Mass Spectrometric and Bioinformatic Analysis of Neuromodulators in *Libinia emarginata*

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Animal nervous systems must be incredibly flexible in order to create a response to a wide variety of internal and external stimuli. One type of process under control by the nervous system includes using central pattern generators to create rhythmic motor patterns, an action which could make the heart contract or push food through the digestive system. In turn, local nervous systems components regulate these patterns by selectively releasing neuromodulators, hormones and peptides whose expression has an effect on nervous system signaling [1]. Furthermore, while animals must possess a host of neuromodulators for different situations, the receptors for these, though not varying much in structure between closely related species, might respond differently to the presence or concentration of certain neuromodulators.

Crustaceans, the subphylum of Arthropods that contains organisms such as crabs, lobsters, and shrimp, make a model group of test subjects due to the fact that they have both an open circulatory system and high conservation among the pericardial organ and the x-organ–sinus-gland system, the neuroendocrine organs responsible for modulating the cardiac and stomatogastric neuromuscular systems [2]. The three species of crabs chosen for this study, *Libinia emarginata, Pugettia producta*, and *Chinoecetes opilio*, are all majoid crabs, belonging to the same genealogical superfamily [3,4]. While *Chinoecetes* and *Libinia* are both omnivorous scavengers, *Pugettia* exists by feeding almost exclusively on kelp [5].

Given the conservation of nervous system components in crustaceans, this experiment seeks to determine if differences in neuromodulation exist due to dietary habits, hypothesizing that *Chinoecetes* and *Libinia* must require more diversity in neuromodulation than *Pugettia* in the stomatogastric nervous system, which is responsible for the digestion of food, since they are omnivorous. As a control comparison, since the cardiac nervous systems should have to respond to similar stimuli among all three species, we hypothesize that there should be more conservation in neuromodulation here.

For my summer project, having adjusted to a completely remote format, I used a bioinformatics-based approach to predict the sequences for target neuropeptides and receptors in both the stomatogastric ganglion (STG) and brain transcriptomes of *Libinia* that had been assembled from previous dissections. Using query sequences gathered from *Homarus americanus* and *Carcinus maenas*, I referenced these against the *Libinia* transcriptome to find well-aligned RNA sequences. Following this, I used the online program ExPASy to translate these RNA sequences into amino acid (protein) sequences. Having identified the proteins, I used a variety of programs to analyze what family each protein belonged to as compared to previously characterized proteins, as well as post-translational analysis of the neuropeptide sequences. With all of this information I helped create a database of putative neuropeptide and receptor sequences for the *Libinia* STG and brain that can be used as a reference in future experiments.

Additionally, Professor Stemmler was able to run a fresh sample of *Libinia* brain tissue through LC-MS/MS, a specific type of mass spectrometry useful for its ability to handle larger molecules such as proteins, allowing for me to confirm or deny the presence of the target neuropeptides whose sequences I derived from bioinformatics. From the preliminary MS results, I was able to confirm the presence of CabTRP, Myosuppressin, and RPCH, while Proctolin and CCAP were absent from the sample. In the future, the Stemmler Lab plans to conduct more experiments with MS backed by the solid bioinformatic data. Additionally, there can be more collaboration between the Stemmler and Dickinson labs to compare results between *Pugettia, Libinia,* and *Chinoecetes*.

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