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**Grant Project Proposal**

**Title:** Atrazine in agricultural run-off pollution has been shown to cause noticeable hormonal changes in the Florida leopard frog (Lithobates sphenocephala sphenocephala), the Florida leopard frog: A study in herbicide and amphibian endocrine disruptors and reproductive system effects and adaptation over a pro-longed period of time.

**Introduction:** The herbicide, atrazine is commonly used on crops like corn and sugarcane to prevent the unwanted growth of weeds around the crop. The biggest issue with using this herbicide is that while it prevents weed damage to the crops, it is also often found as run-off in nearby water reservoirs and in wetlands. This run-off is absorbed by the wildlife of the nearby ecosystem and can have catastrophic effects their endocrine receptors. Studies have found that that species of frogs may absorb this herbicide through their skin and it could affect the homeostasis of the frog’s hormone system.

The endocrine has been reported to be affected in northern leopard frog (Rana pipiens) and green frogs (Rana clamitans). In the journal McDaniel (2008) concluded that some evidence of exposure to endocrine disrupting compounds was noticeable in the data in frog near agricultural areas compared to frogs captured further away from crops. They gathered blood samples and measured a biomarker of exposure to environmental estrogen. There has been some evidence for adaptation in frogs to herbicides. In Boone (2005) the Southern leopard frog (Rana sphenocephala) proved to have higher growth rate during their terrestrial phase considering they had a relatively small larval stage in contaminated water.

The endocrine system is not the only system affected, the reproductive system has been shown to also be comprised. According to Preez, (2008), the research indicated that African clawed frogs (Xenopus laevis) being exposed to atrazine showed reproductive side effects in F2 generation. Another study, T. Oka (2008), conducted experiments on African clawed frog larvae revealing that larvae exposed to E2 chemicals showed a higher ratio of hermaphroditic outcomes in all male ZZ froglets. This research coincides with Hayes (2011), concluding that amphibians and reptiles being exposed to atrazine resulted in an increase size of testicular tubules as well as loss of male germ cells.

**Study System:**

Background: Our research will focus around the Florida leopard frog (Lithobates sphenocephala sphenocephala), which is native to Florida. The leopard frog is an important species in the Florida ecosystem. It is large enough to eat insect pests, but also abundant enough to be eaten by larger predators. The common Southern leopard frog is widespread across the Eastern United States, but the Florida leopard frog is most commonly found in the southeast, Florida. This frog is naturally located in areas ranging from central to north Florida.

**Habitat:** The main habitat environment for the Florida leopard frog is environments with slightly brackish waters. It is known to stay on dry land for periods of time, but always close to a water source. It is a nocturnal amphibian but can sometimes be seen during the day especially after rainfall. (Bridges, 2000)

**Breeding:** The Florida leopard frog normally breed in the winter and spring of each year. It is also common for periods of heavy rainfall to trigger breeding. The eggs can hatch quickly, in about 1-2 weeks. And tadpoles mature from a month and half to 2 and half months.

**Morphology:** The frog is mid- sized ranging from 2-3.5 inches. Normally a light to dark green color with larger dark green to brown spots on its back. It can be identified as well as having light striped on the dorsal ridges, as well as having a light line on the upper lip. It can also be found with spots on its legs and underbelly as well.

**Diet:** Since the Florida leopard frog is mainly found around brackish waters, we can presume that frogs in areas near agricultural crops will be found to have higher levels of atrazine in their systems. Many small rivers lead into large lakes, and sometimes deposit into the ocean. The Florida leopard frogs eats primarily insect, crayfish, and other aquatic vertebrates so this research is very important in determining if the common herbicide will result in a population crash and ultimately extinction, or will the frog adapt to this herbicide to survive. In Katagi (2014), they concluded that frogs are able to absorb chemicals quickly through their skin via water.

Our motivation in our study is to ultimately determine what amount of atrazine will the Florida leopard frog be able to adapt to before major hormonal consequences. Since there is a huge sugar cane industry in Florida we know that it would not be possible to have areas with zero traces of atrazine so we know that frogs currently are adapting. If a certain level of atrazine will cause the Florida leopard frog to decline, i.e all frogs become male or hermaphroditic, then will other species of amphibians will suffer as well. We will focus to see if the Florida leopard frog will be able to adapt to higher concentrations of atrazine through natural selection. In Koprivnikar (2006) their research concluded that the “cercariae of some species is compromised by exposure to atrazine, emphasizing the importance of considering the influence of contaminants on free-living stages of parasites in addressing how environmental degradation may relate to host parasitism”. Even though our focus is around amphibians, this research will prompt future investigations on atrazine and human endocrine and reproductive systems. Boon (2003) summarized that atrazine exposure had negative effects on amphibians including body size and development. On that note, compromised immune systems of frogs lead to a higher risk of other diseases such as lungworm. (Gendron (2003))

**Experiments**: Our research will consist of one lengthy trial, a 10- year period (6 generations), with 3 controlled experiments. The research will record atrazine levels, as well as birth rates, survival rates, growth patterns, reproductive success, and disruptions in endocrine receptors. We will capture wild Florida leopard frogs, approximately 50-60 individuals for each enclosure. Each frog will have to be measured on how much atrazine it has been exposed to. We will use frogs will only trace amounts of atrazine in their system. The outside enclosures will measure 100 X 100 feet and be netted off from outside ground predators. The netting will fence off snakes, cats, and other ground predators but there will still be bird predation. This is to ensure that the population does not become too large and still have natural predation aspects to it. It would be almost impossible to conduct this experiment in a natural environment due to numerous variables in landscape and human interaction. We will be supplying food and other nutrients in addition to whatever is in the natural landscape. The food will not be contaminated, only the water source. In Hanlon (2013) both the food and water source were treated with atrazine, leading to high death rates in frogs.

Our control group (T1) will be Florida leopard frogs with low levels of atrazine, that will not be exposed to atrazine over time. This will be the base model for our other controlled observations. We expect that these frogs will be able to thrive in the controlled environment reproductively but show little adaptation to atrazine over generations. In Kloas (2009) it was recorded that atrazine levels of 0.01 to 100 μg/l did not affect growth in larval development or sexual differentiation.

Our next group (T2) will be Florida leopard frogs with low levels of atrazine that will be exposed to low levels of atrazine through their water supply. We will measure the water quality on a daily basis and replace the water with appropriate levels if, i.e heavy rainfall, or drought. We expect that these frogs will be able to reproduce somewhat successfully but develop adaptation to atrazine through the generations. According to Hayes (2010), the African clawed frogs that were exposed shows a 10% male chemical castration resulting in fewer offspring.

Our last group (T3) will be Florida leopard frogs with low levels of atrazine that will be exposed to higher levels of atrazine through their water supply. We expect that initially these frogs will reproduce successfully, but over time, they will not be able to adapt to create a thriving population. In the journal by Houck (2006) the species Rana pipiens’ immune system adapted to atrazine after being initially introduced, but after a period of time other pathogens combated the frogs’ immune system, decreasing the immune system significantly. We expect that this group will reproduce the poorest and have very few offspring. We fear that having too high of atrazine levels will lead to quick frog mortality, so we will have to assess what is a suitable chemical level for survival.

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