 3 (Simpson's index)	23	26	41

Univariate Linear Regression

Univariate linear regressions were performed on dependent vs. independent variables (Table 5) with JMP statistical software. No regressions of statistical significance were found, all had p-values exceeding 0.1090.

Table 5. Univariate linear regressions

X vs. Y	R^2	p-value
Temperature center vs. shrimp	0.0071	0.7161
Temperature side vs. shrimp	0.0035	0.8003
Current vs. shrimp	0.0000	0.9937
DO vs. shrimp	0.0005	0.9228
DO vs. Simpson's Index for diatoms	0.0418	0.3874
Temperature center vs. snail eggs	0.1295	0.1090
Current vs. snail eggs	0.0687	0.2509
DO vs. snail	0.1285	0.1206
Temperature center Vs. Simpson's Index for diatoms	0.0093	0.6775
Current vs. Simpson's Index for diatoms	0.0094	0.6752

Table 6. Chi-square values with associated p-values. The critical values for the chi-square value to be statistically significant are <0.051 or >7.379.

Diatom	Chi-square value	P-value
U	84.5	<0.05
UU	17.5	<0.05
W	20.2	<0.05
EEE	28.4	<0.05
AA	20.9	<0.05
T	8.1	<0.05
CC	6.1	Not statistically significant (p>0.05)
GGG	15.5	<0.05
X	65.2	<0.05
D	11.1	<0.05
PP	17.5	<0.05
QQ	20.7	<0.05
RR	11.4	<0.05
Y	0.11	Not statistically significant (p>0.05)
VV	12.1	<0.05
F	24.5	<0.05
G	49.6	<0.05
V	14.2	<0.05

Out of the 18 diatom species that met the conditions to be tested with the chi-square statistic, 16 of the species had statistically significant differences in distribution between subsites.

Bar Graphs

Bar graphs were created on SYTAT statistical software of water quality indicator vs. subsite. This was done to see if there were trends between the subsites and the water quality. No clear trends were illustrated with the bar graphs because although the means varied, the error bars were so large that trends could not be established (Figure 4).

Discussion

ANOVA

In the ANOVA statistical calculations, highly statistically significant differences in distribution were found for both snail eggs and diatom diversity between the three subsites, indicating that water quality is not homogenous throughout each stream.

Mann-Whitney U-Test

The Mann-Whitney test did not yield anything of statistical significance. Possibly this is because this test is used to compare pairs of data, and I had three subsites and not two. I tried to use this test because it did not require data to follow the normal curve, but it was not designed for the use of comparing three subsites.

Univariate Linear Regression

The fact that no relationships of statistical significance were found by applying univariate linear regression suggests that more replication was needed in order to discover the trends.

Chi-Square Test

The chi-square test was used to analyze the diatom data in further detail. The fact that 16 out of the 18 diatom species tested with the chi-square test were found to have statistically significantly different distributions at the different subsites is strong evidence supporting that livestock have effects on streams.

Bar Graphs

The bar graphs indicate that more site replication was needed in order to draw stronger conclusions from the study. The fact that the means differ suggests that trends between subsites may exist. With more site replication, the error bars would be reduced, and it would be possible to make statements as to trends within the graphs.

One problem was with the chemical tests for water quality. Neither the litmus paper nor the nitrate chemical test were sensitive enough to indicate differences between samples. Perhaps there would be trends in the pH and nitrate levels if more sensitive tests were used, but perhaps these differences are so small that they are not important. The dissolved oxygen levels were expected to decrease as one passes through the stream portion adjacent to livestock; the fact that they did not may be affected by the varying intensities of the riffles. More extreme riffles will provide the water with greater oxygen content.

As is the case with all observational studies, there were factors that varied from site to site. Due to the nature of the study, I was limited in site selection to sites that had existing livestock adjacent to year-round streams. This basic requirement limited me to seven sites. Beyond the limited number of possible sites, not all the sites had the same livestock. Three sites had cattle, three had pigs, and one had shrimp. Along with not having the same type of animal at each site, the numbers of animals varied from site to site ranging from eight animals in captivity at the wild piggary to 88 animals in captivity at the Agricultural School.

The cattle produced non-point source pollution, which is less concentrated and harder to detect than point-source pollution. The shrimp at site F are confined to ponds with drainage channels and thus give off point-source pollution. The pigs produced point-source pollution due to the way they were confined. Although the pollution from the pigs should have been easier to detect then the cow pollution because it was more concentrated, the pigs were generally farmed in smaller numbers then the cattle. The largest pig farm was at the Agricultural

Figure 3. Photographs (with scale bars) of common diatom species found.

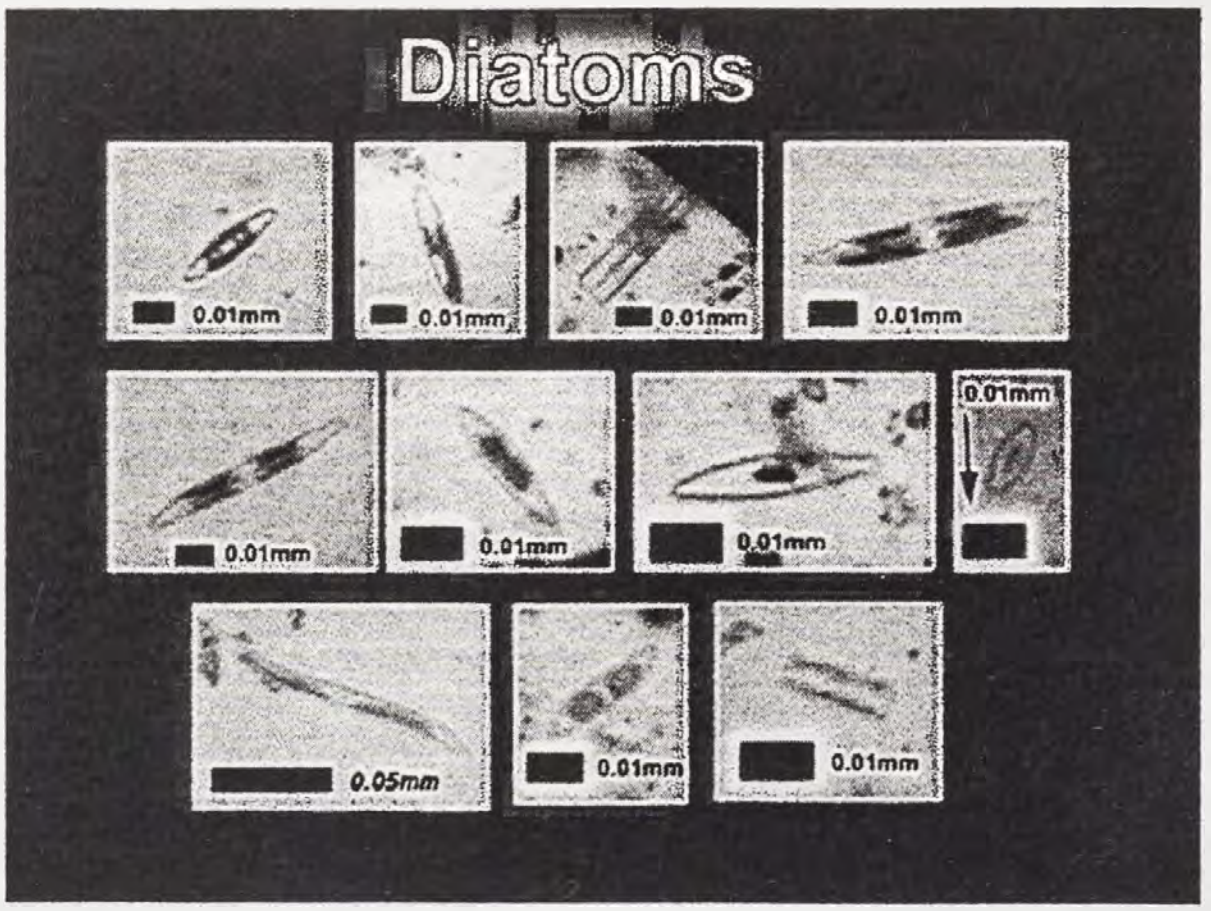
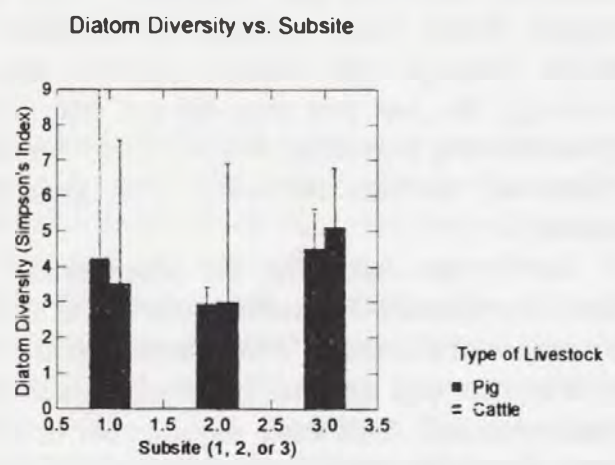
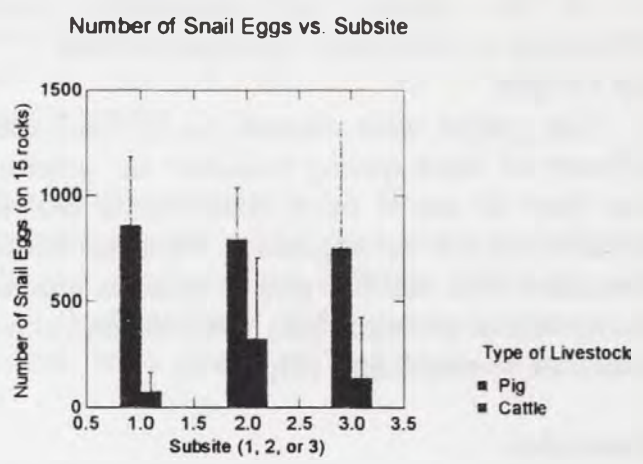
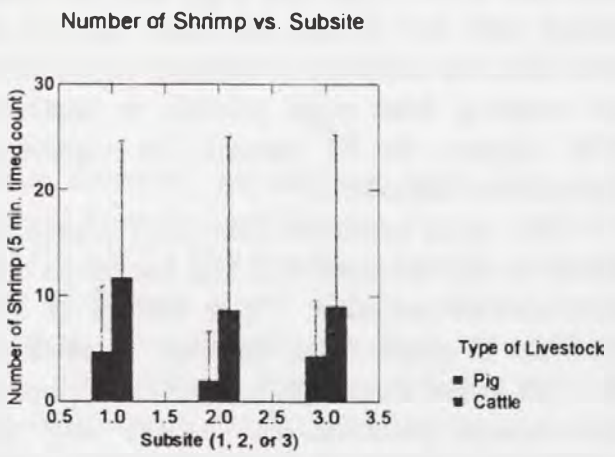
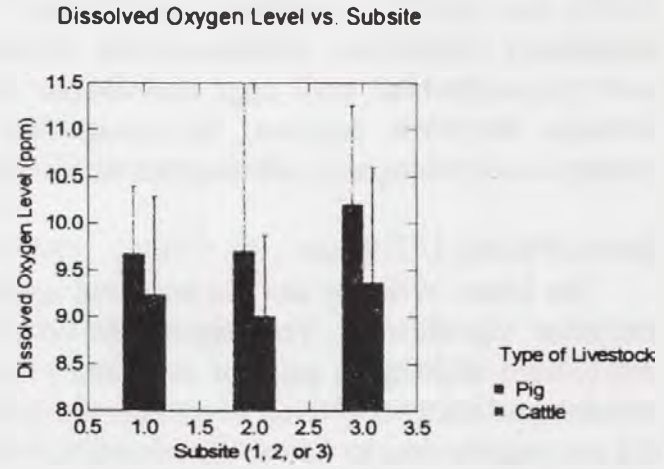


Figure 4. Data of indicators vs. subsite represented by bar graphs with mean error bars.









School, which is the farm that follows the law most stringently. The Agricultural School has a waste treatment program, keeps their pigs 30 meters from flowing water, puts all effluent into treatment pits where it gets filtered and undergoes microbial treatment. Because most of the other piggaries on the island are family farms, they are small enough to avoid being reprimanded for not following the laws. (Agricultural School Director, personal interview).

The pigs at sites C and E are confined to pens and thus have small area: head ratios and give off point source pollution. One of these pig farms, site C, (the Agricultural School) practices waste treatment, while site E does not. The pigs at site I are in two groups, one small group (eight head) of penned pigs, and one larger group (30 head) of free-range pigs within the Afareaitu Valley. Site I is susceptible to a combination of point and non-point source pollution.

Ideally, there would be more sites of cattle, pigs, and shrimp aquiculture, but the study was limited to livestock areas that existed on Moorea. Further work could include livestock on all the Windward Islands in order to have more data to work with. Because there were not more sites for each type of livestock, it was not possible to analyze the effects of eutrophication from different animals due to a low sample size. Analyzing the streams adjacent to different types of livestock separately would eliminate the variability between different animals and indicate if there is variation in effects on streams from different forms of livestock.

The study was representative of livestock on Moorea in that it investigated a cross section of livestock animals. Possibly the good geographical and livestock variation led to problems in finding patterns in the data. In future studies it is recommended that the study sites be narrowed down to the study of only one type of livestock. By narrowing down the livestock varieties, it will enable the researcher to do multiple replications of sampling at each site. This will be useful in determining the statistical significance of the data. In addition, by limiting the streams under investigation to streams adjacent to one type of livestock, many confounding variables will be eliminated. Data should be more conclusive if it is collected multiple times on only a few streams as opposed to once on many streams. With five to ten replications per site, data would be more reliable and more useful for statistical evaluation.

Future studies involving diatoms should preserve the slides as voucher specimens for diatoms specialists to use for diatom identification. Another way of documenting the diatom species is to take digital pictures of them through the microscope. This can be useful because it eliminates the time consuming problem of having to locate diatoms on slides. In order to preserve specimens, permanent preparations can be made by using an oxidizing agent to rid the sample of organic components and then mounting the inorganic cell wall components an a high refractive index. Problems with this method are that all cellular detail is lost and it takes long periods of time to locate the diatoms on the slides.

In this study, as is the case with many studies investigating possible eutrophication of waterways, the history of the land was not taken into account. It is important to keep in mind that past land use influences current diversity of streams. In a study investigating the best indicator of present-day diversity, land use in the 1950's compared to current land use. It was found that the land use which served as the best indicator of current diversity was land use in the past, many of which result in long-term reductions and alterations in diversity (Harding 1998).

A follow up study that could be conducted would be to conduct a very similar study during the rainy season. The data for sites A-H were all collected before the rains came, and the data for site I was collected after a day of rain. Possibly eutrophication effects are seasonal and only apparent in the wet season when there is more runoff. Conditions on Moorea, especially in the streams, are much different in the dry and rainy seasons. In dry seasons, the perennial streams stay fairly constant in terms of water volume. During the rainy season, the streams are subject to flash floods with dramatic surges in water flow. Studies suggest that water quality monitoring of non-point source pollution results are extremely variable depending on the temporal variability of the water chemistry at the time of rains. They suggest that samples should be taken before, during, and after storms. Additionally, they suggest that samples be collected over a time span of several years to account for variability from year to year. It is common for the majority of yearly stream discharge to occur during major rainstorms. The runoff flushed downstream by these rains have the potential to contain high concentrations of non-point source pollution (Tate 1999).

Many of the animal enterprises on Moorea are small and located in the suburban watersheds. Even though only a small number of animals are raised on each farm, the farms still have the potential to pollute adjacent streams. Simple and economical ways of lessening the degree of pollution are to fence off the streams and provide a watering are away from the streams (Godwin 1997).

Previous studies in the tropics have reported positive findings on methods of treating point-source pollution from piggaries. One such water treatment plan is to have a small on site wastewater treatment plant. Such plants can be simple to construct and operate. They have low capital and energy requirements and have shown remove 90% of TCOD and 95% of NH_4-N (Yang, Kongricharoern, and Polprasert 1993). Another study in Malaysia found that in situ solid waste handling management within

pigpens could be successfully implemented to avoid the generation of excessive wastewater. The method investigated allowed for the aerobic digestion of waste in sawdust and thus generated a byproduct of organic fertilizer. This waste management system was also low in capital and energy demands and reduced wastewater discharge (Ong, Choo and Soo 1993). The physical, chemical, and biological effects of piggary wastewater entering tropical streams was studied in Costa Rica. Benthic diatoms were used as biological indicators and it was found that the percentages of the diatom species groups reflected the degree of pollution (Michels 1998). This study found 127 species of diatoms. Trends were found with dominant species; some occurred primarily in pristine sites, while others were more common in effluent pollutes sites (Michels 1998).

The demographics of Polynesia reveals a large proportion of the population is less than 20 years of age (Merceron 1996). The demographic chart illustrates the pyramid of ages pattern characteristic of third world countries (Merceron 1996). With roughly 50% of the population under age 20, Polynesia must prepare itself for exponential growth to continue into the future unless some method of population control is implemented; which is unlikely as families are large and birth control is not emphasized. Polynesians must take measures to ensure enough food is available, while at the same time protecting the natural splendors of Polynesia that provide the staple economy, tourism (cite Laws – Kaneohe Bay, Hawaii). The percentage of Polynesians employed in agriculture and fishing has declined from 46% in 1962 to 14% in 1996 (Merceron, 1996). Workers have entered the more prosperous sectors of the economy- government, tourism, and commerce (Merceron 1996).

Conclusions

The present is a crucial time in Moorea and all of French Polynesia as it prepares itself for independence. Loosing France's subsidies will put a strain on the local economy and may increase local livestock production in order to reduce demands for costly importation. The environmental impact of livestock point and non-point source pollution must be put into monetary terms in order to decide whether the net effect of livestock practices are economical. Depletion of the environment hurts the tourism industry, which brings in more money to Moorea than livestock and agriculture combined (Merceron 1996).

The goal of my study was to explore the anthropogenic effects on streams caused by livestock cultivation by using a range of indicators. Only one of the farms in my study abided by the laws pertaining to the distance animals must be kept from flowing water. For pigs, the minimal distance they must be kept from streams is 30 meters (John Claude Gaulthier personal interview), and of the three pig farms investigated in this study, only one abided by this law (personal observation). It was hypothesized that biodiversity in streams adjacent to livestock will be altered downstream and adjacent to the livestock in respect to biodiversity in the pristine areas upstream from the livestock.

To maintain Moorea's economy, it is important that existing livestock laws are enforced and that livestock owners are educated about simple methods of livestock management and wastewater treatment. Livestock was found to affect water quality of adjacent streams in that statistically significant differences were found between subsites for both diatom diversity and snail eggs. It is recommended that livestock methods be changed in order to prevent the deterioration of the streams and coral at the mouths of the streams.

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Spatial Distribution and Succession of Epiphytes on *Coco nucifera* in Moorea, French Polynesia

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ABSTRACT. Coconut trees are a conspicuous and characteristic part of the South Pacific Landscape. In the past fifty years, a number of coconut tree plantations have been abandoned. These provide for an excellent study of the succession of the epiphytes that use coconut trees as phorophytes. Seven functional epiphyte groups were studied: Bryophytes, Paint Crustose Lichens, Fat Crustose Lichens, Alectorioid Lichens, Cyanobacterial Lichens, Algae and Ferns. Five types of plots were studied, those that had been abandoned for fourty-five years, thirty-five years, twenty-five years, fifteen years, and zero years. Principle Component Analysis confirmed the grouping of the thirteen plots studied within the aforementioned categories. Eight environmental variables of the plots were examined to determine which one had the largest influence on the change in epiphyte composition throughout time. Surrounding canopy cover and canopy height were the two main influential factors determining epiphyte distribution between the plot types as seen through regression and multivariate analysis. Results also showed that Bryophytes are late-successional species and Paint Crustose Lichens and Alectorioid Lichens are pioneer species on *Coco nucifera*. Succession does occur and radically changes the distributions of the epiphyte functional groups on the coconut trees over time. Epiphytes were found on all plot types from the 20% coverage level and above. A two-tiered model was created to explain succession of epiphytes on coconut trees.

Introduction

In 1686, Frederic King began studying bogs and sloughs of Ireland in order to understand their creation. The damning up of rivers created small pools that filled in with grass to eventually create meadows where bushes and trees started to grow. The meadows would then become part of the forest area that surrounded the old river bed. What King observed was the process of succession. Succession is the change in species composition within an ecological community over time. It was not until Halt in 1887 that science first gained the real understanding of the fundamental importance of development in vegetation. He did so by making a systematic study of a region upon this basis and he was the first to grasp the significance of the climax or final community in the process of succession. He said that the distribution of plant communities could be understood only by tracing the development from the first sparse colonies upon bare soil or in water to the now dominant forms of life within a final community. Epiphytes are a polyphyletic group in which succession can occur.

Epiphytes are plants which grow on other plants, mainly trees, while neither harming nor benefiting the host plant. Vascular epiphytes in tropical forests have been studied since as early as the end of the 19th century (Schimper, 1888). Since Schimper s pioneering work, major work on tropical vascular epiphytes has been published at large intervals (e.g., Van Oye, 1924; Went, 1940; Sugden & Robins, 1979). Barkman defined the term epiphyte in 1958: an organism living on a plant or in the dead outer tissues of a plant without drawing water or food from its living tissues.

The epiphytic habit appears a successful adaptation to the tree crown habitat since the number of epiphytic vascular plant species is great. An estimated 29,500 species are epiphytes distributed in 83 plant families (Gentry & Dodson, 1987). In wet tropical rain forest 35% of the number of species may be epiphytes comprising 63% of the number of plant individuals. More research is needed to understand this vital component of rain forests.

Coconut trees are a conspicuous and characteristic component of the landscape of Moorea in French Polynesia. Sixty to one hundred and fifty years ago, coconut tree plantations were established to sell copra and coconuts on the local and world market. These plantations contained only coconut trees and, except for low-lying groundcover, there were no other plant species within the plantations. Starting fifty years ago, it became largely unprofitable for Mooreans to sell coconuts and copra and a large percentage of the coconut plantations were abandoned over the past forty-five years. These abandoned plantations began to re-integrate back into the surrounding tropical forest environment.

Epiphytes are found on many tree species on Moorea, including coconut trees. The abandonment of the coconut plantations provided for a study of succession of epiphyte species that occur on the trunks of the trees as the surrounding environmental factors within the plantations radically changes after it has been abandoned. The focus of this study is the distribution,

abundance and succession of epiphytes on coconut trees, focusing on patterns of epiphyte distribution at different spatial scales and epiphyte abundance among trees differing in size, age, location and undergrowth cover. Possible causal variables for epiphyte abundance and distribution on the coconut trees include time of abandonment, age of trees, height and density of understory canopy, ground cover density, tree diameter and plot slope.

Previous research has shown epiphytes are influenced by five factors: tree and branch size, forest age, substrate type, relative position and microclimate (Wolf, 1993). Microclimate includes light temperature and moisture. According to Lobban, there is a vertical zonation that occurs along the phorophyte or host tree in a tropical forest. Zone 1: fully exposed zone with microepiphytes, zone 2: main zone of epiphytes on large limbs, zone 3: drier upper trunk, zone 4: moister lower trunk with lichens and liverworts and zone 5: damp base of the tree covered with mosses. There are five groups of epiphytes that B. Lyons created in her study: Alectorioids or pendulous lichens, bryophytes which include mosses and liverworts, cyanolichens which are N_2 -fixing macrolichens, other lichens and crustose lichens. Each of these epiphyte functional groups has different environmental requirements for survival. Coconut trees do not fit the normal modal for vertical zonation of epiphytes along the trunk.

Epiphyte succession - Coconut trees on maintained plantations are groomed by the landowners to keep dead leaves off them. Also, other large plants are kept off of the grounds, leaving only grass and other groundcover. When they are abandoned, other plants can move in and will create a different type of environment around the tree. The microclimate becomes less sunny and more wet as the plantation becomes an integral part of the original forest. There will be a succession of epiphytes from dry-loving epiphytes being found in the newer abandoned coconut trees to wet-loving epiphytes being found on older abandoned coconut trees. I propose a two-tier model to explain succession of epiphytes on a coconut tree (Figure 1).

On coconut trees within maintained plantations there should only be epiphytes found on the trees that prefer a dry environment. This population of epiphytes will stabilize until the plantation is abandoned and then as the understory changes, new epiphyte types will begin growing on the trunks. This population will also stabilize over time. There will be a unique flora of epiphytes in the late abandoned coconut groves vs. the early abandoned coconut groves. There will be a vertical zonation of an early abandoned coconut tree plantation that is different than a late abandoned coconut tree plantation. I propose that epiphyte biomass will be determined by the amount of light penetrating the canopy of a plantation, because most epiphytes thrive in wet, shady conditions and in fact need this condition to survive.

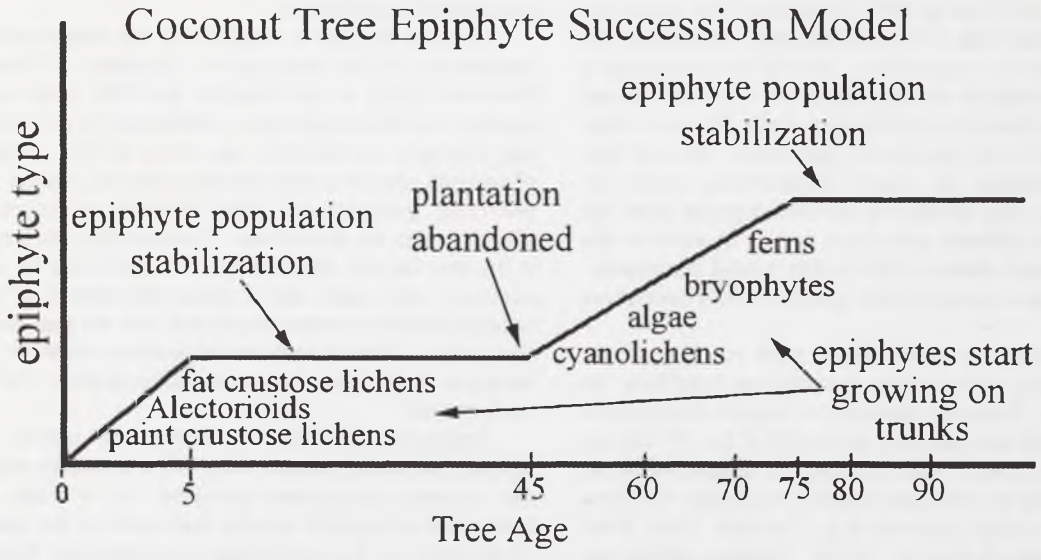


Figure 1. Two-tiered hypothetical model for succession of epiphytes on Moorean Coconut trees.

In this study I quantified the distribution patterns of epiphytic lichens, ferns and bryophytes on *Coco nucifera*. The plots that were studied were divided into five groups based on age of abandonment to test four hypotheses: (i) the distribution and abundance of epiphyte functional groups varies with age of abandonment and percent canopy cover, (ii) there is a unique vertical zonation for coconut trees that differs from the traditional tropical tree epiphyte zonation model, (iii) the two-tiered model will explain the succession of epiphytes that occur on coconut trees in Moorea and (iv) the predefined grouping of the thirteen plots into five groups based on abandonment age is accurate in terms of similarity of environmental and biotic variables. My objectives were to (i) extensively sample trees to quantify patterns of epiphyte distribution at distinct spatial scales (within trees and within the forest canopy), (ii) compare epiphyte abundance among trees differing in age and in age of abandonment, (iii) identify environmental variables correlated with epiphyte abundance and distribution and (iv) to describe the lichen, fern and bryophyte species composition in the coconut tree stands in a successional sequence. This information could improve our understanding of succession within an ecological community and how well a forest is able to recover from the impacts of human-caused environmental change

Materials and Methods

Study Areas

Eight abandoned plantations and five maintained plantations were selected for the study. All plantations were on Moorea and were located in three different areas: the East side of Cook's Bay, Vaianae Bay and Afareaitu (Figure 2). Each site had similar characteristics: Precipitation values, altitude, plantation size, understory vegetation types and soil type were consistent between sites. For maintained plantations, vegetation was either grass or *Wedelia sp.*, a low-growing invasive plant that did not exceed 25 cm in height. For abandoned plantations, the vegetation was mainly Hibiscus trees or transition between Hibiscus trees and Inocarpus trees. A number of abandoned plantations also had Mango trees exceeding 18m. Forest age in abandoned plantations ranged from 15 years old to 45 years old (Table 2). The most significant difference between plantations was slope, which varied from 0% to 25%. All plantations are or were part of large agricultural areas on Moorea and each are within 100m of roads that are used on a routine basis by Mooreans.

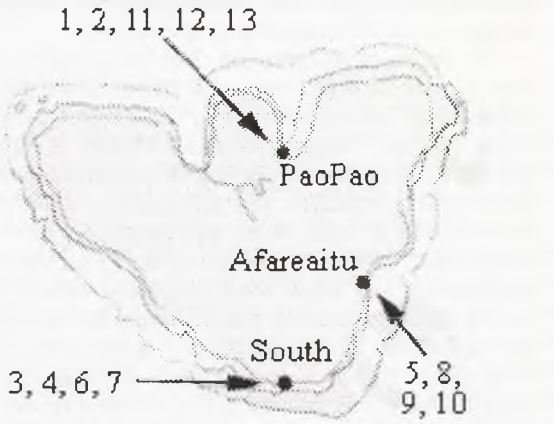


Figure 2. Map of study sites.
PaoPao at S17° 29.585 , W149° 48.981 .
Afareaitu at S17° 32.966 W149° 47.859 .
South at S17° 34.548 W149° 51.037 .

Sampling

The thirteen plots were divided into three groups based on age of abandonment of the plantation: Type 1: 0 years abandoned, Type 2: 15 years abandoned, and Type 3: 25+ years abandoned. The Type 3 plots were further divided into three groups: 3A: 25 years abandoned, 3B: 35 years abandoned, 3C: 45 years abandoned for a total of five different Plot Types. There were five Type 1 plots and 2 plots each of Types 2, 3A, 3B and 3C. Sampling was the same for all plots. Each plot measured approximately 350m in circumference. There were at least thirty coconut trees within this area and this ranged upwards to 100 coconut trees. Eight coconut trees were sampled in each of the plots using random co-ordinates. The majority of coconut trees had an arch to the trunk growth that created a concave side and a convex side of the tree. Only living trees whose convex angle was no less than 75% and whose height was at least 10 meters were chosen.

The occurrence of all fern, lichen and bryophyte species on the trunk below 2.5m in height was recorded on the sample trees. The sampling on the trunks was divided in the concave side and the convex side for the

following reasons: (1) the basal parts of tropical forest trees have generally the highest species richness (Koskinen 1955), (2) The shape of the concave side of the trunk possibly was being exposed to a more humid environment as well as precipitation (3) Prior visual sampling of a group of three coconut trees revealed a sharp difference in epiphyte distribution around the circumference of the trunk, from concave side to convex side and (4) The shape of the trunk would hypothetically create a dry environment on the convex side due to lack of exposure to precipitation.

To obtain cover estimates, 20x50cm sample plots were marked out with transparent plastic sheet along a transect live along the length of the trunk to 2.5m at 50cm, 100cm, 150cm, 200cm and 250cm. This was done on the concave and the convex sides of the trunk. The 20x50cm rectangle was divided into 100 2x5cm subunits, and a single point was randomly placed in each of these. The number of points hit by each species was counted and these point frequency values were used as an estimate of the species' cover percentage on the sample plot. The values on the concave and convex sides of the trunks were combined for many of the analyses done, but in creating a zonation model for coconut trees, this data was not combined in order to be used for separate analysis. Sample points were also chosen at 1000cm and 100cm below the crown of the tree, also called the 2500cm sample point. Species frequency at these points was estimated using binoculars and was not divided into concave and convex sides of the trunk.

Specimens of unidentified crustose lichen species were collected for identification with a microscope. Voucher specimens were deposited at the UC Berkeley Jepson Herberium. Many crustose species could not be identified and were given field id names for ease of sampling and data analysis.

Epiphytes were also classified into functional groups following McCune(1993). Epiphyte functional groups were comprised of species sharing similar ecological roles and morphology. Alectorioids are pendulous lichens belonging to genera *Usnea* and *Alectoria*. Bryophytes include all mosses and liverworts. Cyanolichens are foliose macrolichens which contain cyanobacteria in their thalli. Crustose lichens were divided into two groups according to morphology: (1) paint crustose and (2) fat crustose. The Ferns group included all ferns and the Algae group had only one type of green algae.

The DBH and height of each sample tree were measured. The circumference of the sample tree was measured then the diameter was found from this number for DBH. Height was measured using a clinometer. % Canopy cover above each sample tree was estimated in the 5% classes using a densinometer. Groundcover density 4m around each tree was estimated within the 5% classes. Tree age estimates and age of abandonment of the plantation were gathered from the owners of the plots. Slope of the plot was measured using a clinometer. Precipitation data was obtained from a French Polynesian atlas.

Bark samples were collected from the trunks at 0.5-1.5 m above the ground outside the sample plots. Two or three grams of surface bark in small pieces was incubated in 25 or 37 ml of distilled water for 30 hours and the pH value of the extract was measured with litmus paper.

Data analysis

4.1 Plot Analysis

The thirteen plots were analyzed to compare the relationships to each other using Cluster Analysis and Principle Component Analysis (PCA) in the computer program Systat. Cluster analysis was used to group and show relationships between the thirteen plots. It was based on the environmental variables (DBH, Height, Abandonment Age, Tree Age, Plot Slope, % Canopy Cover, Canopy Height, % Groundcover) and the % cover of each of the 25 epiphytes within each plot (Figure 3). PCA creates an ordinate diagram based on the eight environmental variables and the 25 epiphyte biotic variables for each of the thirteen plots (Figure 4). The variables for each of the plots were graphed onto the ordinate diagram and those plots that were similar to each other cluster more closely to each other. This test was used to determine whether the predefined grouping of the thirteen plots into Types 1, 2, 3a, 3b and 3c was correct or whether this hypothesis would need to be modified. Each of the environmental variables corresponded to a principle component axis, which originated out of the crossing of the X and Y-axis. Those PC axes that were closer to the X-axis are more influential in determining how the plots lie along the X-axis. PC axes closer to the Y-axis do the same for the plots along the Y-axis (Figure 4).

4.2 Plot/Epiphyte Analysis

Data was grouped using two different methods to understand succession on two different levels. The first method involved comparing averaged data from the plots that were grouped into the five different types i.e. Type 1, Type 2, Type 3a, Type 3b and Type 3c versus the average epiphyte data that was grouped into functional groups mentioned previously. To further clarify, in this method, when statistical analysis was used, the data being compared was an average Type X tree versus an epiphyte functional group. There were five average trees being compared against five functional groups. In method two, data of each plot was kept separate and data of each epiphyte distribution

among the plots was kept separate. There were 13 plots being compared against 21 epiphytes. Different statistical analyses were done within each method. Method 1 will be discussed first.

4.2.1 Method One

Individual plots were averaged to generate tree and epiphyte distribution averages. These averages were then averaged with other plots of the same type to generate a tree average for that type of plot. Average epiphyte cover was also calculated for each plot and then for each plot type. Species averages were combined into the functional groups mentioned previously to generate percent cover averages for each functional group within each plot type. Separately, data from the concave side and from the convex side of the trunk was compared to each other for the zonation part of the study.

The original cover values for each species were square root transformed to normalize the data and downweight the influence of very abundant species. Linear regression was then performed between these numbers and eight environmental variables (DBH, canopy cover, canopy height, age of abandonment, tree age, slope and groundcover). A Pearson correlation analysis was used for pairwise examination of tree structure variables (tree height, age and DBH) and epiphyte abundance. A weighted average, W (Whittaker, 1967) was calculated for each functional group as a relative measure of its successional position. It was based on the frequency of the species functional group in each plot type in accordance with the formula:

$$W=(a_1 + a_2 \times 2 + a_3 \times 3 + a_{18} \times 18) / (a_1 + a_2 + a_3 \ldots a_{18})$$

Where a_1 is the frequency of the species functional group in the maintained plantation, a_2 the frequency in a type 2 plantation etc. Groups were ranked according to their W value, those mainly associated with the oldest abandoned plantations having a higher value and vice versa. Furthermore, each group was ranked according to this value W.

4.2.2 Method Two

Data from the individual plots was kept separate in order to compare similarities between them to determine relationships and influences from each environmental variables. Data from each epiphyte variable was also kept separate rather than grouping them into epiphyte functional groups. This method was done in order to use multivariable statistical methods. Multivariable statistical methods take all environmental variables and the influences on each other into account when performing an analysis. Statistical analyses that were run on this set of data include the principle component analysis and the cluster analysis mentioned in section 4.1. Also, a Spearman correlation test was used to compare environmental variables with each other and to compare epiphyte variables with each other. These tests were run using JumpIn by SAS. Multi-variable ANOVA was used to compare all environmental variables with each of the epiphyte variables to determine which had the most influential effect on the epiphyte composition within each Plot Type. This was done using Systat.

Plot #	Plot type	Abandonment	DBH	% GroundCover	Tree Height	Cover Height	Tree Age	% Canopy Cover
1, 3, 5, 7, 11	1	0 yrs	18.65	95.00%	19.39	0	71	0.00%
8,10	2	15 yrs	18.88	75.00%	17.2	8.77	70	24.00%
6, 12	3	25 yrs	15.89	5.00%	18.36	16	65	79.00%
2, 9	3	35 yrs	16.72	12.50%	19.46	18.13	80	70.00%
5, 13	3	45 yrs	19.76	10.00%	19.56	16.75	85	77.00%

Table 1. Plot and tree characteristics for all thirteen study sites.

Results

Stand Characteristics

The trees in the older abandoned plots were generally older than the maintained plantation coconut trees, except for one outlier at Plots 6 and 12. Tree height ranged from 15m to 25m for all trees.

The canopy cover values ranged between 0% and 95% for all trees studied. When averaged together within each plot, the highest canopy cover was 79%. On Type 1 plots, the coconut trees tended to occur in fairly open patches where there was much ground cover which did not exceed more than 50 cm in height. Grass species and *Wedelia sp.* made up the majority of the groundcover for this area. Groundcover decreased with age of abandonment of the plot. pH value was 6.0 for all samples.

A Spearman correlation test revealed a significant positive correlation between abandonment time and cover height and % canopy cover and a negative correlation between groundcover and % canopy cover, canopy height and abandonment time (Table 2). There was also positive correlation between tree height and tree age and a negative correlation between tree height and slope.

Variables	Tree Height (m^2) Corr. coeff	*p*-value	Tree Age Corr. coeff	*p*-value	Plot Slope Corr. coeff	*p*-value	Canopy Cover (%) Corr. coeff	*p*-value	Canopy Height (m2) Corr. Coeff	*p*-value	GroundCover (%) Corr. Coeff	*p*-value
Age	0.568	**0.0427**										
Slope	-0.545	**0.0539**	-0.267	0.3772								
Canopy Cover	0.017	0.9562	0.271	0.37	0.343	0.2514						
Canopy height	0.1074	0.7269	0.44	0.1324	0.224	0.4622	0.872	**<0.0001**				
Ground Cover	-0.082	0.7883	-0.453	0.12	-0.293	0.3312	-0.938	**<0.0001**	-0.941	**<0.0001**		
DBH	0.429	0.144	0.463	0.1111	-0.639	**0.0187**	-0.215	0.481	-0.266	0.3804	0.133	0.6625
Abandonment	0.034	0.9119	0.349	0.243	0.286	0.3427	0.959	**<0.0001**	0.889	**<0.0001**	-0.967	**<0.0001**

Table 2. Spearman correlation values for each environmental variable from the thirteen plots.

Plot Types

Each plot was placed into a predefined Type group (1, 2, 3a, 3b, 3c) which was based on abandonment age. See Table 1. The cluster analysis showed that Plots 3, 1, 7, 5 and 11 cluster together. 10 and 8 were closely related, 9 and 2 were a cluster, 6 and 12 were a cluster and 4 and 13 were a cluster. Each of these clusters match up with the five predefined Types: 1, 2, 3a, 3b and 3C. Cluster analysis showed that the plots do cluster into these five groups (Figure 3). Further analysis with Principle Component Analysis, which takes into account variance within the data and converts values of variables into eigenvectors showed that the plots also cluster according to the predefined Type groups (Figure 4).

	PC1	PC2	PC3
Eigenvalue[A]	4.2651	2.4637	0.5281
Percent[B]	53.3139	30.7960	6.6012
CumPercent	53.3139	84.1099	90.711
Eigenvectors			
DBH	-0.35	0.798	0.448
Height	.084	0.88	-0.36
Age	0.416	0.689	0.981
Slope	0.352	-0.750	0.88
Canopy Cover	0.989	-0.015	-0.24
Canopy Height	0.974	0.087	-0.24
GroundCover	-0.995	-0.0299	-.11
Abandonment	0.959	0.086	-.21

Table 4. Principle Component Values for the eight physical variables of each plot.

[A] Variance explained by component .

[B] Percent of total variance explained.

PCA relies on factors to create the X and Y-axes for the ordinate diagram (Figure 4) and the factors are based on the environmental variables. The environmental variable that is most influential along the X-axis is % canopy cover (Table 4). The X-axis can be defined as a vegetative axis. % Canopy cover and canopy height were both positively correlated with each other along the X-axis and Groundcover was negatively correlated with these two along the X-axis. Abandonment Age was also highly correlated with canopy cover and canopy height. The Y-axis is most influenced by tree height. Positively correlated with it are DBH and Tree Age. Slope is negatively correlated with these three variables (Figure 4 and Table 4).

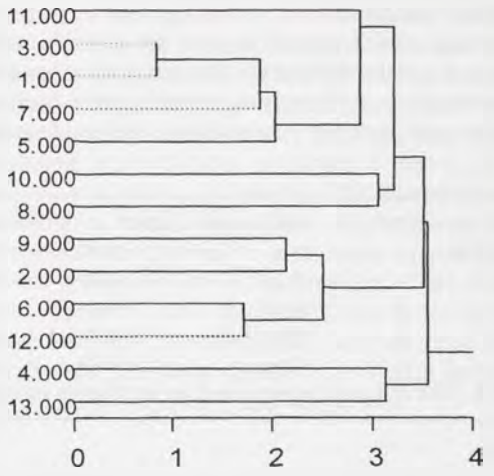


Figure 3. Cluster analysis of Plots 1-13 with distance along horizontal axis showing relationships between plots.

Species Composition and Cover

A total of twenty-five epiphytes were found on all plots. There were six moss species, eleven lichen species, two fern species, five liverwort species, and one alga. The total number of species per stand varied from a minimum of 9 for plot #8 to a maximum of 19 for plot #1. Two species use coconut trees as their main phorophyte. The remaining 23 species occur on a wide

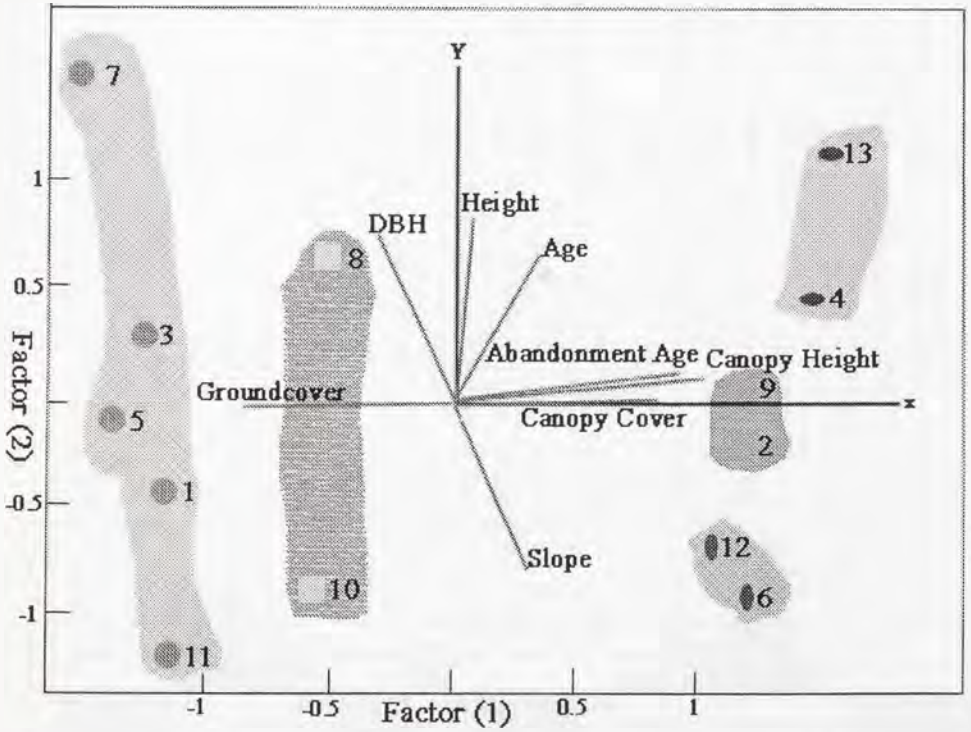
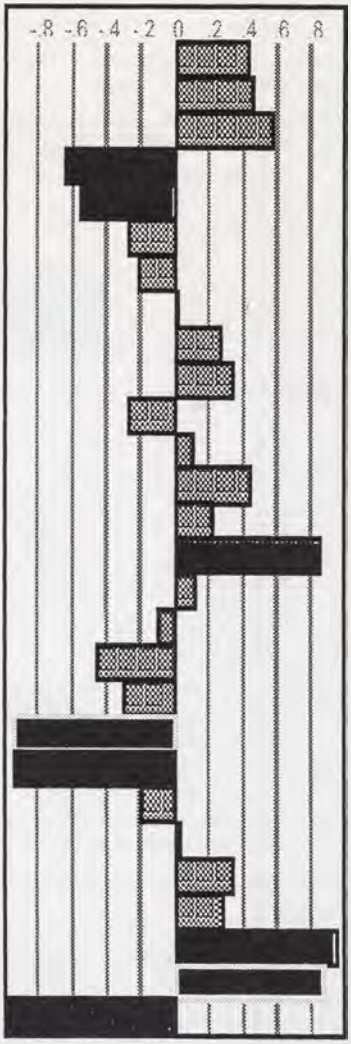


Figure 4. PCA Ordinate Diagram of the thirteen plots showing grouping defined by the five Plot Types. PCA axes are also shown for each environmental variable. Type 1: 7, 3, 5, 1, 11; Type 2: 8 and 10; Type 3a: 6 and 12; Type 3b: 9 and 2; Type 3c: 13 and 4.

Table 3. Spearman Rho coefficients for each environmental variable. Graph on right is of the coefficients.

Nonparametric Measures of

Variable	by	Spearman	Prob>|Rho
Height	DBH	0.4286	0.1440
AGE	DBH	0.4629	0.1111
AGE	Height	0.5684	0.0427
Slope	DBH	-0.6391	0.0187
Slope	Height	-0.5455	0.0539
Slope	AGE	-0.2674	0.3772
Canopy	DBH	-0.2148	0.4810
Canopy	Height	0.0170	0.9562
Canopy	AGE	0.2713	0.3700
Canopy	Slope	0.3429	0.2514
CanopyHeigh	DBH	-0.2656	0.3804
CanopyHeigh	Height	0.1074	0.7269
CanopyHeigh	AGE	0.4400	0.1324
CanopyHeigh	Slope	0.2239	0.4622
CanopyHeigh	Canopy	0.8721	0.0001
Groundcove	DBH	0.1340	0.6625
Groundcove	Height	-0.0827	0.7883
Groundcove	AGE	-0.4531	0.1200
Groundcove	Slope	-0.2930	0.3312
Groundcove	Canopy	-0.9385	<.0001
Groundcove	CanopyHeigh	-0.9414	<.0001
Abandonmen	DBH	-0.1905	0.5331
Abandonmen	Height	0.0341	0.9119
Abandonmen	AGE	0.3486	0.2430
Abandonmen	Slope	0.2865	0.3427
Abandonmen	Canopy	0.9591	<.0001
Abandonmen	CanopyHeigh	0.8889	<.0001
Abandonmen	Groundcove	-0.9676	<.0001



range of phorophytes including Inocarpus and Mango trees.

The lowest number of bryophytes occurred on Type 1 plots and the highest percent were on Type 3C plots (Figure 6). Algae percent cover increased with abandonment time as well as did fern percent cover. Crustose lichens stayed fairly level throughout abandonment time. Viewing the individual functional group cover as a percentage of the total epiphyte cover revealed a large change in the distribution of the epiphytes over time (Figure 5). Paint crustose lichens were 66% of the total epiphyte cover, alectorioids were 12%, fat crustose lichens covered 9%, algae were 6%, cyanolichens were 5% and bryophytes were 1.2% in a Type 1 plot. On a plantation that has been abandoned for 45 years, bryophytes were now 35% of the total epiphyte cover, algae were 32%, paint crustose lichens were 28%, ferns were 1% and cyanolichens and fat crustose lichens were 2% of the total epiphyte cover (t-test for independent samples, $P<0.05$ for all comparisons) (Figure 5).

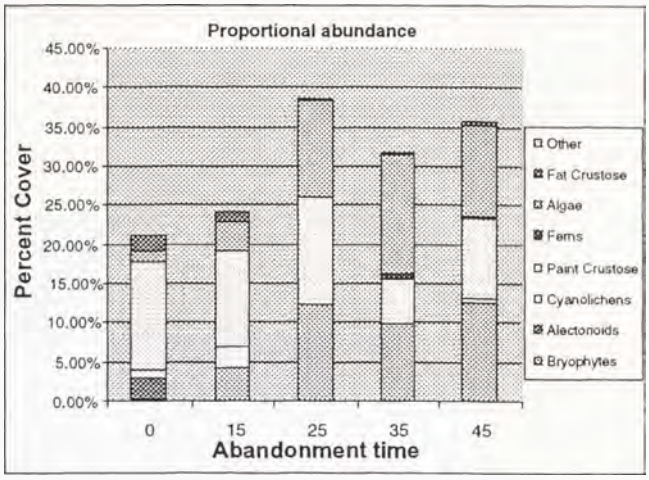


Fig. 6. Proportional abundance of epiphytes per abandonment time.

Effects of Environmental Variables

The linear regression analyses and MANOVA on the environmental variables versus percent cover of each functional group and each epiphyte variable revealed a significant gradient in the data. See Table 3.

Cover height had the highest R2 value, followed by abandonment time, % canopy cover and % groundcover. The rest of the environmental variables were not significant ($P >> 0.05$). For each of the functional groups, regression analyses showed that cover height was the most important factor explaining frequencies.

Type	DBH	% GroundCover	Tree Height	Cover Height	Tree Age	% Cover	Abandonment
R2 Value	0.04	0.89	0.038	0.96	0.28	0.92	0.95
P Value	0.73	0.016	0.75	0.003	0.35	0.009	0.004

Table 3: R_2 values for environmental variables versus bryophyte percent cover

Comparisons of data from the concave side and the convex side revealed a significant difference in epiphyte distribution and frequency between both. The data from each of these sides was averaged together to obtain the epiphyte percent cover estimates for each tree.

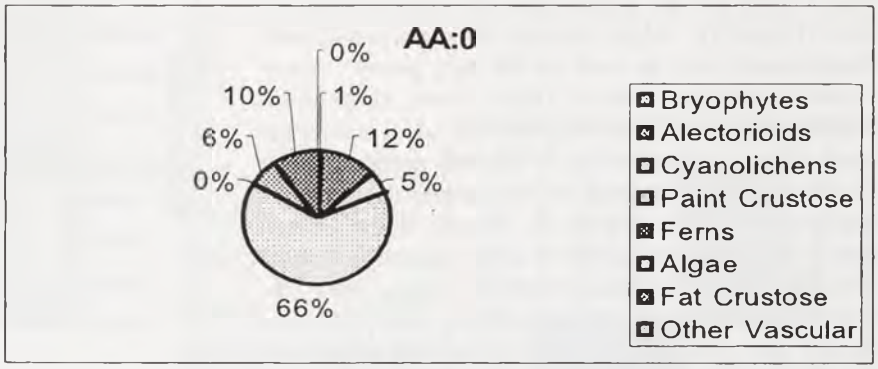
The Whittaker analysis revealed that ferns, byophytes, algae and other vascular plants were most associated with older abandoned plots. Alectorioids and fat crustose lichens were most associated with maintained plots. Paint crustose lichens have a W of 2.46 and this falls in the middle of the data. Cyanolichens were associated with younger abandoned plots also.

Weighted Averages	
Bryophytes	3.696674
Alectorioids	1
Cyanolichens	1.981257
Paint Crustose	2.468842
Ferns	3.745299
Algae	3.63719
Fat Crustose	1.777813

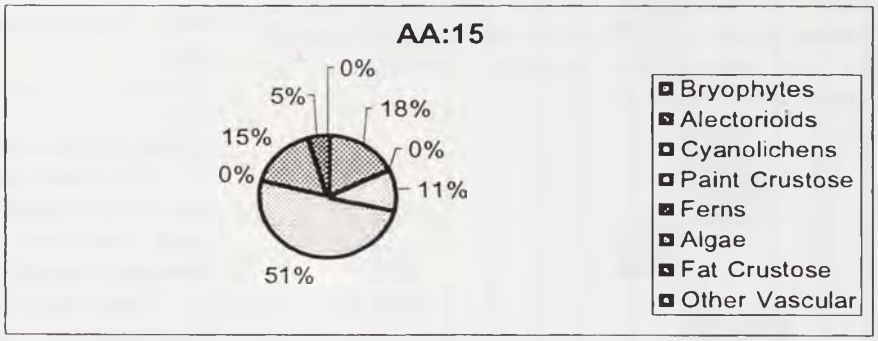
Table 4. Measure of species' successional position

Figure 5. Total Distribution of epiphyte functional groups for each age of abandoned plantation.

Epiphytes:0	% of Total Epiphyte Coverage
Bryophytes	1.20%
Alectorioids	12.34%
Cyanolichens	5.20%
Paint Crustose	64.89%
Ferns	0.17%
Algae	6.28%
Fat Crustose	9.90%
Other Vascular	0.02%
	99.99%



Epiphytes:15	% of Total Epiphyte Coverage
Bryophytes	17.54%
Alectorioids	0.00%
Cyanolichens	11.07%
Paint Crustose	50.94%
Ferns	0.21%
Algae	15.07%
Fat Crustose	5.16%
Other Vascular	0.00%
	99.99%



Epiphytes:25	% of Total Epiphyte Coverage
Bryophytes	31.93%
Alectorioids	0.00%
Cyanolichens	0.27%
Paint Crustose	35.41%
Ferns	0.38%
Algae	31.73%
Fat Crustose	0.28%
Other Vascular	0.00%
	100.00%

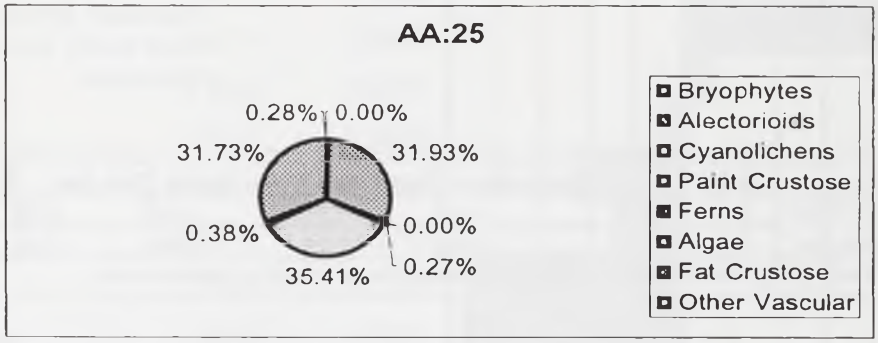
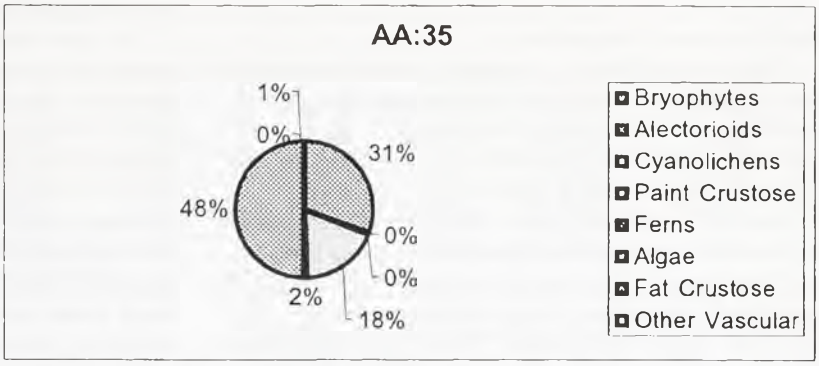
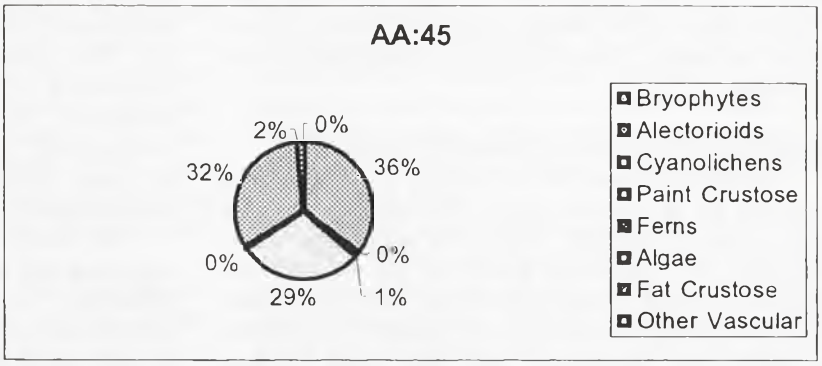


Figure 5 continued.

Epiphytes:35	% of Total Epiphyte Coverage
Bryophytes	30.68%
Alectorioids	0.00%
Cyanolichens	0.33%
Paint Crustose	17.79%
Ferns	2.14%
Algae	48.11%
Fat Crustose	0.13%
Other Vascular	0.82%
	100.00%



Epiphytes:45	% of Total Epiphyte Coverage
Bryophytes	35.24%
Alectorioids	0.00%
Cyanolichens	1.31%
Paint Crustose	28.90%
Ferns	0.45%
Algae	32.29%
Fat Crustose	1.84%
Other Vascular	0.00%
	100.02%



Discussion

Plot Component Composition

Each of the thirteen plots were accurately grouped into five different Plot Types base on abandonment age according to the principle component analysis. The vegetative differences between each plot were the key indicators for predefining each of the thirteen plots into the five different Plot Types. What proved surprising was that the Type 1 plots differed according to Tree age and Tree Height. This may be evidence that in younger abandoned plantations, these environmental variables may be more influential than the vegetative environmental variables, i.e. canopy height, % canopy cover and %groundcover. As abandonment age increases, the forest matrix returns back into these areas. Trees and bushes start to grow up around the coconut trees and the canopy height and canopy covers begin increasing. This continues as abandonment age increases.

Epiphyte Distribution and Community Composition

The abandonment of the coconut tree plantations provided for an excellent study of succession among a large group of epiphytes. Due to the uniqueness of the situation, the two-tiered model of succession was proposed because it was hypothesized that there would be limited epiphyte growth on the coconut trees within a maintained plantation, and that when it was abandoned, the change in environmental factors around the trees would change the epiphyte community composition.

The results show that the height of the surrounding canopy most affected the epiphyte community composition. % Canopy cover and height were highly correlated with each other. These two factors most affected the epiphyte community composition around the basal part of the trunks. This was where the most humid environment was and where bryophytes and ferns would be most likely to grow.

The data suggests that bryophytes and ferns prefer humid environments with little light penetration and that they are shade-tolerant. Looking at the vertical zonation data as the coconut tree rises out of the canopy, the epiphyte abundance drops dramatically to the point where no epiphytes are found within a meter of the crown of the tree. A quick analysis of the leaves of the tree also revealed that there were no plants growing on them.

In other epiphyte community composition studies, the focus has been on comparing young plots to old plots of a certain phorophyte. In this unique situation of abandoned plantations, it can be argued that the coconut trees that have been newly abandoned are similar to young plots of trees in other geographical environments that have not been as exposed for a significant length of time as an older plot has to the environmental variables.

In past studies, phorophyte stand age has been shown to be a major positive influence on epiphyte abundance and diversity (Lesica, et al. 1991; McCune 1993; Neitlich 1993; Esseen et al. 1996). Possible reasons cited for the decreased abundance in young stands include dispersal limitations, unsuitable substrates, and unsuitable canopy microclimate (Sillett and Neitlich 1996). In contrast, a recent study of epiphyte abundance in Gifford Pinchot National Forest found forest age to be less important than structure in explaining patterns of epiphyte abundance.

In this study, abandonment time was a more important factor than tree age for predicting epiphyte abundance. Tree age was only weakly correlated with epiphyte abundance (Table 3) but if we say that abandonment time can be substituted in a way for tree age, then increased exposure time is a factor in determining a more abundance or diverse epiphyte community. As stands age in abandonment time, the heterogeneity of the substrates and microclimate increases creating more specialized niches and microhabitats for species inhabiting the forest canopy.

The study in Gifford Pinchot National Forest suggested that structure explains patterns of epiphyte abundance. In this study, this explanation cannot be used because of the lack of variation in structure of the coconut tree over time. It has one major trunk with no branches and a simple crown of 15-35 arching leaves at its top. This design does not change over the course of the life of a coconut tree so there are no new branches for epiphytes to colonize.

The results also suggest that the decrease in % groundcover may be playing a role in epiphyte community composition. The decrease in groundcover is key evidence for succession also occurring within the understory of an abandoned coconut tree. As the understory grows up around the coconut tree, it becomes thicker with age and less light penetrates to the ground. As a consequence, fewer groundcover plants grow in the soil. Rather, larger trees dominate the forest matrix and shade-tolerant plants are found underneath the forest canopy. These can include ferns and also the late-successional epiphytes found on coconut trees within a 45 year old abandoned plantation such as bryophytes and ferns. The evidence indicates close to 5% groundcover for these Type 3c plantations.

Epiphyte percent cover does indeed increase over time, in fact doubling from a maintained plantation to a plantation abandoned for 45 years. The reason why the two-tiered epiphyte succession model was proposed, though, was because there was a 20% epiphyte cover even on coconut trees within a maintained plantation. No studies were done on recently planted coconut trees,

so the beginning section of the model has not been proven yet, but the results from this study indicate that the rest of the model may be accurate in predicting epiphyte succession among coconut trees in Moorea.

Type	Aband Time	Bryophytes	Alectorioids	Cyanolichens	Paint Crustose	Ferns	Algae	Fat Crustose	Other
1	0 yrs	0.25%	2.61%	0.49%	13.74%	0.04%	1.33%	2.10%	0.00%
2	15	4.23%	0.00%	1.10%	12.27%	0.05%	3.63%	1.24%	0.00%
3A	25	12.30%	0.00%	2.67%	13.64%	0.15%	12.22%	0.11%	0.00%
3B	35	9.74%	0.00%	0.10%	5.65%	0.68%	15.28%	0.04%	0.26%
3C	45	12.58%	0.00%	0.47%	10.32%	0.96%	11.53%	0.66%	0.00%

Table 7. Percent cover of epiphytes at each plot type

Successional patterns

This study indicates that the composition of epiphyte species shows successional change with abandonment time. The most important factors are the increase in % canopy cover and the increase in height of the canopy as the tropical forest begins to reintegrate the plantation back into the matrix over abandonment time. This is in agreement with other studies pointing out the importance of successional stages in explaining species composition (van der Maarel, 1988; van Ruremonde and Kalkhoven, 1991; Tonteri, 1994). This change is principally seen in the increase of bryophyte species and decrease of paint crustose species in late successional coconut tree stands.

This study also shows that late-successional species show very low abilities to colonize nearby maintained plantations. Four out of five of the type 1 plantations were surrounded by large stands of tropical forest that had all the late-successional species found in coconut trees that had been abandoned for over forty years. This pattern is especially clear when comparing the occurrence of lichens that differ with regard to their photobionts, that is green-algal vs. cyanobacterial lichens. Most cyanobacterial lichens were confined to longest abandoned stands. These patterns suggest that certain habitat conditions have to be fulfilled for successful establishment. First, all early pioneer lichens have green-algae photobionts, adapted to habitats characterized by high light intensities. Second, late-successional species are instead adapted to more shady and humid habitats. However, the relative importance of habitat conditions vs. difference in dispersal remains to be clarified for explaining the occurrence of the late-successional species.

The distribution of epiphytes found in this study suggests that the sequence of epiphyte colonization within individual trees does consistently follow the sequence of colonization observed in a series of stand ages. According to Lescica, et al. 1991; McCune 1993; and Sillett and Neitlich 1996, trees growing in exposed conditions of a young stand were initially colonized by crustose lichens, followed by alectorioid lichens. As stand age increases and canopy closure occurs, bryophytes and cyanolichens begin to colonize new growth in the upper and outer canopy. However, epiphyte succession in coconut trees within a tropical environment seems to follow a slightly different pattern. According to the W averages, colonization followed this pattern: (i) Colonization by fat crustose lichens and alectorioids first, (ii) then cyanolichens and paint crustose lichens second followed by (iii) green algae, bryophytes, ferns and other vascular plants. Alectorioid lichens, while proportionally abundance in the upper trunk area of type 1 trees, were sparse in terms of total cover.

Although epiphyte succession was not observed directly, information from the distribution of epiphytes from this study can be used to pose hypotheses on within-stand patterns of epiphyte succession. Since all study trees originated from type 1 plots, the distribution of epiphytes on the type 2-3c plot trees reflect the probable sequence of epiphyte colonization that will occur as the trees become part of the natural tropical forest environment. I hypothesize that as understory trees grow up around the type 1 plot trees that have been recently abandoned, those species that are common on tropical forest trees, bryophytes and ferns, will increase in abundance. In this forest, abundance of these functional groups increases with height of the canopy. Epiphyte communities on trees that do not have forest growing up around them are unlikely to have such a shift as the data from Type 1 plots shows. These trees are approximately the same age as type 3a-c trees yet have a completely different epiphyte community composition (Table 1). Further work remains to be done on a vertical zonation model, which would describe vertical epiphyte composition on a late-successional coconut tree. According to Lobbin, a tropical forest tree contains five vertical zones. Zone 1 is the fully exposed zone with microepiphytes, zone 2 is the main zone of epiphytes on large limbs, zone 3 is the drier upper trunk with crustose lichens, zone 4 is the moister lower trunk with lichens and mosses and zone 5 is the damp base of the tree covered with mosses. For the coconut tree, Zone 2 can be eliminated because of the lack of branches. In zone 1, the visual studies in

this area yield no epiphytes. The other zones do indeed exist for a coconut tree and the data from this research shows evidence of support for these zones. The majority of the bryophytes were concentrated on the lower one meter of the trunk of the tree. Crustose lichens dominated at the fifty percent tree height level. More analysis on the epiphyte percent cover can yield a vertical zonation model in the future.

Conclusion

A major goal of ecology is to be able to predict how communities we are presently disturbing will respond, or recover if the disturbance is stopped, or how much disturbance an ecosystem can absorb and still function. Epiphytes of *Coco nucifera* on the island of Moorea are differentially distributed across abandonment time. Within a vertical zonation model, they vary with plot type, absolute position within the canopy, and relative position within individual trees. The environmental variables most important for predicting epiphyte distribution and abundance are canopy height and % canopy cover and abandonment time. Models of epiphyte succession based on chronosequences of plantation abandonment times within a tropical environment are useful to gauge differences among stands and explain broad patterns in epiphyte distribution, but they may not explain variability in finer spatial scales, particularly within stands with multiple age and size classes of trees.

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Comparative morphology of ten species of cowries (Gastropoda: Cypraeidae) with emphasis on the gastrointestinal system

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ABSTRACT. Both internal and external aspects of the morphology of ten species of Cypraeidae found on Moorea, French Polynesia were documented with drawings and photographs for evaluation of differences between species within the family. These observations showed that while all the species under study had similar morphological themes, there were variations in organ sizes and shapes for each one. Eighty morphological characters were scored to develop two new phylogenies based on (1) morphological data, and (2) morphological data combined with molecular data from Meyers (1998). The phylogenies derived from morphological data differed from those based on molecular data, indicating that morphology could play an important role in elucidating the evolutionary history of cowries. Different types of food material found in the gastrointestinal systems of the ten species were compared in order to determine the diet of each species. Measurements of gastrointestinal organ lengths were compared to the percentages of plant material found in the gastrointestinal tract to determine whether diet type correlated with gastrointestinal organ size. It was found that radular dimensions (length and width) increased proportionally as the percentage of plant material in the diet increased, which may be due to the need for greater processing of plant cells. However, measurements of stomach and intestinal dimensions showed an inversely proportional correlation between size and percent of plant material in the diet. Since most of the animal contents found in the gastrointestinal system were sponge spicules, which are atypical from most animal cells in digestibility, greater stomach and intestine dimensions may be an adaptation to allow for more mucous protection from sponge spicules in the gastrointestinal system.

Introduction

Understanding the comparative morphology of different species of gastropods can elucidate taxonomy, classification, and evolution for the gastropod class as a whole. The anatomy of gastropods has been studied extensively in England, resulting in detailed drawings of all the major organ systems of British prosobranch gastropods (Fretter and Graham 1994), but did not include studies of any of the 170 species (Keen 1971) within the family Cypraeidae. While the conchological features of Cypraeidae from the French Polynesia have been studied extensively (Rives and Salvat 1991), their internal morphology, specifically the gastrointestinal system, has been neglected. At present, information about Cypraeidae internal morphology must be inferred based on anatomical generalizations from studies on species of Cypraeidae from South Africa (Gosliner and Liltved, 1985) or closely related families of gastropods, such as the Triviidae (Gosliner and Liltved, 1982).

The island of Moorea, French Polynesia, is surrounded by reefs that are abundant in *Cypraea* (Rives and Salvat 1991), because dead coral rubble, the preferred habitat of many *Cypraea*, dominates much of the shore waters. As a result of this compatibility with the habitat, as well as the shells' aesthetic qualities which lend themselves for use in jewelry and other craft items (Thiele 1992), cowries are of great economic importance in the French Polynesian tourist industry (Poulsen 1996). Collection of specimens for this study, by means of snorkel, yielded ten species: *C. tigris, C. carneola, C. caputserpentis, C. moneta, C. erosa, C. lynx, C. obvelata, C. isabella, C. leviathon, and C. vitellus*. Of the species collected, one of them, *C. obvelata*, is endemic to Moorea (Burgess 1970).

The goals of this study were to answer questions regarding Cypraeidae morphology, namely, how does the internal and external anatomy, in particular, the gastrointestinal system, compare between different species of *Cypraea*? Accordingly, can generalizations about morphology of the entire family of Cypraeidae based on the studies of closely related species or only a few species of *Cypraea* accurately describe each of the rest of the *Cypraea* species? What can morphological information tell us about the most probable phylogeny of the ten species? If we compare that morphological phylogeny to a molecular phylogeny based on 16s and coxI sequences (Meyers 1998), how will it compare? Finally, how might sizes of gastrointestinal organs such as the radula, stomach, and intestine, have adapted to better accommodate the needs of species with different diet types (e.g., herbivorous versus carnivorous) and do

those diet types have an evolutionary basis for development?

Materials and Methods

Site Selection

Specimens were collected from coral reef habitats of Moorea, French Polynesia that had significant portions of dead coral rubble. All collection sites were accessed by snorkel since SCUBA capabilities were not possible in this study. The sites (Figure 1) included Point Aroa, directly north from the beach lighthouse (S 17°28'433'', W 149°46'549''), mangroves, especially around plant roots (S 17°33'54'', W 149°52'27''), the double reef, southwest from the concrete stairs leading down from the road (S 17°35'33'', W 149°50'24''), and Cook's Bay, in particular the pass and the shore opposite the U.C. Berkeley Gump Research Station (S 17°29'433'', W 149°49'560''). Other locations searched, but yielding no *Cypraea* specimens included: the Temae public beach, the shores directly in front of the pharmacy at Cook's Bay, the junction of Cook's Bay and Opunahu Bay, and Teahuroa.

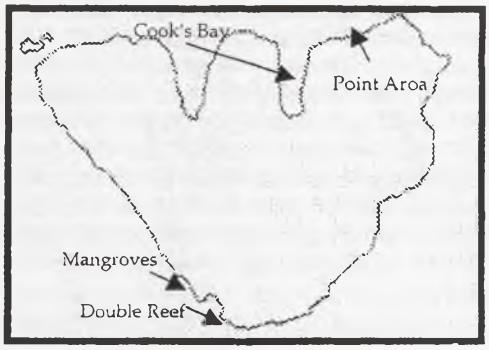


Figure 1. Map of Moorea, showing locations of collection sites.

Collection trips were made both diurnally and nocturnally, and at low and high tides. Though it was observed that the cowries would come out from underneath the dead coral rubble at night, which made for easier collection, it was found that by simply turning over the dead coral rubble during the daytime, the same rate of collection could be obtained during the day as at night.

Sample Sizes

Only one specimen of each of the species *C. vitellus, C. isabella, C. leviathon, C. caputserpentis,* and *C. lynx* were found, therefore comparisons were limited. However, for the other species, *C. tigris, C. carneola, C. obvelata, C. erosa,* and *C. moneta,* numerous specimens of each were found and time allowed for dissection of three each. The results of the measurements and observations for the latter species were composite coded for further analysis.

Dissection Methods

For all *Cypraea* species dissections, the specimens were first photographed while they were alive for optimal visualization of the mantle lobes.

To kill the specimens, they were each submersed in a 90% ethanol solution for a half hour, then placed in a fresh dosage of ethanol for another half hour.

External conchological measurements and observations were recorded, including shell length and width, aperture length and width, presence or absence of protoconch, labrum shine, number and extension past aperture of columellar and labral teeth, and dorsal and ventral shell color and patterns (Figure 2).

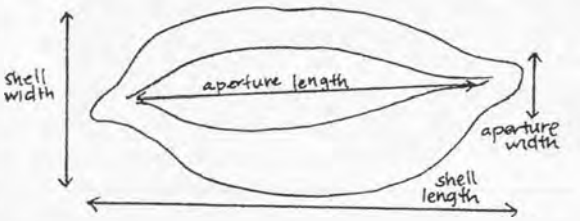


Figure 2. Conchological measurements (zero magnification).

To extract the specimens from their shells, two different methods were used, depending on accessibility of tools. For each shell, the claw of a hammer was positioned in the aperture of the shell and taped to the shell with electrical tape. Then, the shell and hammer were placed in a clear plastic bag to prevent shell pieces from scattering upon breakage. The shell was then hit by the hammer claw on its dorsal side against a cement floor. This produced a vertical crack in the dorsal side of the shell, after which the specimen could be removed from the shell with tweezers or pliers. The second method, which was preferable, was achieved by placing the shell wrapped in tissue, to avoid slipping, in a vice and slowly squeezing the shell until it cracked. This was repeated on both dorsal and ventral sides to optimize the surface area cracked. Shell pieces were then peeled away with tweezers or pliers to extract the specimen housed inside.

The specimen was then photographed, drawn, measured, and observed with the use of a dissecting microscope, to score the remaining characters for use in developing the phylogeny.

For each specimen, the intestine and stomach were cut with scissors longitudinally to expose the

gastrointestinal contents. The contents were then extracted with tweezers and examined on a slide for visualization under a compound microscope at 100x and 400x magnification (Figure 3). Percentages of gastrointestinal content types found in each specimen were estimated based on detailed visual scanning of all contents under the compound microscope.

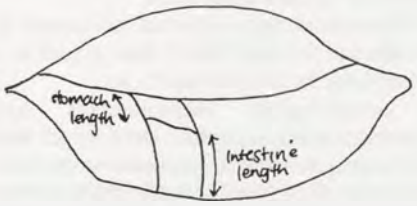


Figure 3. Internal measurements (zero magnification).

Finally, under a dissecting microscope, the radula was extracted from each specimen by peeling back the mantle edge surrounding the buccal mass, cutting directly between the tentacles through the mouth and esophagus into the bulge of the radular sac, which housed the radula (Figure 4).

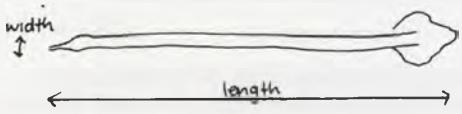


Figure 4. Radular measurements (zero magnification).

Selection of Outgroups

Five outgroups were selected because their families were evolutionarily close to *Cypraea*, but were still from a different family, and also accessible for dissection (except in the case of the *Trivia ovulata* specimen whose character states were gleaned from morphological papers about the species). Four Naticidae and one Trividae were chosen. The Naticidae were: *Polinices lewisii* (found at Half Moon Bay, California), *Polinices incei* (found 7/3/00 in Queensland Australia at the north end of Five Rocks Beach, north of Yeppoon in the sand, 22°49'S, 150°49'E), *Polinices sordidus* (found 6/20/00 in Queensland, Australia, at Shorncliffe, north of Brisbane, 27°19'S, 153°06'E, in muddy sand), and *Eunaticina papilla* (found 6/24/57 in Marin, California, 2 miles northwest of Tomales Point in sandy mud). The Triviidae was: *Trivia ovulata*.

Selection of Morphological Characters for Phylogeny

A total of eighty characters were scored for the ten species of Cypraeidae. The characters were selected based on their satisfaction of four criteria: 1) Discrete states, meaning that there was either a presence or absence of the character, or if it were in the form of a measurement, the values could be graphed and found to inhabit distinct ranges that could be labeled as separate character states. 2) Independence, meaning that variance of one character did not affect the state of another character. 3) Homology, meaning similarity in structure or evolutionary development of organs or structures in the series of species under examination. 4) Genetically based, meaning that the environment did not determine the state of the character (Landau 1986).

Examples of characters that were considered for the phylogenies, but not scored, included shell length and shell width because visual observations indicated that they may not vary independently of one another.

Data Analysis

Phylogemes were obtained with the computer programs MacClade 4.0 and PAUP by entering the character states for the ten *Cypraea* species and five outgroups (first only the morphological data, then only the molecular data, then both combined) into the program's data table. The molecular data used, 16s and coxI sequences, were from a Meyer's (1998) previous phylogenetic study. A heuristic search was then requested for the most parsimonious phylogenetic tree with 100 replicates and no outgroups designated.

Correlations between percent plant in diet and gastrointestinal organ lengths were found by conducting linear regression on the raw data for all the specimens studied.

Results

Morphological Descriptions for the Ten Cypraea Species

I found ten species of Cypraea, selected data for which is detailed in Appendix 1. All specimens were found either under or around dead coral rubble, the habitat Shepherd and Thomas reported them to be most commonly found (1989). While the species were all similar with respect to location of organs such as the intestine, stomach, mouth, radula, and foot, some notable morphological differences were found: *C. tigris* was distinguished by its large size. It was more than twice the size of any other species. Two black salivary glands surrounded the esophagus. *C. carneola* was distinguished by an enlarged and red buccal area. A bright yellow bursa copulatrix was also found in the female specimens. *C. obvelata* was by far the most abundantly found species and always had its

characteristic oval white shell. *C. moneta* had a mouth that was slightly enlarged with respect to its foot size, compared to the other nine species studied. *C. erosa* also had an enlarged mouth, however it was orange. *C. isabella* had a distinctive black mantle covering with no visible papillae.

Cypraea tigris (Linneus 1758)
(Figure 6)

- *Location and Quantity Found*

A total of three specimens were collected at the pass of Cook's Bay and the shore opposite the Gump Research Station, from 0.5-4 meters in depth.

- *Distribution*

Cypraea tigris is known to be found throughout French Polynesia and the Indo-Pacific (Rives and Salvat 1991).

- *Shell Features*

The shell has a white base, with 0.5 to 2 cm diameter brown spots on the dorsal side which increase in density towards the crest. A tan colored vertical stripe is present at the dorsal-most point of the shell. The ventral side, including the tooth area, is completely white.

- *Mantle*

The mantle is translucent to reveal brown and white spotting from the shell and has raised fleshy papillae.

- *Unique Gastrointestinal Features*

Two salivary glands are present surrounding the esophagus. The larger one is black and the smaller one is lavender, however they appear to be fused.

- *Gastrointestinal Contents*

Golden brown algae, green algae, filamentous green algae, and diatoms were found in the stomach, which indicate *C. tigris* is an herbivore.

- *Miscellaneous Unique Features*

The osphradium could be easily seen on the underside of the mantle edge adjacent to the radular sac.

Cypraea carneola (Linneus 1758)
(Figure 7)

- *Location and Quantity Found*

A total of nine specimens were collected at the Cook's Bay pass, shore opposite the Gump Research Station, mangroves, and Point Aroa, from 1-4 meters in depth.

- *Distribution*

Cypraea carneola is known to be found throughout all the French Polynesian and Indo-Pacific islands (Rives and Salvat 1991).

- *Shell Features*

The shell has horizontal orange and yellow or red bands across its dorsal side. The entire periphery of the dorsal side of the shell is composed of a solid yellow or orange band. The ventral side, including the tooth area, is yellow with violet denticles.

- *Mantle*

The mantle is smooth, deep brown and opaque with fleshy finger, white or brown papillae.

- *Unique Gastrointestinal Features*

The buccal area is enlarged and red.

- *Gastrointestinal Contents*

The intestine and stomach contained sponge spicules, coralline algae plates, carpocysts from red/brown algae, and green filamentous material, indicating the *C. carneola* is an omnivore.

- *Miscellaneous Unique Features*

A bright yellow bursa copulatrix was found in female specimens.

Cypraea obvelata (Lamarck 1810)
(Figure 8)

- *Location and Quantity Found*

A total of nine specimens were collected at the Cook's Bay pass, shore opposite the Gump Research Station, Point Aroa, mangroves, and the double reef at 1-2 meters in depth. They were the most abundantly found species. Many more were found than were collected.

- *Distribution*

C. obvelata is known to be found in French Polynesia on the Jarvis, Society, Marquesas, Tuamotus, and Austral islands (Rives and Salvat 1991).

- *Shell Features*

The shell is white, with the dorsal side having an orange outlined oval with a deep gray center. Some dorsal shells had slight yellowing and thickening at maturity. The ventral side, including the tooth area, is white as well.

- *Mantle*

The mantle is opaque with a black and white stripe base pattern and wart-like papillae.

- *Gastrointestinal Contents*

The intestine housed a plethora of sponge spicules and siliceous algae, indicating that *C. obvelata* is an omnivore.

Cypraea caputserpentis (Linneus 1758)
(Figure 9)

- *Location and Quantity Found*

A single specimen was found at the double reef, in 1 meter of water.

- *Distribution*

C. caputserpentis is known to be found on all the French Polynesian islands and the Indo-Pacific (Rives and Salvat 1991).

- *Shell Features*

The shell has a light brown base on its dorsal side, as Cate found in Eastern Pacific specimens of the same species (1969), spotted with white dots. It has a deep

brown frame that surrounds the periphery. The ventral side is dark brown, which blends to become white at the tooth area.

- *Mantle*

The mantle is semi-translucent with fleshy finger-like papillae.

- *Gastrointestinal Contents*

The intestine contained sponge spicules, diatoms, golden brown algae, and green algae clumps, signifying it is an omnivore. In a 2mmx2mm space within the intestine, approximately eight siliceous algae could be seen with the naked eye.

Cypraea moneta (Linneus 1758)
(Figure 10)

- *Location and Quantity Found*

Eight specimens were found in the mangroves and Cook's Bay pass, at approximately 1-2 meters in depth. Second to only the *C. obvelata*, these were also abundantly found and not all collected when found.

- *Distribution*

C. moneta is known to be found on all the French Polynesian and Galapagos islands, as well as the Indo-Pacific (Rives and Salvat 1991).

- *Shell Features*

The shell is triangular in shape, as opposed to the typical oblong shape of most *Cypraea*, with raised bumps on its sides. It showed thickening at maturity. It is completely white with the exception of a deep grayish-green oval center that is crossed by 1-2 horizontal, thin, yellow stripes.

- *Mantle*

The mantle has black and white striped pattern and fleshy finger-like papillae.

- *Unique Gastrointestinal Features*

The white mouth was slightly enlarged in relation to its foot size, as compared to the other nine species.

- *Gastrointestinal Contents*

Green algae clumps and green filamentous material were found in the intestine, indicating that *C. moneta* is possibly an herbivore.

- *Miscellaneous Unique Features*

The radula was found to turn from hard yellow at its anterior end to soft pink towards its posterior end.

Cypraea erosa (Linneus 1758)
(Figure 11)

- *Location and Quantity Found*

Five specimens were found directly across Cook's Bay from the Gump Station and at the mangroves in waters 1-3 meters in depth.

- *Distribution*

C. erosa is known to be found on all the French Polynesian islands and the Indo-Pacific (Rives and Salvat 1991)

- *Shell Features*

The shell has its periphery surrounded by a beige frame with orange short stripes and spots on it. The dorsal side has white spots on a green base. There is a white vertical line on the dorsal side that is lined up exactly opposite to the aperture on the ventral side. The ventral side is beige. A brown spot is located at the two midsections on the periphery.

- *Mantle*

The mantle has a black opaque spotted base pattern with wart-like papillae.

- *Unique Gastrointestinal Features*

C. erosa has an enlarged orange mouth, similar to that of *Cypraea moneta*.

- *Gastrointestinal Contents*

C. erosa had the most diversity in terms of gastrointestinal contents. It contained diatoms, sponge spicules, siliceous algae, and golden brown algae.

Cypraea vitellus (Linneus 1758)
(Figure 12)

- *Location and Quantity Found*

One specimen was found at the shores across from the Gump Station at 2 meters depth.

- *Distribution*

C. vitellus is known to be found on all the French Polynesian and Indo-Pacific islands (Rives and Salvat 1991).

- *Shell Features*

The shell has brown and tan horizontal stripes across its dorsal side, with white 1-2mm sized spots randomly on top, as Cate found in Eatern Pacific specimens of the same species (1969). It has a deep brown frame on its periphery. Its ventral side fades from pink on the outer edges to white at the tooth area.

- *Mantle*

The mantle has deep brown opaque base color with fleshy finger-like white papillae.

- *Gastrointestinal Contents*

The intestines contained sponge spicules and diatoms, indicating that *C. vitellus* is an omnivore.

Cypraea isabella (Linneus 1758)
(Figure 13)

- *Location and Quantity Found*

One specimen was found at the mangroves, at a depth of 1 meter.

- *Distribution*

C. isabella is known to be found in the Indo-Pacific, Society, Marquesa, Tuamotu, and Gambier islands (Rives and Salvat 1991).

- *Shell Features*

The shell has black thin vertical lineson its tan-maroon base-colored dorsal side, as Cate found in Eastern Pacific cowries (1969), two deep red spots at its top and bottom orange ends. The ventral side is white.

- *Mantle*

The mantle covering is distinctive because it is completely black and opaque with no visible papillae.

- *Gastrointestinal Contents*

Some green algae clumps were found in the intestine, which was very small and shriveled, however, small sponge spicules were found. This indicates that *C. isabella* may have been a carnivore.

Cypraea leviathon (Schilder and Schilder, 1937)
(Figure 14)

- *Location and Quantity Found*

One juvenile specimen was found at the shores opposite the Gump Station, at 1.5 meters in depth.

- *Distribution*

C. leviathon is known to be found throughout the islands of the Pacific East, Society, Marquesas, Tuamotus, Australia (Rives and Salvat 1991).

- *Shell Features*

The shell has horizontal brown and tan stripes on its dorsal side that become horizontal as toward the end opposite the still-visible protoconch. Since it was a juvenile, the shell did not close completely on its ventral side, resulting in no external teeth on its shell.

- *Mantle*

The mantle is translucent and has multiple-finger-like papillae.

- *Gastrointestinal Contents*

The intestine contained sponge spicules, diatoms, green filamentous material, and siliceous algae.

Cypraea lynx (Linneus, 1758)
(Figure 15)

- *Location and Quantity Found*

One specimen was found in the mangroves at 0.5 meters in depth.

- *Distribution*

C. lynx is known to be found on all the islands of French Polynesia and the Indo-Pacific (Rives and Salvat 1991).

- *Shell Features*

The shell has horizontal brown and tan wide bands on its dorsal side with dark brown spots randomly on top. It was a tan base on its ventral labral side and is white on its ventral columnar side.

- *Mantle*

The mantle is semi-translucent, granular, with fleshy finger-like papillae.

- *Gastrointestinal Contents*

The intestine contained a diversity of diatoms, sponge spicules, and golden brown algae.

Correlation of Diet Type with Gastrointestinal Organ Size

A sample of the gastrointestinal system contents (Figure 16) of the ten species revealed that four species, namely, *C. tigris, C. leviathon, C. moneta,* and *C. obvelata*, showed predominantly herbivorous diet preferences, while three others, namely, *C. isabella, C. vitellus, C. caputserpentis,* showed predominantly carnivorous diet preferences. These findings confirm previous studies that have shown Cypraeacea to include both herbivores, some grazing on algae or detritus, and some carnivorous, eating sessile colonial animals such as sponges (Shepherd and Thomas 1989).

When linear regression was executed comparing percent plant found in each species' diet with the lengths and widths of each species' radula, stomach, and intestine, the correlation between percent plant in diet and stomach length produced statistically significant results (P value=0.045). These results indicated that there was a negative correlation between the two factors (Figure 18).

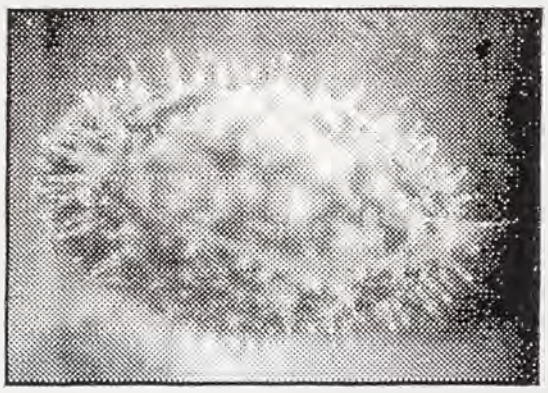
Though not statistically significant, comparisons between percent plant in diet and both radula length (P value=0.652) (Figure 19) and width (P value=0.525) (Figure 20) showed a positive correlation and comparisons between percent plant in diet with stomach width (P value=0.414) (Figure 21), intestine length (P value=0.092) (Figure 22), and intestine width (P value=0.484) (Figure 23) showed a negative correlation.

Phylogeny determined by morphology

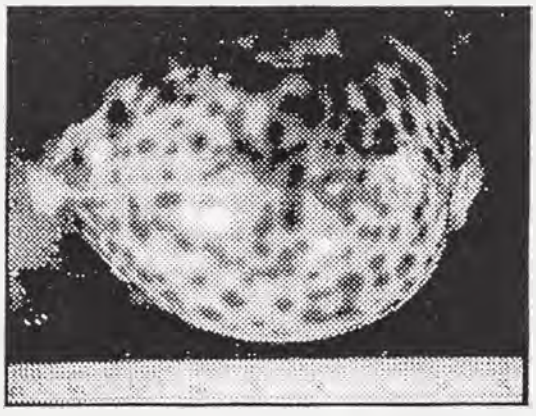
The eighty morphological characters that were scored (see Appendix 2 and 3) for all ten species of *Cypraea* and the five outgroups were used to create a morphologically based phylogeny. Two equally parsimonious phylogenies were produced (Figure 24). A strict consensus tree was formed from these two phylogenies (Figure 25).

A molecular based phylogeny (Figure 26) based on nucleotide sequences that were previously found for 16s and cox1 genes (Myers 1998) was also established.

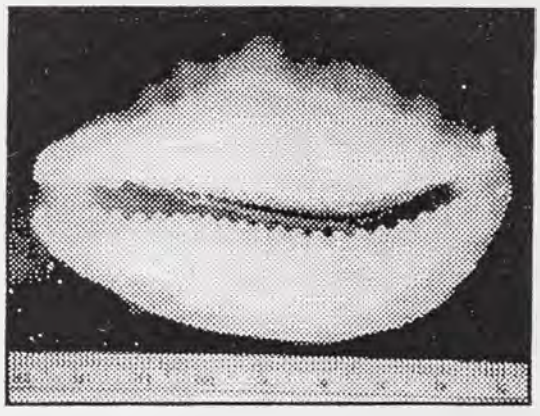
Figure 6. *Cypraea tigris* External and Internal Morphology



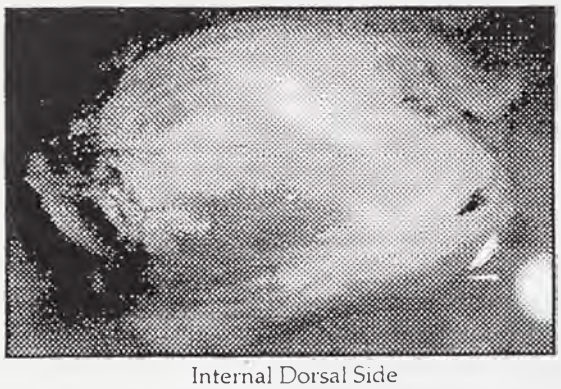
Live specimen with mantle fully extended.



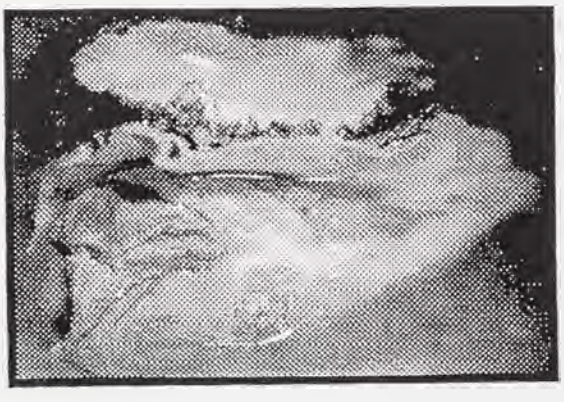
External Dorsal Side



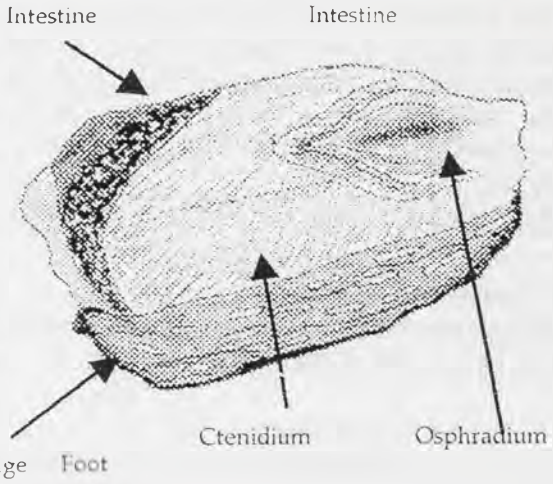
External Ventral Side



Internal Dorsal Side



Internal Ventral Side



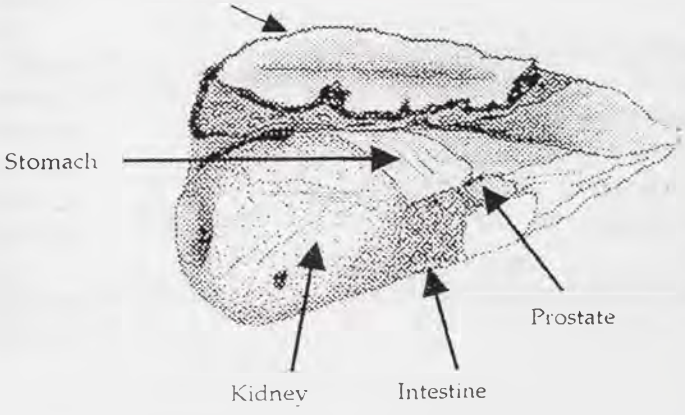
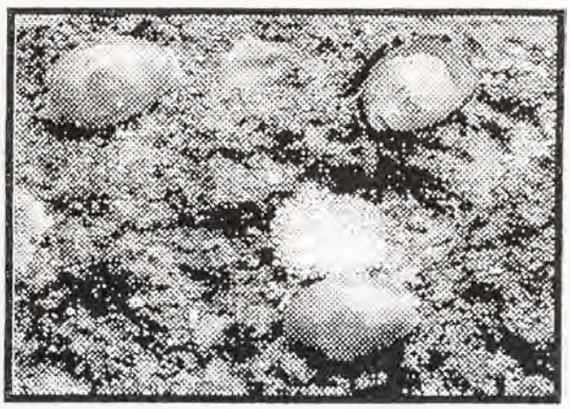
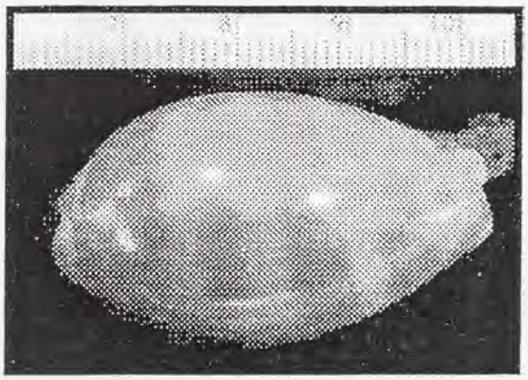


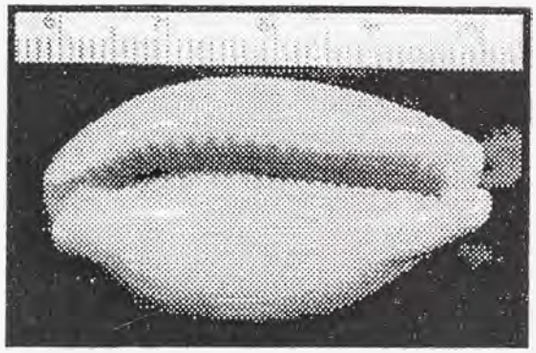
Figure 7. *Cypraea carneola* External and Internal Morphology



Live specimens around eggs under dead coral rubble.

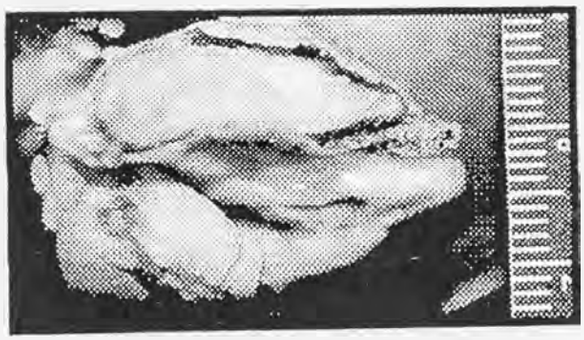


External Dorsal Side

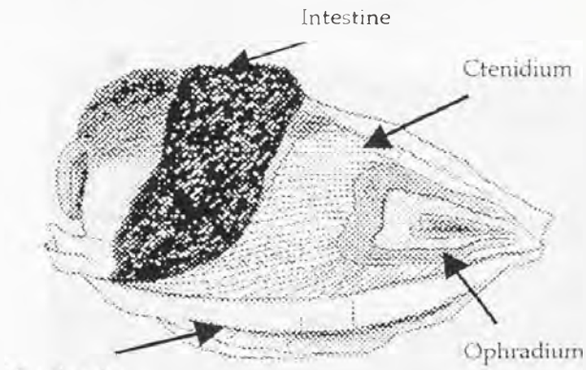


External Ventral Side

Internal Dorsal Side



Internal Ventral Side



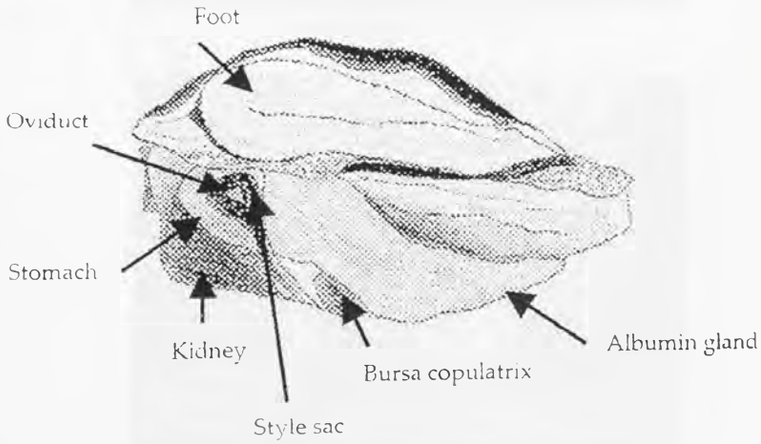
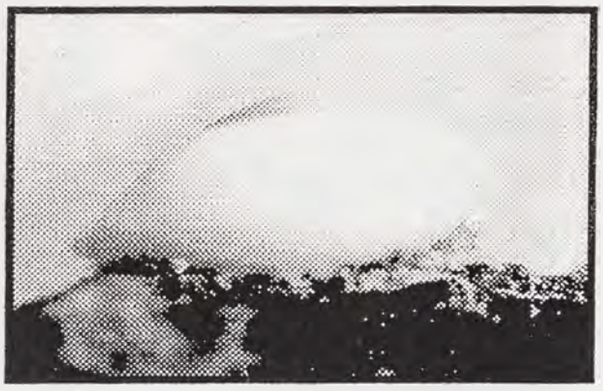
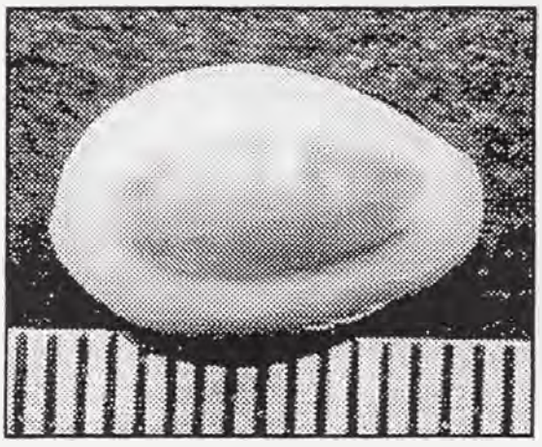


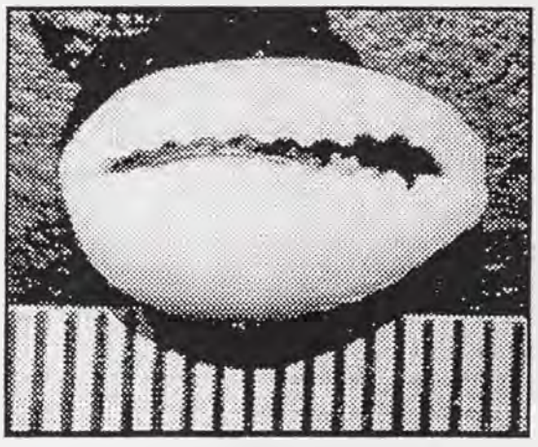
Figure 8. *Cypraea obvelata* External and Internal Morphology



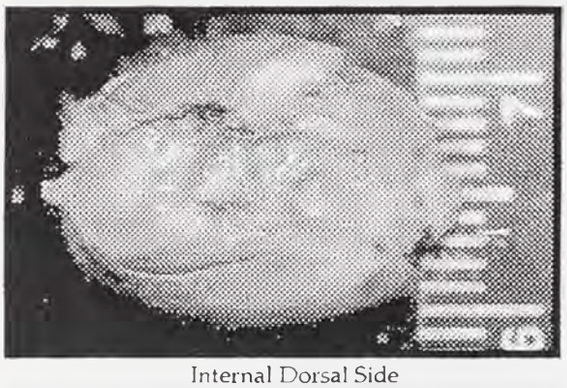
Live specimen found under dead coral rubble.



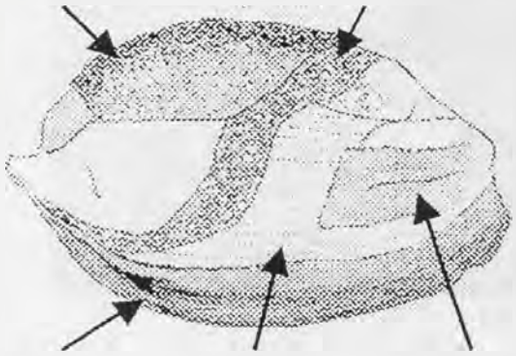
External Dorsal Side

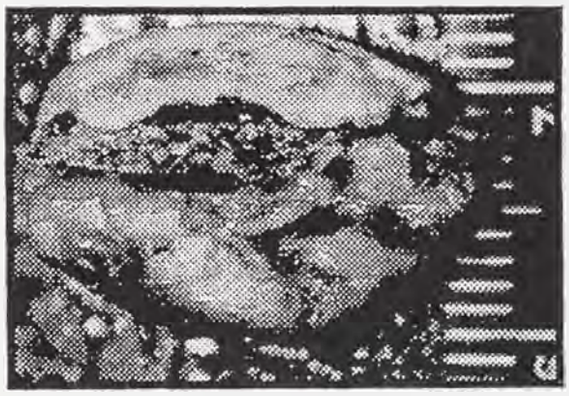


External Ventral Side



Internal Dorsal Side





Internal Ventral Side

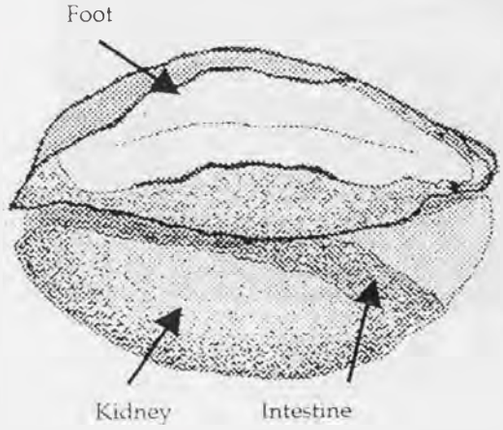
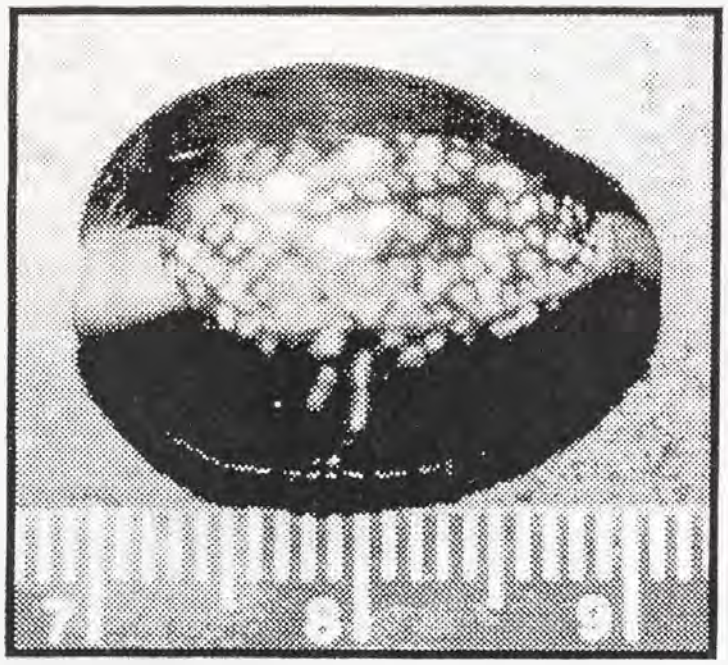
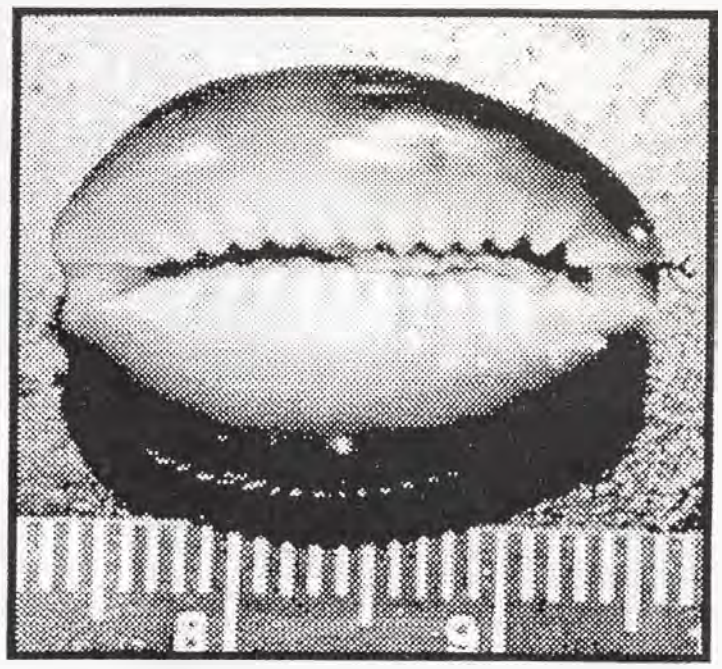


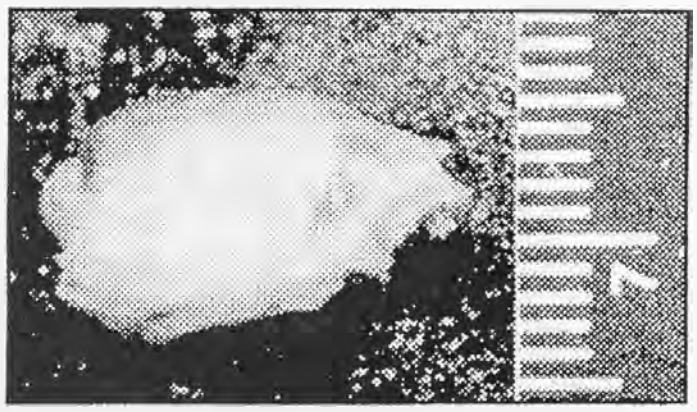
Figure 9. *Cypraea caputserpentis* External and Internal Morphology



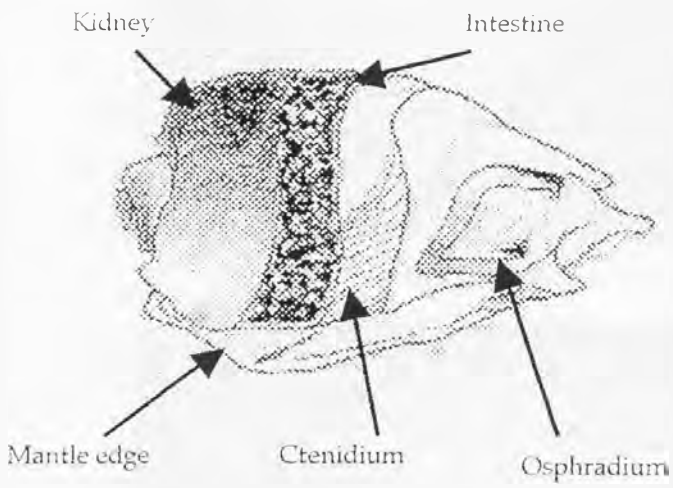
External Dorsal Shell

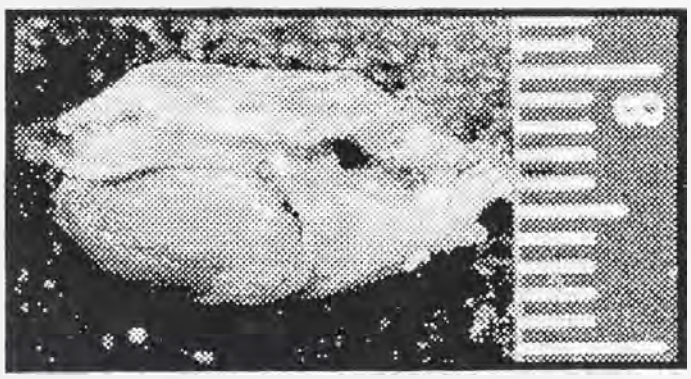


External Ventral Shell



Internal Dorsal Side





Internal Ventral Side

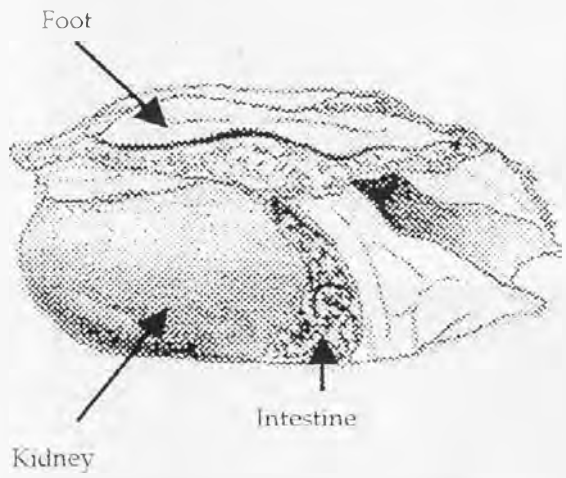
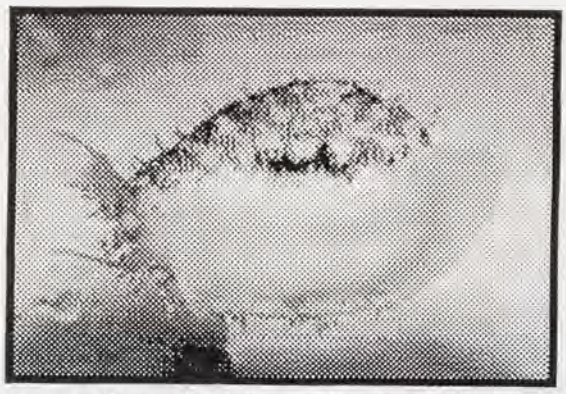
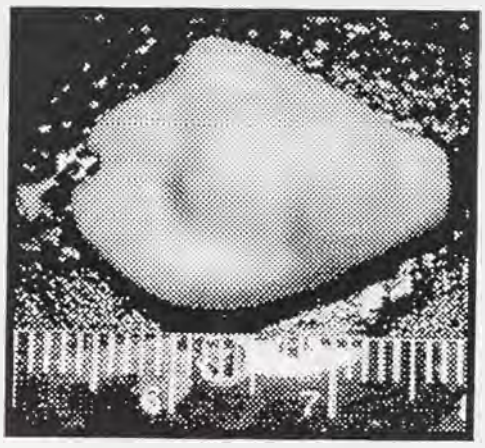


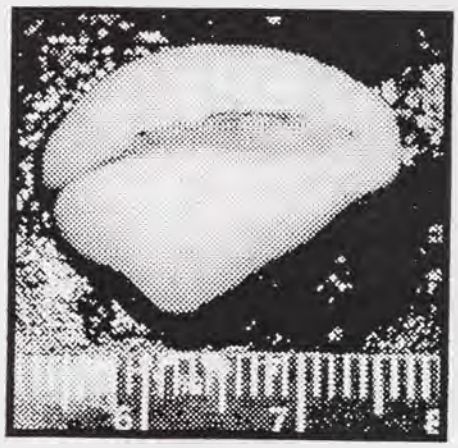
Figure 10. *Cypraea moneta* External and Internal Morphology



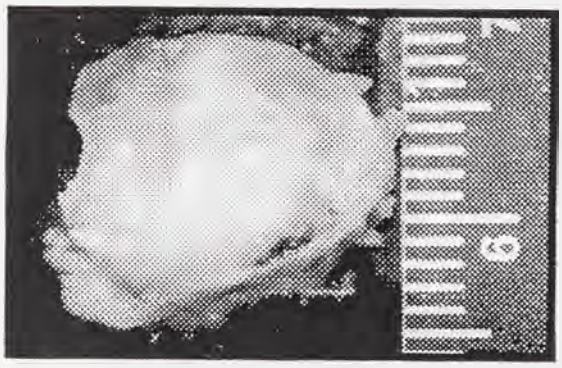
Live specimen with mantle, siphon, and tentacles fully extended.



External Dorsal Shell

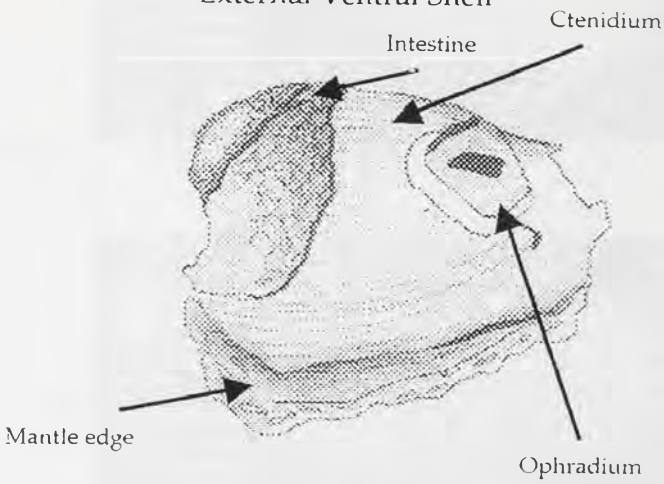


External Ventral Shell



Dorsal Side

Ventral Side



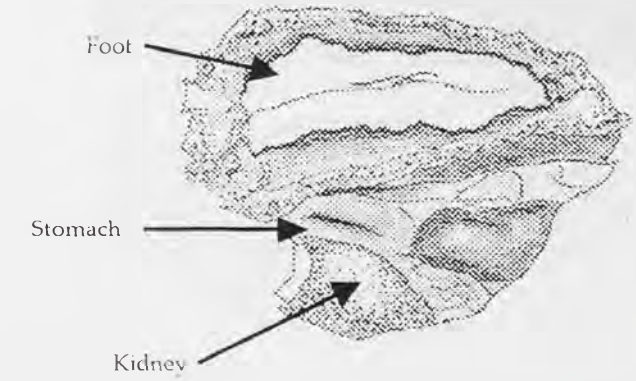
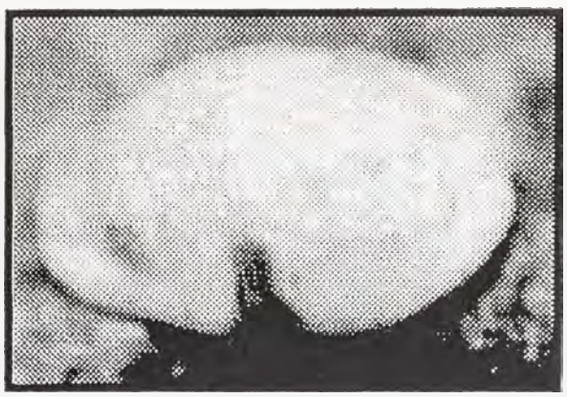
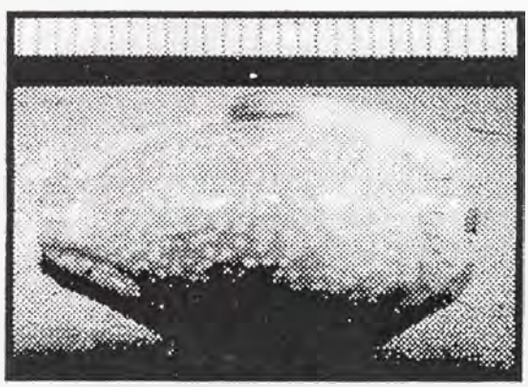


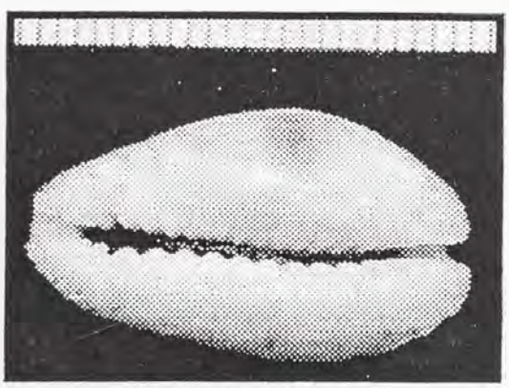
Figure 11. *Cypraea erosa* External and Internal Morphology



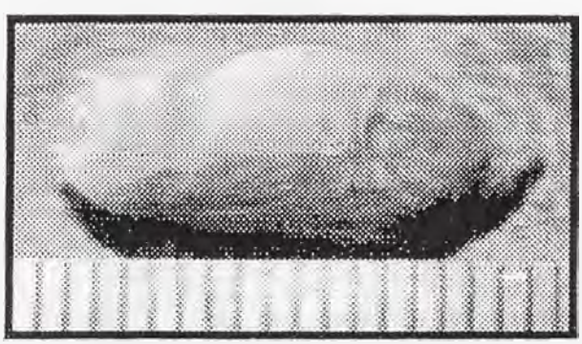
Live specimen found under dead coral rubble.



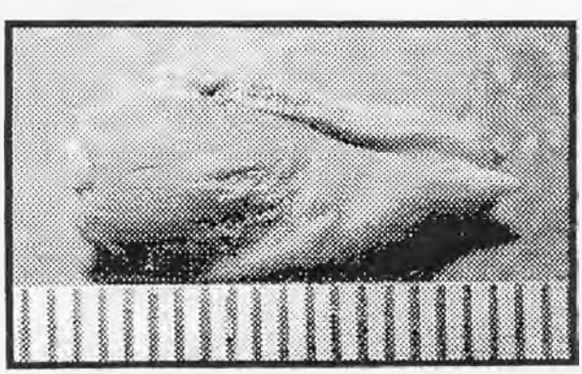
External Dorsal Side



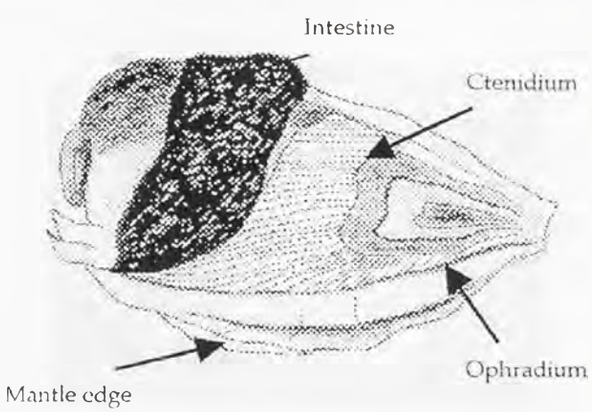
External Ventral Side



Internal Dorsal Side



Internal Ventral Side



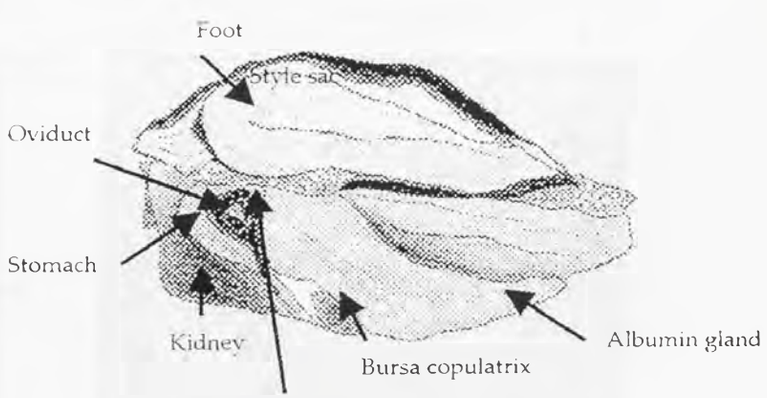
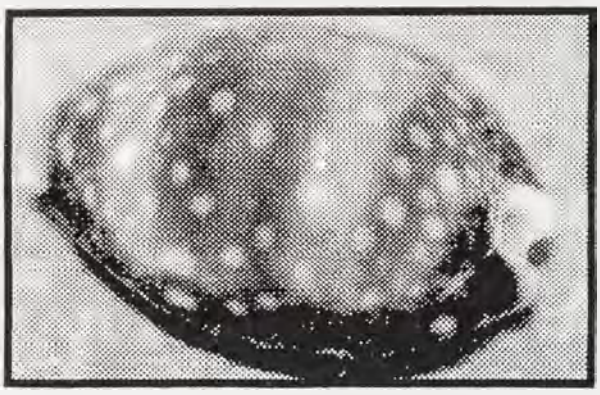
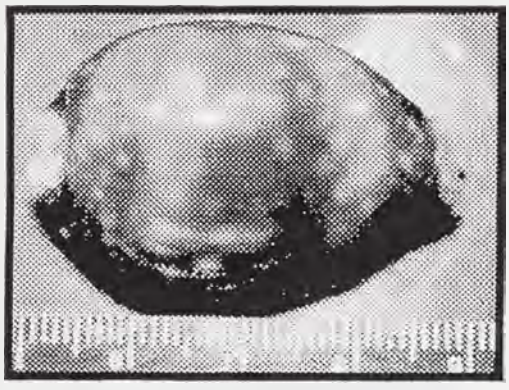


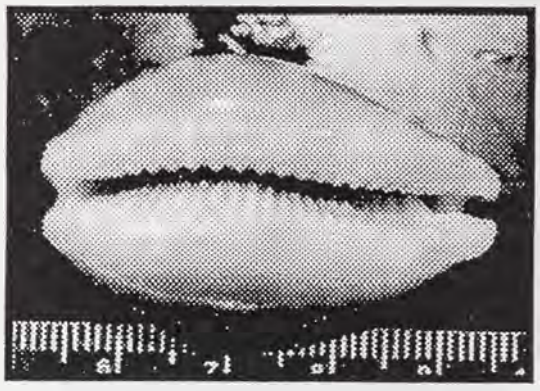
Figure 12. *Cypraea vitellus* External and Internal Morphology



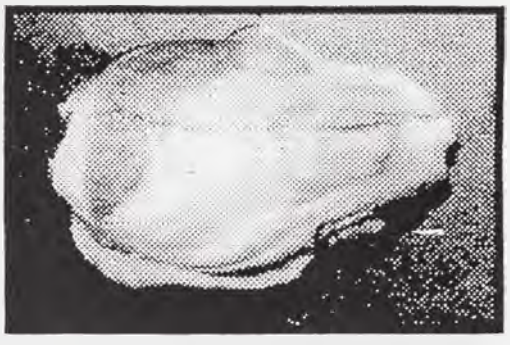
Live specimen found under dead coral rubble.



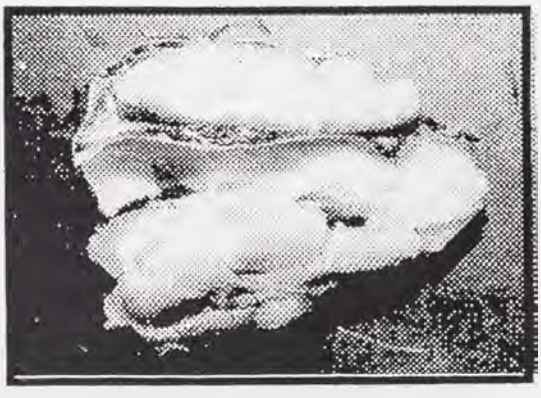
External Dorsal Side



External Ventral Side



Dorsal Side



Ventral Side

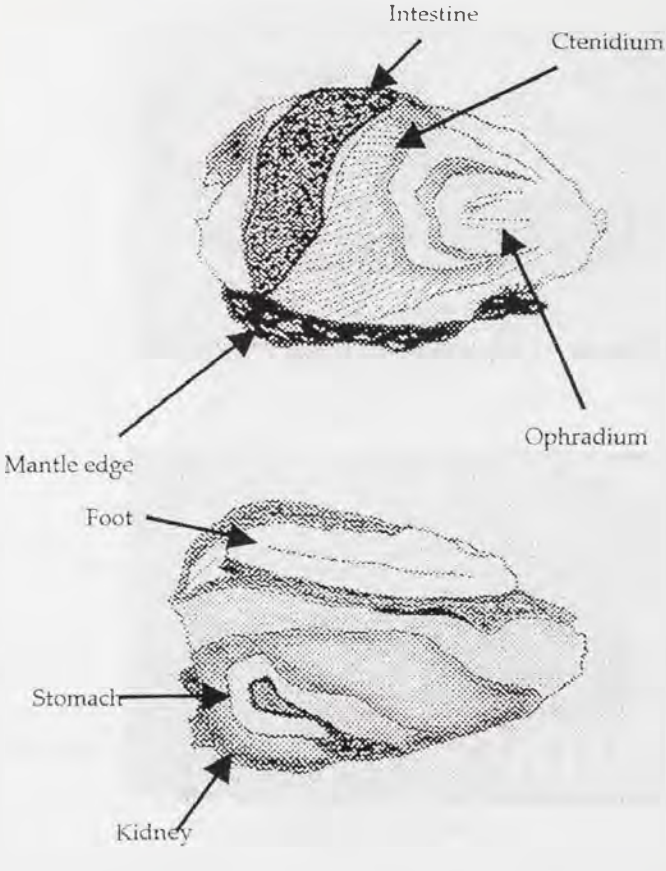
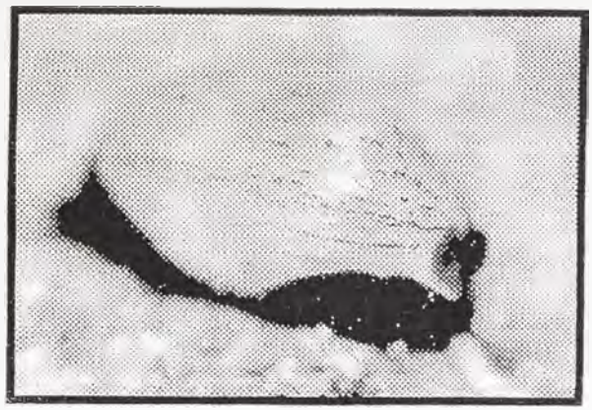
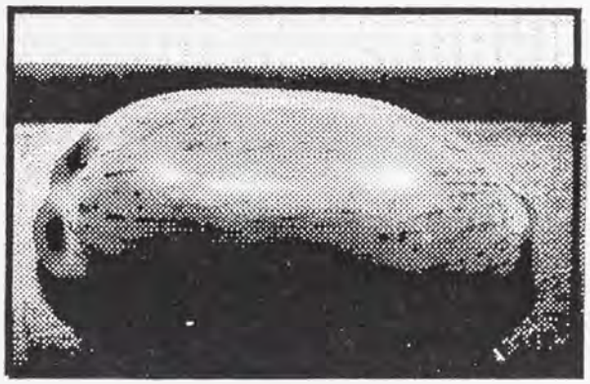


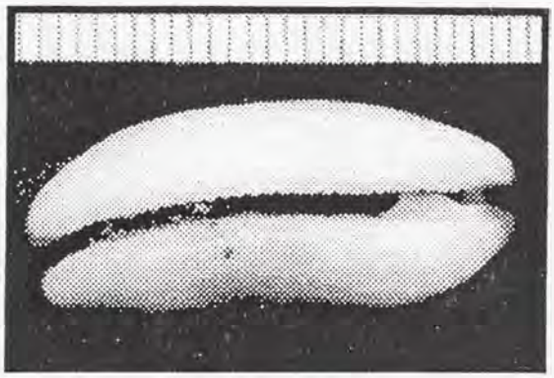
Figure 13. *Cypraea isabella* External and Internal Morphology



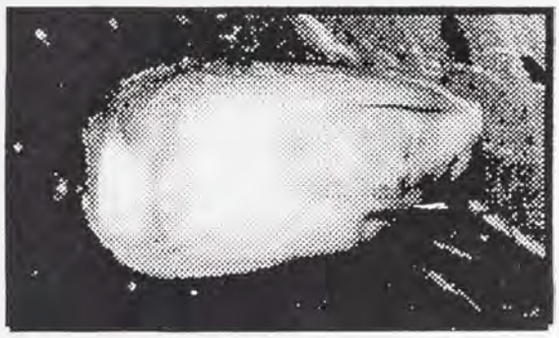
Live specimen found under dead coral rubble.



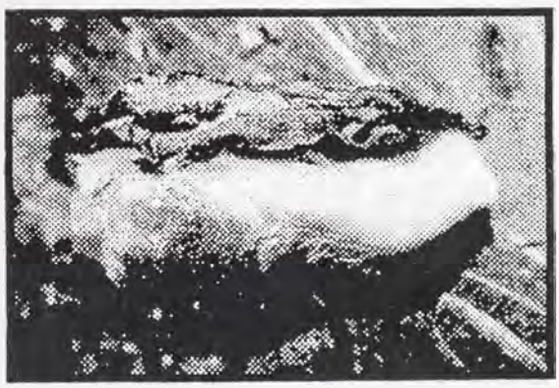
External Dorsal Side



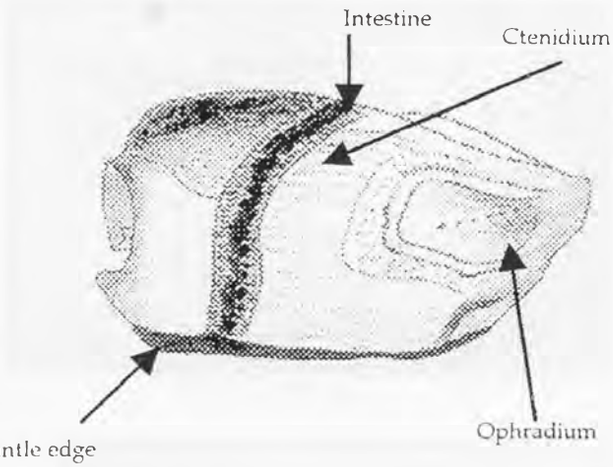
External Ventral Side



Dorsal Side



Ventral Side



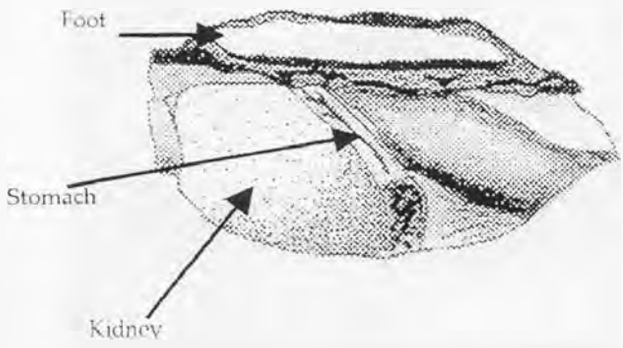
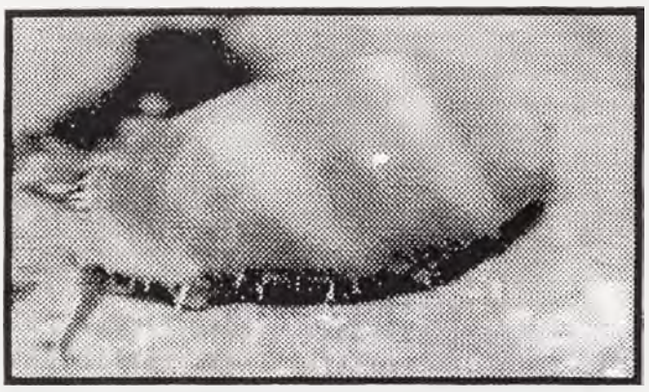
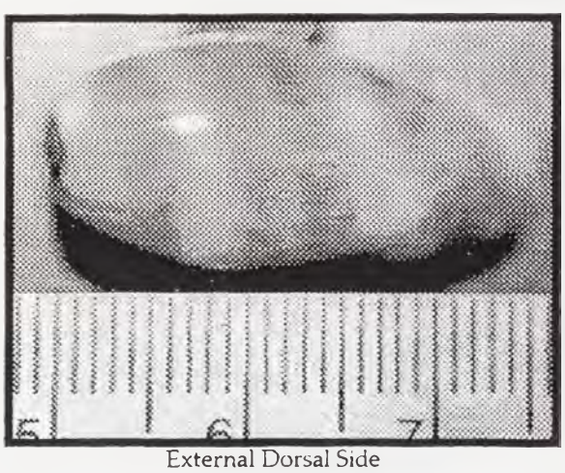


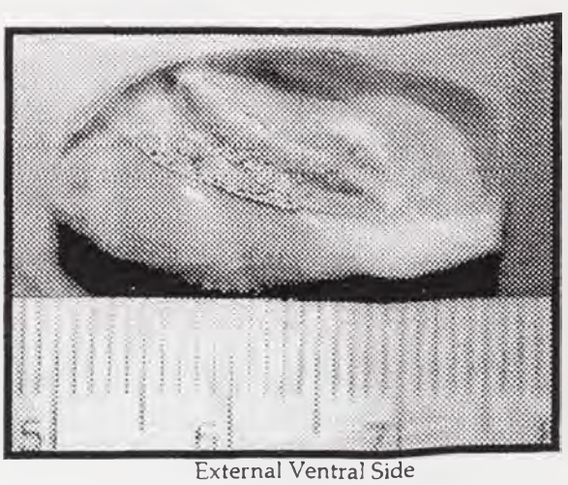
Figure 14. *Cypraea leviathon* External and Internal Morphology



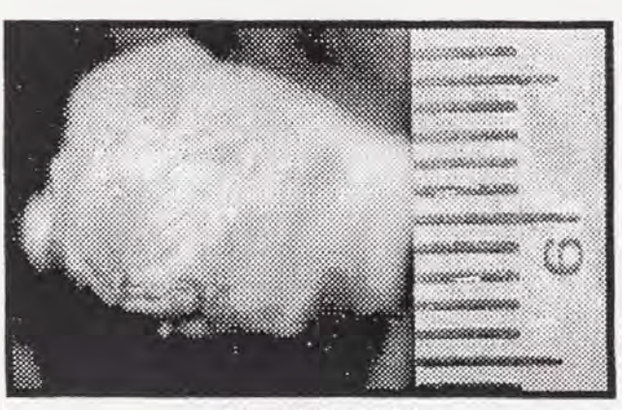
Live specimen found under dead coral rubble.



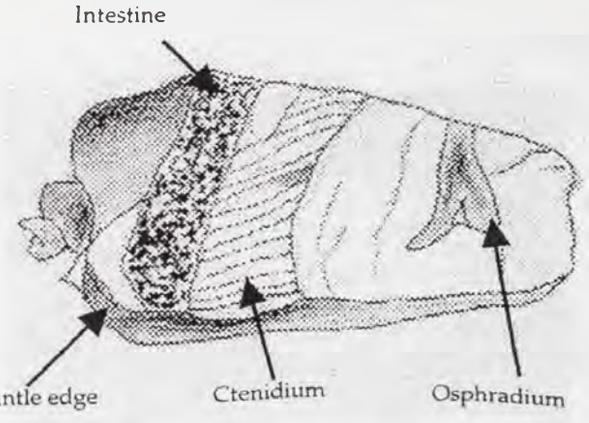
External Dorsal Side

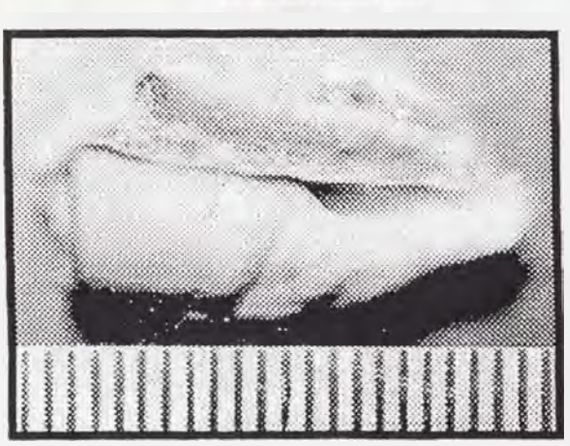


External Ventral Side



Internal Dorsal Side





Internal Ventral Side

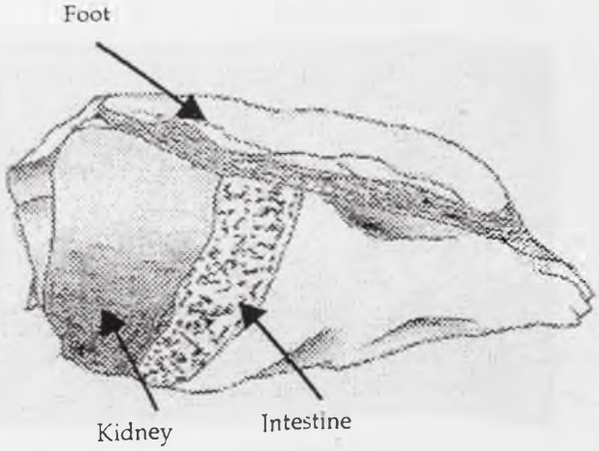
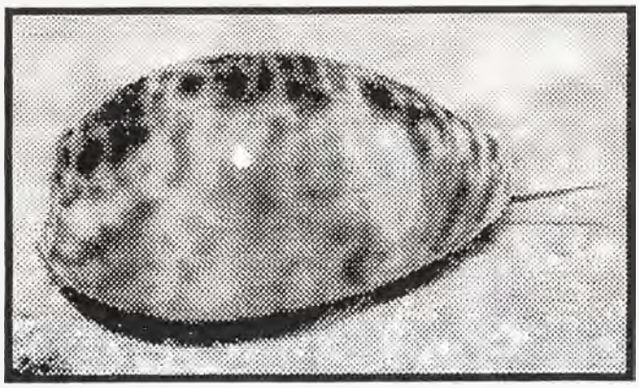
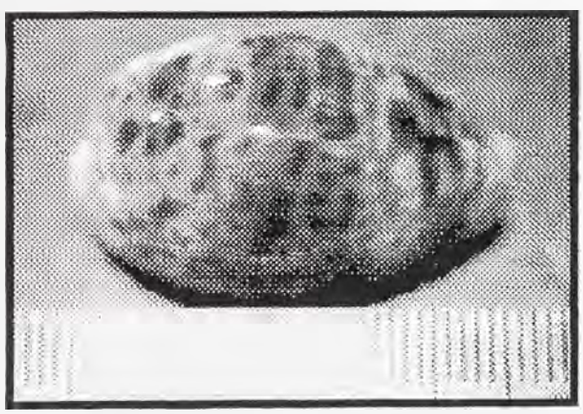


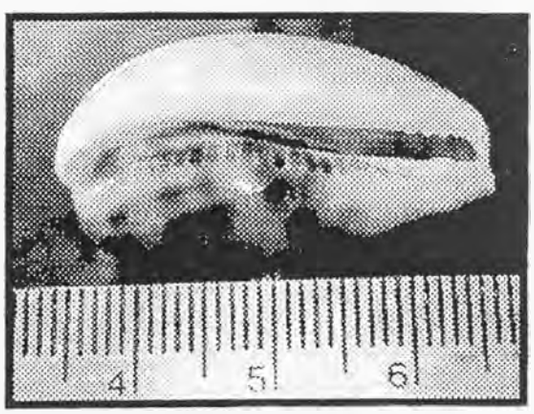
Figure 15. *Cypraea lynx* External and Internal Morphology



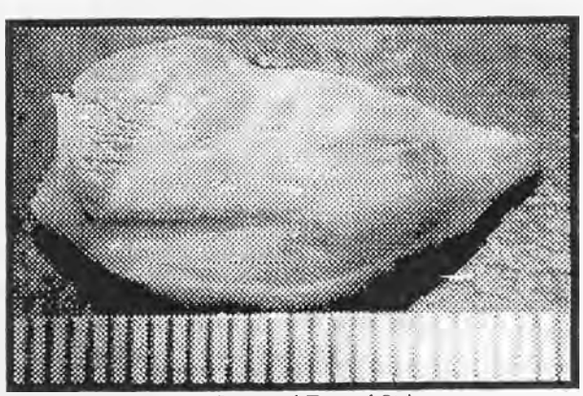
Live specimen found under dead coral rubble.



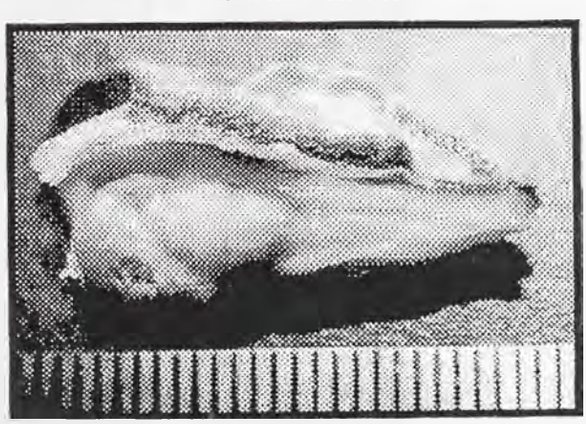
External Dorsal Side



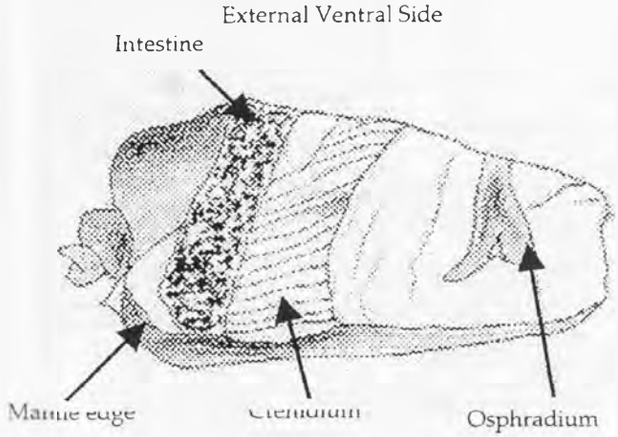
External Ventral Side



Internal Dorsal Side



Internal Ventral Side



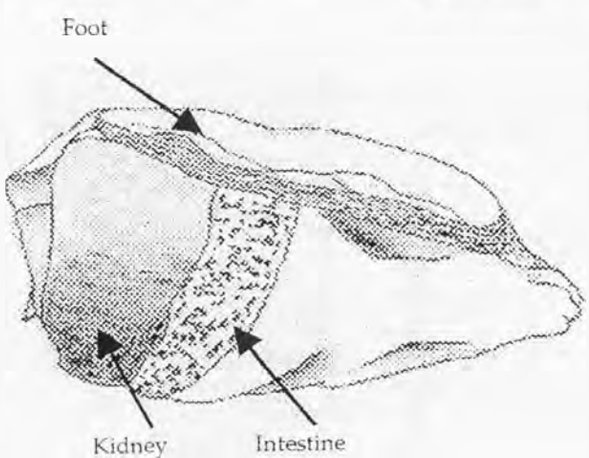
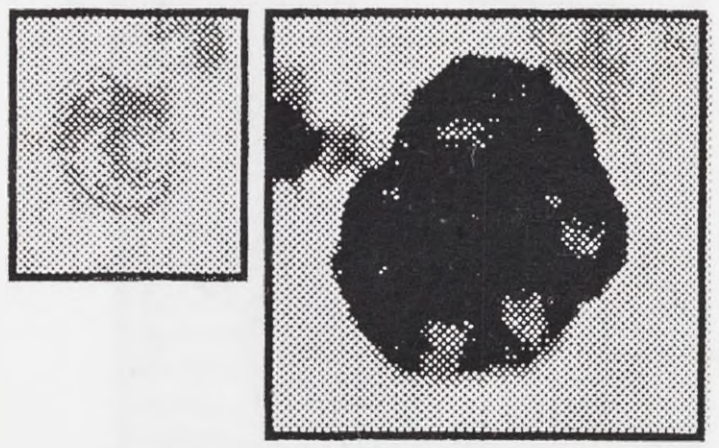
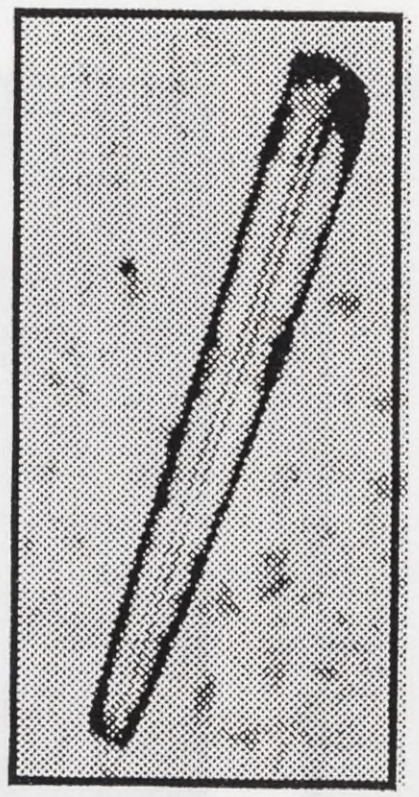


Figure 16. Gastrointestinal System Contents

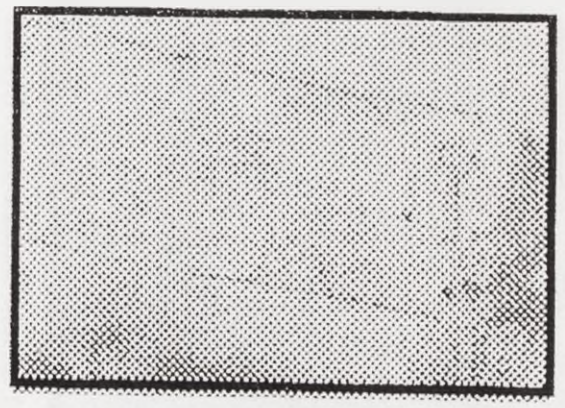
Foraminifera

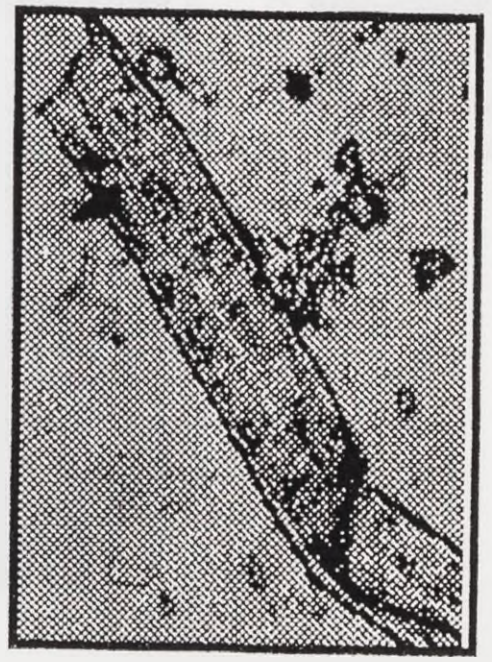


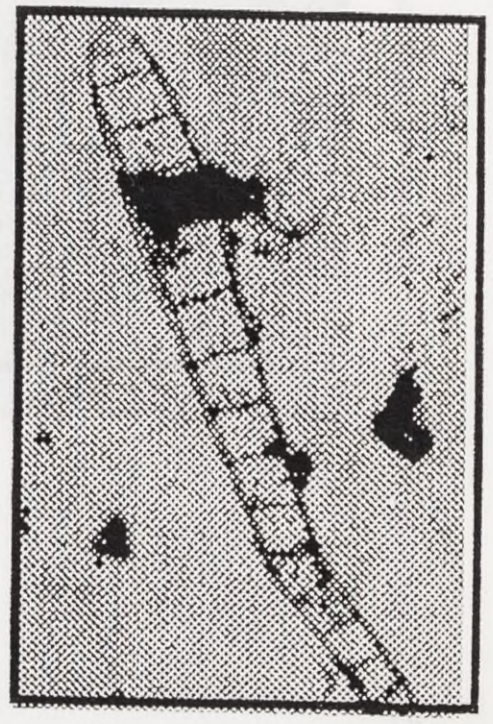
Spicules



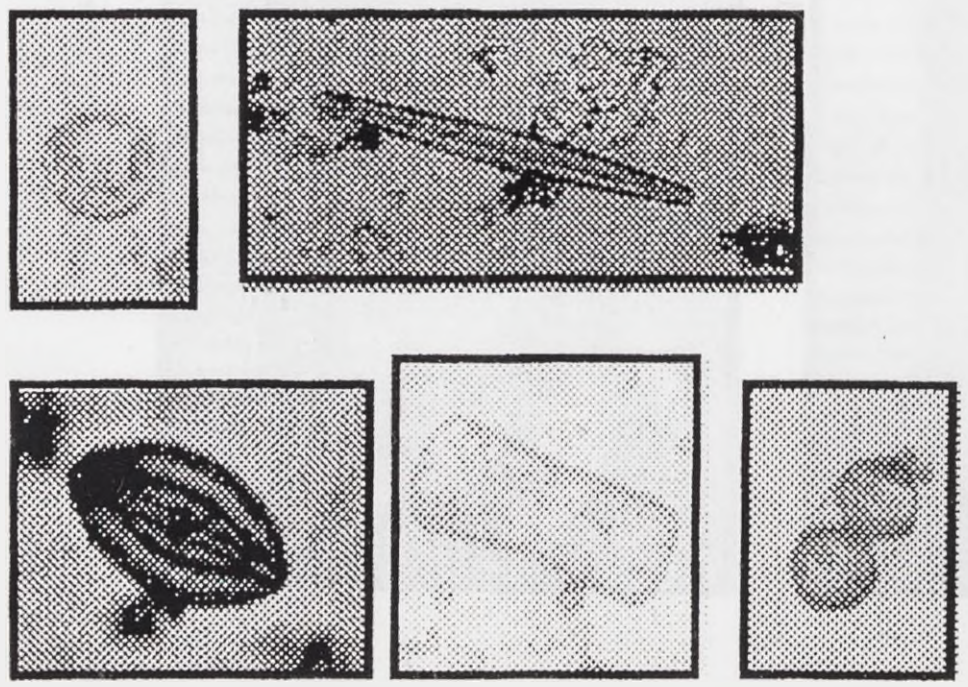
Filamentous Algae







Diatoms



0.24 mm

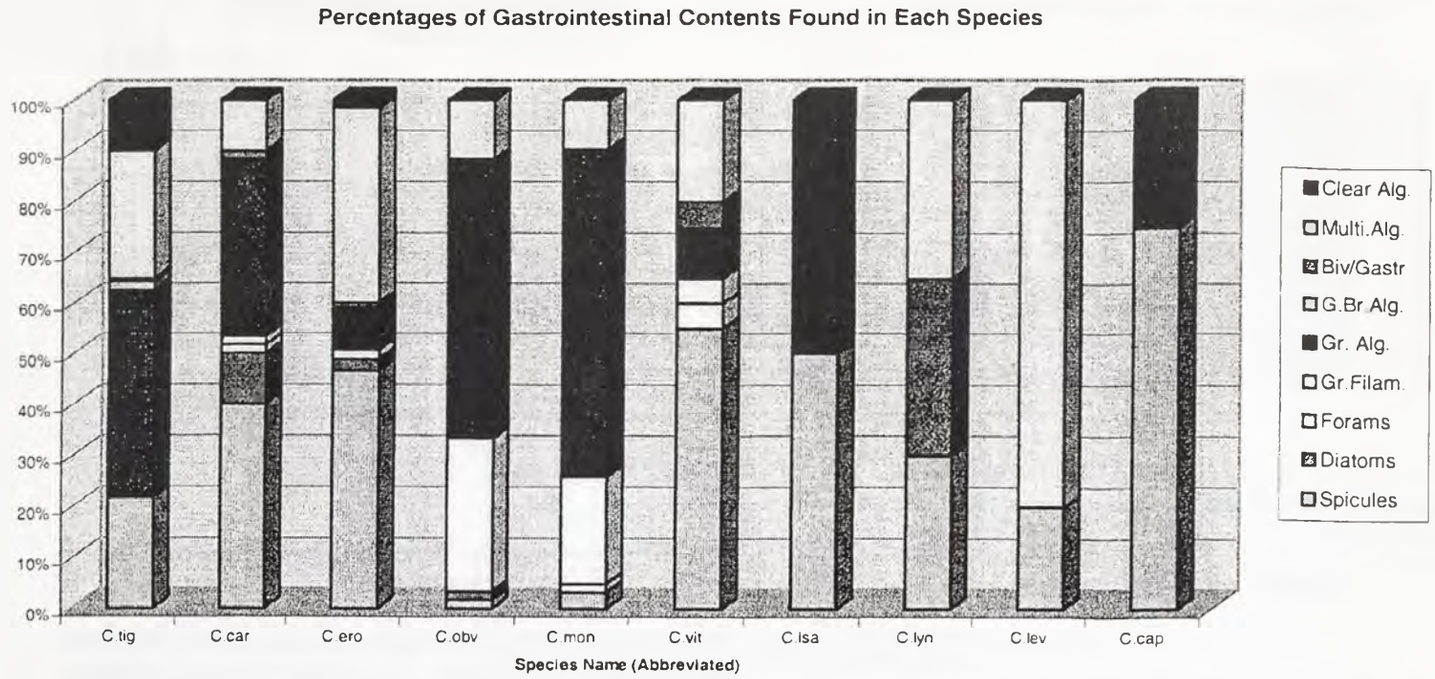


Figure 17. Percentage of gastrointestinal content types found in each species. Key for abbreviations: "Clear Alg" = algae with transparent walls. "Multi. Alg." = multicellular algae. "Biv/Gastr" = bivalves or gastropods. "G.Br.Alg" = golden brown algae. "Gr.Alg." = green algae. "Gr.Filam." = green filamentous algae. "Forams" = foraminifera. "Diatoms" = diatoms. "Spicules" = sponge or tunicate spicules.

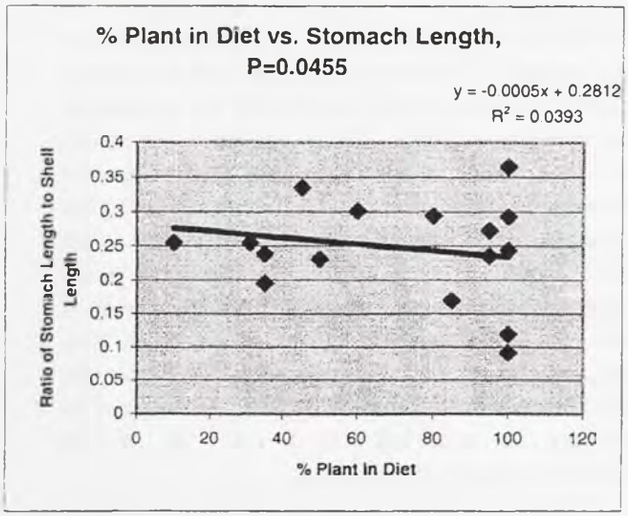


Figure 18. Percent plant in diet versus ratio of stomach length/shell length.

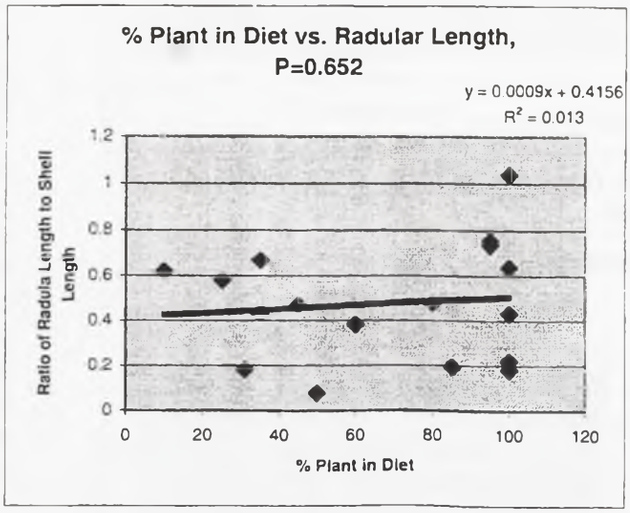


Figure 19. Percent plant in diet versus ratio of radular length to shell length.

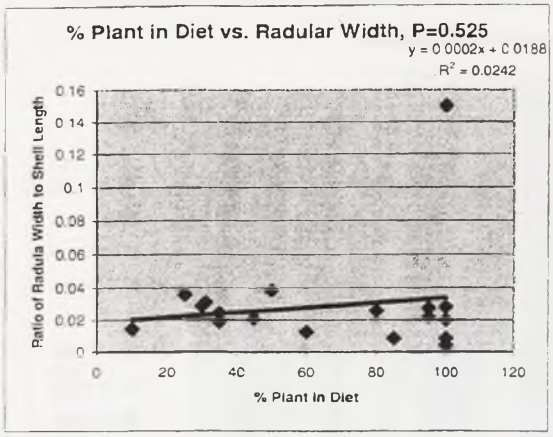


Figure 20. Percent plant in diet versus ratio of radular width to shell length.

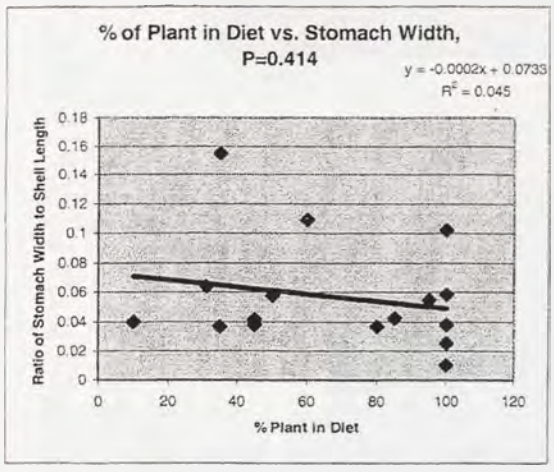


Figure 21. Percent plant in diet versus ratio of stomach width to shell length.

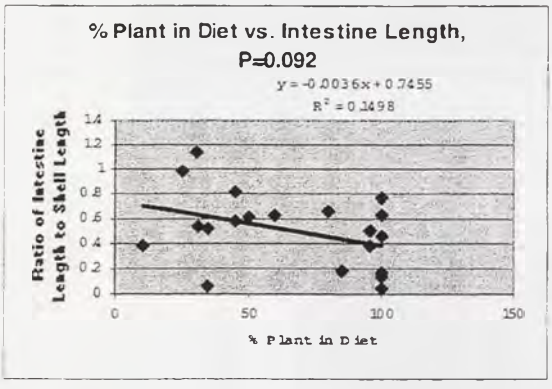


Figure 22. Percent plant in diet versus ratio of intestine length to shell length.

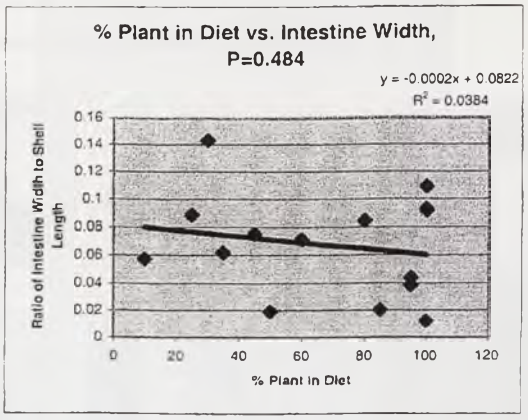


Figure 23. Percent plant in diet versus ratio of intestine width to shell length.

The molecular phylogeny was similar to the morphology-based phylogeny in the three following relationships: 1) *Cypraea erosa* and *Cypraea obvelata*, 2) *Cypraea tigris* and *Cypraea isabella*, and 3) *Cypraea carneola* and *Cypraea lynx*. However, all the other phylogenetic relationships differed between the two phylogenies.

To establish a comprehensive phylogeny, a final phylogenetic tree was developed based on both the morphological and molecular data (Figure 27). In all molecular analyses, the nucleotide sequences for *Cypraea carneola* were substituted by the sequences for the closely related *Cypraea propinqua* and molecular data for *Trivia ovulata* were substituted by sequences from the closely related *Trivia solandri* because molecular data were only available for *Cypraea propinqua* and *Trivia solandri*. One should note that this substitution prevented completely accurate comparisons since (as this study indicates) variation between species within a family can exist that make it sub-optimal to assume that information about one species may be applicable to another species. However, it was determined that some kind of comparison to molecular data was preferable to none at all, so the substitutions were made in this case.

Phylogenetic Mapping of Diet Type

To examine if there was an evolutionary basis for diet type, the species that exhibited clear preference for a carnivorous or herbivorous diet type (indicated by the majority of the diet being for either animal or plant material) were charted on the phylogeny based on both morphology and molecular data (Figure 28). Results indicated that there is no evolutionary conservatism in diet type. Therefore, it is doubtful that diet type among different species had similar evolutionary roots. However, it is also important to note that since only ten

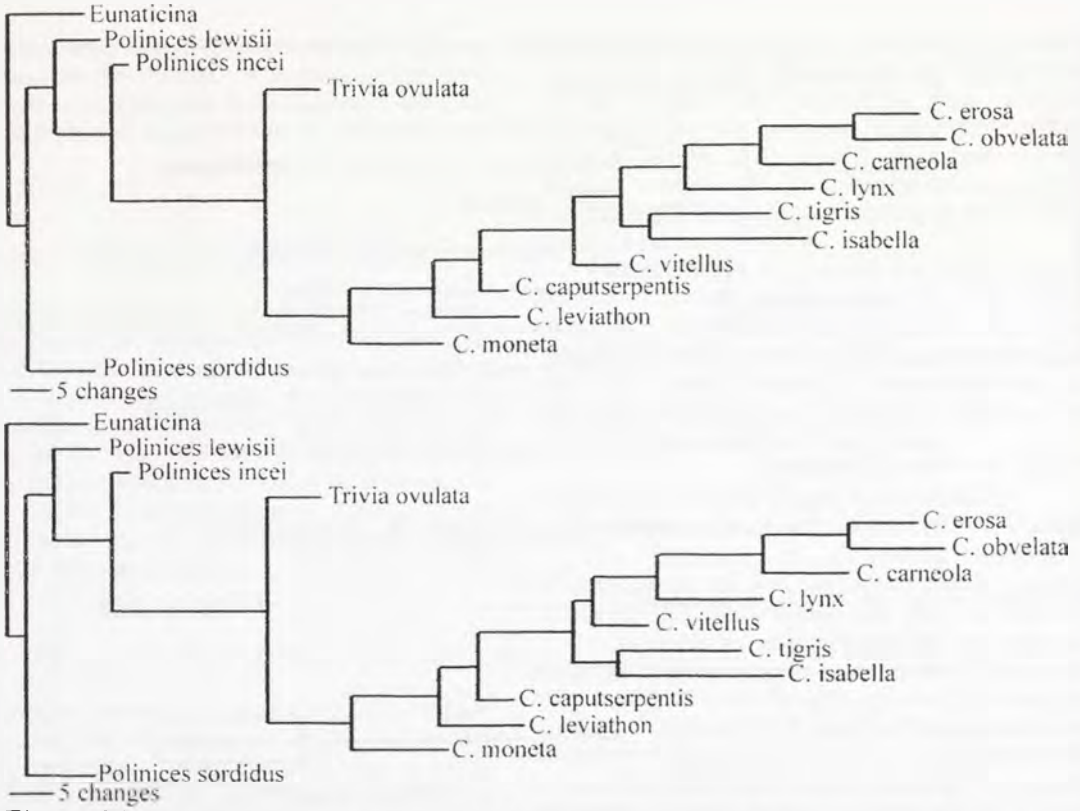


Figure 24. The two most parsimonious phylogenetic trees obtained from morphological data.

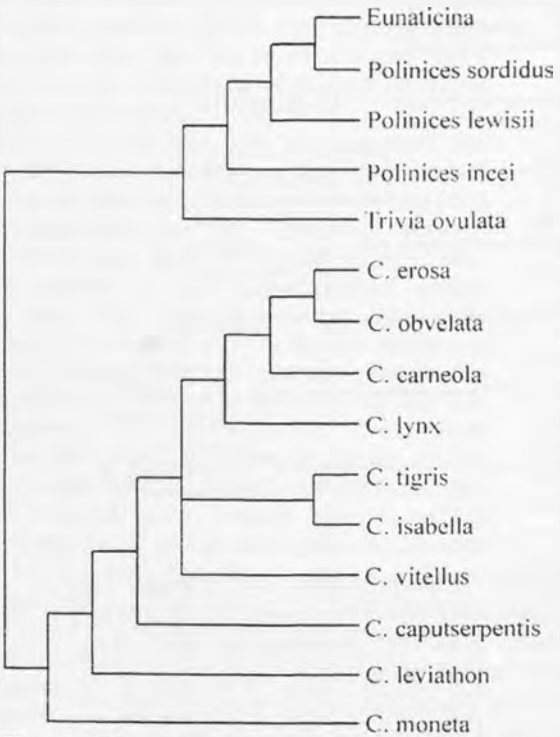


Figure 25. Strict consensus tree of original two phylogenetic trees based on morphologicaldata.

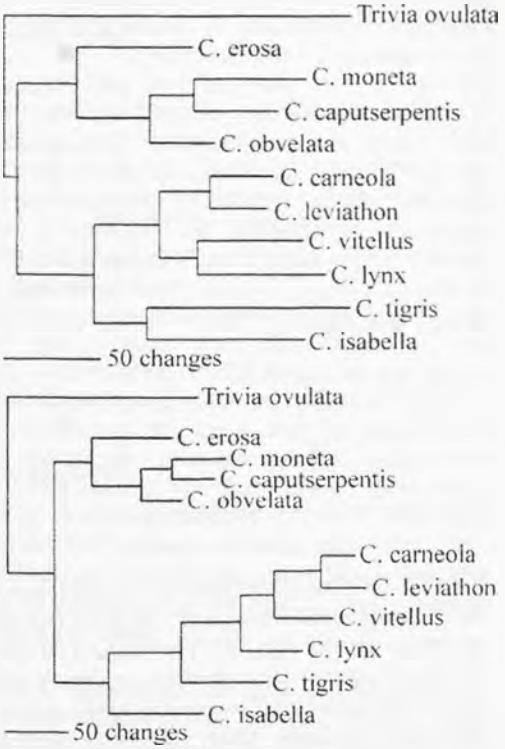


Figure 26. The two phylogenic trees based on molecular data (Meyers 1998).

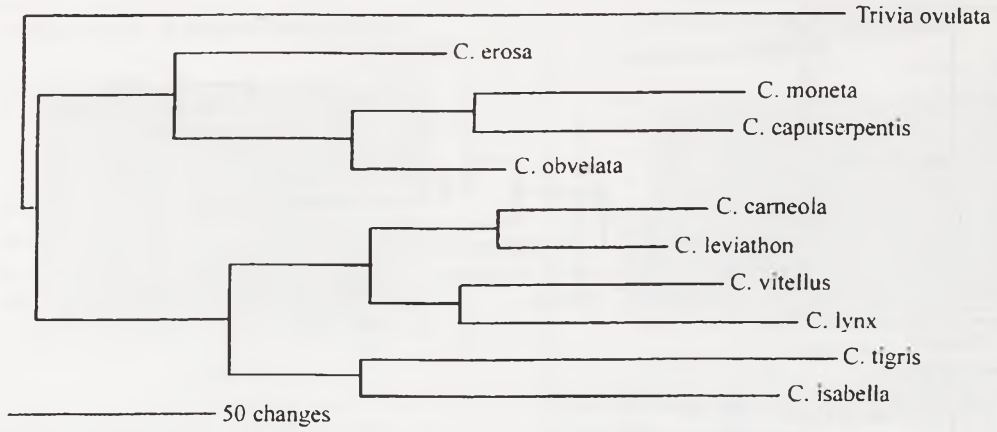


Figure 27. Phylogenetic tree based on morphological and molecular data combined.

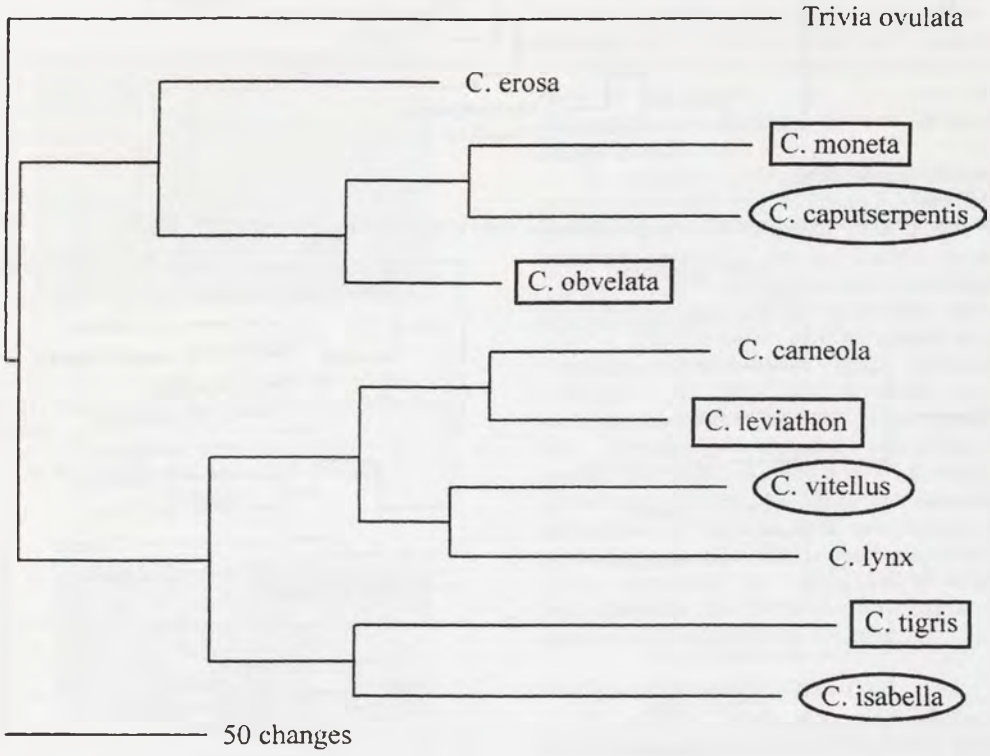


Figure 28. Plot of diet type on the phylogenetic tree based on morphological and molecular data shows no apparent evolutionary basis for diet type. Predominantly carnivorous species (indicated by a preference greater than 50% for animal material) are labeled by a circle. Predominantly herbivorous species (indicated by a preference greater than 50% for animal material) are labeled by a circle. Predominantly herbivorous species (indicated by a preference greater than 50% for plant material) are indicated by a square. Other species not labeled did not show a preference greater than 50% for either plant or animal material.

not the most closely related species among the *Cyprea*, it is still possible that if a study with more species were conducted, it is possible that an evolutionary basis for diet type could still be found.

Discussion

Comparative morphology among the ten Cypraea species

Comparative examination of the internal anatomy of the ten species of *Cypraea* showed that while all species had the same general morphology and location of organs in the body (for example, the osphradium was always anterior to the ctenidium, which was always anterior to the intestine and kidney), there were slight differences in their actual shapes and sizes from species to species. Similar to the conchological differences that make each species unique, the internal anatomy of each species was also distinct and characteristic of the species.

Correlations between gastrointestinal structure and contents

The positive correlation between the percent plant in the diet and radular length and width suggest that there was an adaptive advantage to having a larger radula when a species ate more plant material. A possible explanation for this finding may be that since plant cell walls are more rigid than animal cell walls due to cellulose content, species that consume more plant material in their diet than others will also have a larger radula to better scrape and break down the higher plant content in their diets.

Though it initially may have been expected that radular trends would echo those of the intestine and stomach, the fact that they did the opposite prompted a different explanation for the correlation between intestine and stomach size with percent plant in diet. Since the majority of the gastrointestinal system contents, other than plants, consisted of animal contents, one can conclude that an inverse relation of stomach and intestine size with percent plant in diet implies a positive correlation of stomach and intestine size with percent animal content in diet. Examination of the main type of animal content in the ten species examined showed that sponges were the dominant type of animal material eaten. Sponge spicules, which indicate the amount of sponge eaten even though they may not be digested themselves, have a different structure than most animal cells in that they are long, sharp, and pointed. As a result, one might speculate that this structure would normally be a source of damage to the gastrointestinal system as it passes through the body. However, the presence of a larger stomach and intestine in animals that have larger amounts of these spicules may be their way of producing more protective mucous to prevent damage to the gastrointestinal system. Since the correlation was statistically significant for the stomach length, but not for the intestine length, it is possible that since one of the stomach's prime roles is to secrete mucous, its length had a stronger negative correlation with percent plant in diet than intestine size did.

Furthermore, it is possible that while the radular correlations with percent plant in diet were not statistically significant, the hypothesis that they are enlarged in species with a higher percent plant in diet may still be valid because the radulae are well known for their morphological malleability. Further studies could support this preliminary finding.

Comparison of morphological and molecular phylogenies

The presence of differences between the morphology-based and molecular-based phylogenies indicated that morphology did play a role in the evolutionary history of the Cypraeidae that could not be reflected in the purely molecular data. It also suggested that molecules and morphology data do not always confirm each other in all aspects of phylogenetics since morphology is an external phenotype that can be shaped to suit environmental needs, whereas molecules are genotypes passed through generations of a species. However, comparison of the phylogeny based on both molecular and morphological data with the phylogenies established by Meyers (1998) independent of morphological data revealed that one of the two molecular-based phylogenies was actually identical to the phylogeny with morphological data added. This indicated that, while morphological data did have an influence on the phylogeny different than the molecules did alone (shown by the presence of differences between the morphological and molecular phylogenies), the sheer number of molecular data outweighed most of the morphological contributions when the data for both character types were combined. However, the similarities in three species relationships among the two phylogenies lended confidence to the validity of those segments in both phylogenies that expressed those particular evolutionary relationships. It is important to note that while some of the characters examined were purely morphological in nature, there were some (such as gastrointestinal system contents) that were more ecological in nature. Nonetheless, all of these characters could still be used to create the phylogenies, according to Luckow and Bruneau (1997).

Comparison to outgroups: Naticidae and Triviidae

Since the Naticidae and Triviidae families both showed up on the phylogeny as being most distantly related to all ten of the Cypraeidae species, the

monophyly of *Cypraea* is supported. This finding indicates that the ten *Cypraea* species are indeed more related to each other than to other families of gastropods, as would be expected.

Recommendations for Future Studies

In order to better understand the possible relationships between adaptations of gastrointestinal organs to diet type, phylogenetic relationships of cowries, and morphological similarities and differences between individual species within the Cypraeidae family, the following are recommendations for future studies. Examining other organ systems, such as the reproductive and nervous systems, could further elucidate the kinds of anatomical similarities and differences that exist from species to species within a family and serve to better describe each of the species in detail. Characters obtained from these additional studies could also be added to the morphological characters found in this study to create more comprehensive morphology-based phylogenies. These new phylogenies could then be compared to see if they change to agree more or less with molecular-based phylogenies. More specimens of each of the species examined in this study could be examined to obtain more statistically significant data. Similar studies of a more diverse species pool as well as inclusion of additional reproductive morphological characters in the study, such as total number of offspring, number of veligers/capsule, number of eggs, and number of capsules/egg mass, could provide further evidence to support or disprove findings on gastrointestinal organ size correlations to diet type, improve the evaluation of morphological differences within the Cypraeidae family, and aid our ability to build an accurate phylogenetic tree for the Cypraeidae. Finally, repeating these studies for other organisms can reveal whether gastrointestinal system adaptations to diet type are universal.

Conclusion

The fact that internal anatomy differed from species to species within Cypraeidae allowed us to conclude that generalizing about the morphology of individual species from knowledge of the Triviidae or even other species of Cypraeidae was insufficient to accurately describe the morphology of each individual species. Accurate characterization required detailed studies of each species separately.

Furthermore, since the two phylogenies based on morphological and molecular data produced similar relationships for three pairs of species, the validity of those phylogenetic relationships was supported. However, discrepancies between the two phylogenies indicated that morphology did have an influence on the evolutionary development of the ten species examined in this study that molecular information alone did not address.

Finally, a statistically significant negative correlation between percent plant in diet with stomach length supported the hypothesis that with lower percentages of plant in the diet, animal material, in particular, sponge spicules, consumed a larger portion of the diet which may have caused the stomach to adapt to protect from spicule damage by increasing in size. Though not statistically significant, a similar correlation found for intestine size may be due to similar causes, and a positive correlation between percent plant in diet and radular size may be due to a need for better scraping abilities in species with higher plant content diets. These findings contribute to our understanding of the manner in which the gastrointestinal system may adapt to meet an organism's dietary needs.

Acknowledgments

Dr. Jere Lipps, Dr. Vince Resh, Dr. Jaime Bartolome, Sarah Rieboldt, and Aimee Betts guided me at all stages of my project development. Dr. Brent Mishler helped me to conduct phylogenetic analyses. Dr. Carole Hickman allowed me to use her lab for dissections upon my return to UC Berkeley. Dr. Chris Meyers provided advice and data from his own phylogenetic work on *Cypraea*. Paul Roberge, Ken Peer, and Matt Epstein were excellent assistant cowrie hunters in the field. Audrey Aronowsky, my snorkeling partner and mentor, helped me every step of the way and engendered in me a wonderful passion for snails. Thank you to all of you and the entire Moorea class for making this an unforgettable learning experience.

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Appendix 1.

SELECTED DATA FOR 10 *CYPRAEA* SPECIES							
SPECIES NAME/SPECIMEN # (if >1):	*E. papillae*	*P. lewisii*	*P. incei*	*P. sordidus*	*T. ovulata*	*C.erosa #3*	*C. moneta #3*
Shell Features:							
shell shape: max width/max height	31.1/36.1mm	34.1/40mm	12.2/22.2mm	24.5/30mm	13.5/18.9mm	13.4/23.2mm	11.3/21.85mm
aperture: max length/max width	11.3/23.7mm	16.6/25.1mm	8.5/13mm	11.4/19.2mm	?	1.5mm/19.4mm	1.3/15mm
Denticles:							
# columellar teeth	n/a	n/a	n/a	n/a	14	14	13
# labral teeth	n/a	n/a	n/a	n/a	12	15	11
Foot:							
dorsal foot color	brown	tan/brown	beige	gray	?	deep brown	white
ventral foot color	tan	white	white	white	?	white	white
Tentacles:							
length	3.6mm	5mm	3.3mm	3mm	?	2mm	3mm
color	brown/black	beige	beige	wht, w/ little gray	?	white/reddish	white/black tinge
height							
color	tan	white	white	white	?	white, red tint	white
Radula:							
height	10.7mm	12.3mm	?	3.5mm	?	9.15mm	11.4mm
width	0.5mm	0.9mm	?	0.1mm	?	0.3mm	0.5mm
fraction in radular sac	20%	100%	?	100%	?	20%?	see above
#rows of teeth	81	69	?	45	37-44	90	102
GastroIntestinal System Size:							
stomach length	29.6mm	43.1mm	9mm	9.2mm	?	7.2mm	10mm
stomach diameter	2mm	2.8mm	2.7mm	1.8mm	?	2.6mm	2.5mm
intestine length	?	?	?	?	?	15mm	11mm
intesting width	?	?	?	?	?	1.7mm	1.2mm

C. moneta #2	*C. obv. #3*	*C. obv. #2*	*C. carn #3*	*C. tigris #3*	*C. erosa #2*	*C. carn. #2*	*C. erosa #1*	*C. tigris #1*
12.7/12mm	13.3/17.3mm	10.1/14.8mm	20/31mm	49.6/72.6mm	18.7/23.3mm	20.5/31.7mm	15.4/25.2mm	50/80mm
1/11.2mm	0.9/12.6mm	0.9/11.6mm	2.6/26.4mm	5/58mm	1.4/20.8mm	2/24.4mm	1.5/19.7mm	5.6/55mm
						black		
10	10	11	19	19	14	20	13	20
10	10	11	21	20	15	22	15	21
black spotty	black	black stippled	brown	?	black	white	black	?
white	white	white	beige	?	beige	white	white	?
1.5mm	1.8mm	2mm	2.2mm		3.8mm	5mm	?	?
white/black tinge	black	white, black tinge	black	gray	black	black	dark pink	?
white	white	gray out/white inside	black	black	white	black	slightly black	?
10.6mm	13.8mm	13.4mm	21.6mm	50mm	11.2mm	15.1mm	11.2mm	?
0.25mm	0.4mm	0.5mm	0.5mm	2.2mm	0.5mm	0.7mm	0.5mm	?
33% on bulge	20%	100%?	25%	10%	25%	33%	33%	?
88	121	73	205	129	81	169	95	53
5.4mm	5mm	4.3mm	8.9mm	28.7mm	8mm	8.5mm	8mm	25mm
0.6mm	1mm	1mm (of one bend)	1.4mm	4.6mm	0.9mm	1.33mm	1mm	8mm
8.5mm	9.2mm	6.9mm	13.5mm	49.5mm	19.5	16mm	14mm	3.7mm
0.7mm	0.7mm	0.8mm	2mm	7.3mm	1.8mm	3.2mm	?	?

C. obv. #1	*C. mon. #1*	*C. caput. #1*	*C. isab.#1*	*C. vit. #1*	*C. carn. #1*	*C. tigris #2*	*C. lev. #1*	*C. lynx #1*
17.85/22.8mm	17.8/25.2mm	15.6/22.5mm	14/26mm	21.7/41mm	26/42mm	57.75/83.0mm	14.9/27.2 mm	19.6/32.3mm
1/20mm	1.8/18mm	1.5/25.4mm	1.46/23.9mm	2.2/33.55mm	2.25/36mm	10/70mm	7.8/25.7 mm	2.6/29.25mm
10	9	13	31	19	21	26	n/a	20
11	10	11	29	22	24	22	n/a	21
black	black	spotty black	black	black	black	black	brown	black
white	white	white	black	white	white	white	tan	white
?	?	3mm	2mm	4mm	3mm	5mm	3.5mm	4.4mm
white	white	black tips?	black	yellow?	black	white	black	black
black	?	black	black	black	black	black	black	black
19mm	13mm	13mm	2mm	17.75mm	1mm	14.3mm	13mm	21.5mm
0.75mm	0.5mm	0.8mm	1mm	1mm	1mm	2.45mm	0.7mm	0.6mm
90%	66%	100%	?	66%	100%?	100%	100%	100%
130	96	83	14	110	17	43	112?	142
?	7mm	?	6mm	8mm	?	20mm	8mm	7.7mm
?	1.5mm	?	1.5mm	1.5mm	?	5mm	1mm	5mm
14mm	10mm	22mm	16mm	2.6mm	40mm	42mm	18mm	16.7mm
2mm	?	2mm	0.5mm	?	5mm	?	2.3mm	2mm

Appendix 2

Character State Descriptions and Definitions

Note: For characters 1,2,9,10,11,25,37,44,45,46,47,48,56,57,58, and 59, character states were determined by plotting all the data points for each of the specimens examined on a scatterplot. These graphs produced distinctive regions or clusters of data points, allowing for determination of each region as a separate character state. Graphs for each of these characters follow the character state descriptions. In the cases where divisions of character states may have been ambiguous, statistical analysis was conducted on the data sets to find the mean +/- one standard deviation. Then, data points were assigned to character states based on whether the data was +/- a standard deviation from the mean.

1. Shell Shape is defined by the ratio of the maximum width of the shell divided by the maximum height in millimeters. 0=Ratio <1.30. 1=Ratio 1.30-1.78. 2=Ratio >1.78.

2. Aperture ratio is defined by the ratio of the maximum length of the aperture divided by the maximum width. 0=Ratio less than 10. 1=Ratio between 10-15. 2=Ratio greater than 15.

3. 0=Spire umbilicate. 1=Spire not umbilicate.

4. 0=Protoconch covered with callus. 1=Protoconch not covered with callus.

5. 0=Labrum naturally has shiny finish. 1=Labrum naturally has dull finish.

6. 0=Difference in aperture width not apparent. 1=Aperture is widest at anterior end of ventral side of shell.

7. 0=No columellar or labral teeth. 1=Columellar teeth are finer than labral teeth. 2=Labral teeth are finer than columellar teeth.

8. 0=No columellar or labral teeth. 1=Neither columellar nor labral teeth extend beyond the aperture. 2=Columellar teeth extend beyond aperture. 3=Both columellar and labral teeth extend beyond aperture.

9. 0=No columellar teeth. 1=Number of columellar teeth is less than 17. 2=Number of columellar teeth is between 17 and 28. 3=Number of columellar teeth is greater than 28.

10. 0=No labral teeth present. 1=Number of labral teeth is less than 18. 2=Number of labral teeth is between 18 and 25. 3=Number of labral teeth is greater than 25.

11. 0=No labral or columellar teeth present. 1=Ratio of labral to columellar teeth less than 1. 2=Ratio of labral to columellar teeth equal to 1. 3=Ratio of labral to columellar teeth greater than 1.

12. 0=Light shell base color. 1=Dark shell base color.

13. 0=Different colored or patterned shell frame at base of dorsal side of shell.1=No frame at base of dorsal side of shell.

14. 0=Unspotted dorsal shell. 1=Spotted dorsal shell.

15. 0=No lines on dorsal shell. 1=Lines on dorsal shell.

16. 0=Light colored ventral side of shell. 1=Dark colored ventral side of shell.

17. 0=More than 35 leaflets on ctenidium. 1=Less than 35 leaflets on ctenidium. States were chosen through observations made during dissections that leaflets varied around this cut-off evenly.

18. 0=Thick and wide foot. 1=Thin and long foot.

19. 0=Posterior thinning of foot absent. 1=Posterior thinning of foot present.

20. 0=Light dorsal foot color. 1=Dark stippled dorsal foot color.

21. 0=Light bottom of foot color. 1=Dark bottom of foot color.

22. 0=Short and wide siphon. 1=Long and thin siphon.

23. 0=Uneven siphon at apex. 1=Even siphon at apex.

24. 0=Light colored siphon. 1=Dark colored siphon.

25. 0=Tentacle length less than 2.5mm. 1=Tentacle length between 2.5 mm- 3.7mm. 2=Tentacle length greater than 3.7mm.

26. 0=Light colored tentacles. 1=Dark colored tentacles.

27. 0=Straight tentacles. 1=Curved tentacles.

28. 0=Parallel width tentacles. 1=Slightly tapered tentacle width. 2=Tapered width tentacles.

29. 0=Eyes at base of tentacle. 1=Eyes at midpoint of tentacle. 2=Eyes at tips of tentacles.

30. 0=No mantle present. 1=Translucent mantle. 2=Opaque mantle.

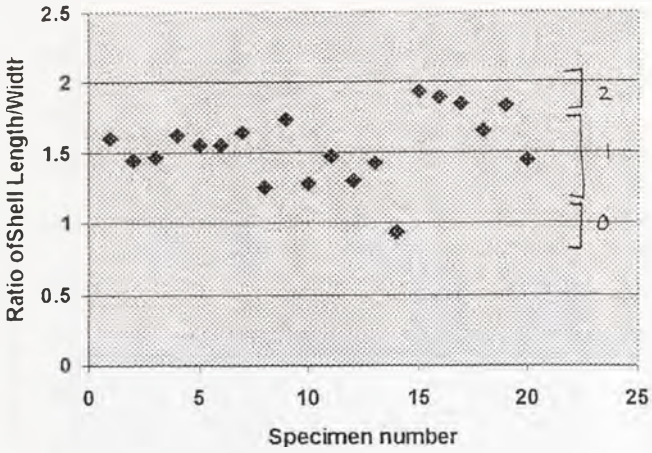
31. 0=No mantle present. 1=Smooth mantle. 2=Granular mantle.

32. 0=No mantle present. 1=Light base color of mantle. 2=Dark base color of mantle.

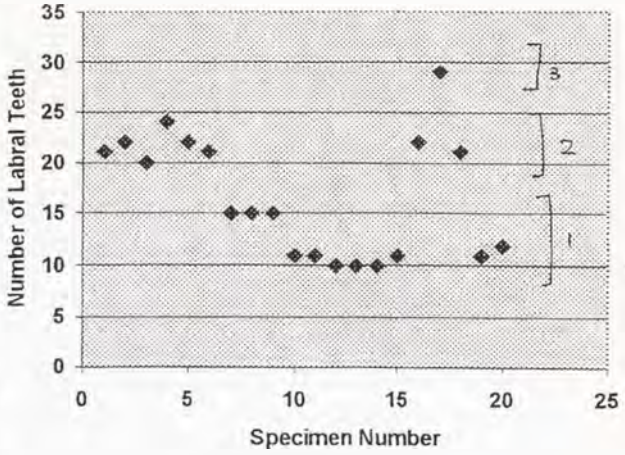
33. 0=No papillae visible. 1=Wart-like papillae. 2=Fleshy finger-like papillae.

34. 0=Mantle complex directed to right side of specimen's body (with ventral side up). 1=Mantle complex directed to left side of specimen's body (with ventral side up).
35. 0=Osphradium anterior to ctenidium. 1=Osphradium posterior to ctenidium.
36. 0=Kidney posterior to ctenidium. 1=Kidney anterior to ctenidium.
37. 0=Leaflets on one section of osphradium less than 17. 1=Leaflets on one section of osphradium greater than 17.
38. 0=Light colored esophagus. 1=Dark colored esophagus.
39. 0=Narrow esophagus. 1=Wide esophagus.
40. 0=Non-glandular esophagus. 1=Glandular esophagus.
41. 0=Radula coiled around itself. 1=Radula coiled around radular sac.
42. 0=No lamellae present on esophagus. 1=Lamellae present on esophagus.
43. 0=Radula short and wide. 1=Radula elongate and narrow.
44. 0=Radula length less than 10mm. 1=Radula length between 10mm and 15mm. 2=Radula length greater than 15mm.
45. 0=Radula width less than 1.5mm.1=Radula width greater than 1.5mm.
46. 0=Ratio of radula length:width less than 10. 1=Ratio of radula length:width between 10 and 30. 2=Ratio of radula length:width greater than 30.
47. 0=Less than 50% of radula wrapped around radular bulge. 1=More than 50% of radula wrapped around radular bulge.
48. 0= Rows of teeth less than 80. 1=Between 80-120 rows. 2=More than 120 rows.
49. 0=Rachidian tooth mono-cusped. 1= Rachidian tooth tri-cusped. 2= Rachidian tooth penta-cusped. 3=Rachidian tooth multi-cusped (more than 5 cusps).
50. 0=Rachidian tooth center cusp reduced. 1=Rachidian tooth center cusp enlarged (or large mono-cusped tooth).
51. 0=Inner lateral teeth not visible. 1=Inner lateral teeth bi-cusped. 2=Inner lateral teeth tri-cusped. 3=Inner lateral teeth penta-cusped. 4=Inner lateral teeth multi-cusped.
52. 0=Inner lateral tooth not visible. 1=Inner lateral tooth center cusp reduced. 2=Inner lateral tooth center cusp enlarged (or one of two bi-cusped teeth enlarged).
53. 0=Outer lateral teeth mono-cusped. 1=Outer lateral teeth bi-cusped. 2=Outer lateral teeth tri-cusped.
54. 0=Outer lateral tooth not visible. 1=Outer lateral tooth center cusp reduced. 2=Outer lateral tooth center cusp enlarged (or one of two bi-cusped teeth enlarged).
55. 0=0 degrees of radula row curvature. 1=0.5 degree of row curvature. 2=1 degree of radula row curvature.
56. 0=Stomach length less than 15mm. 1=Stomach length greater than 15mm.
57. 0=Stomach diameter less than 3.5mm. 1=Stomach diameter greater than 3.5mm.
58. 0=Intestine length less than 30mm. 1=Intestine length greater than 30mm.
59. 0=Intestine diameter less than 3mm. 1=Intestine diameter greater than 3mm.
60. 0=Mouth reduced. 1=Mouth enlarged.
61. 0=Light colored mouth. 1=Dark colored mouth.
62. Gastrointestinal contents: 0=Sponge spicules absent. 1=Sponge spicules present.
63. Gastrointestinal contents: 0=Tunicate spicules absent. 1=Tunicate spicules present.
64. Gastrointestinal contents: 0=Cyanobacteria absent. 1=Cyanobacteria present.
65. Gastrointestinal contents: 0=Diatoms absent. 1=Diatoms present.
66. Gastrointestinal contents: 0=Foraminifera absent. 1=Foraminifera present.
67. Gastrointestinal contents: 0=Green filamentous material absent. 1=Green filamentous material present.
68. Gastrointestinal contents: 0=Green algae clumps absent. 1=Green algae clumps present.
69. Gastrointestinal contents: 0=Golden brown algae absent. 1=Golden brown algae present.
70. Gastrointestinal contents: 0=Gastropod larvae or bivalves absent. 1=Gastropod larvae or bivalves present.
71. 0=Echinospira absent. 1=Echinospira present.
72. 0=Mantle does not cover shell. 1=Mantle covers shell.
73. 0=Bi-pectinate osphradium. 1=Tri-pectinate osphradium.
74. 0=Operculum present. 1=Operculum absent.
75. 0=Shell resorption (uneven calcification of shell). 1=No shell resorption (even recalcification of shell).
76. 0=Propodium present (keeps shell glossy). 1=Fused foot.
77. Gastrointestinal system: 0=(A) absent. 1=(A) present.
78. Gastrointestinal system: 0=(B) absent. 1=(B) present.
79. Gastrointestinal system: 0=(C) absent. 1=(C) present.
80. Gastrointestinal system: 0=(D) absent. 1=(D) present.

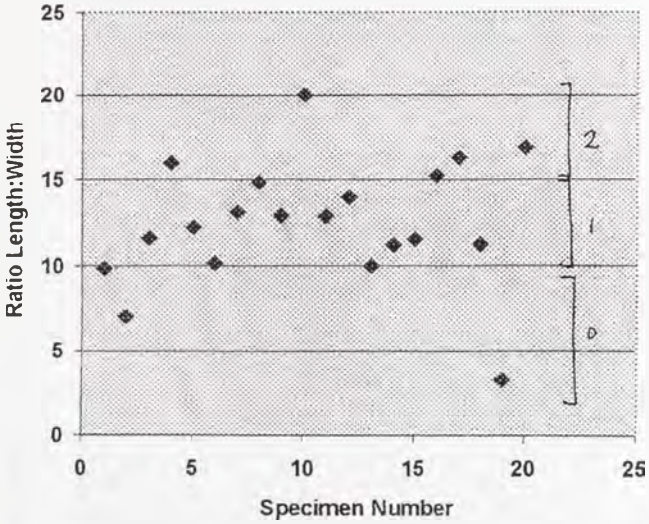
Character 1. Shell Ratio



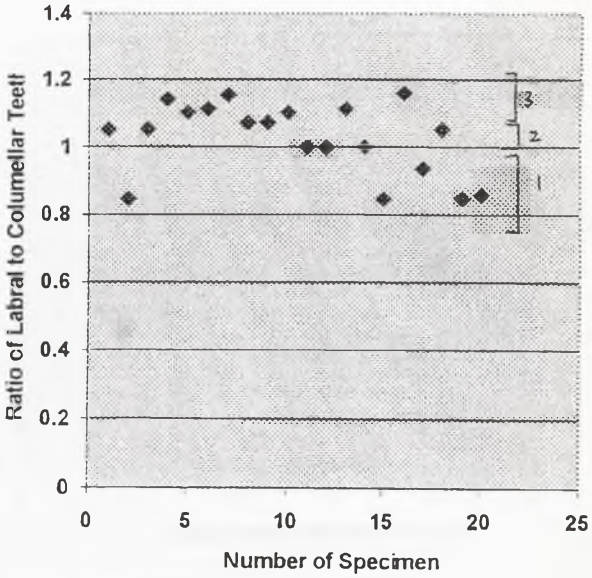
Character 10. Labral teeth



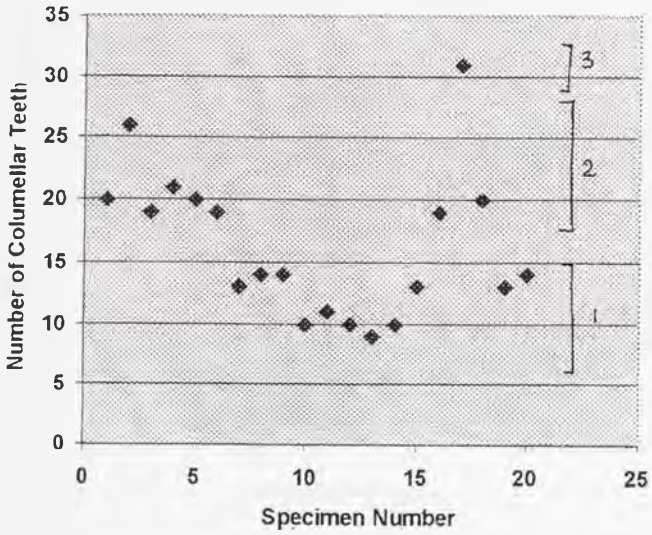
Character 2. Ratio of Aperture Length:Width



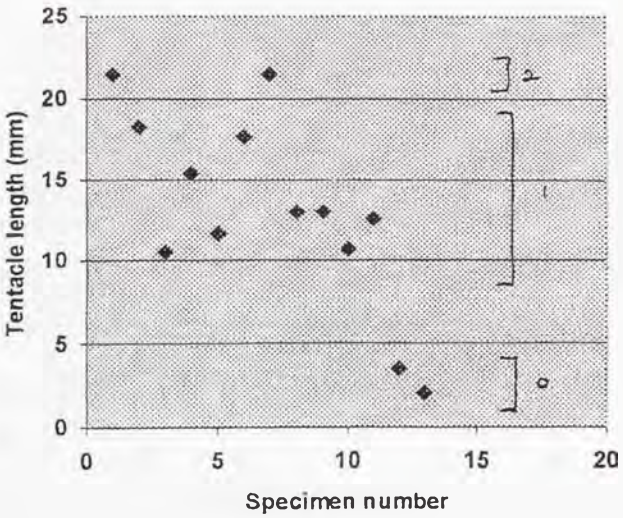
Character 11. Ratio of Labral to Columellar Teeth



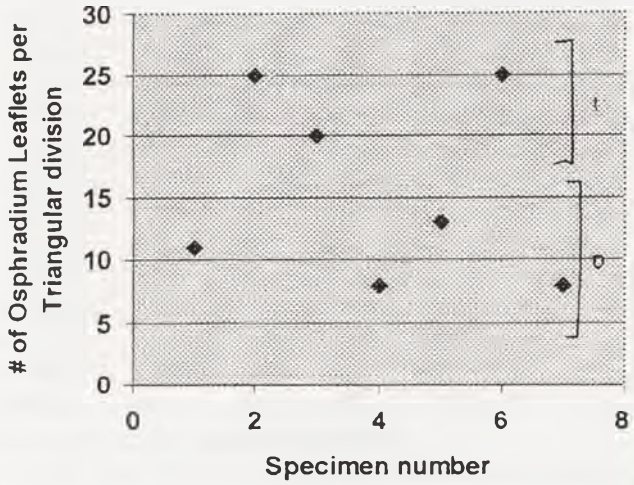
Character 9. Columellar teeth



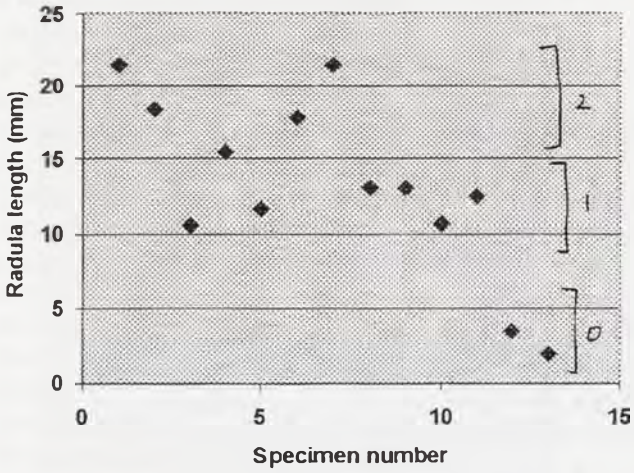
Character 25. Tentacle Lengths



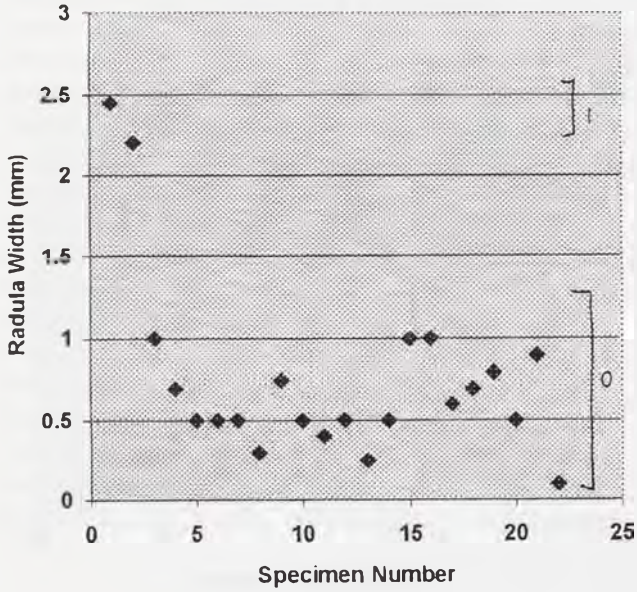
Character 37. Osphradium Leaflets



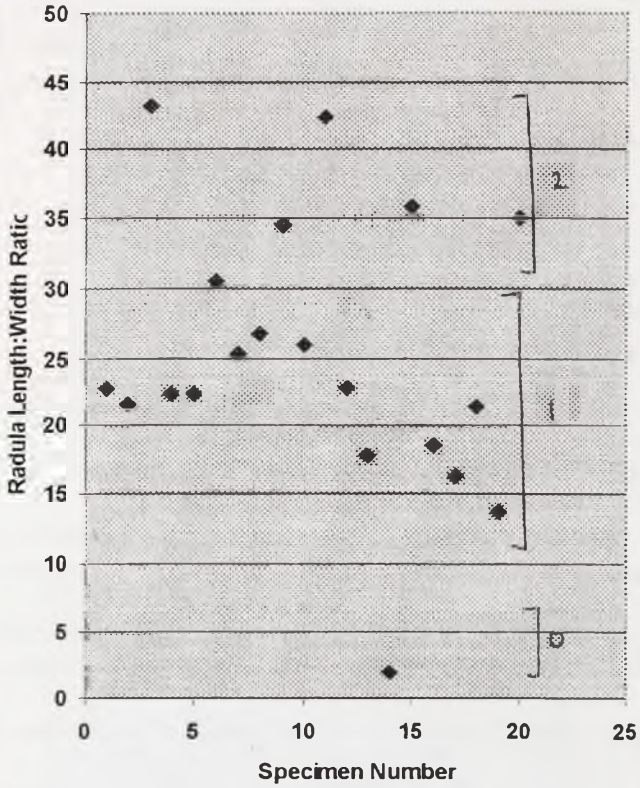
Character 44. Radulae Lengths



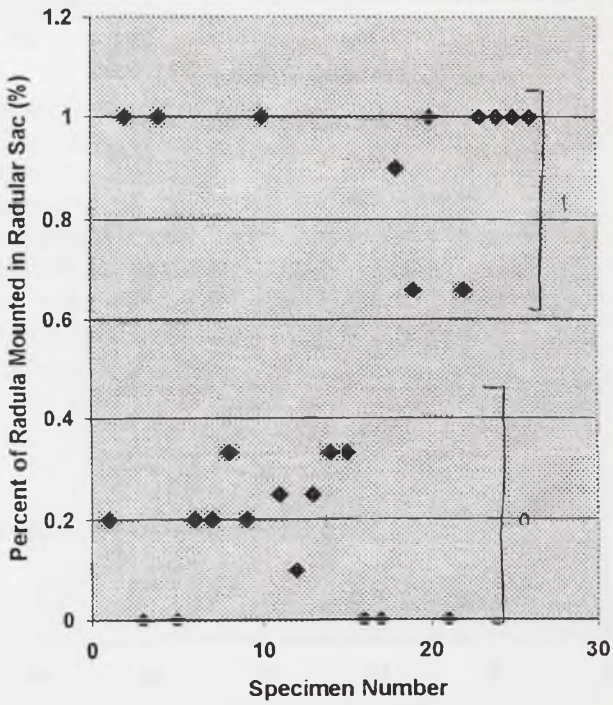
Character 45. Radulae Widths



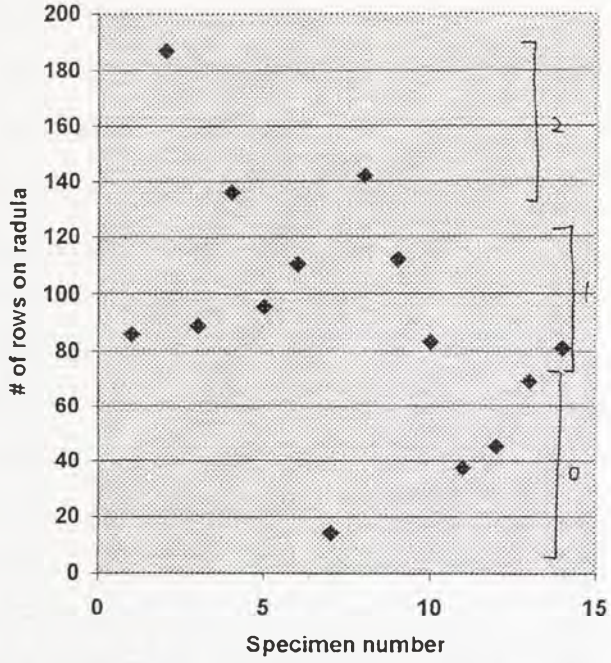
Character 46. Radulae Length:Width Ratios



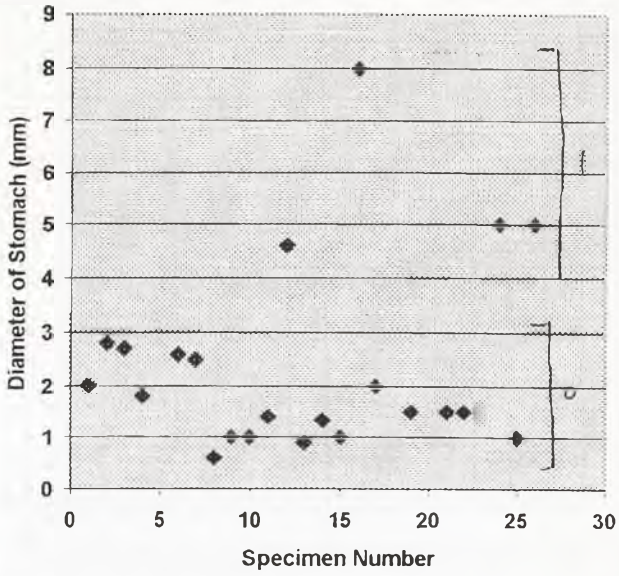
Character 47. Percent of Radula Mounted in Radular Sac



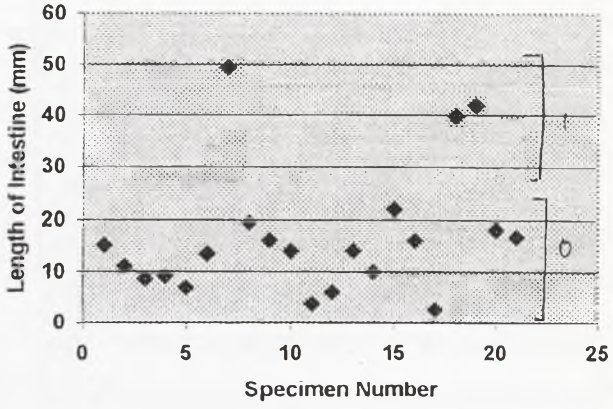
Character 48. Radulae Rows



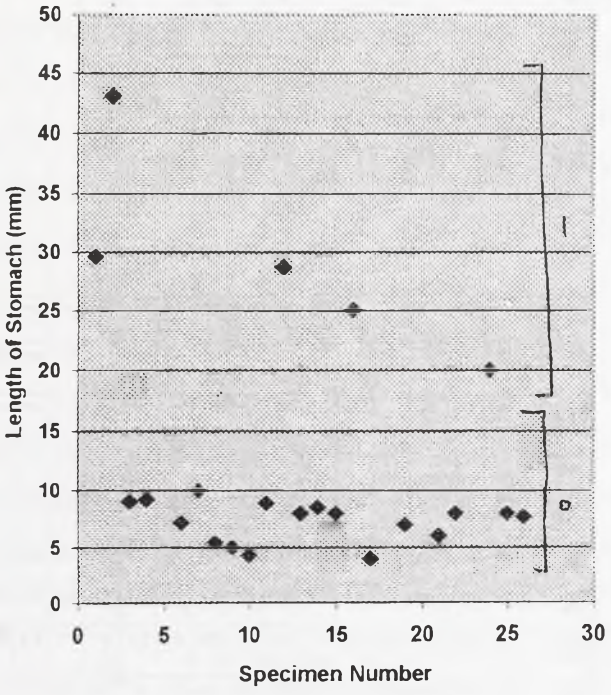
Character 57. Stomach Diameter



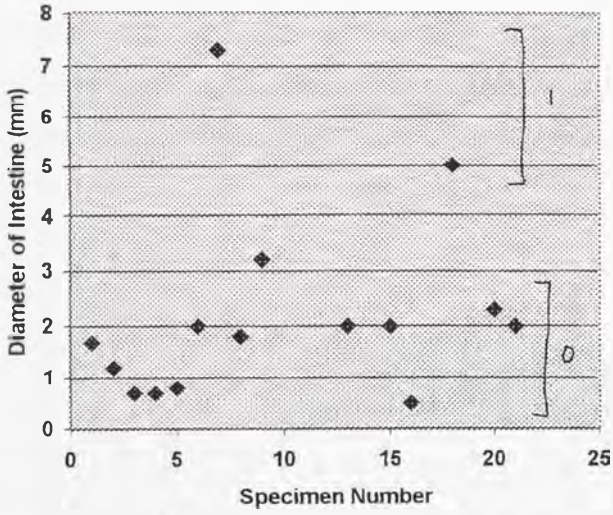
Character 58. Intestine Length



Character 56. Stomach Length



Graph 59. Intestine Diameter



Appendix 3. Character State Chart

Character State Number:	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
Species Name:																											
Eunaticina papillae	0	0	0	1	1	0	0	0	0	0	0	0	1	0	1	0	0	0	0	1	1	?	?	?	1	1	0
Polinices lewisii	0	0	0	1	1	0	0	0	0	0	0	1	1	0	1	0	0	0	0	0	0	0	1	0	2	0	0
Polinices incei	2	0	0	1	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	?	?	?	1	0	0
Polinices sordidus	0	0	0	1	1	0	0	0	0	0	0	1	1	0	1	1	0	0	0	1	0	?	?	?	1	0	0
Trivia ovulata	1	?	1	0	0	1	2	3	1	1	1	0	1	0	0	0	0	?	?	0	0	?	?	0	?	?	0
C.erosa	1	1	1	0	0	1	2	3	1	1	3	0	0	1	1	0	1	0	0	1	0	0	0	1	1	1	0
C. moneta	1	1	1	0	0	1	2	3	1	1	1	0	1	0	1	0	1	1	1	1	0	1	0	0	0	0	0
C.obvelata	1	2	1	0	0	1	2	3	1	1	3	0	1	0	1	0	1	1	1	1	0	1	0	0	0	0	0
C.carneola	1	1	1	0	0	1	2	3	2	2	3	1	0	0	1	1	1	0	0	1	0	0	0	1	1	1	0
C.tigris	1	0	1	0	0	1	2	3	2	2	1	0	1	1	0	0	0	1	1	1	0	0	?	0	2	0	0
C. isabella	1	2	1	0	0	1	1	3	1	1	1	1	0	1	0	0	0	?	?	1	0	0	1	0	1	1	1
C.isabella	2	2	1	0	0	1	1	2	3	3	1	1	0	1	1	0	0	1	1	1	1	0	0	1	0	1	0
C.vitellus	2	2	1	0	0	1	2	3	2	2	3	1	0	1	1	0	0	1	1	1	0	0	1	0	2	0	1
C.leviathon	2	0	1	1	0	1	?	?	?	?	?	1	1	0	1	1	0	1	1	1	1	0	1	0	1	1	0
C.lynx	1	1	1	1	0	1	2	2	2	2	3	1	1	1	1	0	0	0	0	1	0	0	0	0	2	1	0

28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62
2	2	0	0	0	0	1	0	0	?	1	1	0	0	0	1	1	0	1	0	1	1	1	1	2	0	0	0	1	0	?	?	?	?	0
2	2	0	0	0	0	1	0	0	?	0	1	0	0	1	1	1	0	1	1	0	1	1	0	0	0	0	2	1	0	?	?	1	0	1
2	2	0	0	0	0	1	0	0	?	0	1	0	?	1	1	?	?	?	?	?	?	?	?	?	?	?	?	0	0	?	?	0	0	1
2	2	0	0	0	0	1	0	0	?	?	0	?	1	0	0	0	0		1	0	1	1	0	0	0	0	0	0	0	?	?	0	0	1
0	0	2	1	2	2	0	0	0	1	0	?	?	?	?	?	?	0	?	?	0	3	1	4	1	0	0	?	?	?	?	?	?	?	?
1	1	2	2	2	?	0	0	0	?	0	0	0	0	1	1	1	0	1	0	1	1	1	2	2	2	2	1	0	0	0	0	1	1	1
0	0	1	1	2	2	0	0	0	0	0	0	0	0	1	1	1	0	1	0	1	1	1	2	2	2	2	1	0	0	0	0	0	0	0
?	1	2	2	2	1	0	0	0	1	1	1	0	0	1	1	2	1	1	1	2	1	1	2	2	2	2	0	0	0	0	0	1	1	1
2	0	2	1	2	2	0	0	0	0	1	1	1	0	1	1	2	1	0	0		2	1	3	2	2	2	1	0	0	0	1	1	1	1
1	0	1	2	2	1	0	0	0	0	1	1	1	0	1	1	2	0	0	1	1	1	1	2	2	2	2	2	1	1	1	1	1	1	1
2	0	1	1	1	2	0	0	0	0	1	1	1	1	1	1	1	0	1	1	1	1	1	2	2	2	2	2	?	?	0	0	0	0	1
1	0	2	1	2	1	0	0	0	?	1	1	1	0	0	0	0	0	0	0	0	0	1	2	2	2	2	0	0	0	0	0	0	1	1
1	0	2	2	2	2	0	0	0	0	1	0	1	1	1	1	2	0	1	1	1	1	1	2	2	2	2	2	0	0	0	?	0	0	1
2	0	1	1	1	2	0	0	0	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	2	1	2	2	0	0	0	0	1	1	1
0	0	1	2	1	2	0	0	0	1	1	0	1	1	1	1	2	0	2	1	2	1	1	2	2	2	2	1	0	1	0	0	0	0	1

63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80
0	0	?	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0
0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	1
0	0	?	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0
0	0	?	0	1	0	0	0	0	0	0	0	1	0	1	0	0	0
?	?	?	?	?	?	?	?	1	0	0	0	1	1	?	?	?	?
0	0	1	1	1	1	1	0	0	1	1	1	0	1	1	0	0	0
0	1	0	0	1	1	0	0	0	1	1	1	0	1	0	0	0	0
0	0	1	1	1	0	0	1	0	1	1	1	0	1	1	0	1	0
1	0	1	1	1	0	0	1	0	1	1	1	0	1	0	1	1	0
0	0	0	0	1	1	0	0	0	1	1	1	0	1	1	0	0	0
0	0	1	0	0	1	0	0	0	1	1	1	0	1	1	0	0	0
0	0	?	0	0	1	0	0	0	1	1	1	0	1	0	0	0	0
0	0	1	0	1	?	0	1	0	1	1	1	0	1	0	0	0	1
0	0	1	0	1	1	0	0	0	1	1	1	0	1	1	0	0	0
0	0	1	1	0	1	1	0	0	1	1	1	0	1	0	1	0	0

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