

# Investigating the roles of neuropeptides on gut contractions in the American lobster *Homarus americanus* and in the red swamp crayfish *Procambarus clarkii*



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## INTRODUCTION

Neuropeptides modulate physiological processes. Although many have been identified and their effects on the organism have been determined, there are many neuropeptides whose roles have yet to be determined.

In crustaceans, little information is known about the role that neuropeptides play in controlling musculature in the gut region. Determining the effects of different neuropeptides on the gut region is important for controlling gut movements and for better understanding the physiological processes of the gut.

## BACKGROUND

### Lobster (*Homarus americanus*)

- Infraorder: Astacidea
- Decapod crustacean
- Lives in saltwater
- Average length: 12 inches



*H. americanus*

### Crayfish (*Procambarus clarkii*)

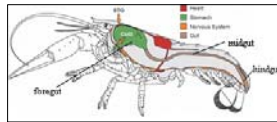
- Infraorder: Astacidea
- Decapod crustacean
- Lives in freshwater
- Average length: 3 inches



*P. clarkii*

### Crustacean Anatomy

- the gut region is divided into three parts:
  - foregut
  - midgut
  - hindgut



### Neuropeptides

- small segments of proteins, composed of amino acids
- signaling molecules found in the nervous system of organisms
- activate and inhibit physiological processes
- effects are important when studying regulation

### Orcokinin

- family of neuropeptides with a conserved N-terminal region
- NFDEIDRSGFGFN [Asn<sup>13</sup>]
- First discovered in the crayfish *Orconectes limosus*
- Found in the nervous system, the brain, and the hindgut of *H. americanus*
- Found in the brain of the crab *Cancer borealis*
- Stimulates gut contractions in *O. limosus*



### CabTRP Ia

- Full name: *Cancer borealis* tachykinin-related peptide Ia
- APSGFLGMRamide
- Found in the midgut of the crabs *C. magister*, *C. borealis*, and *C. productus*.
- Unknown function physiologically, but hypothesized importance in feeding behavior of fed versus unfed crabs

### Val<sup>1</sup>-SIFamide

- VYRKPPFNGSIFamide
- Found in the brain, the stomatogastric nervous system (STNS), and the posterior midgut cecum (PMC) of *H. americanus*
- Affects part of the STNS that controls food flow from the foregut to the midgut

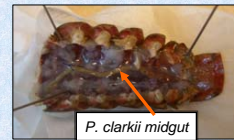
## OBJECTIVE

To determine the effects of orcockinins, CabTRP Ia, and Val<sup>1</sup>-SIFamide on the mid and hindgut of lobster and crayfish.

If activation or inhibition is found, to determine what these results mean for feeding patterns.

## METHODS

- The animals were anesthetized with ice for 15 to 30 minutes.
- The tail was removed and was pinned down in a dissection dish in physiological saline (10-12°C for lobster, 18-20°C for crayfish).
- The ventral side of the animal and the skeletal muscle was removed to expose the midgut and the hindgut.

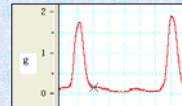


*P. clarkii* midgut



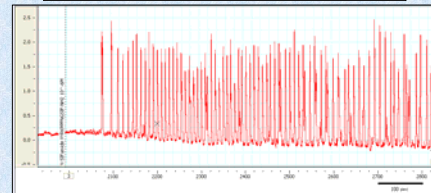
Force transducer

- A string was tied around the midgut of the animal and was connected to a force transducer.
- The force transducer converted mechanical movements of the string into a recording on the computer.
- Peptides at a concentration of 10<sup>-6</sup>M were applied and movements were recorded.
  - Proctolin as a positive control
  - Orcokinins, CabTRP Ia, Val<sup>1</sup>-SIFamide



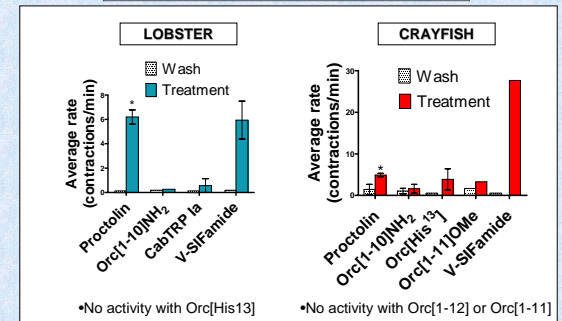
## RESULTS

Representative Trace of Mid and Hindgut Contractions  
*H. americanus*



Val<sup>1</sup>-SIFamide at a concentration of 10<sup>-6</sup>M stimulates gut contractions

## SUMMARY OF RESULTS



•Proctolin and Val<sup>1</sup>-SIFamide at a concentration of 10<sup>-6</sup>M increase the average contraction rate in both *H. americanus* and *P. clarkii*.

## CONCLUSIONS

- Val<sup>1</sup>-SIFamide at 10<sup>-6</sup>M increases the average contraction rate for the lobster and crayfish
- Orcokinins seem to activate the gut of the crayfish, but not the gut of the lobster
- More replicates needed to determine role of CabTRP Ia

## FUTURE WORK

- Complete testing of orcockinin [Asn<sup>13</sup>] to determine its effects on the mid and hindgut of the lobster and crayfish
- Isolate midgut from hindgut to test individual responses
- Couple immunohistochemistry with MALDI-FTMS for identification and localization of orcockinin, CabTRP Ia, and Val<sup>1</sup>-SIFamide in the mid and hindguts of lobster and crayfish

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