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Project Title: Symbiosis in two marine invertebrates.

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Summary

Marine invertebrates acquire photosynthetically-fixed carbon by forming symbioses with algae. These symbioses exist primarily in shallow tropical oceanic regions where clear waters and low nutrient levels provide maximal advantage for the symbiosis (reviewed in Fournier 2013). The associations are common in Porifera (sponges) and Cnidaria (hydroids, corals, sea anemones and jellyfish) but appear to be uncommon or absent in other invertebrate phyla. Those organisms that host symbionts harvest photosynthetic products released by the algae at substantial rates (Yellowlees et al. 2008). However, hosting algal symbionts also carries risks for the host because environmental perturbations, especially high temperature or irradiance, lead to the production of reactive oxygen species causing host damage resulting in the breakdown of the symbiosis (reviewed in Venn et al. 2008). The dominant algal symbionts in marine invertebrates are dinoflagellates of the genus *Symbiodinium*, which are represented by nine clades (A-I) and an undetermined number of subclades (Pochon and Gates 2010). Most recent studies have concentrated on cnidarians, and more specifically corals, because of the impact of global climate change resulting in coral bleaching (reviewed in Davy et al. 2012). We have focused our attention on understanding the host-symbiont interactions in the important reef-building Millepore corals (fire coral) at two thermally different locations in the Caribbean (Bahamas and Belize). Additionally, we have initiated a study in which we identified the presence of *Symbiodinium* in a photonegative intertidal gastropod (snail) of the genus *Nerita*.

Millepore-Symbiodinium Project

Coral reefs in the Caribbean offer an ideal setting to explore host-symbiont dynamics because the Caribbean Sea has warmed on average by 0.27°C per decade over the period from 1985-2009 (Chollett et al. 2012). Additionally, there are considerable temperature differences across the Caribbean region, with northern areas showing cooling and western areas warming. These warming trends are sufficient to push corals outside of their range of thermal tolerance. Record high temperatures in 1998 and 2005 resulted in Caribbean-wide mass coral bleaching events (Wilkinson and Souter 2008). Increased temperatures and bleaching events are also linked to increases in the prevalence of coral disease (Ruiz-Moreno et al 2012). This is a particular concern in the Caribbean, which is considered a disease “hotspot” (Harvell et al 2007).

The vast majority of research exploring the coral-*Symbiodinium* relationship has focused on Scleractinian (hard) corals, while ignoring the important reef framework building Millepores (fire coral) (Reviewed in Davy et al. 2012). The purpose of our research is to examine the diversity of the Millepore-*Symbiodinium* symbiosis at two thermally different Caribbean reef locations. Our lab is using quantitative polymerase chain reaction (qPCR) with *Symbiodinium* clade-specific rDNA primers (Ulstrup and van Oppen, 2003, Correa et al. 2009) to identify Millepore symbiont diversity at two different Caribbean locations. The two sites are San Salvador, The Bahamas which has a yearly ocean temperature range of 22-28°C and South

Water Caye, Belize which has a yearly ocean temperature range of 26-30°C (www.nodc.noaa.gov/General/temperature.html).

Our initial results (Samayoa et al. 2017) from San Salvador, The Bahamas (N = 49) and South Water Caye, Belize (N=35) indicate that *Symbiodinium* clade B is the only dominant clade present in Millepores on San Salvador reefs while both clades A (74%, N=26) and B (26%, N=9) are dominant in Millepores on reefs surrounding South Water Caye. Venn et al. (2008) have shown that at thermally variable inshore sites clade A predominates in *Condylactis gigantean* (tropical sea anemone), but at sites that are cooler and more thermally uniform, clade B dominates. This is consistent with our data showing that clade A is dominant in Belize Millepores, which have likely experienced more thermal stress events than Millepores residing on reefs in The Bahamas. Loram et al. 2007 showed that at elevated temperatures clade B fixed carbon at a lower rate and that *C. gigantea* containing clade B symbionts were more susceptible to bleaching and symbiont expulsion at elevated sea temperatures.

Both geographic locations contained background clades. In the Bahamas, 67.3% (33/49) Millepore colonies contained a single background clade, 20.4% (10/49) of the colonies contained multiple background clades and 12.2% (6/49) contained no background clades. In contrast, 62.8% (22/36) of the Belize Millepores contained a single background clade, only 2.8% (1/35) contained multiple clade backgrounds and 34.3% (12/35) contained no background clades. Currently our individual reef and species sample sizes are not large enough to determine if correlations exist between the presence of background clades and geographic location, reef site or Millepore growth form.

Background levels of clade C were present in 30.6% (16/49) of Millepores collected in The Bahamas and only 2.8% (1/35) of the Belize Millepores. Rowan and Knowlton (1995) showed that the Caribbean coral *Montastraea annularis* was dominated by clades A and B in shallow, well illuminated reef sites and by clade C in deep and poorly illuminated locations. Since all of our Belize samples were collected from shallow reef locations and 17/49 of our Bahamian samples were collected from deeper reefs (6-9 meters) this could explain the low abundance of clade C in Belize samples. This explanation can easily be tested once we analyze more Millepores collected at deeper reef locations from both sites.

Methods and Means to be Utilized

Symbiodinium DNA will be isolated from Millepore coral samples as described in Tepper et al. 2012. Quantitative polymerase chain reaction (qPCR) will be used to identify Millepore-specific *Symbiodinium* clades from samples collected in February 2017 from reefs located in San Salvador, The Bahamas and South Water Caye, Belize (Ulstrup and van Oppen 2003, Correa et al. 2009, Samayoa et al. 2017).

Gastropod (snail)-*Symbiodinium* Project

Despite our extensive knowledge of coral symbiosis, very little is known about *Symbiodinium* associations with other invertebrates. One of the more interesting symbiotic associations occurs with numerous marine mollusk species. For example, “solar-powered” nudibranchs (sea slugs) have been identified as having *Symbiodinium* in their tissues. These carnivores obtain their symbionts by feeding on sponges, anemones, hydroids and corals (FitzPatrick et al. 2012). Nudibranchs store *Symbiodinium* in epithelial cells of the branched digestive system allowing the host to adjust the symbionts exposure to sunlight (FitzPatrick et al 2012). Nudibranchs can receive up to 50% of the total photosynthetically fixed carbon via translocation (Hoegh-Guldberg and Hinde 1986) allowing the host to survive food shortages of weeks to several months (Burghardt and Wagele 2006).

Is there evidence to support photosymbiosis in shell-bearing mollusks? Tridacnid bivalves (giant clams) that contain brightly colored exposed mantles are packed with *Symbiodinium* (Carlos et al. 2000). Tridacnids that lacked large numbers of *Symbiodinium* possessed significant decreases in fitness (Leggat et al 2003). The algal symbiont translocated an estimated 95% of the fixed carbon to the host providing giant clams with a nutritional advantage over non-symbiotic bivalves (Hawkins and Klumpp 1995).

Little direct evidence exists to support photosymbiosis in marine shell-bearing gastropods (snails). Berner et al. (1986) reported that a variety of gastropods collected from the Red Sea contained endozoic microalgae in the upper whorls of their shells. However, there is no evidence that these gastropods were benefiting nutritionally from their symbionts. Banaszak et al (2013) reported that acquisition of *Symbiodinium* at the translucent veliger larval stage significantly enhanced survival and growth of *Strombus gigas* (queen conch). When adult *S. gigas* were examined they found healthy and mitotically-active *Symbiodinium* located in the digestive tract, gills, mantle, foot, nephridium and proboscis that displayed low photosynthetic efficiencies. They suggested that acquisition of *Symbiodinium* during larval development is beneficial, but the maintenance of the symbiont in adult tissues might be due to the lack of the host's ability to eliminate the symbiont. Hence, the symbiont might confer a net cost to the adult host by acquiring nutrients heterotrophically (Banaszak et al. 2013).

In February of 2016, students in my 485 research course, taught at the Gerace Research Centre (GRC) in The Bahamas, explored whether common intertidal nerites (snails) contained *Symbiodinium*. Nerites are very abundant on rocky shores and graze on hard wet surfaces feeding on algae. Nerites display photonegative behavior and retreat into rock crevices avoiding wave action, sunlight, and high temperatures (Bovbjerg 1984). My students collected *Nerita versicolor* (four tooth nerite, N=10) and *Nerita peloranta* (bleeding tooth nerite, N=12). DNA was isolated from snail tissue at GRC and samples were transported to Cornell College. We used qPCR with clade-specific primers for *Symbiodinium* clades A, B, C and D (Ulstrup and van Oppen 2003, Correa et al. 2009, Samayoa et al. 2017) to determine if symbionts were present in adult nerite tissues. Our preliminary results showed that all samples contained a high abundance of clade B. No background clades were found in any of the samples. Additionally, we showed the presence of clade B in gill tissue (N=2) and digestive gland (N=5) of *N. peloranta* and *N. tessallata* (checkered nerite). As far as we are aware, *Symbiodinium* has not been reported in these photonegative shelled-gastropods.

Symbiosis typically provides a benefit to the cooperating parties. We hope to begin the initial steps in elucidating whether there are metabolic interactions between algal symbionts and adult nerite gastropods.

Methods and Means to be Utilized

I propose to explore the relationship between adult nerites and *Symbiodinium*. I will expand the collection of these common nerites at three different rocky intertidal sites in The Bahamas (N=20 for each nerite species at each site) to begin a more systematic exploration of the symbiont diversity as a first step toward understanding the role symbiosis plays (if any) in these important grazers. I plan to isolate DNA at GRC (February 2017) and quantify the *Symbiodinium* clades present in digestive tract, gills, mantles, foot and nephridium using qPCR protocols established in my lab (Samayoa et al. 2017).

I would also like to determine if the symbionts are providing photosynthetic nutrients to the adult nerite. An initial step in answering this question is to determine if *Symbiodinium* present in adult nerite tissues are photosynthetically active. My lab plans to approach this question by determining if specific *Symbiodinium* photosynthetic genes are transcriptionally active in symbionts residing in adult nerite tissues. Using a section of the same tissue samples

collected for DNA analysis, I plan to isolate total RNA (SV Total RNA Isolation System, Promega) and use clade-specific primers in conjunction with reverse transcriptase qPCR (qRT-PCR) to quantify the transcription of photosynthetic reaction center genes *psbA*, which encodes the D1 protein of photosystem II, and *psA*, which encodes the p700 protein of photosystem I (McGinley et al. 2012). Boldt et al. (2008) have shown that beta-actin and PCNA (proliferating cell nuclear antigen) are excellent normalization genes for the expression of the photosynthetic reaction center genes and control for exogenous RNA contamination.

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