Changes in Anti-Predatory Behavior after Corticosterone Treatment in *Rana clamitans* Tadpoles

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Allegheny College

Department of Neuroscience

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A Senior Comprehensive Project in Partial Fulfillment of the Requirements for a Bachelor of Science Degree from Allegheny College

I hereby recognize and pledge to fulfill my responsibilities as defined in the Honor Code and to maintain the integrity of both myself and the College community as a whole.

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Pledge

Approval by the Senior Thesis Committee:

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Dr. Mumme

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Dr. Clark

Abstract

The hormone corticosterone has an inhibitive effect on growth in tadpoles. This protein, when given in excess, causes tadpoles to grow shorter than normal. Tadpoles have a standard method for responding to a predator, increasing distance from the predator and reducing movement. This response may or may not be affected by internal hormone levels and tail length. In this experiment, I manipulated corticosterone levels in tadpoles of the green frog, *Rana clamitans*, in order to study the anti-predatory behavioral changes caused by the excess hormone. Tadpoles were housed in one of two levels corticosterone solution or in fresh water before being presented with a predator, *Anax junius*, the common green darner dragonfly larvae. There was a marginally significant ($p = 0.0675$) effect of the CORT on tadpole distance from the predator. Low CORT tadpoles maintained a farther distance from the *Anax* than the control tadpoles, while the high CORT tadpoles maintained a closer distance than the controls. Results suggest a non-linear threshold effect of CORT on anti-predator behavior in green frog tadpoles.

Introduction

Rana clamitans, known as the green frog, is a species of frog native to the eastern half of the United States and Canada. Generally, green frogs can be found close to water, in ponds, marshes, streams, and rivers, but during heavy rainfall, they may move into wooded areas. Green frog larvae have a variety of predators, with dragonfly larvae, *Anax longipes* and *Anax junius* (Marino and Werner, 2013), and fish, *Lepomis gibbosus* (Szuroczki and Richardson 2012), being the most commonly used experimentally. In the presence of a predator, tadpoles can be

found avoiding areas where the predator was seen, as well as hiding in the vegetation, but other behaviors have been associated with response to a predator. Brief bouts of fast swimming with little displacement, sharp twisting of the body in any direction, and flicking of the tail are also behavioral responses to a predator (Szuroczki and Richardson 2012).

Tadpoles of most species of frogs are able to detect chemical and visual signals, either from a predator or other tadpoles, which warn them that a predator is nearby. Not all tadpoles react in the same way, but the way most tadpoles respond to predatory cues is to reduce movement and stay at a distance from where they detected the presence of a predator (Kiesecker et al. 1996). There are many types of predators for tadpoles, the most common being fish (bass, trout, carp), birds (herons, kingfishers), and insect larvae.

Kiesecker et al. (1996) exposed tadpoles to either chemical or visual cues, or both, to determine a baseline anti-predation response in *Bufo boreas* to multiple possible predators – *Notonecta* spp. (backswimmers), *Lethocerus americanus* (waterbugs), *Thamnophis sirtalis* (garter snakes), *Taricha granulosa* (roughskin newts), and *Oncorhynchus mykiss* (rainbow trout). The *B. boreas* tadpoles responded to chemical cues from backswimmers, waterbugs, and garter snakes, but not to the newts or trout. Visual cues produced little to no response, mainly to garter snakes. Chemical cues are the primary source for predator recognition, and the tadpoles were able to differentiate between predatory and non-predatory chemical signals. Other species of tadpoles, *Rana temporaria* and *Bufo bufo*, have exhibited anti-predatory responses to chemical cues from water that has been conditioned with predators that were fed different diets (Marquis et al. 2004). These tadpoles reduced their physical activity significantly more when presented with chemical cues from crushed conspecifics or water conditioned with a

predator that was fed conspecific tadpoles. Predators, as well as other prey, release chemical signals that notify tadpoles of a potential threat in the region.

Growth and stress hormones can affect a tadpole's response to a predator. Corticosterone (CORT) is a steroid hormone that is involved in stress response. Inducing stress, as well as externally manipulating CORT levels, are effective at increasing internal CORT levels in Pacific Treefrog (*Hyla regilla*) tadpoles (Belden et al. 2005). Externally manipulating CORT levels is a method to decrease growth rate in some species of tadpoles (Hasan and Ahmed 2012). After being housed in solutions of CORT, *Bufo melanosticus* tadpoles grew an average of 4.32 mm shorter than tadpoles kept in fresh water. Length of the tadpole, specifically tail length, is important for tadpoles attempting to escape a predator (Maher et al. 2013). Long-term exposure to external CORT caused tadpoles to grow longer tails, and these tadpoles also had higher survival rates than tadpoles that were given short-term treatments with CORT.

Growth hormone (GH) and prolactin (PRL) are proteins that can induce growth in tadpoles. Intraperitonial injections of mammalian prolactin and growth hormone resulted in tadpoles that were longer than non-injected tadpoles (Brown and Frye 1969). Between the two mammalian proteins, prolactin caused tadpoles to grow longer than growth hormone. However, in *Rana temporia* tadpoles, ovine growth hormone was more effective than ovine prolactin at stimulating growth (Enemar et al., 1968). Behavior is also affected by growth hormone. Trout that had been treated with ovine GH had higher foraging rates, were more active, and displayed less predator avoidance (Johnsson et al., 1996).

Predators can evoke morphological and behavioral responses in some animals. *Rana pipiens*, Northern leopard frog tadpoles, when exposed to caged predators regularly, grew

larger tail fins (Hossie et al. 2010). Tadpoles that were treated with metyrapone, a corticosterone synthesis inhibitor, and exposed to the caged predator had smaller tail fins. These physiological changes suggest that corticosterone plays a role in the tadpoles' response to predator risk.

In this experiment, I treated greenfrog tadpoles with corticosterone. Exposure period to CORT was short, therefore effects on growth were minimal. After the treatments, the tadpoles were exposed to a predator and their behavior was compared to that non-treated tadpoles.

Tadpoles and Predators

73 *Rana clamitans* tadpoles were collected from ponds in Crawford County, Pennsylvania. Tadpoles were fed fish food flakes. Six predators, all *Anax junius* (Common Green Darner dragonfly) larvae, were collected from the same pond were the tadpoles were collected.

Testing Tanks

10 gallon glass tanks were used to house and test the behavioral response in the tadpoles. One side of the test tank contained a mesh cage to store the dragonfly larva while testing. The bottom of the tank had distance markings to estimate the distance from the tadpoles to the predator.

Hormones

Corticosterone was used to induce tadpole growth. There were two experimental groups, 0.1 μg/mL and 0.5 μg/mL CORT. Tadpoles were housed in the CORT water for 17 days prior to testing. Water was freshened with these solutions every two days.

Testing.

Tadpoles were individually exposed to dragonfly larva in the testing tanks for a period of 5 minutes. Predators were placed in the test tank 20 minutes prior to testing in order to ensure chemical cues from the dragonfly larvae filled the tank. Estimated distance from the predator to the tadpole will be recorded once each minute. Testing trials took place on October 9th, 11th, and 14th.

Results

A total of 45 tests were run on randomly selected *Rana clamitans*. The greenfrog tadpoles responded to exposure to *Anax junius* by avoiding the predator. Although a makeshift shelter was used originially to give the tadpoles the option of hiding or keeping a safe distance, it had to be removed as a result of all the tadpoles choosing to hide in the shelter.

Time spent in each treatment tank over the course of testing did not have a significant overall effect on the tadpoles' distance from the predator (Figure 1). From the first test trial to the third, the control and low CORT tadpoles kept a steady average distance from the predator, their trendlines are about parallel to the x-axis. High CORT tadpoles saw a small increase in average distance between the first and last trials. There were marginally significant differences ($p = 0.0675$) between treatment groups. Tadpoles in the control group stayed at an average distance of 6.31 inches from the predator. The low CORT tadpoles maintained a farther distance of 6.91 inches, while the high CORT tadpoles came the closest to the predator with an average distance of 6.03 inches (Figure 2).

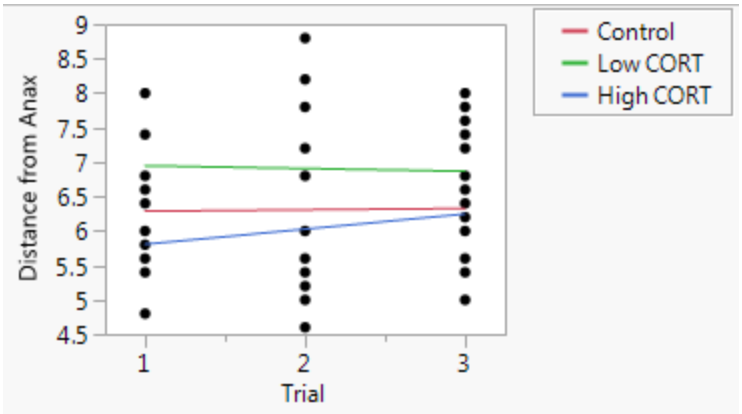


Figure 1. Distances of all tadpoles from predator, separated by trial date.

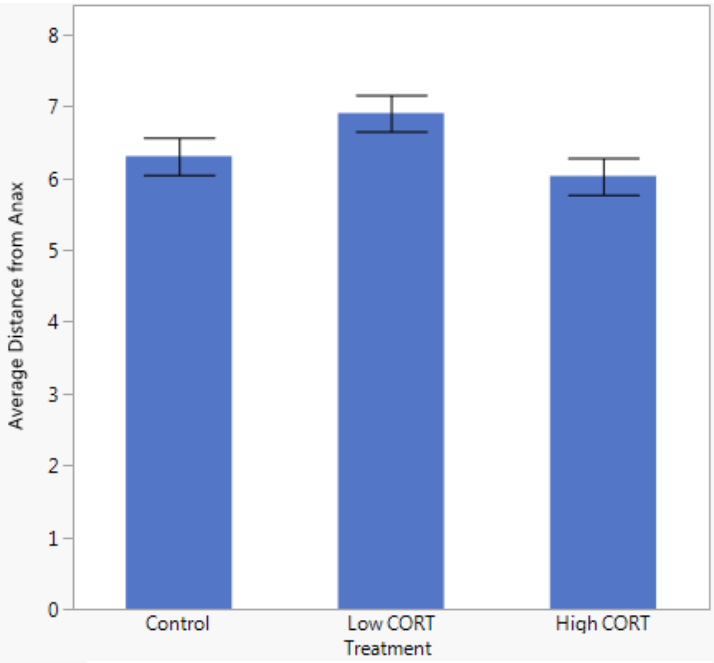


Figure 2. Average distance of the tadpoles from each group and treatment date. Error bars represent the standard error.

Discussion

It is known that corticosterone is a growth inhibitor in some species of tadpoles (Belden et al. 2005). Growth inhibition by corticosterone includes both length and body mass reduction when compared to tadpoles with natural corticosterone levels. Behavioral effects of exogenous corticosterone were tested in this study, with no significant results confirming the hypothesis

that the corticosterone-treated tadpoles would maintain a farther distance from a predator than an uninhibited tadpole.

The study did reveal what could be a non-linear dose-dependent effect on behavior, where a higher than normal level of corticosterone could produce the desired effect, while too much could have an opposite effect. The low CORT (0.1µg/ml) group of tadpoles maintained a longer distance from the *Anax* than the control group, while the high CORT (0.5µg/ml) group remained closer to the predator than the control. A similar, although reversed, effect is seen on mass of *Rana pipiens* tadpoles treated with Growth Hormone (GH) (Brown and Frye 1969). This study observed the effects of prolactin and growth hormone on metamorphosis of the frog tadpoles. The hormones delayed metamorphosis and decreased the length and mass the tadpoles grew to before metamorphosis. From 0.5µg GH to 1.0µg GH, there is a drop in the effect on mass, but when increased to 5.0µg GH, the tadpoles showed an increasing change in mass. There could be a threshold that changes the effects of certain hormones. Thyroxine is hormone that stimulates synthesis of carbamyl phosphate synthetase in *Rana catesbeiana*. There is a linear effect with dosage, up until 0.05 µg thyroxine/ml, and above that level there is a spike in death (Paik and Cohen 1959). A hormonal threshold could explain the increase in distance from the control to the low CORT group and the decrease in distance from the control to the high CORT group.

During testing, the problem of the shelter being too accommodating to the tadpoles arose, which could have had an effect on the tadpoles. Removal of the shelter decreased similarities to a natural setting where there are places for the tadpole to hide. This was

necessary in order to obtain the data, but a more realistic test tank could yield more compelling results. Without a shelter to hide under, the tadpoles have less options when responding to a risk, which could elicit unnatural responses. Future experiments could use a more natural mesocosm during testing to allow the tadpoles to react in a more natural manner.

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