DIVERSITY AND SPECTRAL RESPONSE OF MICROBIAL COMMUNITIES ON FOSSILIFEROUS LIMESTONE PLATFORMS IN MOOREA, FRENCH POLYNESIA

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Abstract. Extremophiles are organisms that survive extreme environmental differentials. Cyanobacterial mats on fossiliferous limestone platforms in the intertidal zone of oceanic islands are subject to wave stress and intense UV radiation. The distribution of each mat was studied to infer causal factors of zonation. Line transects delineated zonation in visibly distinct cyanobacterial mats. Spectrometry determined spectral transmittance of a mat and an inhabited rock. Six distinct mat types, coralline algae and Littorina coccinea snails were quantified. For a 20 meter platform, mats with yellow-green, black-green moss-like and brown morphologies were most abundant (over 50% cover) closest to the lagoon (up to 2 meters from the lagoon), the middle of the platform (from 1 meter to 14 meters from the lagoon), and closest to the beach (from 6 meters to 20 meters from the lagoon) respectively. L. coccinea were found closest to the beach (between 6 meters from the lagoon and the beach). Spectrum of a black-green moss-like slice shows the mat type blocks 90% of low visible wavelength light. Future studies will be done on identifying the microbial communities of each mat and desiccation tolerances as possible causes of community stratification.

Key words: microbial mats, cyanobacteria, zonation, intertidal, Moorea, French Polynesia

INTRODUCTION

Few organisms live in locations subject to extreme temperature, radiation and humidity differentials. Those organisms, called extremophiles, are found in hyperarid deserts (Nienow et al. 1988), hypersaline pools (Des Marais 2003), and deep-sea vents (Miroshnichenko 2006). The most commonly known extremophiles are microbes, but there are eukaryotes that favor extreme environments. Aerobic organisms can be considered extremophiles living in highly oxygenic environments, as reactive oxygen is toxic to cells (Rothschild 2007). Extremophiles are of interest to the planetary science community, as the environments that humans consider to be extreme on Earth were natural conditions previously in Earth's history and remain natural on other worlds such as Mars (Billi et al. 2011), Enceladus (McKay et al. 2008) or Europa (Lipps and Rieboldt 2005). Extremophiles are also of equal interest to evolutionary scientists as conditions we consider extreme today were prominent during the early development of life, shaping the evolution of modern organisms (Westall et al. 2002).

One of the oldest microbes on Earth is the progenitor of plants: cyanobacteria, or blue-green algae. Fossiliferous cyanobacteria have been found in stromatolites, cemented biofilm structures, that date back to up to 3500 million years ago (Golubic and Seong-Joo 1999). Cyanobacteria are the first organisms to develop oxygenic photosynthesis, causing a massive increase of oxygen content in the Earth's atmosphere. Because of their early origin in Earth's history, they are adept extremophiles and are found in a multitude of habitats ranging from valleys in Antarctica (Sabbe et al. 2004) to motus in the Pacific (Richert et al. 2006). Cyanobacterial colonization is limited by transmittance of inhabited rock in the photosynthetically active region (PAR, 400-700nm) and available water-accumulation measures.

There are two predominant forms of cyanobacteria-containing communities: microbial mats and biofilms. Biofilms are bacterial colonization associated with a solid surface (Stolz 2000). Cyanobacterial biofilms can be found in places like hyperarid deserts where heterotrophs are unable to survive desiccation stresses. These biofilms survive intense radiation by inhabiting the undersides and pores of rock substrates (Warren-Rhodes 2007). Rock substrates provide selective absorption of UV, though endoliths have been observed to screen UV while maintaining photosynthetic processes (Cockell et al. 2004, Herrera et al. 2009, Wong et al. 2010, McKay et al., 2012). Subsurface environments, known as "hypolithic" environments, are thought to accumulate water while passing enough light for photosynthesis (Pointing et al. 2007, Warren-Rhodes et al. 2006). Some cyanobacteria live inside the substrate, an "endolithic" habitat, and thrive on accumulated water in the pores (Nienow 2009).

A microbial mat is a composition of a variety of microbes that usually functions as an ecosystem (Stoltz 2000). Mats are characterized by "stratification" of different types of microbial layers that can represent trophic layers. Microbial mats almost always have cyanobacteria as the dominant producers (Des Marais 2013). These mats can adapt topologically to optimize nutrient flow and spectral quality of transmitted light (Steeman 1975, Jorgenson 1990). Mats can include biofilms, but are generally more complex than a single biofilm (Stoltz 2000).

Aging volcanic islands have fossiliferous limestone platforms, also referred to as conglomerate platforms (Letchworth 2010), that are composed of cemented coral piled onto the barrier reef by extreme storms and hurricanes (Murphy 1992). These platforms are karstically eroded, forming pools and depressions (Waljeski 2003). Platforms are exposed to stressful environmental conditions including high insolation, changing salinity and desiccation. However, there are microbial mats, invertebrates and other organisms that are able to survive the extreme conditions on the platforms (Letchworth 2010, Firestone 1998).

The Society Islands are a hotspot archipelago in proximity to a M2 tidal amphidrome (Stillman 2013). Limestone platforms are found in Moorea, French Polynesia at the seaward edges of motus such as Tiahura and Temae (Chang 2006). As tidal amplitudes vary less than in California, studies in Moorea on intertidal mats are easier to replicate in a short time span. Previous studies have examined invertebrate (Firestone 1998) and benthic microbial mat (Letchworth 2010) diversity in karstically eroded pools. In addition, Letchworth (2010) observed several extremely desiccation-tolerant microbial mats on the platform at Tiahura, including endolithically-associated cyanobacteria. As living coral substrates are known to confer UV protection to coral endoliths (Shashar et al. 1997), the cemented coral platform might offer similar benefits to endolithic cyanobacteria.

Fossiliferous limestone platforms are subject to wave action and intense UV radiation. I hypothesized that thinner filmy mats will be closest to the lagoon and that thicker moss-like mats will be further from the lagoon. The mats closest to the lagoon were submerged at high tide, so a mossy topology would collect oceans salts and thus a filmy morphology would be optimal. The mats further from the lagoon would be better optimized for collecting fresh water by having a thick moss-like texture. I also hypothesized that black-green colored mats would be most abundant furthest from the lagoon and block UV radiation, as a black-green coloration absorbs radiation and would be a useful adaptation in the highly desiccated region furthest from the lagoon. As each mat is macroscopically different according to Letchworth (2010), I expect they will look different from each other at the microscopic scale.

METHODS

Study site

This study was conducted in October and November 2013 at the fossiliferous limestone platform northwest of Temae Public Beach in Moorea (Fig. 1). Each transect was taken at

FIG. 1. Map of Moorea, French Polynesia. Temae is highlighted in gray and the range transects were taken is in red. Courtesy of the Geospatial Innovation Facility, University of California, Berkeley.

0800 HST. Transect, tide, and illuminance were recorded.

Platform zonation

Zonation was quantified using four line transects from the lagoon-limestone interface to the beach-limestone interface. The cover of each visibly distinct mat in a 13 cm x 13 cm quadrat was recorded at one meter intervals starting from the lagoon-limestone interface. The number of Littorina coccinea snails was also recorded. I modified an identification system based on one by Letchworth (2010) to characterize each mat distinctly (Appendix A). As dry mats appeared differently than wet mats, the quadrat would be wetted using ocean water to ensure consistent identification. I applied linear regression analysis to estimate if there was a significant correlation between specific mat cover and distance from lagoon using Python (Python Software Foundation).

As the color change in the mat is a possible measurement of photosynthesis recovery, I photographed the quadrat before and after wetting. I chipped off four pieces of limestone for later microscopic observation within 0.2 meters of the quadrat distance.

Microscopic characterization

To assess microscopic appearance of each visibly distinct mat, I examined the limestone chips under a dissecting microscope and photographed microscopic colonization appearance of epilithic and endolithic mats (Appendix A).

Light transmittance

I used an OceanOptics USB2000 Miniature Fiber Optic Spectrometer to quantify the spectral transmittance of chips collected from the platform. Sunlight was used as a light source.

RESULTS

Study site

Four transects were measured on four days. Measured brightness remained between 104000 and 137000 Lux. The transects were measured for platform lengths of 20.9 meters, 22.5 meters, 19.7 meters, and 13 meters.

Platform zonation

The green-yellow mat preferentially covered the lagoon-facing edge. It was observed between the lagoon-platform interface and the middle of the platform (49% platform length from lagoon). The black-green moss-like mat preferentially covered the middle of the platform. It was found in the region between the lagoon-platform interface and 90% platform length with respect to the lagoon. The brown mat covered the beach-facing edge of the platform. It was found in the region between 9% and 93.9% platform length with respect to the lagoon. L. coccinea snails were found on the beach-facing edge of the platform (from 29.5% platform length from the lagoon to the beach-limestone interface). There is no significant preferred range of the green endolithic mat, the black-green crustose mat, the blue-gray cyanobacteria mat and red coralline algae.

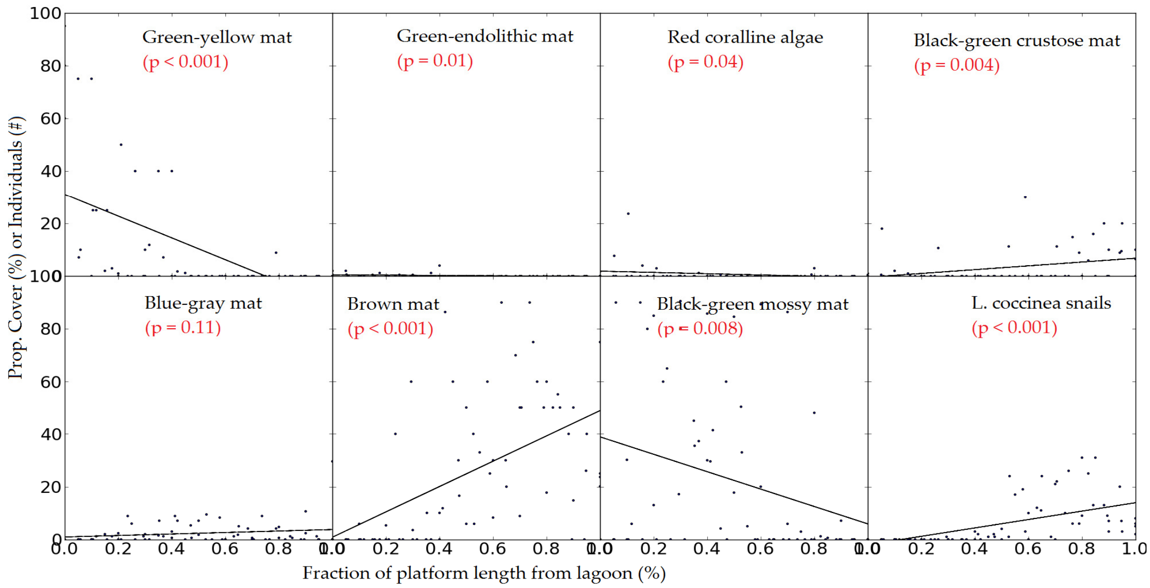

FIG. 2. Zonation of each microbial mat with reported p-value for a linear fit.

The maximum observed proportion cover of each mat was 4% green-endolithic, 30% black-green crustose, 29.6% blue-gray, 23.7% coralline algae, 95% green-yellow, 90% brown, and 90.5% black-green moss-like. A maximum of 31 L. coccinea snails were observed.

Species richness across the platform did not vary significantly for each transect, remaining between 1 and 6 species (Fig. 3). The average species richness was 3.13 ± 0.23.

Microscopic characterization

Each visibly distinct mat appeared different from each other at the microscopic scale (Appendix A).

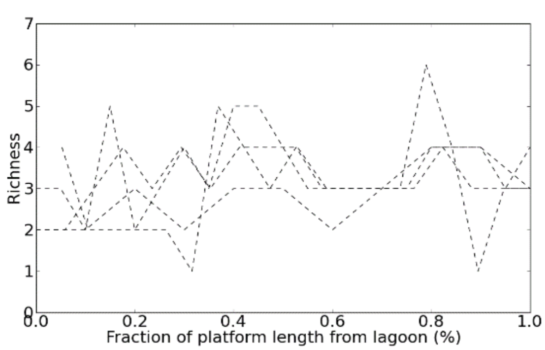

FIG. 3. Species richness of the platform.

Light transmittance

A section of black-green moss-like mat blocked low visible solar wavelengths, transmitting 10% at 425 nm, while passing high visible wavelengths (80% at 735 nm) (Fig. 4). There is a decrease in transmission at 690 nm, which is a known chlorophyll a absorption wavelength (Rabinowitch and Govindjee 1970). The increases in transmission at 760 nm and 660 nm coincide with oxygen absorption lines in the atmosphere, and hence are not due to the mat (Sokolik 2008).

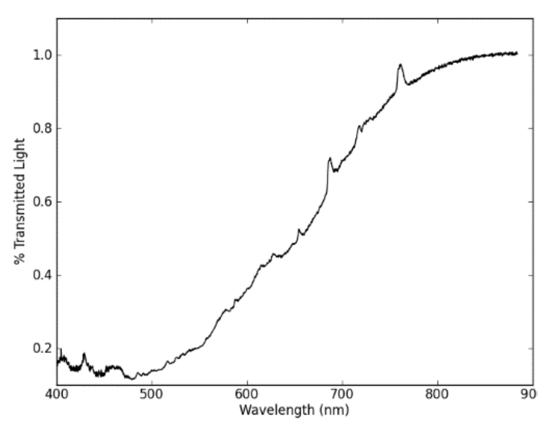

FIG. 4. Spectral transmittance of a 0.25 cm thick section of black-green moss-like

DISCUSSION

There were clear differences between the brown, black-green moss-like, and green-yellow microbial mats. These mats divided the platform into zones, but they did not follow the hypothesized patterns. The black-green moss-like mat was mostly found adjacent to the green-yellow zone, closer to the middle of the platform. As this region was not preferentially wetted with either runoff or ocean spray, some other factor could be causing the zonation.

Black-green coloration also did not confer preferential growth at the zone closest to the beach, as the brown mat and snails were the most abundant species found from the middle of the platform to the beach. While the black-green mats did not look different when dehydrated, the wet appearance of the brown mat was observably markedly greener (Fig. 5). As a green coloration is due to the presence of chlorophyll a, it is possible that the chlorophyll is inactive until wetted. This could be a desiccation tolerance adaptation that makes brown mats better suited to the beach-facing edge of the platform.

L. coccinea snails prefer the beach-facing edge of the platform. This region is subject to less wave action and greater presence of brown mats. These factors can be studied in greater detail in future classes.

The morphological differences between mat types are possibly due to the composition of the community of cyanobacteria in the mat. As measured salinities of pools at another fossiliferous limestone platform in Moorea ranged between 37-40 ppt (Letchworth 2010), the mats at Temae might be exposed to similar salinity levels. According to Dr. Des Marais (NASA Ames, Pers. Comm.), filamentous cyanobacteria species are more predominant in fluctuating salinity pools with salinity below 70 ppt. Thus filamentous cyanobacteria species might be more common. Further studies on the zonation can identify the species complement of each mat.

As the black-green moss-like mat blocks low visible wavelengths, it likely blocks UV radiation. This spectrometry technique can be applied to compare spectral transmittance of each mat. Endoliths are limited by UV radiation and PAR availability. Lagoon water and epilithic biofilms would confer additional protection while blocking and/or using that part of the spectrum. Future studies will examine quantification of endolithic cover in rocks from different zones and transmittance of each mat. This can be used to validate a model to predict endolithic zones at the platform (Jolitz and McKay 2013)

FIG. 5. Dry (left) and wet (right) brown mat in a 20 cm x 20 cm quadrat.

ACKNOWLEDGMENTS

I would like to thank Dr. Brent Mishler, Dr. Jonathon Stillman, Dr. Stephanie Carlson, Dr. Vince Resh, and Dr. George Roderick of UC Berkeley for coordinating and teaching the UC Berkeley "Tropical Island Biology and Geomorphology" class. I would also like to thank Dr. Des Marais for advising me on microbial mats, Dr. Chris McKay for key project insight and Dr. Richard Quinn for instructing me on the use of the OceanOptics USB2000 Spectrometer (NASA Ames Research Center). Finally, I would like to thank my classmates and Ben Jolitz for assisting me with collections.

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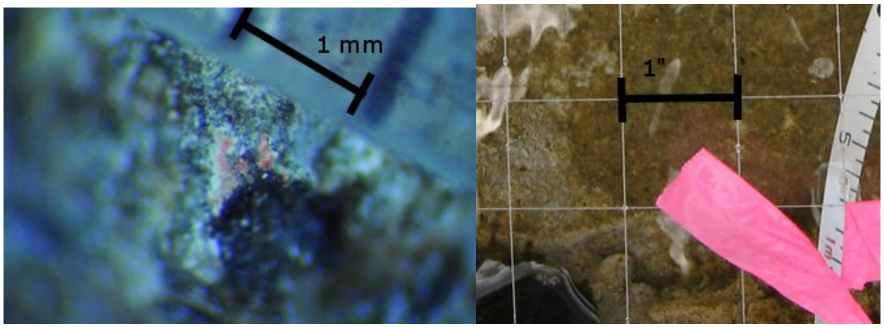
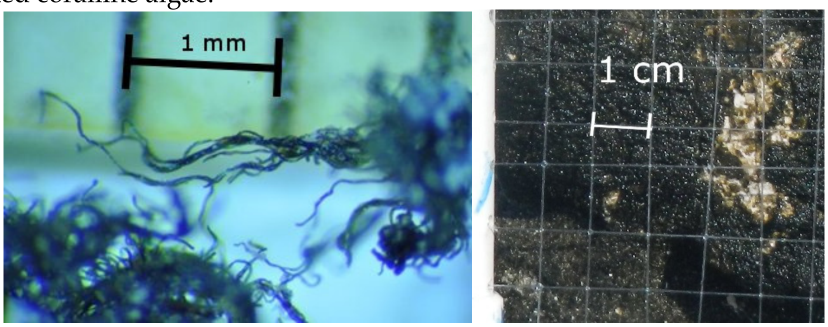
APPENDIX A

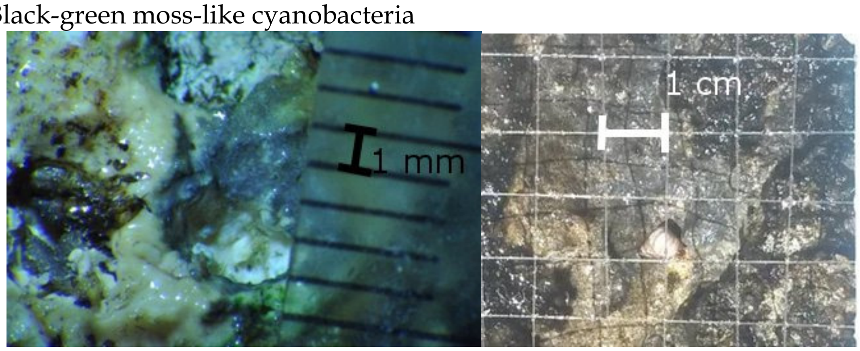
Microscopic observation on left and macroscopic observation (in a wetted quadrat) on right.



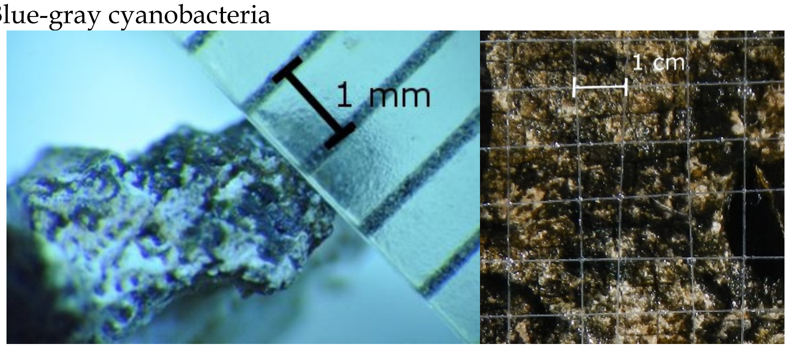
Red coralline algae.



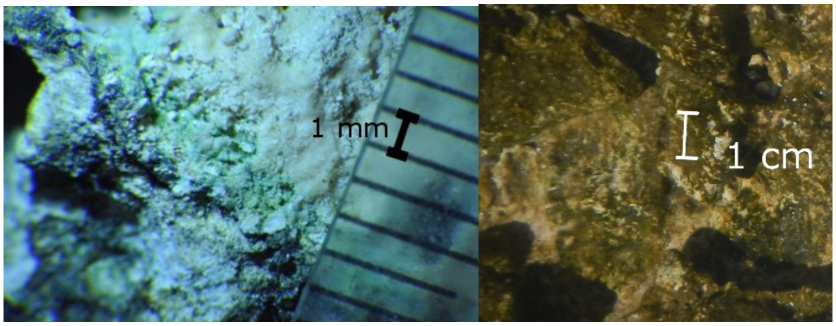
Black-green moss-like cyanobacteria



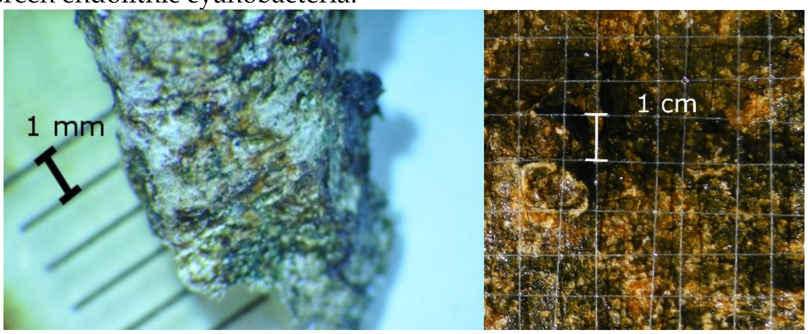
Blue-gray cyanobacteria



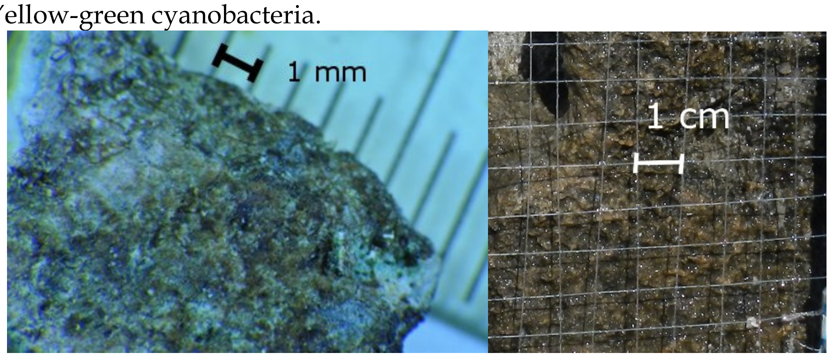
Black-green crustose cyanobacteria.



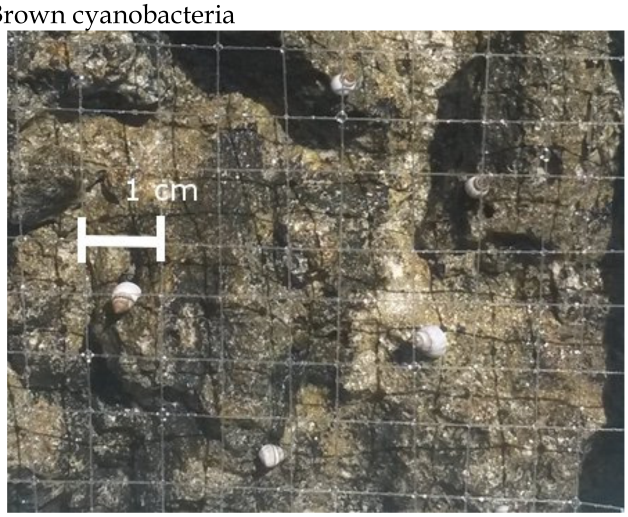
Green endolithic cyanobacteria.



Yellow-green cyanobacteria.



Brown cyanobacteria



Littorina coccinea snails