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

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

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

INTRODUCTION

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

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

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

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

METHODS

Study Site

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

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

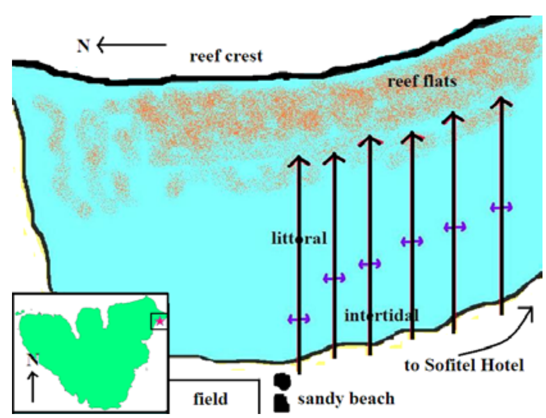

FIG. 1. Temae Public Beach located on the northeast tip of Moorea. Aerial view of study site depicting belt transects run perpendicular to the shore.

Mapping Distribution

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

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

Mat Collection & Analysis

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

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

Current Flow Study & Analysis

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

RESULTS

Mapping Distribution, Collection, & Analysis

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

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

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

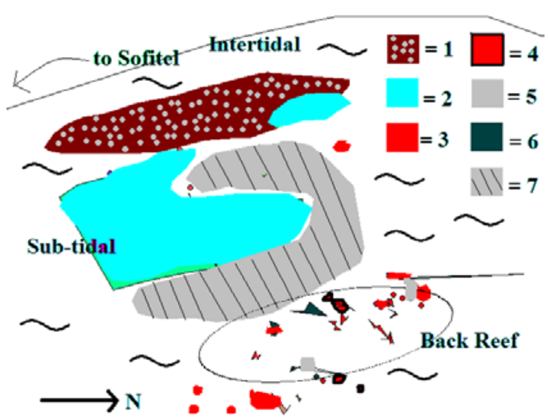

FIG. 2. Approximate distribution of 7 mat phenotypes. Areas marked indicate boundaries within which mats were found; it does not indicate density.

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

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

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

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

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

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

Current Flow Study

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

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

DISCUSSION

Composition & Mapping Distribution

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

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

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

*1 = thinnest, 4 = thickest

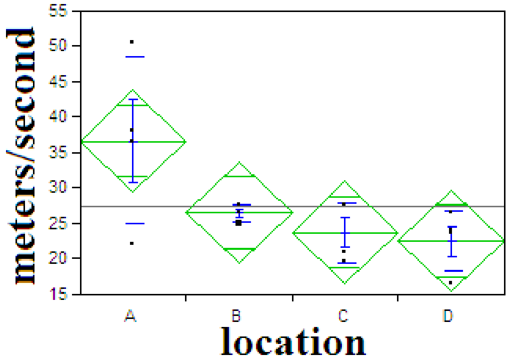

FIG. 3. One-way ANOVA results for rates of flow by location. $p = 0.0404$ ($F = 3.7822$, ≤ 0.05); therefore, the differences between rates of flow are overall statistically significant

supralittoral counterparts (Hoffmann 1999).

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

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

Current Flow Study

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

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

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

Herbivory

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

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

CONCLUSION

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

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

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

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LITERATURE CITED

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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