UNIVERSITY of NEW HAMPSHIRE

Graduate School Dissertation Year Fellowship Application Cover Page

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Applicant Information					
Name:		**UNH ID** *(not SSN)*:			
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Current Program of study:					
Have you reached candidacy? (If no, indicate when)					
Dissertation Title:					

Referee Information	
Referee 1	
Name:	
Email:	
Title:	☐ Graduate Program Coordinator ☐ Dissertation Chair ☐ Dissertation Committee Member*
Referee 2	
Name:	
Email:	
Title:	☐ Graduate Program Coordinator ☐ Dissertation Chair ☐ Dissertation Committee Member*

*__PLEASE NOTE__: A dissertation committee member can only provide a letter of reference IF the Graduate Program Coordinator (GPC) and your Dissertation Chair are the SAME PERSON. If not, letters from both the GPC and Dissertation Chair are **REQUIRED**.

Statement of Significance

It is well known that microbes play an important role in the health of many important coral reef organisms such as corals and sponges; however, detailed information on the taxonomic and functional composition of many of these microbial communities is not known. My research on the ecology and physiology of the microbial community of the sponge *Xestospongia muta* will provide valuable insight into microbially-mediated nutrient cycling and host-microbe interactions. This information will be useful to scientists and managers who are working to better conserve and manage coral reefs, particularly in the face of environmental threats such as climate change.

Our understanding of the close, or symbiotic, relationship between microbes and important coral reef organisms has rapidly gained attention in the scientific community. Several studies have shown that complex microbial communities are in symbiosis with key reef members, including sponges and corals [1], and that these communities have functional roles in the ecology, biogeochemistry and overall health and functioning of coral reefs. Furthermore, it has been shown that these microbial communities respond to shifts in environmental conditions with potentially detrimental results to the host [2,3]. There is still much we do not know about the functional roles of many of these microbes or the fundamental relationship between the microbes and the host or even the full taxonomic and functional diversity of these microbial communities. Further characterization of these communities will improve our understanding of the healthy functioning of coral reefs and how reefs will respond to future environmental changes. In this regard, I propose to examine the taxonomic and functional diversity of microbes associated with the prominent reef sponge *Xestospongia muta*, in an effort to better understand physiological processes and nutrient cycling occurring on coral reefs.

Well documented that coral reefs around the world are being degraded due to multiple factors [2,4]. In a report by the World Resources Institute, it was documented that 75% of the world's coral reefs are considered threatened when local threats and thermal stress are considered [5]. One of the key findings of the report is that changes in climate and ocean chemistry (e.g., ocean acidification) represent significant and growing threats [5]. These threats not only affect corals but other vitally important coral reef members such as sponges, which influence the structure and function of coral reefs [6]. The sponge I propose to study, *X. muta*, is abundant on Caribbean reefs and due to the large size and the high abundance of this sponge, it is

likely that microbially-mediated nutrient cycling within *X. muta* influences nutrient availability on coral reefs in the Caribbean. A better understanding of the contribution of important reef members such as the sponge *X. muta,* and its symbiotic bacteria, to nutrient cycling on the coral reefs will increase our understanding of coral reef resilience to environmental changes.

Thus far 26 bacterial phyla and both major groups of Archaea have been documented in sponges [7], and this number continues to grow with the use of next generation sequencing technology (i.e., pyrosequencing). The use of metagenetics has revolutionized the way we view microbial diversity and its application to sponge microbial ecology has revealed previously unknown levels of bacterial diversity in sponges [7,8]. Metagenetics is particularly useful for documenting rare bacteria that would otherwise not be identified using cloning techniques, but may have important roles in sponge biology, nutrient transformations, and disease [8,9].

Of particular interest in coral reef ecosystems is the potential for microbially-mediated nitrogen transformations by sponges or their associated microbes. Nitrogen is a limiting nutrient in tropical waters and symbiotic microbes appear to have an important role in the availability of dissolved inorganic nitrogen (DIN) on coral reefs [1,10,11]. For example, nitrogen fixation adds 'new' nitrogen to the system by converting molecular nitrogen (N_2) to ammonia (NH_3), which can then be assimilated by organisms. For many sponges, which nitrogen transformations occur and the extent to which they influence nitrogen cycling in the sponge and on the reef is not known. Furthermore, there have been virtually no studies examining how environmental stressors may affect these nitrogen transformations.

My goals are to first characterize the microbial community of *X. muta* using a metagenetic approach with 454 pyrosequencing and to compare the community among different locations in the Caribbean (Florida Keys, Cayman Islands, Bahamas) and over a depth range of

approximately 9 – 91 m. I have recently performed a pilot study with one *X. muta* sample for pyrosequencing and have documented a diverse community of bacteria, including groups not previously observed in this sponge, and I have just received pyrosequencing data from a natural experiment where replicate sponge samples were collected from different geographic areas that I will be analyzing soon. I am also examining the potential for nitrogen transformations in *X. muta* using nutrient analyses and genetic tools including amplifying, cloning, and sequencing of relevant genes involved in nitrogen cycling (*nif*H, nitrogen fixation; *amo*A, nitrification; *nir*S, denitrification; 16S, anammox). Additionally, I will be sequencing the metatranscriptome (all of the mRNA) of the sponge microbial community, which captures at the time of sampling those genes that are active, which should provide insight into nitrogen cycling as well as other previously undescribed physiological functions or host-microbe interactions. This will be the first metatranscriptome study from a sponge microbial community to the best of our knowledge and will provide unique insight into the functional diversity of this community. My last goal is to analyze results of an environmental stress experiment, which was performed during the summer of 2011, where sponges were exposed to relevant increases in temperature and concentrations of CO_2 (and therefore differences in seawater pH) over 4 days in order to document any stress response by the sponge, and examine any changes in nitrogen transformations.

Methods

Sponge samples (pieces approx. 2 x 4 cm, n = 3 for each) have been collected from Little Cayman Research Center (LCRC) near Little Cayman, Cayman Islands, Lee Stocking Island (LSI), Bahamas, and Key Largo (Conch Reef), Florida. At LCRC samples were collected over a depth gradient of ~9-91 m, while at LSI samples were collected from ~9-61 m, and in Key

Largo, samples were collected from 15 m only. For pyrosequencing we will use MID tags (V6 and V8 regions) to amplify 16s rRNA genes of Eubacteria and Archaea, using replicate (n=3) *X. muta* samples and water column samples from each of the three locations (Florida Keys, Bahamas, Cayman Islands). Total RNA will be extracted using the Qiagen RNeasy kit and run on a Bioanalyzer (Agilent) to determine quality. All sequencing reactions (DNA and RNA) will be sequenced at the University of Illinois and we will collaborate with the Genome Center at UNH for assistance with bioinformatic data processing and analysis.

To examine nitrogen transformations that may occur in the sponge I will use PCR with primers specific to genes for each transformation as described above to provide genetic evidence for these processes in the sponge. Water samples (~ 30 ml) of the current will be collected from the osculum of the sponge and ambient water adjacent to the sponge and will be analyzed for NH_3, nitrite plus nitrate (NO_x) and phosphorous at the Woods Hole Oceanographic Institute, Woods Hole, MA.

Implications

A better understanding of the variability in the complex microbial communities inhabiting reef organisms and their response to stress will allow us to better predict how future environmental changes will impact coral reefs. The research I propose here will provide important insight into the symbiotic microbial community of one of the most prominent organisms found on Caribbean coral reefs, *Xestospongia muta* and the biogeochemistry of inorganic nitrogen occurring in this dominant member of Caribbean coral reefs. The results of this research will also be relevant to effective management and conservation of coral reefs in the face of increasing anthropogenic impacts.

References

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Schedule

[redacted] Department of Molecular, Cellular, and Biomedical Sciences, UNH

My work plan for the academic year 2012-2013 is developed based on the structure of my proposed dissertation entitled as "Characterization of the microbial community of the giant barrel sponge, *Xestospongia muta*."

May-July 2012	May-June: Submit manuscript for publication based on my work on the taxonomic characterization of microbial symbionts of the sponge *X. muta* (Chapter 2). Also, continue analysis on gene expression of microbial symbionts of *X. muta*, and finish fieldwork (Bahamas). July: Prepare manuscript for my work on the metatranscriptome (gene expression) of *X. muta* microbial symbionts (Chapter 3). Continue work on nitrogen cycling in the sponge and environmental stress experiment.
August-October 2012	Submit metatranscriptome manuscript for publication. Continue work on nitrogen cycling and environmental stress experiment, and prepare manuscripts for both.
Nov 2012-Jan 2013	Submit manuscripts on nitrogen cycling (Chapter 4) and the environmental stress experiment (Chapter 5) for publication. Work on manuscript revisions as necessary. Begin compiling manuscripts into dissertation format.
February-April 2013	Manuscript revisions, and compiling and formatting of dissertation. March 1-17: Complete reformatting Introduction/Chapter 1 (currently published as a review on nitrogen cycling in the marine environment) and finish compiling manuscripts into dissertation format. March 18-31: Submit draft dissertation to committee for review. Prepare powerpoint presentation for defense. April 1-14: Dissertation defense, revise and edit dissertation. April 15-21: Drop of dissertation draft copy for formatting review by the Graduate School. April 22-30: Present final copy of the dissertation to the Graduate School for binding.