

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

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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 ocean temperatures rise, 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 that 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. There, 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* in hemocytes (cells) found in circulatory fluid 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 from the lobster were collected, and anticoagulant was added to prevent the blood from clotting to allow for analysis. Plasma was then removed to isolate the cells, and AMPs were then extracted and analyzed using high performance liquid chromatography (HPLC) following an adaptation of a previously developed method to make use of fluorescence detection to increase sensitivity. From here semi-quantitative analysis allowed the concentration of *Hoa-D1* to be found. In each trial for this experiment there was one lobster that was injected with a combination of LPS and saline and one lobster that was injected only with lobster 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. Six trials of this experiment were conducted using two lobster pairs, meaning there were three data replicates for each pair. After each pair's first trial, all lobster health deteriorated, leading to inconclusive data for subsequent trials.

In the first trial for each pair, I found that one day after LPS injection, the concentration of *Hoa-D1* in cells drastically increased. This could suggest that as an immune response more *Hoa-D1* was produced in 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, as previous data showed a drop in *Hoa-D1* post LPS injection. Additionally, analysis of the plasma collected during the time course experiment could provide insight as to the function of *Hoa-D1* in the immune system of lobsters.

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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.