NSF OCE 1608709 Biogeography of a marine defensive microbial symbiont: relative importance of host defense vs. abiotic factors

The type of data collected for Hypothesis 1 will be a combination of population survey, molecular, potential predation, and chemical defense data. Data from the surveys will be stored on servers at GSU. Duplicate voucher specimens preserved in ethanol will be stored in two -20°C freezers at GSU. For the Q-PCR, at least 3 technical replicate experiments will be performed with 10 biological replicates. Quality controls on real time Q-PCR data include analysis of variation between replicate samples and amplification efficiencies, and statistics will be performed on the data. The potential predation data will be stored on servers at GSU. Four strips will be deployed on each side of the docks. Experiments will be repeated 3 times per day for 5 days to account for temporal and tidal variation. Results will be analyzed using the appropriate statistical test (PASW Statistics 18). For comparison of chemical defenses in symbiotic and aposymbiotic *B. neritina*, 20-25 individuals of each type of predator will serve as replicates for each feeding assay. Consumption of control food items/pellets will be observed before and after each test food item or extract pellet is offered to ensure that the predator is willing to feed and is not satiated, respectively. Purified bryostatins will be stored at -80°C in the dark to prevent degradation. For bryostatin quantification, standard curves will be generated using multiple injections of known concentrations of purified bryostatins. Data on host/symbiont and predator species at each site will be deposited in the National Oceanographic Data Center (NODC), the Ocean Biogeographic Information System (OBIS), and the Biological and Chemical Oceanography Data Management Office (BCO-DMO) for public access.

For Hypothesis 2, survivorship and morphological data (numbers of zooids, ovicells) will be stored on both servers at GSU and dedicated hard drives. Six – 10 colonies of each type will be used as biological replicates. Results will be analyzed using the appropriate statistical test (PASW Statistics 18). For Hypothesis 3, survivorship, morphological, molecular, and microscopy data will be collected and stored on both servers at GSU and dedicated hard drives. These data will be deposited in the BCO-DMO and made available for public access.

All experimental data will be stored on servers at Georgia State University to which the co-PI (at Randolph-Macon College) will be provided secured access for uploading and retrieving data. We will store samples of symbiotic and aposymbiotic animals collected and generated in this study at -80°C. Excess DNA extracts will also be stored at -80°C. Original data and limited, available materials will be available for use by other researchers upon review and signature of a Materials Transfer Agreement by the GSU Offices of Legal Affairs and Research Integrity within a reasonable time frame. All DNA sequences obtained in this research will be deposited in the National Center for Biotechnology Information (NCBI).

The PI and Co-PI will hold monthly Skype meetings to discuss progress and plan future experimentation. The PI, Co-PI, post-doc, and graduate student and as many undergrads as possible, will meet either in VA or in NC for field work and experimentation once per year. The post-doc, graduate student, and the undergraduates will give presentations on their work and we will discuss results and future plans as a group. It is expected that the PI, post-doc, and graduate student will collect primarily from the Southern sites, and monitor NC transplants and mesocosms, whereas the Co-PI and RMC undergraduates will primarily collect from Northern sites, and monitor VA transplants.