Semi-Quantitative Analysis of a Defensin-Type Antimicrobial Peptide in Hemolymph from the Lobster *Homarus Americanus* as a Function of Molt Status

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This summer, I worked on a project in Elizabeth Stemmler's lab studying antimicrobial peptides (AMPs) in the American Lobster (*Homarus* americanus.) Antimicrobial peptides (AMPs) play an essential role in the nonspecific innate immune responses of lobsters. AMPs have potential implications in medicine and could help to further our understanding of crustacean immune systems. Unlike humans, lobsters do not have adaptive immune systems. Instead, they rely on an innate immune system that uses these AMPs to combat any pathogens that the lobster encounters.

AMPs, small, cationic molecules fight off bacterial pathogens by disrupting negatively charged bacterial membranes. This antimicrobial activity is especially important during the molt cycle, in which lobsters shed their protective exoskeletons and become more susceptible to infections. Climate change and commercial aquaculture practices have negatively impacted the temporality of the lobster molting process in recent years. Therefore, quantifying AMPs throughout different molt cycles serves as important groundwork for crucial future studies related to lobster health, especially in response to anthropogenic sources of stress and disease. The knowledge gained from studying AMPs can also provide useful insight for developing novel antibiotics that are more effective against resistant bacteria.

In the American lobster, AMPs are synthesized in hemocytes (blood cells) found in lobster circulatory fluid. So far, few lobster AMPs have been characterized and studied. So my main focus for this summer was employing various quantifying tools to the lobster AMPs we have characterize in our lab: one defensin-like peptide (*Hoa*-D1). Little is known about the changes to concentration in response to different stresses. In this study, we focus on the development of a semi-quantitative method for the analysis of *Hoa*-D1 to assess concentration changes throughout the *H. americanus* molt cycle to learn about lobster immunity via its AMP expression levels. The results provide an excellent resolution of *Hoa*-D1 from other cellular components and is being applied to the analysis of hemocytes from lobsters at different points in the molt cycle. I was surveying toddler lobsters that are house in the basement of Druckenmiller. After collecting blood sample (hemolymph), we isolated hemocytes and extracted the AMPs using Solid Phase Extraction (SPE) amongst other protocol used. Then, we used the HPLC to separate the different AMPS and identify *Hoa*-D1 and quantify it. This approach resulted in excellent resolution of Hoa-D1 from other cellular components and is being applied to the analysis of hemocytes from lobsters at different points in the molt cycle. We were able to find microscopic images and used Countess, an instrument that counts cells on a special slide, to determine if hemocytes concentration varies with molting cycle. Our initial comparison between

adults and toddler lobsters shows there is a difference, however furthermore analysis would provide more information. I would like to thank the Fellowship office and the Henry L. and Grace Doherty Charitable Foundation Coastal Studies Research Fellowship for making this project possible.