

Investigating Neuropeptides and Receptors in *Pugettia producta*: a Bioinformatics and Transcriptome Data Mining Approach
Isabel Petropoulos, Class 2023

During the summer of 2020, I worked with Professor Dickinson using bioinformatics and transcriptome data mining to explore the role peptide and receptor sequences (in the brain and stomatogastric ganglion (STG)) play as a possible contributing mechanism for behavioral flexibility in the crab *Pugettia producta*. Behavioral flexibility is the ability an organism has to interact appropriately with changes in its environment and is mediated by modulation.

While I specifically looked at the crab *Pugettia*, other students in the Dickinson Lab focused on *Libinia emarginata*. Both crabs are part of the same superfamily Majoidea, but *Pugettia* is a specialist feeder while *Libinia* is an opportunistic feeder. Due to the limited diet of *Pugettia*, the Dickinson lab predicted that *Pugettia* has less neuromodulation in the STNS (stomatogastric nervous system) which controls the foregut movement necessary for feeding, compared to opportunistic feeders such as *Libinia*. Physiological recordings confirm that *Pugettia* is less responsive to exogenously applied neuromodulators than opportunistic feeders. My research this summer took the first step in determining whether the differential expression that was observed during physiological recordings in *Pugettia* is due to the differences in ability to respond to modulators. Bioinformatics and transcriptome data mining enabled us to explore and determine sequences of neuropeptides and receptors in the brain and STG that can be used in the future to test this hypothesis, consider how these sequences differ between *Pugettia* and *Libinia*, and tentatively predict the processing of neuropeptides.

Regarding our techniques, we followed a simple bioinformatics workflow focusing on five neuropeptides and receptors for those peptides. Each neuropeptide or receptor search began by using protein query sequences collected from previously annotated brain and eyestalk transcriptomes to search a public access CLC BLAST search tool (University of Hawaii). The BLAST returned sequences (notated by TRINITY numbers) that we used to search the *Pugettia* transcriptome. The *Pugettia* transcriptomes (brain and STG) allowed us to have a snapshot of the RNA present in *Pugettia* and determine which proteins *Pugettia* cells are expressing. I then translated the nucleotide sequences gathered from the transcriptome to a protein sequence (Expasy translate tool). Homology- based BLAST searches (Flybase and NCBI non-redundant arthropod protein BLAST) were then used to validate the identity of the sequence searched. We then utilized other online programs and databases to further analyze the structural features of the neuropeptide and receptor protein sequences. Below is a table summarizing some of my results.

Neuropeptide (Brain)	Neuropeptide STG	Present (P) Not Present (NP)	Bioactive Peptide (Brain)	Bioactive Peptide (STG)	Receptor (Brain)	Receptor (STG)	Present (P) Not Present (NP)	Confidence
Proctolin	Proctolin	P/P	RYLPT	RYLPT	Proctolin	Proctolin	P/P	Moderate
Myosuppressin	Myosuppressin	P/NP	QDLDHVFLRFG	N/A	Myosuppressin	Myosuppressin	P/P	Moderate
CCAP	CCAP	NP/P	N/A	PFCNAFTGCG	CCAP	CCAP	P/P	High
CabTRP	CabTRP	P/P	APSGFLGamide	APSGFLGMRamide, TPSGFLGMRamide	CabTRP	CabTRP	P/P	High
RPCH	RPCH	P/NP	pQLNFSPGWamide	N/A	RPCH	RPCH	NP/NP	Low

I am extremely grateful to Professor Dickinson, Professor Stemmler, the Student faculty research fellowship, and Bowdoin’s resources for the opportunity to be part of this exciting project.

Faculty Mentor: Patsy S. Dickinson

Funded by the Student faculty research fellowship supported by the National Science Foundation