The Effects of 17β-Estradiol on Gonadal Morphology in *Acris blanchardi*

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Abstract: Endocrine disrupting compounds, like 17β-estradiol (E2), contaminate wildlife habitats,which leaves organisms vulnerable to compounds that have adverse effects on their gonadal development. Amphibians are the most vulnerable to these compounds due to the high permeability of their skin. In our study, we will be observing the effects 17β-estradiol has on the gonadal morphology in *Acris blanchardi*. The frogs will be exposed to E2 concentrations dependent on the treatment group (concentrations) throughout the larval phase until metamorphosis. With increasing concentrations of E2 we expect to see a movement towards complete sex reversal to phenotypic females marked by varying other gonadal abnormalities.

Keywords: Gonadal Morphology, Sex Reversal, Estradiol, E2, Mesocosm

Introduction

Endocrine disrupting compounds (EDCs) can have a detrimental effect on wildlife as anthropogenic contaminants enter natural waterways, soil, and overall habitats (Mosconi et al. 2002). Amphibians are being exposed to different types of EDCs from animal waste, sewage, and plant decomposition (Leech et al. 2009). 17β-Estradiol, the EDC we will be using, mainly comes from animal waste (Park et al. 2005). When 17β-Estradiol contaminates water in aquatic environments, it can remain for several days to months under anaerobic conditions (Leech et al. 2009). This EDC has been observed to cause alterations or abnormalities in reproductive organs, cause abnormalities in overall gross morphology, and obstruct larvae development, such as inhibit time to metamorphosis (Park et al. 2005). The gonads of amphibian tadpoles are identical and composed of an outer cortex and inner medulla. In an evolving embryo, the inner medullar and outer cortex arise from the epithelium at the center position of the mesonephric kidney and support the germ cell (Reeder et al. 1998). Different genetic components will decide to differentiate the medulla into testicular tissue, or the cortex into ovarian tissue (Reeder et al. 1998). Exposure to EDCs can influence the genetic processes during gonadal development (Reeder et al. 1998). Young gonads are highly reactive to compounds imitating hormones, and sex determination can become reversed entirely. Immature male frogs can also develop an ovotestis, a gonad with testicular and ovarian characteristics (Reeder et al. 1998). These physiological disruptions can lead to lower fitness rates, population imbalances, and mortality (Park et al. 2005).

We will be studying the effects of 17β-Estradiol on the gonadal morphology in Blanchard's Cricket frogs (*Acris blanchardi*) starting from early life exposure. We want to know if the EDC will cause complete sex reversal or hermaphroditism once the frogs reach sexual maturity. After we determine the effects of the gonadal morphology, we will be looking at the male to female ratios in the population.

Methods

In our research, we are striving to provide more information about the effects E2 has on amphibians under environmentally relevant conditions. We will be raising tadpoles from eggs oviposited by captured amplexed pairs. The amplexed pairs will be captured around local ponds in Stillwater, Oklahoma. Once the pairs are obtained, we will frequently inspect the pairs

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Figure 1: These are the mesocosms the tadpoles will be raised in until they are sexually mature adults. The mesocosms are sealed off with netting to ensure the live specimens do not escape. Photo taken by: Shauni D'Na Windle

Table 1: This table represents the concentrations of 17β-estradiol in micrograms/liter that will be used for exposure on the frogs.

Dosages for 17β-estradiol (µg/L)
0.0
0.020
0.066
0.218
0.719
2.37

to see if eggs have been laid. The eggs will be raised at our experimentation site while the parental pairs are sacrificed and sent for histology. The histology from the parental pairs will establish background data. This background data will be able to show if the parental pairs had previous exposure to EDCs or if manipulations in their gonadal morphology are present. We will be raising and housing the tadpoles in constructed mesocosms relating to their natural environment (Figure 1). The mesocosms consist of a dry land portion and reservoir made by 20L stainless steel tubs buried inside the ground. The reservoir will serve as the medium of E2 treatment. We chose to use the static renewal method to ensure consistent exposure of E2. The water will be renewed every four to five days, along with a fresh dosage of E2 correlating with the initial concentration used. The concentrations are based on a previous study done by Wolf et al. (2010), we had to manipulate the concentrations based on the size of our chosen species (Table 1). The trials will last until a few weeks after the organisms reach full metamorphosis. Once the frogs are reproductively viable, they will be sacrificed by the usage of tricaine methanesulfonate (MS-222), and examined via gross for morphology and gonadal changes. The frogs will then be sent to a secondary lab to undergo histologic examination for our endpoints.

Expected Results

We currently do not have any data due to the breeding season of Blanchard's Cricket frogs and abnormal weather. The results we expect should resemble previous studies that concluded estradiol exposure causes gonadal changes such as mixed-sex, ovotestis, or complete sex reversal (Wolf et al. 2010).

Table 2: This table shows the genotypes of male, female, and sex reversed male to female genotypes. When a male reproduces with a sex-reversed female (previously male), the offspring will result in all males.

Male Genotype: ZZ
Female Genotype: ZY
Sex Reversed (M to F) Genotype: ZZ

Discussion

In addition to focusing on the gonadal changes after exposure to E2, we are going to study the male to female ratio in the population. The complete sex reversal of male amphibians will result in all male offspring in future clutches (Table 2). The result of all male offspring eventually will start to cause an imbalance in the population. When we complete the E2 study, it will contribute to a better understanding of how EDCs affect gonadal morphology in Blanchard's Cricket frogs and their population imbalances. In the future, we plan to replicate this study by using environmentally relevant levels of atrazine.

Literature Cited

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