The Innate Immune System of the Lobster, *Homarus americanus*: Characterization of Hemocytes Throughout the Lobster Molt Cycle

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Lobsters have become an animal of significance in our society, specifically in Maine. Ecologically, they are mid-trophic consumers, making them part of the energy transfer from primary consumers to predators. In 2018, the lobster industry generated more than $500 million in Maine. It has also created around 39,500 jobs in Maine alone, as 80% of the lobsters in the United States come from Maine (Greene et al. 2020). Lobsters have also become integral parts of coastal communities. Like most animals, lobsters have stressors in their environment that make them vulnerable. These include global warming, pollution, diseases, and molting. Molting is the process that lobsters go through where they lose their shell and grow. When lobsters lose their shell, they become soft-shelled and extremely vulnerable to stressors. There are four stages of molting; intermolt, pre-molt, ecdysis, and post-molt. At different points in these stages, lobsters become more exposed and need to protect themselves in other ways.

The lobster immune system is of interest because they only have an innate immune system. This means that their responses are non-specific. Lobsters respond to immune stressors through different cellular functions. Their cells can perform adhesion (clotting), encapsulation (surrounding the foreign invader and killing it), or exocytosis (releasing certain defenses from the cell). In exocytosis, antimicrobial peptides can be released from the cell. Antimicrobial peptides are small, cationic peptides that are amphiphilic. They have the ability to bind to larger molecules and cause enough damage to kill them (Lei et al. 2019).

Lobster hemolymph (blood) is composed of three types of hemocytes (cell types). Granulocytes are the largest followed by semi-granulocytes and lastly hyaline cells. The Stemmler Lab has identified one antimicrobial peptide, called *Hoa-D1* that is localized to semi-granulocytes and granulocytes (Vu et al. 2018). There is still a lot to be found about the different compositions of each type of hemocyte.

This summer, I focused on optimizing the separation of the three hemocyte types in order to perform proteomics on each cell type to determine their protein composition. I used a Percoll density gradient to create layers with the cell types and separate them from one another and from the plasma in the hemolymph. This involved extracting hemolymph samples from lobsters and mixing them with an anticoagulant to prevent the hemolymph from clumping together. This mixture was placed on top of a pre-made Percoll gradient. This gradient had different densities of Percoll, a low viscosity liquid with colloidal silica particles that allow the cells to travel through the layers. The anticoagulated hemolymph was placed on top of the Percoll and centrifuged the cell types. The densest cells (the granulocytes) end up beneath the 80% Percoll layer, the semi-granulocytes were between the 80% and 60% layer and the hyalinocytes were between the 60% and the 30% Percoll layers. Any cellular debris and plasma were above the 30% layer.

The separation of the three cell types allows for further research to be performed on each cell type by extracting and analyzing AMPS and proteins. Shotgun proteomics, where the mixture of proteins is digested with trypsin and analyzed by mass spectrometry, was applied to the Percoll separated cell types. Data analysis is ongoing but provided preliminary evidence of differences in protein content. Learning about how the lobster hemocyte cell types differ can help to inform the understanding of how the lobster immune system responds to stressors.

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