

Preliminary Report of EPHE – IRCP – CRIOBE / SNH & SPDD grant

Sponge-Microbe Symbioses in French Polynesia (Criobe, Moorea)

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During our stay at the CRIOBE (USR 3278 CNRS-EPHE-UPVD) from August 30th to September 11th 2014 as part of the project titled “Sponge-Microbe Symbioses in French Polynesia”, Dr. Cole Easson and I collected replicate individuals of 10-12 sponge species from 12 sites on the Northern coast of Moorea (Figure 1).



Sampling:

Samples were collected using a combination of SCUBA from CRIOBE vessels, shore-based SCUBA, and snorkel. Sites included bay, lagoon, motu, reef crest, channel openings, beaches, reef slopes, and docks/man-made structures.

We used previously published literature (Adjeroud and Salvat, 1996; Adjeroud, 1997; Desmet, 2009; Hall et al., 2013) on sponges in French Polynesia and, in particular, Moorea to focus our collections at sites previously shown to support locally diverse (1-6 species) sponges. Indeed, sites within the bays (especially the west sides) contained diverse assemblages of sponges, many of which were in shaded or otherwise semi-cryptic habitats such as underneath ledges or within caverns. These species included members that, by preliminary identification appear to be within the following genera: *Stylissa*, *Leucetta*, *Dysidea*, *Lamellodysidea*, *Cinachyrella*, *Haliclona*, *Chondrosia*, and at least 5 species of which IDs are currently TBD (see panels in Figure 2).

In the lagoon site in shaded locations around the Intercontinental Resort we also found many individuals of the pink *Haliclona* sp. This species was also found at a bay site around Hotel Kaveka. We also observed large individuals of a *Dysidea* or *Lamellodysidea* sp. at the motu and within the bays. Individuals from these genera were of multiple growth forms and colors. Finally, at a site on the western side of the island in about 5-10 m of water, we found approximately 6 species of sponges growing amongst coral rubble, under ledges, and within crevices. These species were extremely unique and many of these have been targeted by the Moorea Biocode and the Porifera Tree of Life project as potential new records to Moorea or even new species (see TBD examples in Figure 2).

Goals and Objectives:

Our goal was to survey diverse sponge-microbe symbioses throughout Moorea by carrying out the following objectives:

Objective 1: What is the current status of sponge-microbe symbioses throughout this area and how are these symbioses impacted by anthropogenic stressors?

Objective 2: What is the interconnectivity between anthropogenic stressors and holobiont productivity and the efficiency of symbiont nutrient assimilation and nutrient transfer to host sponges?

To carry out objective 1, replicate samples (3-5) of each of the species above were collected at sites when observed. Sponges were brought back to the lab, where they were photographed and processed to obtain samples for the abundance of cyanobacterial symbionts (measured by chlorophyll a analysis; Freeman and Thacker, 2011), microbial community profiling, and histology and molecular

barcoding for species identification. Samples were also frozen for future stable isotope ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) analysis to assess whether these species are obtaining their C and N from internal symbiont sources or local sources from the water column (Freeman and Thacker, 2011). Finally, all individuals were screened using a portable spectrometer to obtain an action spectra corresponding to the wavelengths absorbed by each species. These spectra allow us to assess whether different species are hosting symbionts and whether these symbionts are capable of absorbing different portions of the PAR spectrum.

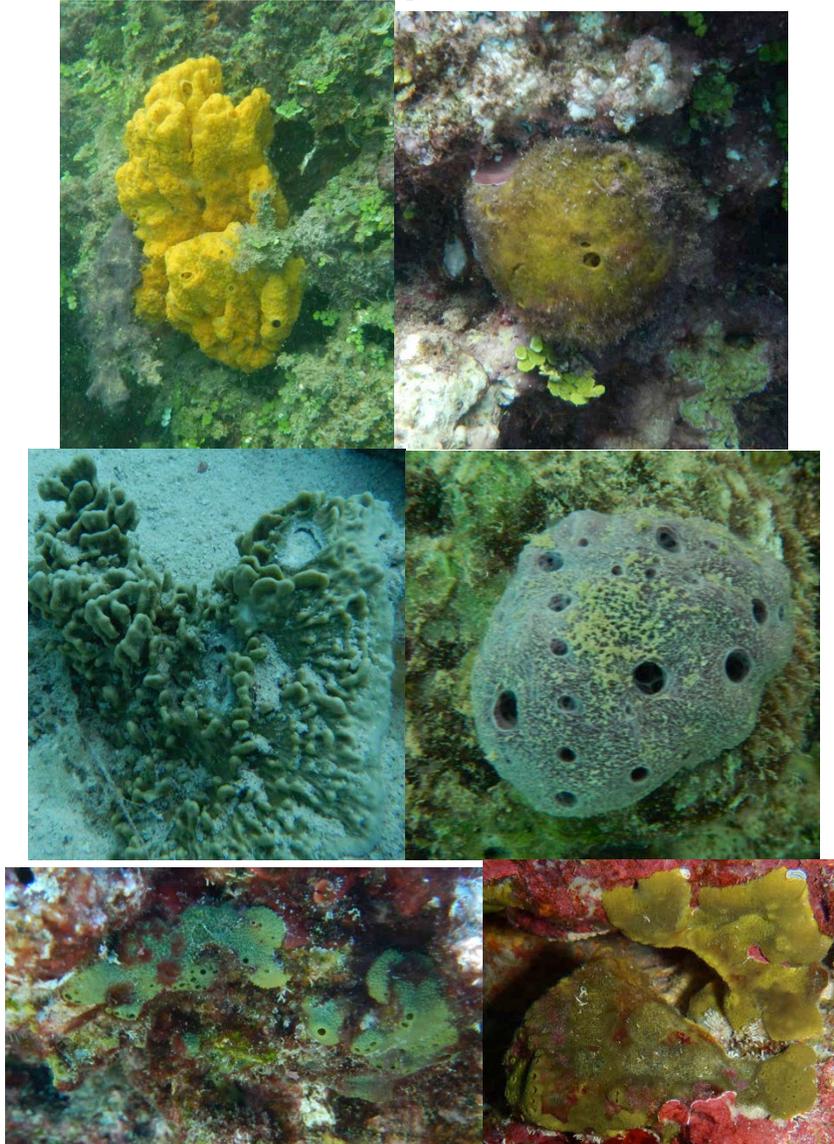




Figure 2: Representative photographs of sponges collected from Moorea. Tentative identifications clockwise from top left corner: *Stylissa* sp., *Cinachyrella* sp., *Haliclona* sp., TBD1, *Leucetta* sp., encrusting *Dysidea/Lamellodysidea* sp., TBD2, massive/fan-shaped *Dysidea/Lamellodysidea* sp.

Although we originally proposed sampling across a gradient of proximity to human impact, it was difficult to sample the same species at diverse sites. One species (*Leucetta* sp.) was sampled at multiple locations, but this species is a calcareous sponge that likely lacks an abundant symbiont population. Instead, we will be able to compare symbiont populations within sponges from different sites (specifically bay versus lagoon and reef slope) to observe how microbial community composition changes across sites in Moorea.

The lack of similar species across disparate sites also led to difficulties achieving the second objective as originally proposed. We were, however, able to carry out an experiment using *Dysidea* sp and *Lamellodysidea* sp collected from 3 disparate sites (Opunohu Bay, Motu, and the lagoon surrounding the Intercontinental).

Preliminary analysis at CRIOBE (by placing diverse sponges in EtOH) suggested that these two species hosted an abundant community of photosymbionts (presumably cyanobacteria). We thus used these species in an incubation experiment as originally described in our proposal (Freeman et al., 2013). These incubations were carried out in light [translucent] bottles containing filtered (0.70 μm GF/F Whatman) seawater enriched with 98 Atom % $\text{NaH}^{13}\text{CO}_3$ [1.18 mM] and 98 Atom % $\text{Na}^{15}\text{NO}_3$ [0.117 mM]. Productivity was measured by taking initial and final dissolved oxygen measurements (in $\text{mg O}_2 \text{ L}^{-1}$) in the light bottles and productivity is reported as Net Primary Productivity (NPP). Using these methods we show that these species from around Moorea (green bars in Figure 3) all host photosymbionts (such as cyanobacteria) that are capable of compensating for the respiration of host and symbiont cells (production of O_2 via photosynthesis more than compensates for the consumption of O_2 via respiration). Importantly, these photosymbionts appear to be more productive than 2 out of 3 Caribbean sponges used in similar experiments (blue bars in Figure 3). This provides initial evidence that some sponges from Moorea host symbionts capable of photosynthesis.

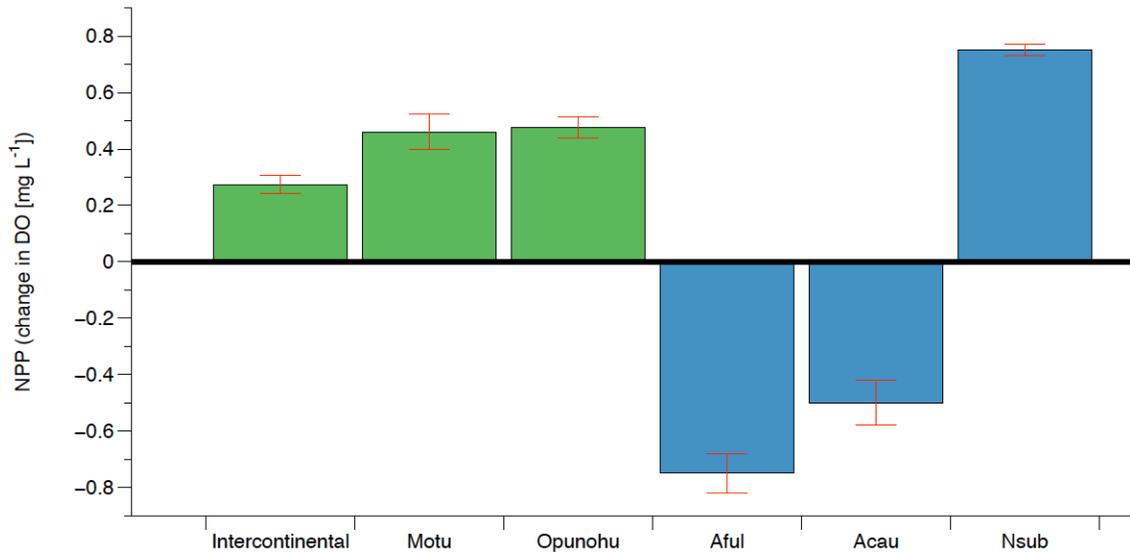


Figure 3: Net Primary Productivity (NPP) of *Dysidea* and *Lamellodysidea* (green bars) from sites in Moorea compared to the NPP of 3 species from the Caribbean (Blue bars).

Back at the Smithsonian Marine Station in Fort Pierce, FL, sponge and microbial fractions will be isolated from bulk sponge tissue. Host and symbiont fractions will be analyzed and expressed as Atom Percent Excess (APE) values, which compare the Atom % of the heavy isotope (¹³C and ¹⁵N) in enriched samples to that found in nature (initial samples). These analyses will allow us to assess the assimilation of ¹³C- and ¹⁵N-based substrates and the transfer of symbiont-derived nutrients to the host.

Conclusions:

We located and collected approximately 12 species of sponges on the North coast of Moorea. These species were distributed mostly in semi-cryptic locations within these systems, except for *Dysidea* and/or *Lamellodysidea* sp, which were found in exposed locations. These latter two species host productive communities of photosymbionts, as revealed by preliminary chlorophyll extractions and incubations in light bottles. It is likely that these hosts obtain a predominant portion of their C from their microbial communities.

Future Directions:

To complete this study, we will conduct the following analysis with all individuals collected:

- 1) Chlorophyll a analysis to assess the abundance of photosymbionts within these sponges
- 2) Isotope analysis to determine whether these sponges are obtaining their C and N from symbiont or heterotrophic sources (by filter-feeding on external microbes within the water column)

- 3) Histology and barcoding to ensure accurate identification of species collected
- 4) Processing of data from spectrometer
- 5) Microbial community profiling via either T-RFLP analysis and/or next generation sequencing via the IonTorrent platform at the National Museum of Natural History in Washington D.C.

We anticipate publishing 2 articles from this research, the first of which should be submitted later next year.

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