"The Quantification of Antimicrobial Peptides Throughout the Molt Cycle of the American Lobster, *Homarus americanus*"

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The American Lobster, *Homarus Americanus,* is an important species economically and culturally in the northeastern United States. According to the National Oceanic and Atmospheric Administration, 132 million pounds of lobster were caught in Maine in 2016 (roughly worth 540 million dollars). Because of the value of the American Lobster, their wellbeing and health are of interest in research. In recent years, there have been many mass die offs of lobsters due to disease. These mass deaths are linked to increased stress on the lobsters due to warming water temperatures (Pearce & Balcom, 2005). Therefore, understanding lobster immune systems could provide insight into this phenomenon.

Unlike people, lobsters do not have adaptive immune systems. Instead, they rely on an innate immune system to combat any pathogens that they encounter. This innate immune system is largely composed of antimicrobial peptides (AMPs) from hemocytes (blood cells) within their hemolymph (blood). In order to observe how these AMPs work within the lobster, I have studied changes in AMPs over the molt cycle of the American Lobster. The molt cycle is the process in which lobsters shed their old shells and continue to grow throughout their life. Because of the stress involved with this cycle, it is believed that immune system changes may occur including changes in AMPs. I hypothesized that AMPs could change in quantity or identity throughout this process. There could be more or less AMPs within the system or the types of AMPs could fluctuate.

In order to observe these changes, I had to develop a reliable way to determine which stage of the molt cycle a lobster was currently in. I developed a multiple criteria method in which I used shell hardness, hemolymph color, and pleopod staging in order to place a lobster within one of four molt stages. Pleopod staging involved observing the pleopods, or "swimmers", found under the tail of the lobster under a microscope in order to look for epidermal retraction. This broad approach allowed me to accurately place my samples within a specific molt cycle.

This grant has allowed me to gather these samples in preparation for continuing this project as my honors research this academic year. I am able to study these AMPs using samples of hemocytes harvested from lobster hemolymph. It was a priority to focus on sample gathering rather than analysis this summer due to adult lobsters completing the molt cycle only during the summer. Using flow cytometry this summer, I was able to look at the distribution of hemocyte within my samples through different stages (which could indicate AMP quantity changes). I was also able to use a hemocytometer to calculate a total cell count from these samples (another measure of AMP quantity). During the academic terms, I plan to use high performance liquid chromatography to quantify different types of specific AMPs present in my samples. Using all of these measures, I hope to create a full picture of the changes these AMPs may undergo throughout the molt cycle in relation to a possible increase in immune response due to stress. This information could contribute to our understanding on crustacean immunology, mammal immunology (we also have AMPs), and even possible advances in novel antibiotics by understanding this mode an organism defending itself against pathogens.

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References:

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