**Neuromodulation in the crabs *Pugettia producta* and *Libinia emarginata*: Neuropeptide Identification via Mass Spectrometry**

**Eva Dowd, 2022**

Neuropeptides are small protein-like structures produced and released by neurons across a wide variety of animal nervous systems (1). Because their release is regulated, neuropeptides have a modulating effect on their target cells, such as neurons, glial cells, and tissues, which manifests as changes in the behavior and cognition of individual organisms (1). These changes occur to "pattern-generators" in the central nervous system, which regulate rhythmic movements like walking (2). So, the impact of neuropeptides within these systems can be long term and adaptive (2).

Crustaceans, the taxon to which the crabs *Pugettia producta* and *Libinia emarginata* belong, have been used historically to study neuropeptides and have resulted in breakthroughs on their characterization and their role in the nervous system (2). The crustacean nervous system has a relatively simple structure, but it still allows for advanced behavior through its release of neuropeptides (2). Even removed from the organism, the crustacean nervous system still responds to neuropeptides when they are applied externally; under these conditions, it is possible to study the impact of various neuropeptides on system modulation.

The stomatogastric nervous system (STNS) is a useful place to study the effects of neuromodulators as its pattern generators are crucial for feeding and dietary behaviors in crustaceans (3). The stomatogastric ganglion (STG) is a piece of the STNS made up of just 30 neurons that control two pattern-generating circuits relevant to digestion (3). Our original purpose for this study was to investigate via RNA transcriptome and mass spectrometric (MS and MS/MS) analysis if the dietary differences between *P. producta* and *L. emarginata* resulted in either (1) different concentrations of neuropeptides in either the brain or the STG or (2) different physiological responses to neuropeptides applied to their nervous systems. Due to COVID-19, we were unable to be physically present in the lab this summer, so I primarily focused on RNA transcriptome analysis for *L. emarginata* and was able to do MS analysis for the *L. emarginata* brain only. With this data, we compared the neuropeptide results for the RNA transcriptome data with the MS and MS/MS data for the *L. emarginata* brain, in order to understand the presence of neuropeptides in the RNA versus in the brain tissue itself. We also compared the results of RNA transcriptome analysis of neuropeptides and receptors in the brain versus in the STG.

In our analysis of *L. emarginata,* we primarily looked at five neuropeptides and their receptors: proctolin, crustacean cardioactive peptide (CCAP), *Cancer borealis* tachykinin-related peptide (CabTRP), myosuppressin, and red pigment-concentrating hormone (RPCH). In the MS data for the brain, neither proctolin or CCAP were detected, although they appeared in the RNA transcriptome data. Interestingly, proctolin and CCAP have been shown to illicit very similar responses in the cardiac ganglion in *Cancer borealis*; perhaps their functional similarity caused them to disappear as a pair (4). CabTRP had the strongest presence in both the MS and the transcriptome data. Although all five neuropeptides appeared in the brain, only proctolin and CabTRP were detected in the STG transcriptome. Receptors for proctolin, CabTRP, CCAP, and myosuppressin were present, but not for RPCH. This lack of RPCH receptors is interesting as this neuropeptide was shown to have an impact on the STG in *Cancer borealis* (5).

In the future I hope to be able to perform more MS and MS/MS analysis to confirm these RNA transcriptomics findings.

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