Does the Concentration of the Antimicrobial Peptide (AMP) *Hoa*-D1 Increase in Response to an Immune System Challenge to the Lobster, *Homarus americanus*?

Mia Cawley '27

Lobsters (*Homarus americanus)* play an integral role in the identity of Maine, acting as an important cultural and economic resource for coastal communities. Yet, with threats such as climate change, the lobster's immune system is increasingly vulnerable. As the temperature of oceans rises, lobsters are left more susceptible to disease. Unlike humans, lobsters do not have adaptive immunity to develop antibodies and protect themselves against environmental threats, pollutants, and diseases. Lobsters only have innate immunity. One way innate immunity can help to respond to stress and immune system challenges is through the release of antimicrobial peptides, or AMPs. These small, positively charged peptides are drawn to the negatively charged outer membrane of bacteria by electrostatic attraction. Then, the hydrophobic properties of the peptide allow it to penetrate the membrane of bacteria to destroy it. While these peptides are commonly found in all organisms, Professor Stemmler's research team has identified the only AMP reported in lobsters, called *Hoa*-D1 (Vu et. al 2018).

Over the summer I ran a time course experiment to discover if the concentration of the antimicrobial peptide *Hoa*-D1 changes in response to an immune system challenge. To challenge the immune system the lobster was injected with lipopolysaccharide (LPS). LPS is a sugar that simulates the effects of bacteria in lobsters. This acts as a stressor that may trigger an immune response in lobsters, such as an increase in the concentration of *Hoa*-D1 to combat the effects of LPS. Lobster hemolymph (blood) acts as an indicator of lobster health, so to test for the concentration of *Hoa*-D1, hemocytes (cells) from the lobster were collected, and anticoagulant was added to prevent the blood from clotting so that it could be analyzed. Plasma was then removed to isolate the cells, and AMPs were then extracted and analyzed using high performance liquid chromatography (HPLC). From here semi-quantitative analysis allowed the concentration of *Hoa*-D1 to be found. In this experiment there was one lobster that was injected with a combination of LPS and saline and one lobster that was injected only with saline to act as a control. Comparing the concentration of *Hoa*-D1 among lobsters that were injected with a bacteria simulant and lobsters that were not helped foster an understanding of the relationship between the concentration of AMPs and the lobster's immune system.

I found that three hours after LPS injection, the *Hoa*-D1 concentration in cells drastically decreased, and remained low for every other timepoint at which cells were collected. This suggests that as an immune response the *Hoa*-D1 was released from cells, confirming that this peptide plays an important role in the immune system of lobsters. In the future replication of this data would be useful in analyzing trends, especially for the control lobster injected with saline, which showed some variation in *Hoa*-D1 levels. Additionally, analysis of the plasma collected during the time course experiment could provide insight as to where the *Hoa*-D1 goes after it is released from cells.

Faculty Mentor: Professor Elizabeth Stemmler
Funded by the Henry L. and Grace Doherty Charitable Foundation Coastal Studies Research Fellowship

References

Vu GH, Do D, Rivera CD, Dickinson PS, Christie AE, Stemmler EA. Characterization of the mature form of a β-defensin-like peptide, Hoa-D1, in the lobster *Homarus americanus*. Mol Immunol. 2018;101:329-43.