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The goal of this research project was to gain a greater understanding of the metamorphic cue of the serpulid polychaete *H. elegans*. These polychaetes are a common organism found in biofouling communities throughout tropical and subtropical marine harbors (see Nedved and Hadfield 2009 for review). *H. elegans* are one of the most troublesome biofouling organisms and are thus an ideal model organism (ten Hove 1974; Hadfield et al. 1994; Nedved and Hadfield 2009). Working as an EDEN intern this summer allowed me to expand upon my previous preliminary work at the Kewalo Marine Laboratory under the guidance of Dr. Michael G. Hadfield. This summer I was able to prepare a manuscript focusing on the effect of catecholamines in the metamorphosis of *H. elegans*, as well as learn developmental biology techniques to study the genes involved in the metamorphic response. My research last summer focused on the effect of catecholamines on the settlement and metamorphosis of *H. elegans*, using settlement assays to test various compounds present in the biosynethic pathway of invertebrates.

First, I tested the effect of the catecholamine breakdown product, hydrogen peroxide (H_2O_2) on the metamorphosis of *H. elegans*. A major difficulty in using artificial inducers is due to the fact that in alkaline aqueous solutions, catechols rapidly oxidize to quinones (Pires and Hadfield 1991). This reaction produces hydrogen peroxide, which has been shown to induce partial metamorphosis in other marine invertebrates. In my settlement experiments this summer with *H. elegans*, I was able to determine hydrogen peroxide caused toxic and lethal effects over the range of 10^{-4} M to 10^{-5} M, and concentrations 10^{-6} M and lower had no effect on the larvae, thus hydrogen peroxide is not an inducer of metamorphosis for this organism.

My preliminary research from last summer proposed that the metamorphic cue could be induced through treatments of L-DOPA, dopamine, epinephrine, and norepinephrine. This summer I expanded upon this by testing a variety of α- and ß-adrenergic receptor antagonists and agonists. By testing a variety of chemicals that mimicked or blocked the biological response, I was able to determine that the metamorphic cue in *H. elegans* is stimulated by action on the α-adrenergic receptor. The studies I was able to complete as an EDEN scholar allowed me to prepare a manuscript on the data I collected.

I used the computer software Geneious to create a protein alignment of several different genes including tyrosine hydroxylase, which is the enzyme in the biosynthetic pathway responsible for converting L-Tyrosine to L-DOPA; for adrenergic receptors (including both α- and ß- genes); and for dopamine. I used the GenBank sequences of approximately 20 organisms, including several closely related invertebrates, to create this alignment. Using this alignment, I made degenerate PCR primers to try and obtain bands for the genes involved.

The next steps involved culturing larvae in the lab according to the protocol established by Nedved and Hadfield (2009). I did a TriReagent RNA extraction (see protocols) on competent larvae at 6 days old. PCR analysis was run on the cDNA generated. I ran degenerate primer PCR on this cDNA using a temperature gradient on the PCR machine and run gel electrophoresis to identify bands. I was able to obtain bands for tyrosine hydroxylase as well as for the adrenergic receptor. The tyrosine hydroxylase bands were apparent at 50˚ (Fig. 1) while the adrenergic bands were apparent at 62˚. I prepared and submitted my tyrosine hydroxylase product for direct DNA sequencing at the University of Hawaii, however due to the fact that there are very likely multiple sequences in this product, the sequence did not come out clear. The next steps involve cutting out these bands in the gel and cloning the product. The post doctorate, Dr. Brian Nedved, is hoping to finish this study and clone these bands I obtained this fall. If I could continue my work at the lab, I would like to clone the products I obtained for the tyrosine hydroxylase as well as the adrenergic receptor. In conclusion, the research I was able to do this summer as an EDEN scholar was critical in helping me learn valuable developmental biology skills and helped me immensely in gaining experience as a researcher.

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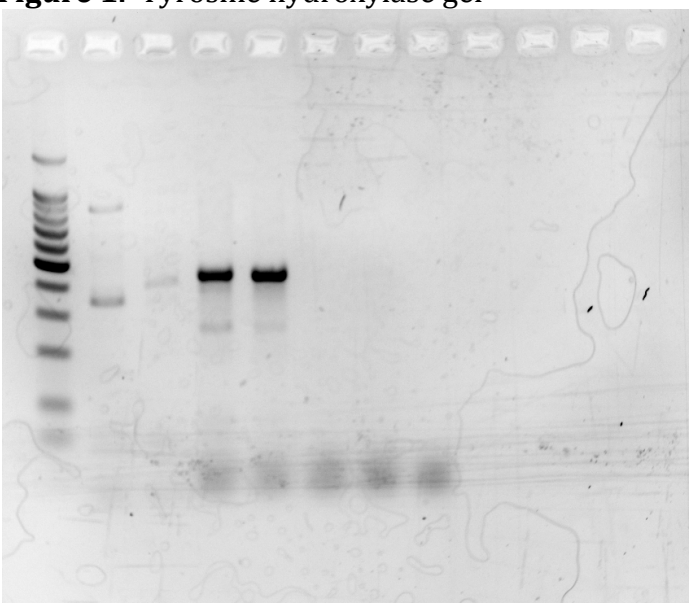
Figures

Figure 1. Tyrosine hydroxylase gel



From left to right: DNA ladder, cDNA postive control using Tubulin primers, negative control using water and Tubulin primers, tyrosine hydroxylase at 50˚ with a 10x PCR product concentration from round 1, tyrosine hydroxylase at 50˚ with a 50x PCR product concentration from round 1, tyrosine hydroxylase at 50˚ with water as a negative control.