The Role of Molluscan Insulin-Related Peptides in Regulating the Phenotypic Plasticity and Response to Predators in Snails

Introduction: Researchers have long been interested in the effects predator prey interactions have on the stability and efficiency of ecosystems (Volterra, 1926). Increasing the number of predators or the number of prey not only affects the prey and the predator directly, but other organisms connected in the food web (Volterra, 1926). Predators are most often thought to have lethal effects on prey, but they can also exert non-lethal effects such as reallocating energy to avoid predators resulting in decreased reproduction (Lima, 1998) and altering phenotypic characteristics to increase the odds of survival (Salice and Plautz, 2011). For example, in many taxa ranging from tadpoles to fish to snails, interactions with predators can alter morphological and physical characteristics (Relyea, 2001). Snails, specifically, exhibit increased shell thickness, decreased aperture size, and decreased reproduction after being exposed to crayfish, which are natural predators to snails (Salice and Plautz, 2011). These phenotypic changes increase the crush resistance of snail shells, which enhances the snail's defense against predators. Although the morphological and physiological response to predators has been well described in the literature (Lima, 1998, Salice and Plautz, 2011), the mechanistic regulation of phenotypic plasticity in snails is unclear. In mammals, growth and development is regulated by hormones, including insulin (Hamano *et al,* 2005). In snails, similar hormones known as molluscan insulin-related peptides (MIPs) are suggested to determine growth and reproduction (Hamano *et al,* 2005). Hence, I hypothesize that as snails are exposed to predation, MIP gene expression will increase because MIP regulates growth, reproduction, and shell morphology in snails.

Methods

Experimental Design: To test my hypothesis, I will compare snails living in the presence or absence of predator cues. I will create a "predator cue" by combining crayfish water with crushed snails to stimulate a predator response in snails (*Physa* acuta; Salice and Plautz, 2011). I will collect crayfish from Teal Ridge Wetlands in Stillwater, Oklahoma, and house them in 10 L glass aquaria. I will take three snails 3-4 mm long from a culture stock and place the snails in a glass jar with 500 mL of dechlorinated tap water; each jar will represent an experimental unit (n=8 units per treatment). I will renew snail water 3x a week, and add 1 mL of predator cue to the predator cue treatment with each renewal. I will feed snails cooked lettuce *ad libitum*, supplemented with spirulina and tetramine (Salice and Plautz, 2011). Morphology, growth, and reproduction will be measured weekly, while gene expression will be measured after 21 and 35 d (n = 8 units per treatment per time-point) post exposure to the predator cues.

Growth, Shell Morphology, Reproduction: I will measure change in snail mass using a digital scale, and measure shell thickness and aperture size using digital calipers. Shell width and length will be determined using a digital microscope to take a picture of the snail, then analyzed using ImageJ software. I will measure reproduction by counting egg casings and the number of eggs in each casing (Salice and Plautz, 2011). I will test for differences in snail size and reproduction between treatments using a repeated-measures ANOVA.

MIP Gene Expression: I will homogenize snail soft tissue and isolate total RNA using methods validated in our lab. Total RNA will be reverse transcribed into cDNA. Using primers developed in our lab, I will quantify gene expression by RT-qPCR. Data will be analyzed using a two-way ANOVA.

Expected Results and Conclusion: I expect to find a relationship between increased predator cues and MIP gene expression. Snails exposed to predator cues will produce more MIP, resulting in increased growth, changes in shell morphology, and possibly a change in reproduction. While studies have individually looked at how an increase in predator cues or an increase in MIP affect snail's growth, this will be the first study to examine how predator cues effect MIP, and evaluate whether this hormone regulates phenotypic plasticity in snails.

References:

Hamano, K., Awaji, M., Usuki, H. 2005. cDNA structure of an insulin-related peptide in the Pacific oyster and seasonal changes in the gene expression. *Journal of Endocrinology*. 187:55-67

Lima, S. L. 1998. Nonlethal Effects in the Ecology of Predator-Prey Interactions. *BioScience*. 48(1):25-34

Relyea, R. A. 2001. Morphological and Behavioral Plasticity of Larval Anurans in Response to Different Predators. *Ecology*. 82(2):523-540

Salice, C., J., Plautz, S. C. 2011. Predator-induced defences in offspring of laboratory and wild-caught snails: prey history impacts prey response. *Evolutionary Ecology Research.* 13: 373-386

Volterra, V. 1926. Fluctuations in the abundance of a species considered mathematically. *Nature.* 118:558-560