Sponges of the Caribbean: linking sponge morphology and associated bacterial communities

Ericka Ann Poppell

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ABSTRACT

SPONGES OF THE CARIBBEAN: LINKING SPONGE MORPHOLOGY AND ASSOCIATED BACTERIAL COMMUNITIES

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A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at the University of Richmond

University of Richmond, May 2011

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The ecological and evolutionary relationship between sponges and their symbiotic microflora remains poorly understood, which limits our ability to understand broad scale patterns in benthic-pelagic coupling on coral reefs. Previous research classified sponges into two different categories of sponge-microbial associations: High Microbial Abundance (HMA) and Low Microbial Abundance (LMA) sponges. Choanocyte chamber morphology and density was characterized in representatives of HMA and LMA sponges using scanning electron microscopy from freeze-fractured tissue. Denaturing Gradient Gel Electrophoresis was used to examine taxonomic differences among the bacterial communities present in a variety of tropical sponges. The results supported the hypothesis that choanocyte chamber density is greater in LMA sponges than in HMA sponges. Distinct microbial differences were observed between HMA and LMA sponge species. Our results provided insights into the role that symbionts play in shaping the trophic ecology of these sponges.
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I certify that I have read this thesis and find that, in scope and quality, it satisfies the requirements for the degree of Master of Science.

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SPONGES OF THE CARIBBEAN: LINKING SPONGE MORPHOLOGY AND ASSOCIATED BACTERIAL COMMUNITIES

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B.S., Virginia Commonwealth University, 2007

A Thesis
Submitted to the Graduate Faculty
of the University of Richmond
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1. Introduction

1.1 General Justification for Thesis

Marine sponges contribute a significant proportion of biomass to many benthic communities throughout the oceans of the world, and they have a major influence on benthic-pelagic processes. Sponges have been the focus of much recent interest, mainly due to the fact that they form close associations with a wide variety of microorganisms. While significant advances in our understanding of these associations have been made in recent years, many gaps remain in our knowledge of the structure and stability of these associations. For example, the scientific community lacks a clear picture of the extent of microbial diversity as well as factors that influence this diversity in the host sponge. Furthermore, we know very little about the role that symbionts play in shaping the feeding ecology of sponges, which is critical to understanding the ecological and evolutionary consequences of the relationship. Sponges are sessile, filter-feeding organisms that are extremely efficient at obtaining nutrients from the surrounding water column despite and very simple body plan (Reiswig et al. 1971; Vogel, S. 1977; Pile et al. 1996). Sponges are able to actively pump water throughout their tissues via a unique aquiferous canal system using many flagellated cells called choanocytes (Amano et al. 1996). Clusters of these choanocytes forming choanocyte chambers produce a current that generates the movement of large quantities of water through the sponge body (Boury-Esnault et al. 1985; Reiswig et al. 1974; Langenbruch et al. 1983, 1986). Indeed, some sponges are capable of pumping amounts close to 24 m$^3$ kg$^{-1}$ sponge day$^{-1}$ (Vogel 1977). Due to these pumping capabilities and their physiological activities, sponges can
Among certain species, these microbes can make up 40% of the sponge biomass (Wilkinson et al. 1978). Collectively, these microbes actively demonstrate a wide range of diverse metabolic pathways, such as photosynthesis, methane oxidation, nitrification, and nitrogen fixation, among other processes (Bayer et al. 2008; Hoffmann et al. 2005; Wilkinson 1983).

Important questions remain regarding the relationship between microbial symbiont density and diversity and the ecology and evolution of the host sponge. Determining whether symbionts influence sponge morphology and physiology has important implications for our understanding of marine community structure and function. The work presented in this thesis had two major goals. The first was to compare and contrast the morphological characteristics of tropical sponges with distinct symbiotic strategies. The second was to determine whether the microbial communities found in sponges harboring high densities of symbionts differed from microbial communities in sponges with low densities of symbionts.

1.2 Thesis background, development and summary

Past examinations of taxonomically diverse sponge species using transmission electron microscopy identified two different types of sponge-microbe associations (Vacelet and Donadey, 1977). One group of sponges, labeled high microbial abundance (HMA) sponges, contains dense tissue with abundant and diverse microbial communities; the other group of sponges, low microbial abundance (LMA) sponges, have highly irrigated (?) tissue and contained few microbes, some of which were of a single morphotype (Hentschel et al., 2006). Factors shaping and contributing to this interesting
dichotomy in microbial symbiont strategies is poorly understood. Currently, we lack an understanding of how differences in sponge morphology (e.g., choanocyte chamber size, volume and density) affect the actual pumping rates between HMA and LMA sponges.

An important study conducted by Turon et al. (1997) considered clearance rates and microarchitecture of the aquiferous systems of two sympatric sponge species, *Crambe crambe* and *Dysidea avara*. The aim of this study was to discern any noticeable differences between the two species in structure and efficiency of the filtering abilities. If differences in microarchitecture were found between these species, then it may relate to differences in filtration efficiency and a dependency on particle size. This study did reveal that the two sympatric species demonstrated important variations in structure and filtration efficiency in sponges, which correlates with diverse biological strategies. The clearance rates for *C. crambe* were always lower than for *D. avara* given the morphological differences of the aquiferous system (e.g., size of ostia, thickness of choanosome layer, size of choanocyte chambers). Wilkinson et al. (1978) suggested that smaller ostia, choanocyte chambers and long canals could result in higher retention rates of bacteria. However, it was assessed that *C. crambe* has small choanocyte chambers and ostia but had the lowest clearance rates (Turon et al. 1997). The results also suggested that the sponge with higher growth and turnover rates had much higher clearance rates than the slow-growing form, which would indicate an adaptive variability concerning the filtering abilities of these sponges.

Recent work conducted by Weisz et al. (2008) confirmed these predictions given that HMA sponges displayed a slower pumping rate than LMA sponges. These
differences in microbial abundance suggested a morphological explanation for the differences in pumping rate, which provides evidence of significant differences between HMA and LMA sponge morphology and physiology. Even though it is not yet understood if the presence of bacteria is responsible for these morphological and physiological differences among different sponge species, these correlations could provide important evidence regarding the relationship between microbial abundance and the evolution of sponge body plans.

More recent studies have conducted broad surveys of stable C and N isotopic ratios between both HMA and LMA sponges (Peterson et al. 1987; Weisz 2006). These ratios are used to understand the nutrient cycling input from the associated microbes within these two groups of sponges and to identify what food sources are being consumed by the sponge. These microbes are thought to actively transform nitrogen compounds (Southwell et al., 2005). Interestingly, research that examined a variety of sponge species showed that the HMA sponges contained low $\delta^{15}N$ levels within their tissues and LMA sponges contained high $\delta^{15}N$ levels (Weisz, 2006). The results suggested that the HMA sponges are less heterotrophic and are relying on their associated microbes for energy input required for growth and development. Conversely, the LMA sponges are thought to be more heterotrophic, considering these sponges have a small microbial load present in their tissue. Molecular tools were used to investigate the relative presence or absence of microbes and how it related to low and high N levels in different sponge species. There is an obvious association between $\delta^{15}N$ levels and microbial association density and diversity (see Fig. 2.3, Weisz, 2006).
Further investigation among the HMA/ LMA and High/ Low $\delta^{15}$N groups of sponges considered the importance of sponge pumping rates. In this particular study, 8 sponge species were sampled to examine the range of pumping rates under natural conditions. Research has previously suggested that the dense tissue in the HMA sponges would result in smaller water canals, which would contribute to a decrease in water flow (Vogel et al., 1978). Dye measurements were used in order to quantify the pumping rates of both HMA and LMA sponges. The results suggested that the HMA sponges displayed a slower pumping rate than the LMA sponges (see Fig 1.6, Weisz, 2006).

The previous finding of different microbial abundance suggests that there is a morphological explanation for the differences in pumping rates. These results also provide evidence of significant differences between HMA and LMA sponge morphology and physiology (Weisz, 2006). Even though it is not yet understood if the presence of bacteria is responsible for these morphological and physiological differences of the different sponge groups, these correlations provide important evidence for future research. Overall, previous research suggests the differences in tissue density, $\delta^{15}$N levels and pumping rates between HMA and LMA sponges demonstrates a significant role of the associated microbes and their impact on morphology, physiology and transformation of nutrients of their host sponge.

These current trends provide enough evidence to examine the morphological structure of the choanocyte chamber among a variety of marine sponges to better understand its influence on functional processes. To achieve this, I examined the relationship between choanocyte chamber density and pumping rate, and the relationship
between choanocyte chamber density and bacterial density. Additionally, I investigated potential differences in the microbial communities found in selected sponge groups with varying bacterial densities.

In this study we present the results of an extensive sampling of both HMA and LMA sponge groups and insights into their associated microbial communities. The sponge species used were collected near Summerland Key, FL from distinctly different locations that include an offshore patch reef and a shallow, near-shore seagrass habitat. The 11 different sponge species included in this study were previously classified as either belonging to the HMA sponge group or the LMA sponge group. Despite all of the work that has been done to understand the abundance and diversity of microbes associated with many different sponge species, there is still much to discover about these associated communities and how this influences all aspects of sponge biology, specifically morphology. It is important to determine whether differences exist in community structure/composition that might explain the significantly different isotopic signatures observed between the HMA and LMA species. Thus, the goal of this work was to compare choanocyte chamber densities to the density and diversity of microbial communities harbored by the sponge host. To further explore possible explanations for the differences in sponge microbial loads, isotopic signatures, and flow rates we compared the microbial communities using a combination of molecular approaches. In addition, we compared the relative abundance of microbial load between the HMA sponge group and the LMA sponge group by measuring the microbial species diversity within the community and explored the microbial community structure using multivariate
techniques. We hypothesized that choanocyte chamber density/size was greater in LMA sponges than in HMA sponges.

Overall, this research provides a detailed understanding about the morphology and dynamics of associations between sponges and their microflora in tropical habitats. The results also provide insights into physiological and ecological consequences of the sponge-associated microbial communities. This research allowed for the identification of similarities and differences among microbial communities harbored by certain sponge species. The research also permitted estimates of the roles associated bacteria play in host sponge metabolism.

2. Methods

Sponge Collection and Processing

Several sponge species were collected at 15 m in the summer of 2008 at the CVFI reef site, Summerland Key, FL (24° 39.601' N, 81° 22.775' W) (Table 1). Other sponge species were collected from Niles Channel and the 'Mote Flats' in a water depth of ~1.5 m in the summer of 2008 (Table 1). Samples were cut from the sponge with a diving knife and placed into a mesh collection bag and transported to the lab in running seawater, within 2 hours of collection.

In the laboratory, each sponge sample was placed onto a submerged cutting surface and was quickly cut with a sterile scalpel into long, thin strips (taking note to sample equal amounts of both pinacoderm and choanoderm). The strips (~40 mm X 5 mm) were placed directly into a 15ml Falcon tubes containing 5ml of 2% OsO4, 4.5ml FSW buffer and 0.5ml of 2.5% glutaraldehyde and were stored at RT overnight. To
ensure proper fixation and preservation of choanocyte chambers for subsequent scanning electron microscopy (SEM), it was noted that OsO4 penetrates 0.5mm of tissue/hr (Johnston and Hildemann 1982) so samples were left for no longer than 16 hours in fixative. The samples were stored in fresh seawater (FSW) buffer and kept at RT after fixation. For subsequent molecular work, additional samples were cut with an EtOH-sterilized scalpel and placed directly into 1.5ml Eppendorf tubes and stored at -20°C.

2.1 Sponge morphology

Following standard procedures (Johnston and Hildemann 1982), tissues were dehydrated in an ethanol series as follows: three washes for 15 minutes in 30%, 70%, 90%, and two washes for 20 min in 100%. Following dehydration, the samples were freeze-fractured in liquid nitrogen (Johnston and Hildemann 1982). All liquid was removed in a SAMDRI-795 critical point drier and 3-4 pieces of tissue were mounted on aluminum stubs using a silver adhesive and placed into the dryer oven for 1 hour. Specimens were then sputter coated in a DENTON VACUUM Desk IV with a 40:60 gold: palladium mix. Samples were viewed on a JOEL 6360 LV scanning electron microscope.

To accurately represent choanocyte chamber densities among all 11 sponge species used in this study, Image J software was used to determine the specific area of choanocyte chambers per total area of each SEM image, the fracture plane was identified in each image, and the proportion of mesohyl devoted to choanocyte chamber formation was estimated. For each species, the number of scanning electron micrographs analyzed represented the sample size. For statistical analysis of each sponge species, the overall
choanocyte chamber proportion, the average collar cell head width and spherical indices of choanocyte chambers (all in µm) were determined.

2.2. Microbial ecology

Genomic DNA was extracted from each sponge using a modified CTAB protocol (Enticknap et al., 2004). The universal 16S rDNA gene primer pairs (PRBA338F-GC and PRUN518R) (Muyzer 2001) and (1055F and 1406R-GC) (Wang et al. 2008) were used for the PCR amplification of bacterial 16S rDNA genes. Negative controls (PCR’s without any DNA template) were included for each 16S rDNA gene amplification reaction. The cycle profile included: an initial denaturing step at 95°C for 2 min; 35 cycles of denaturing at 95°C for 1 min; primer annealing at 53 and 63°C for 30s and elongation at 72°C for 1 min, followed by a final extension step at 72°C for 5 min. The PCR mix consisted of 5µl of 10X reaction buffer, 2.5µl of each primer, 6µl of 25mM MgCl₂, 4µl of dNTP mix, 0.25µl Takara Taq DNA polymerase, 2µl DNA template and sterile water up to 50µl.

Denaturing Gradient Gel Electrophoresis (DGGE)

DGGE was performed using a Bio-Rad DCode Universal Mutation detection system (Bio-Rad, Müenchen, Germany) on a 10% (wt/vol) polyacrylamide gel in 1X TAE using a 30-70% denaturing gradient. Electrophoresis was performed for 17 h at 70V and 60°C. The gels were stained for 30 min in 1X TAE spiked with 3µl of ethidium bromide (0.5 µg ml⁻¹), destained for 25 min in dH₂O and visualized and photographed with a GelDoc System (GelDoc 2000, Bio-Rad). A mixture of PCR products from five common bacterial species were applied at edges of the gels as markers. Selected DNA bands from
the LMA sponges were carefully excised to prevent risk of neighboring band
contamination using an ethanol-sterilized scalpel and stored at –20°C. To recover DNA
from DGGE bands, 25µl of TE buffer was added to bands and placed at 4°C overnight. A
total of 4µl of eluted DNA was used for re-amplification with primers PRBA338F-GC
and PRUN518R using the PCR conditions described above.

The re-amplified PCR products from the excised DGGE bands were purified
(MinElute Gel Extraction Kit, Qiagen), ligated into the TOPO II vector and transformed
in E.coli competent cells (Invitrogen) following the manufacturer’s protocol and plated
onto LB Ampicillin (50 mg/ml) agar. Plates were incubated overnight at 37°C. White
colonies were randomly picked from the plates and patched to fresh LB Amp plates to
ensure that colonies were pure isolates. At least four transformants were separately grown
overnight in a 5ml LB broth medium supplied with 5µl of Ampicillin (50 mg/ml).
Plasmid DNA from these cultures were isolated and amplified using the M13 forward
and M13 reverse sequencing primers (Invitrogen) to check for appropriate insert size.
The plasmid DNA was sequenced at VCU’s facility.

Sequences from the selected DGGE bands of the LMA species were compared to
sequences in GenBank (National Center for Biotechnology Information), and aligned
using Clustal (Thompson et al., 1994). The closest sequence matches in GenBank was
recorded along with the associated bacterial phyla.

To assess microbial diversity and evenness in the community, DGGE profiles of
HMA and LMA sponge species were examined, and we established a minimum band
intensity level for the analysis. The band intensities were plotted using Image J software
and subsequently calculated by measuring the area under each band peak. The DGGE band intensities were plotted and measured twice to reduce statistical error. The proportion of band intensities were calculated and from these values two diversity indices and two evenness indices were determined (Simpson’s and Shannon’s diversity) (Simpson 1949; Shannon & Weaver, 1949). The diversity and evenness data were grouped based on microbial load, either HMA or LMA. A simple t-test was used to determine if any significant differences existed among the two sponge groups. The DGGE profiles were also used to examine the structure of the microbial communities across the selected HMA and LMA sponge groups. A standardized method of counting individual bands among all sponge species was implemented and subsequently entered into a dissimilarity matrix. We used Ginkgo software as a multivariate analysis tool, which is oriented mainly towards ordination and classification of ecological data and to spatially display a distributional plot across the sponge taxa.

3. Results

3.1 Morphology of sponge mesohyl/choanocyte chambers

Bacterial symbiont density was visually assessed from scanning electron micrographs (Figs 1-10). Bacterial densities fell into two categories; high microbial abundance (HMA) (Fig.1) and low microbial abundance (LMA) (Fig. 2). Morphological characteristics varied across the sampled sponge taxa, which were sampled from a range of sponge families. Differences among the sponges included choanocyte chamber size and shape, flagella length and collar head width (Figs. 3-10).
Visual analysis of SEM images allowed a clear distinction between HMA and LMA morphology. The microbes were densely packed in the HMA sponges and exhibited a high tissue density. The two HMA (high $\delta^{15}N$) species, *Agelas clathrodes* and *A. conifera*, have nearly circular choanocyte chambers (Fig. 6a & b). The chambers appear to contain fewer cells (that may even be less densely packed) than other sponges (Fig. 6). The collar cell head widths ranged from 1.9-2.1 $\mu$m wide, with spherical indices of 0.96 and 1.03, respectively (Table 2).

The four HMA species that come from the group (low $\delta^{15}N$) (*Chondrilla caribensis f. hermatypa*, *Aplysina cauliformis*, *Smenospongia aurea* and *Calyx podatypa*) have choanocyte chambers that appear less circular and more oblong. Within each species there appears to be a characteristic and species specific choanocyte chamber shape (Figs. 3-5, 7). The individual choanocytes appear to be well spaced in these sponges except for *C. caribensis f. hermatypa*, which appear to contain copious compressed choanocytes within the choanocyte chamber (Fig. 4). The collar cell head widths and associated apparatus of these four sponge species vary extensively, with diameters ranging from 2.1-2.3 $\mu$m wide and spherical indices ranging from 0.96-1.16 (Table 2).

The five LMA species (*Amphimedon compressa*, *Scopalina (Ulosa) ruetzleri*, *Tedania ignis*, *Niphates digitalis* and *Spheciospongia vesparium*) have circular choanocyte chambers (Figs. 8,9,10). Compared to the other sponges in this group, *N. digitalis* appears to have tightly compact choanocytes within the chambers (Fig. 10a). The collar cell head widths range from 2.2-3.4$\mu$m and spherical index ranges from 1.02-1.30
Sponges in the LMA group showed a distinct arrangement of connected choanocyte chambers and canal systems. Interestingly, *S. vesparium* appeared to have the most area of mesohyl with very few visible microbes (Fig. 8). The mesohyl among the other four LMA species was much less apparent and devoid of microbes (Figs. 9a and 10a,b). *S. ruetzleri* had the most disconnected body structure (Fig. 9b).

The density of choanocyte chambers within the sponge mesohyl was found to significantly differ (*t* = 2.263; *p* < 0.05) between HMA and LMA sponge groups (27% vs. 16% respectively, Fig. 16a). No significant differences in collar cell head widths or spherical indices were detected between the HMA and LMA sponge groups. (*t* = 2.263; *p* > 0.05, Fig. 16b,c, respectively). Though based on only 3 values, pumping rates (taken from Weisz et al. 2008) were correlated with symbiont load (Table 2). That is, our HMA sponge had much lower pumping rates than the 2 LMA sponges. Interestingly, *S. vesparium*, considered within the LMA sponge group, had a choanocyte chamber proportion of 0.30 (the highest of these three sponges) and a pumping rate of 1.527 L/s/kg (Table 2).

### 3.2 Microbial diversity and community structure

The bacterial diversity within both HMA and LMA sponges was examined using DGGE analysis of PCR-amplified partial 16S rDNA fragments (Fig. 11). The LMA sponge group showed in the green box includes six randomly chosen species and the HMA sponge group displayed in the blue box and also includes six randomly chosen species. Within the HMA group, a yellow box indicates two sponge species that
displayed a difference in trophic status (high $\delta^{15}$N) from the other HMA sponges (low $\delta^{15}$N).

DGGE analysis revealed that the microbial communities varied substantially between the HMA and LMA sponge groups. The HMA sponges all appear to host massive and diverse microbial communities, with a much greater GC-content than the DGGE banding pattern of the LMA sponge group (Fig. 11). In the LMA group, five sponge species display a dominant member of the bacterial community, with greater banding intensity (Lanes Ne, Nd, Cv, Ti, Cvf) (see Fig. 11). As described above, this pattern supports the hypothesis that there are two different sponge groups defined by the microbial community they host.

Another application of DGGE, specifically for 7 randomly chosen LMA sponge species, showed a pattern of few populations of bacterial associates, suggesting a species-specific microbial community. Moreover, each species appeared to have one high-density microbial strain that was different from the other species. The orange boxes (see Fig. 15) indicate predominant community members (Ti1, Cp1, Cv1, Ne1&2, Ac1, Nd1 and Ib1 (Ib1 band not shown in Fig. 15)) that were excised from the gel and sequenced to obtain phylogenetic information. In total, 16 clone inserts that were sequenced showed high affinity (94-100% homology) to known bacterial phyla (Table 3). Three of the bands (Nd1, Ne1 and Ne2) appeared to be related to the $\alpha$-Proteobacteria, three (Ac1, Cv1 and Ib1) appeared to be related to $\gamma$-Proteobacteria, Chloroflexi and Bacteriodetes, respectively (Table 3). The 16S fragment represented by band Ti1 is closely related to an uncultured bacterium clone Mann16S_E04, and the phylum is unidentified. Additionally,
C. plicifera presented problems with sequencing (Cp1) and did not provide any results for analysis (Table 3).

The Shannon index and the reciprocal of the Simpson index were used to determine diversity and evenness indices for both HMA and LMA sponge groups. A two-tailed t-test revealed significant differences between the diversity indices of the LMA sponge group and the diversity test (Shannon's/Simpson's) (t-value=2.015; p<0.05) and the diversity indices of the HMA sponge group and the diversity test (t-value=1.943; p<0.05; Fig. 13). Another two-tailed t-test showed significant differences between the evenness indices of the LMA sponge group and the evenness test (EH/ED) (t-value=2.015; p<0.05) and the evenness indices of the HMA sponge group and the evenness test (t-value=1.943; p<0.05; Fig. 14).

Analysis of the DGGE by NMDS (Fig. 12) showed a clear grouping pattern based on microbial association between the LMA sponges (circled in red) and the HMA sponges (circled in blue). Within the LMA sponge group, the data showed a distinct divide between the sponge species collected from the bay (circled in brown) and those from the reef (circled in orange). Within the HMA sponge group, there is a clear pattern based on trophic status between the High δ^{15}N sponges (circled in green) and the Low δ^{15}N sponges (circled in yellow).

4. Discussion

The ecological and evolutionary relationship between sponges and their symbiotic microflora remains poorly understood. Some sponge species harbor extraordinarily dense populations of microbes (high microbial abundance (HMA)
sponges), while other species maintain bacterial populations at very low levels (low microbial abundance sponges (LMA)).

I was interested in determining whether the symbiont status of the sponge was correlated with differences in ultra-structural characteristics of a variety of sponge hosts. Specifically, I examined aspects of choanocyte chamber density and morphology. My hypothesis was that symbiont communities provide some level of trophic input, and this would lead to a reduced reliance on heterotrophic feeding achieved through filtering large quantities of water. I predicted that HMA sponges would have lower concentrations of choanocyte chambers, and the choanocytes would themselves be modified compared to LMA sponges. I was motivated to test these hypotheses because these data would help shed light on the observation that pumping rates differ between HMA and LMA sponges (Weisz et al. 2007).

I was also interested in studying the differences in community structure of microbes harbored by HMA and LMA sponges. Broadly speaking, I was interested in addressing the question, are the communities found in HMA sponges structured differently than those found in LMA sponges? While the distinction between HMA (also known as bacteriosponges) and LMA sponges has been known for many years (e.g., Reiswig 1981), the ecological characteristics of the microbial communities (e.g., species richness, evenness) are unknown. This type of information might help us understand how these symbioses are formed and maintained.

The examination of choanocyte chamber and mesohyl morphology across the sampled marine sponge taxa revealed that HMA sponges have considerably greater
microbial loads than LMA sponges and denser tissues. Scanning electron micrographs of the mesohyl of *A. cauliformis*, *S. aurea*, *A. clathrodes*, *A. conifera* and *C. podatypa* (Fig. 3, 5-7 respectively) showed a significant abundance of bacteria and a variety of morphotypes. In contrast, micrographs of *T. ignis*, *U. reutzleri*, *N. digitalis* and *A. compressa* (Fig. 9 &10 respectively) showed a major reduction in microbial load. As described previously, (Vacelet & Donadey 1977, Hentschel et al. 2006; Weisz 2008), these findings support the hypothesis that two different life strategies exist in sponges.

Interestingly, my results revealed some exceptions to the proposed dichotomous nature of HMA and LMA sponges. For example, micrographs of the supposedly HMA sponge *S. vesparium* (Fig. 8) indicated that the bacterial load was remarkably low, and that this sponge was more correctly considered a LMA species. Another example found *C. caribensis f. hermatypa*, another supposedly belonging to the HMA group, had a low microbial load within the mesohyl (Fig. 4, Table 2). Interestingly, this species had a choanocyte chamber density of 0.22, which was the highest in the HMA group. In contrast, the HMA sponge *C. podatypa* (Fig. 7) revealed a significant presence of bacteria within the mesohyl surrounding choanocyte chambers. It was proposed in a previous study by Vacelet and Donadey (1977) that sponge species containing massive bacterial communities (HMA sponges) also contain dense tissue. However, we found some ‘dense’ tissue LMA sponges (e.g., *S. vesparium* Fig. 8).

In a recent study by Weisz (2008), *S. vesparium* was found to have irregular *in situ* flow rates, with occasional slow and absent flow rates. The pumping rate reported for this sponge was much higher compared to the HMA sponge *A. conifera* (Fig. 6b), but
much lower compared to the LMA sponge *N. digitalis* (Fig. 10a; Table 2). Vogel (1978) suggested that species having denser tissues would result in small water canals and an increased resistance to water movement. Consequently, this increased resistance should cause a decrease in flow rate, assuming pressure remains the same. Our data showed that *S. vesparium* had the highest choanocyte chamber density among all HMA sponges examined (0.30; Table 2), resulting in a larger proportion of mesohyl devoted to choanocyte chamber formation. It seems acceptable to consider other factors determining microbial load in these sponges besides tissue density and filtering capacity. Moreover, sponges within the same symbiont status may exhibit varying physiological processes. Unfortunately, flow rate data was not available to report along with every sponge species we examined morphologically. Future work could involve measuring flow rates for these HMA sponges and subsequently compare these rates to the sponge morphology.

Choanocyte chamber density is significantly different between the HMA and LMA sponges examined (Fig. 16a). No significant difference was detected between symbiont status and other morphological attributes (collar cell head width, spherical index) (Figs. 16b-c). In a recent study, the choanocyte from different sponge species was distinguished by the differences in size, shape and structure of the basal apparatus (Gonoboleva et al. 2009). Choanocyte shape is thought to not only vary based on species or taxonomic grouping, but also depending on the choanocyte location within the chamber itself (Boury-Esnault et al., 1984, Eerkes-Medrano and Leys, 2006). Taxon-specific variation in choanocyte characteristics may override the changes that result from symbiont communities. However, it may be possible to test for symbiont influence on
these ultrastructural characteristics by comparing a single species with variable symbiont loads (e.g. *Chondrilla caribensis* or *Cymbastella*).

Unfortunately, very few recent studies combine molecular and microscopy methods (Thoms et al 2003; Schmitt et al 2007; Weisz et al 2007). By combining these data with molecular profiles, it may be possible to clarify what processes are likely to contribute to the differences in HMA/LMA sponges. In a recent study, observations of dense mesohyl/microbial load among the HMA sponges lead to more diverse microbial processes and that these microbial communities may form distinct niches within the sponge mesohyl (Schlappy et al. 2010).

To gain a better understanding of microbial diversity, DGGE banding patterns (Fig. 11) showed distinct microbial differences between the HMA and LMA sponge species. Notably, a large number of high GC content bacteria were found in HMA sponges that were absent from LMA sponges (see lower portion of gel, Fig 11), which may provide some information about the taxonomy of the bacteria present in the HMA communities. For example, *Actinomycete* bacteria have a high GC ratio, and they often play an important role in decomposition of organic material.

It remains to be seen whether these bacteria are the component of the microflora present in sponges that influence trophic status of the host (e.g., Weisz 2006). Although a great amount of research has been done on the microbial communities associated with many sponge species, much remains to be learned about microbial diversity and factors that influence it. These diverse bacterial communities also suggest a significant and potentially varying impact on the physiological characteristics of their host sponges. As
shown by Weisz et al. (2007), HMA sponges have slow pumping rates, but these sponges still take up dissolved organic nutrients from the water column (Yahel et al. 2003) and can utilize a large amount of organic matter from the water much more efficiently, which reduces the demand for pumping to meet the nutrition requirements.

The seven LMA sponges displayed relatively simple microbial communities and possessed one dominant bacterial strain, shown by relatively intense bands (Fig. 11). These prominent bands among the LMA sponges were chosen to be sequenced (Fig. 15). All bands that were sequenced showed high homology (94-100%) with previously reported sequences from other sponge-associated microbes (Table 3). The individual sequences revealed LMA sponges harbored bacterial phyla affiliated with the Gamma- and Alphaproteobacteria, Chloroflexi and Bacteriodetes.

In DGGE analysis, the basic assumption is every band within a DGGE fingerprint represents a unique type of 16S rDNA gene. It is possible, due to PCR-inherent biases, to experience limitations with this technique (von Wintzingerode et al., 1997). Problems, such as poor band resolution or the co-migration of similar sequences (Muyzer 2001), potentially disrupt the reliable estimation of the number of phylotypes (i.e. richness). Despite these limitations, molecular techniques such as DGGE have revolutionized our understanding of microbial diversity. Nonetheless, many recent microbial diversity studies have demonstrated a lack of replication and statistical procedure reporting in molecular ecological research (Morris et al. 2002). It has been suggested that spatial and temporal heterogeneity of bacterial communities is unavoidable due to the small size and generational times of these microorganisms, and so appropriate sampling techniques are
required (Taylor et al. 2004). To overcome these limitations, I was careful during visual inspection of the DGGE band patterns, to ensure that all banding patterns were counted at least twice.

The NMDS data for the eight HMA sponges and the seven LMA sponges support the hypothesis that these sponges express important differences in characteristics of the microbial community based on trophic status and geographic location of the host (Fig. 13). For example, near-shore and off-shore sponges differed in important ways. (Fig. 13). The DGGE patterns generated from the LMA sponge group indicated that distinct microbial associates dominated the community, which was concomitantly associated with a low level of bacterial diversity (Fig. 11 & 15). These data might be explained by a variety of processes (e.g., host preference for one type of symbiont, competitive exclusion for that host niche by the dominant species), but greater work is needed to tease apart the processes in operation in LMA sponges.

Sponges are important largely from an ecosystems perspective due to the benthic-pelagic coupling point produced by their specific method of filter feeding. Research focused on understanding the dynamics of the sponge-microbe association has important implications for our understanding of marine community structure and function. As major consumers on coral reefs, and as important nutrient re-cyclers, sponges undoubtedly rely on their symbionts for physiological purposes. Greater work is needed to decipher the role the microbes play in the sponge. It is also important not only to identify individual members of associated microbial communities, but also to have a clear understanding of the ecological roles these associated symbionts play concerning
metabolic processes of the host sponge. Sponges appear to be reservoirs of elusive marine microorganisms, many of which are currently uncultured (Olson et al. 2005). Thus, sponges represent a repository of biological diversity on coral reefs, and yet we know virtually nothing of the species that reside in these organismal habitats.

It is also beneficial to clarify differences in bacterial densities and if these bacterial associations provide a mutualistic association with the hosts. When considering environmental change, the effects will most likely alter microbial communities and ultimately disrupt the ability for sponges to function normally and to produce ecologically important compounds within the benthic community. If the scientific community is able to provide a clear picture concerning the dynamics and conditions of sponge-microbe associations, then the development of strategies to meet these challenges will be possible.

Given the role that sponges play in ecosystem-level process on coral reefs, it is exceptionally important to understand the connection between sponge physiology and morphology in the context of the complicated symbiotic associations involving bacteria. The research presented here has provided some evidence that there are significant differences in morphology among sponge species that is likely influenced by the microbe associations, which in turn has major implications for our understanding of sponge feeding strategies. I present data that highlight several important questions that should be addressed so we better understand the central role sponges play in coral reef dynamics, which is becoming more pressing as patterns of environmental change on a global scale are influencing coral reefs like those studied here.
References


Figure 1.
Ultrastructure of HMA (high $\delta^{15}$N) species *Agelas conifera* (A.) *Calyx podatypa* (B.) and HMA (low $\delta^{15}$N) *Aplysina cauliformis* (C.). Scale bar, 10$\mu$m (B&C) and 5$\mu$m (A), respectively. b=bacteria, ch=channel, cc=choanocyte chambers (site of sponge feeding), c=choanocyte, mv=choanocyte microvilli, fl=flagella, sp=spicules
Figure 2.
Ultrastructure of LMA species *Tedania ignis* (A.) and *Niphates digitalis* (B). Note that these species have a substantial proportion of their mesohyl tissue devoted to feeding structures. Scale bar 10µm. ch=channel, cc=choanocyte chambers (site of sponge feeding), c=choanocyte, sp=sponge cell.
Figure 3. *Aplysina cauliformis* (Order Verongida, Family Aplysinidae). Scanning electron micrograph illustrating bacterial symbiont density within the mesohyl and choanocyte chamber morphology. b=bacteria, cc=choanocyte chambers (site of sponge feeding), c=choanocyte, mv=choanocyte microvilli, fl=flagella.
Figure 4.
*Chondrilla caribensis f. caribensis* (Order Chondrosida, Family Chondrillidae).
Scanning electron micrograph illustrating bacterial symbiont density within the mesohyl and choanocyte chamber morphology. b=bacteria, cc=choanocyte chambers, c=choanocyte.
Figure 5.

*Smenospongia aurea* (Order Dictyoceratida, Family Thorectidae). Scanning electron micrograph illustrating bacterial symbiont density within the mesohyl and choanocyte chamber morphology. cc=choanocyte chambers, c=choanocyte, b=bacteria.
Figure 7.
*Calyx podatypa* (Order Haplosclerida, Family Phloeodictyidae). Scanning electron micrograph illustrating bacterial symbiont density within the mesohyl and choanocyte chamber morphology. cc=choanocyte chambers, c=choanocyte, sp=spicule, b=bacteria.
Figure 8. *Spheciospongia vesparium* (Order Hadromerida, Family Clionidae). Scanning electron micrograph illustrating bacterial symbiont density within the mesohyl and choanocyte chamber morphology. cc=choanocyte chambers, c=choanocyte, ch= channel, me=mesohyl.
Figure 9
*Tedania ignis* (A.) and *Ulosa ruetzleri* (B.) (Order Poecilosclerida, Family Myxillina and Esperiopsidae, respectively). Scanning electron micrograph illustrating bacterial symbiont density within the mesohyl and choanocyte chamber morphology. cc=choanocyte chambers, fl=flagella, c=choanocyte, me=mesohyl.
Figure 10.
*Niphates digitalis* (A.) and *Amphimedon compressa* (B.) (Order Haplosclerida, Family Niphatidae). Scanning electron micrograph illustrating bacterial symbiont density within the mesohyl and choanocyte chamber morphology. cc=choanocyte chambers, fl=flagella, c=choanocyte, sp=spicule, fl=flagella.
Figure 11.

DGGE banding patterns comparing HMA and LMA bacterial communities. LMA sponge species are located in the green box, and HMA species are in the blue box. The yellow box within the HMA group indicates high dN15 sponges, all other HMA sponges are low dN15. All LMA sponges have high dN15 isotopic signatures. Sponge species examined: *Niphates erecta* (Ne), *N. digitalis* (Nd), *Amphimedon compressa* (Ac), *Callyspongia plicifera* (Cp), *C. vaginalis* (Cv), *T. ignis* (Ti), *Cliona varians forma varians* (Cvf), *Agelas clathroides* (Acl), *A. conifera* (Acon), *Xestospongia muta* (Xm), *Ircinia campana* (Ic), *I. felix* (If), *Ectoplasia ferox* (Ef), *Aplysina cauliformis* (Aca).
Figure 12.
Scatterplot diagram for two-dimensional NMDS ordination of two sponge main sponge groups based on microbial load. LMA group (red ellipse), which contains two species sampled from a nearshore location (brown ellipse) and five species sampled from an offshore reef location (orange ellipse). HMA group (blue ellipse), which contains sponges with high $\delta^{15}N$ signatures (green ellipse) and low $\delta^{15}N$ signatures (yellow ellipse).
Figure 13. Relationships between diversity test types and the level of diversity among bacterial families between two sponge types (high microbial abundance (HMA), and low microbial abundance (LMA)). A statistical comparison was performed using a student t-test. Significant differences were detected between LMA group (t-value = 2.015; p<0.05) and between the HMA group (t-value = 1.943; p<0.05).
Figure 14.
Relationships between evenness test types and the level of evenness among bacterial families between two sponge types (high microbial abundance (HMA), and low microbial abundance (LMA)). A statistical comparison was performed using a student t-test. Significant differences were detected between LMA group (t-value = 2.015; p<0.05) and between the HMA group (t-value = 1.943; p<0.05).
Figure 15.
DGGE banding patterns of bacterial gene fragments using V3 and UNI primer sets. Fragments are amplified from LMA sponge species only; (Tedania ignis (Tign), Callyspongia plicifera (Cpli), C. vaginalis (Cvag), Niphates erecta (Nere), Amphimedon compressa (Acom), N. digitalis (Ndig) including a bacterial reference marker (M)) all sampled from CVFI 1 offshore reef site. Orange rectangles show the DGGE bands that were excised and indirectly sequenced.
Figure 16.

A.

![Graph A]

Proportion of choanocyte chamber presence within mesohyl tissue between LMA and HMA sponge species. Histobars represent means (±SE); the red and green bars are significantly different (t-value=3.73, df=9; p<0.05).

B.

![Graph B]

Average collar cell head width (µm) between LMA and HMA sponge species. Histobars represent means (±SE); the red and green bars are not significantly different (t-value=2.18, df=9; p>0.05).

C.

![Graph C]

Average spherical indices between LMA and HMA sponge species. A spherical index of 1.0 indicates a regularly circular choanocyte chamber. Histobars represent means (±SE); the red and green bars are not significantly different (t-value=1.08, df=9; p>0.05).
Table 1.
Sponge species collected near Summerland Key, Florida.

<table>
<thead>
<tr>
<th>Species</th>
<th>Collection date(s)</th>
<th>Collection site</th>
<th>Depth (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Agelas clathrodes</em></td>
<td>6/08</td>
<td>CVFI reef</td>
<td>12</td>
</tr>
<tr>
<td><em>Agelas conifera</em></td>
<td>6/08</td>
<td>CVFI reef</td>
<td>12</td>
</tr>
<tr>
<td><em>Agelas schmidtii</em></td>
<td>7/08</td>
<td>Blake’s Reef</td>
<td>12</td>
</tr>
<tr>
<td><em>Amphimedon compressa</em></td>
<td>6/08</td>
<td>CVFI reef</td>
<td>12</td>
</tr>
<tr>
<td><em>Aplysina cauliformis</em></td>
<td>7/08</td>
<td>CVFI reef</td>
<td>12</td>
</tr>
<tr>
<td><em>Aplysina fulva</em></td>
<td>7/08</td>
<td>Blake’s reef</td>
<td>21</td>
</tr>
<tr>
<td><em>Callyspongia plicifera</em></td>
<td>6/08</td>
<td>CVFI reef</td>
<td>12</td>
</tr>
<tr>
<td><em>Callyspongia vaginalis</em></td>
<td>6/08, 5/09</td>
<td>CVFI reef</td>
<td>12</td>
</tr>
<tr>
<td><em>Calyx podatypa</em></td>
<td>7/08</td>
<td>Blake’s Reef</td>
<td>21</td>
</tr>
<tr>
<td><em>Chondrilla caribensis f.</em></td>
<td>6/08</td>
<td>CVFI reef</td>
<td>12</td>
</tr>
<tr>
<td><em>Chondrilla caribensis f.</em></td>
<td></td>
<td>Niles Channel Flats</td>
<td>1</td>
</tr>
<tr>
<td>hermatypica (reef)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Chondrilla caribensis f.</em></td>
<td></td>
<td>Niles Channel Flats</td>
<td>1</td>
</tr>
<tr>
<td>*caribensis (mangrove/bay)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Ectyoplasia ferox</em></td>
<td>7/08</td>
<td>CVFI reef</td>
<td>12</td>
</tr>
<tr>
<td><em>Iotrochota birotulata</em></td>
<td>6/08</td>
<td>CVFI reef</td>
<td>12</td>
</tr>
<tr>
<td><em>Ircinia campana</em></td>
<td>6/08, 5/09</td>
<td>Niles Channel Flats</td>
<td>1</td>
</tr>
<tr>
<td><em>Ircinia felix</em></td>
<td>7/08</td>
<td>CVFI reef</td>
<td>12</td>
</tr>
<tr>
<td><em>Ircinia strobilina</em></td>
<td>7/08, 5/09</td>
<td>CVFI reef</td>
<td>12</td>
</tr>
<tr>
<td><em>Ircinia variabilis</em></td>
<td>6/08</td>
<td>Niles Channel Flats</td>
<td>1</td>
</tr>
<tr>
<td><em>Niphates digitalis</em></td>
<td>6/08, 5/09</td>
<td>CVFI reef</td>
<td>12</td>
</tr>
<tr>
<td><em>Niphates erecta</em></td>
<td>6/08, 5/09</td>
<td>CVFI reef</td>
<td>12</td>
</tr>
<tr>
<td><em>Smenospongia aurea</em></td>
<td>7/08</td>
<td>Blake’s Reef</td>
<td>21</td>
</tr>
<tr>
<td><em>Spheciopongia ve parium</em></td>
<td>6/08</td>
<td>Mote Flats</td>
<td>1</td>
</tr>
<tr>
<td><em>Tedania ignis</em></td>
<td>6/08, 5/09</td>
<td>Niles Channel Flats</td>
<td>1</td>
</tr>
<tr>
<td><em>Scopalina (Ulosa) ruetzleri</em></td>
<td>7/08, 5/09</td>
<td>CVFI reef</td>
<td>12</td>
</tr>
<tr>
<td><em>Xestospongia muta</em></td>
<td>6/08, 5/09</td>
<td>CVFI reef</td>
<td>12</td>
</tr>
<tr>
<td><em>Cliona varians f. varians</em></td>
<td>6/08</td>
<td>Mote Flats</td>
<td>1</td>
</tr>
</tbody>
</table>
Table 2. Summary of sponge morphological characteristics.

<table>
<thead>
<tr>
<th>Sponge sp.</th>
<th>Order</th>
<th>Family</th>
<th>Symbiont status a</th>
<th>$\delta^{15}$N b</th>
<th>$\delta^{13}$C b</th>
<th>Morphology</th>
<th>Depth of collection (m)</th>
<th>CC Density c</th>
<th>Collar Cell head width (µm) d</th>
<th>Spherical Index of CC (µm) d</th>
<th>Pumping Rate (L/s/kg) dry e</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agelas clathrodes</td>
<td>Agelasida</td>
<td>Agelasidae</td>
<td>HMA</td>
<td>4.98</td>
<td>-17.44</td>
<td>Lobate/tube</td>
<td>12</td>
<td>0.118±0.008 (n=22)</td>
<td>1.904±0.064 (n=13)</td>
<td>0.96±0.078</td>
<td>N/A</td>
</tr>
<tr>
<td>Agelas conifera</td>
<td>Agelasida</td>
<td>Agelasidae</td>
<td>HMA</td>
<td>4.46</td>
<td>-18.22</td>
<td>Lobate</td>
<td>12</td>
<td>0.126±0.008 (n=27)</td>
<td>2.122±0.106 (n=22)</td>
<td>1.03±0.053</td>
<td>0.395</td>
</tr>
<tr>
<td>Chondrilla caribensis f. hermatypa</td>
<td>Chondrosida</td>
<td>Chondrillidae</td>
<td>HMA</td>
<td>1.53</td>
<td>-18.87</td>
<td>Encrusting</td>
<td>12</td>
<td>0.217±0.017 (n=13)</td>
<td>2.197±0.072 (n=21)</td>
<td>1.16±0.100</td>
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<tr>
<td>Aplysina cauliformis</td>
<td>Verongida</td>
<td>Aplysinida</td>
<td>HMA</td>
<td>1.95</td>
<td>-17.90</td>
<td>Branching</td>
<td>12</td>
<td>0.128±0.015 (n=11)</td>
<td>2.370±0.049 (n=28)</td>
<td>0.96±0.061</td>
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<td>Smenospongia aurea</td>
<td>Dictyoceratida</td>
<td>Thorectidae</td>
<td>HMA</td>
<td>2.25</td>
<td>-16.56</td>
<td>Lobate</td>
<td>21</td>
<td>0.154±0.021 (n=8)</td>
<td>2.144±0.070 (n=15)</td>
<td>1.04±0.053</td>
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<td>Calyx podatypa</td>
<td>Haplosclerida</td>
<td>Phloeodictyidae</td>
<td>HMA</td>
<td>1.75</td>
<td>-17.78</td>
<td>Encrusting</td>
<td>21</td>
<td>0.202±0.014 (n=18)</td>
<td>2.256±0.056 (n=18)</td>
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<td>Amphimedon compressa</td>
<td>Haplosclerida</td>
<td>Niphatidae</td>
<td>LMA</td>
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<td>-17.15</td>
<td>Branching</td>
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<td>0.247±0.016 (n=25)</td>
<td>3.361±0.072 (n=42)</td>
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<td>Niphates digitalis</td>
<td>Haplosclerida</td>
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<td>LMA</td>
<td>4.20</td>
<td>-16.57</td>
<td>Vase</td>
<td>12</td>
<td>0.219±0.020 (n=14)</td>
<td>2.757±0.082 (n=19)</td>
<td>1.05±0.029</td>
<td>4.101</td>
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<td>Sphieiospongia vesparium</td>
<td>Hadromerida</td>
<td>Clionidae</td>
<td>LMA</td>
<td>4.44</td>
<td>-13.60</td>
<td>Massive/vase</td>
<td>1</td>
<td>0.302±0.015 (n=21)</td>
<td>2.372±0.069 (n=23)</td>
<td>1.13±0.074</td>
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<td>Scopalina ruetzleri</td>
<td>Poecilosclerida</td>
<td>Esperiopsida</td>
<td>LMA</td>
<td>5.77</td>
<td>-19.48</td>
<td>Encrusting</td>
<td>12</td>
<td>0.340±0.028 (n=8)</td>
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<td>Tedania ignis</td>
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<td>Myxillina</td>
<td>LMA</td>
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<td>0.219±0.016 (n=32)</td>
<td>2.385±0.034 (n=35)</td>
<td>1.10±0.045</td>
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</tr>
</tbody>
</table>

a Symbiont status indicates whether the species are in the HMA sponge group or the LMA sponge group.
b Measurements of $\delta^{15}$N and Avg. $\delta^{13}$C isotopic signatures (Weisz 2006).
c CC density indicates the proportion of mesohyl devoted to choanocyte chamber formation + SEM. n = total number of images examined.
d Collar cell head width indicates proportion of collar cell head width (10 collar cell heads were measured per image)+SEM. n = total number of images examined.
e Spherical index of CC indicates the x axis / y axis dimensions (10 CC were measured per image) to obtain an overall spherical index +SEM. An index closer to 1.0 represents a regularly circular CC.

*Pumping rate values included from Weisz et al. 2008 study.
Table 3. Results of sequence analysis of chosen DGGE bands from LMA sponges. Sponge samples collected from CVFI I (offshore reef site) Summerland Key, FL.

<table>
<thead>
<tr>
<th>Sponge Species (LMA)</th>
<th>DGGE band</th>
<th>Size (bp)</th>
<th>Closest sequence match in GenBank (BLAST)</th>
<th>Phylum</th>
<th>% homology</th>
<th>E-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Amphimedon compressa</em></td>
<td>Ac1</td>
<td>159</td>
<td>Uncultured γ-proteobacterium clone 929-C13</td>
<td>γ-Proteobacteria</td>
<td>94%</td>
<td>3e-61</td>
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<tr>
<td><em>Callyspongia plicifera</em></td>
<td>Cp1</td>
<td></td>
<td>Problem with sequence</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Callyspongia vaginalis</em></td>
<td>Cv1</td>
<td>139</td>
<td>Uncultured Chloroflexi bacterium clone A95</td>
<td>Chloroflexi</td>
<td>100%</td>
<td>8e-66</td>
</tr>
<tr>
<td><em>Iotrochota birotulata</em></td>
<td>lb1</td>
<td>132</td>
<td>Uncultured Sphingobacteriales bacterium clone HF10_31F02</td>
<td>Bacteroidetes</td>
<td>96%</td>
<td>5e-63</td>
</tr>
<tr>
<td><em>Niphates digitalis</em></td>
<td>Nd1</td>
<td>135</td>
<td>Uncultured Rhodobacteracea bacterium clone Ndsurf32</td>
<td>α-Proteobacteria</td>
<td>99%</td>
<td>6e-62</td>
</tr>
<tr>
<td><em>Niphates erecta 1</em></td>
<td>Ne1</td>
<td>135</td>
<td>Hyphomonadaceae bacterium GSW-23 Uncultured α-proteobacterium clone 3m04AISB5R</td>
<td>α-Proteobacteria</td>
<td>99%</td>
<td>6e-62</td>
</tr>
<tr>
<td><em>Niphates erecta 2</em></td>
<td>Ne2</td>
<td>130</td>
<td>Uncultured α-proteobacterium isolate DGGE gel band</td>
<td>α-Proteobacteria</td>
<td>99%</td>
<td>3e-60</td>
</tr>
<tr>
<td><em>Tedania ignis</em></td>
<td>Ti1</td>
<td>136</td>
<td>Uncultured bacterium clone Mann16S_E04</td>
<td>Unidentified</td>
<td>98%</td>
<td>8e-61</td>
</tr>
</tbody>
</table>
**VITA**

Ericka Poppell was born in Waterville, Maine on June 28th, 1980. She graduated from Lancaster High School in Lancaster, Virginia in 1998. She attended Virginia Commonwealth University in Richmond, Virginia and graduated with a Bachelor of Science degree in Biology in 2007. She entered the Master of Science in Biology program at the University of Richmond in the fall of 2007. At UR, she worked in an Evolutionary Ecology laboratory under the guidance of Dr. Malcolm Hill, Ph.D. She completed her Master of Biology in May of 2011.