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### **Nature of the Problem**

Environmental contaminants that are introduced to Texas waterways via industrial and agricultural runoff threaten the habitat and livelihood of Texas wetlands ecosystems. Aquatic environments are particularly sensitive to pollution and provide indications of heavy metal poisoning, endocrine disruption, and similar effects. Many of these contaminants do not result in death at ecologically relevant doses but instead accumulate in animal tissues resulting in the disruption of animal reproduction, immune function, as well as endocrine and other systems. Harmful and often multigenerational effects have been discovered in invertebrates, fish, amphibians, birds, and reptiles associated with wetlands in the United States and around the world. Although the agents responsible for these effects are known in some cases, the physiological mechanisms and method of disruption are not well understood and in need of extensive research. As awareness concerning environmental contaminants has expanded among the scientific community and the public, researchers have begun to examine certain species which are both sensitive to biologically active contaminants and possess observable responses. These sentinel species are indicators of the presence, danger, and biological activity of contaminants-canaries in a mineshaft.

Among reptiles, the American alligator, *Alligator mississippiensis*, has notably arisen to sentinel species status. The alligator was once extirpated from most of its natural range due to factors such as habitat destruction and especially over hunting (1). Having benefited from state and federal protection as a Texas endangered species, the alligator has become an important aquaculture species and has reestablished itself in much of its former range, reemerging to face a new generation of environmental threats.

Because the alligator is a top predator, there may be an increased risk of lipid-soluble contaminant bioaccumulation. This is the same phenomenon that was seen among bald eagles experiencing reproductive failure due to the bioaccumulation of the chlorinated organic pesticide Dichlorodiphenyl Trichloroethane (DDT). In Florida, researchers have begun extensive study of endocrine disruption among American alligators in several lakes exposed to environmental contaminants. Reported effects include population declines and developmental and endocrine abnormalities (2). Juvenile alligators living in Lake Apopka have a much smaller average penis size (24% decrease) and lower plasma testosterone (70% decrease) (3). These effects have been linked to a spill of several pesticides, including DDT. This claim was further substantiated by evidence that chemical combinations exhibited a synergistic effect on estrogen receptors and some may bind progesterone receptors (4). Alligator steroidogenesis is also affected by agricultural chemicals, including the herbicide most commonly used in the United States, atrazine (5). Later studies have shown that feminizing effects are not limited to lakes with a major pesticide spill, but some effects are also visible among alligators in Lakes Griffin, Jessup, Orange, and Okeechobee when compared to animals of the Lake Woodruff National

Wildlife Refuge (6). Furthermore, non-reproductive physiological systems are also altered, including thyroid function (7, 8).

In order to investigate the effects of environmental contaminants, one must first understand the workings and control of the physiological systems that may be disrupted. These physiological and regulatory processes enable organisms such as the alligator to survive in natural surroundings. One such essential regulatory system is the biological clock. Alligators, like nearly all other organisms, express circadian rhythms that regulate the timing of behavioral, biochemical, and metabolic events on a daily basis. The circadian clock is also essential for timing important seasonal and annual events like the reproductive cycle. The neural and behavioral aspects of biological timing represent a largely unexplored branch of alligator reproductive biology, and may provide essential clues regarding the regulation of alligator reproduction. This knowledge, in turn, provides the necessary framework to improve aquaculture, examine the health of Texas alligators, and see the influence of environmental contaminants on Texas wetlands.

There is substantial variation among vertebrate species in the organization of circadian components. Since most vertebrate circadian knowledge concerns mammals and birds, investigation of other classes is essential to understanding the regulatory physiology of the animals that are most directly affected by environmental contaminants in wetland habitats. Unlike birds and mammals, modern Alligators lack a functional pineal gland and are remarkably similar to their Oligocene ancestors, which possessed a similar overall morphology and are thought to have had a common aquatic ecology (9). Alligators exhibit rhythmicity in daily and seasonal cycles of behavior. Adult alligators utilize both terrestrial and aquatic habitats, regularly moving from water to land to bask in the morning and from land to water to forage in the evening. There is evidence from controlled semi-natural conditions that this amphibious behavior is regulated by an internal circadian rhythm (10). The overall objective of this work will be the characterization of the circadian system of *Alligator mississippiensis*, including the identification of its components and the use of behavioral and physiological analyses to elucidate its organization and regulatory mechanisms.

### **Research Objectives**

The first objective will be the characterization of the alligator's ability to entrain to environmental conditions. It will be necessary to demonstrate that alligators will entrain to LD cycles and free run in DD in this laboratory. Juvenile alligators are diurnally active and demonstrate robust locomotor activity rhythms when maintained in the laboratory. They entrain to light-dark (LD) cycles and free run in total darkness (DD) with a period of slightly more than 24 hours (11). Circadian outputs that have been described in reptiles include locomotor activity, body temperature, and behavioral thermoregulation (12). Behavioral rhythms will be measured by using Dataquest III software and intraperitoneally implanted telemeters to collect locomotor activity and body temperature data from animals in temperature and light-cycle controlled experimental chambers. There is some evidence that temperature cycles may provide an important time cue for crocodylians (10). Alligators will be examined in the laboratory to determine whether

they will entrain to a temperature cycle in DD. If so, the interactions of different time cues can be investigated experimentally by manipulating environmental cues for animals in the aforementioned chambers, for example by opposing LD and temperature cycles.

It possible that certain environmental contaminants provide a false chemical time cue through the aryl hydrocarbon receptor (AhR). The AhR is a bHLH-PAS (basic-helix-loop-helix) protein, several of which have recently been implicated as central molecular clock components. The AhR induces transcription through ligand-specific activation. There are several naturally-occurring ligands as well as xenochemicals such as polycyclic aromatic hydrocarbons, heterocyclic amines, and the environmental contaminant 2,3,7,8-tetrachlorodibenzo-p-dioxin, or TCDD. Dioxins will be administered in water under controlled conditions to determine whether there is an effect on the alligator clock. Implanted telemeters will record dioxin-induced effects as an alteration in circadian period .

The American alligator plays an increasingly important role in Texas water resources as a valuable aquaculture species, keystone species of Texas wetlands, and important sentinel species. Relatively little study has been conducted to date on the neural and physiological mechanisms of survival and reproduction in this species. Studies such as this one will enable better utilization of the alligator as an aquaculture species as well as provide insights in to the mechanisms of regulatory physiology that are threatened by the accumulation of environmental contaminants. These insights will facilitate better management of Texas wetlands by allowing researchers to better use living resources both to provide an early warning sign of contamination and as a relative gauge of environmental danger to wetland habitats.

## References

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### **Academic Qualifications**

Immediately prior to my current research I participated in a rotation involving two projects: the characterization of the avian (chick) vSCN and a bird song control project using the house sparrow. I assisted with electrolytic lesions of the vSCN, pressure injections of CTB subunit into the vSCN for tracing, and transcardial perfusions of the chick and house sparrow. I participated in field capture of house sparrows and learned to collect behavioral and physiological data using the Dataquest III telemetry data acquisition system.

I also worked under the direction of Tim Scott investigating the illness of farm-raised alligators suspected of lead poisoning. I mounted intestinal parasites and identified them to species. The population density and diversity of intestinal flatworms were used as biological markers for overall alligator health. These data were compared to control animals and previously described normal parasite load parameters for wild and captive populations.

My first rotation was with Duncan MacKenzie, a fish thyroid regulation specialist. It is not known whether thyroid hormones in teleost fishes are regulated by a negative feedback loop as in other vertebrates; in fact, some evidence suggests that the hypothalamus may suppress thyrotropin production in teleosts. I administered Triiodothyronine (T3) to red drum in the diet in order to test the hypothesis that thyrotropin secretion in the adenohypophysis is controlled by a negative feedback loop, ultimately regulating circulating T4 levels. Because of the difficulty of measuring circulating thyrotropin in fish, thyrotropin synthesis is measured in the secretory cells of the pituitary via mRNA. Additionally, I helped test several genes for T3 sensitivity. We induced hypothyroidism and hypothesized that gene expression would reappear following

submersion in different T3 solution concentrations, and used northern blots to measure mRNA levels as an indicator of gene expression.

### **Intended Career Path Statement**

I plan to pursue a DVM, PhD, and laboratory animal board certification in order to establish a career in laboratory animal medicine and research. I am extremely interested in aquatic ecosystems in particular and plan continued involvement with environmental concerns and research questions involving the behavioral and regulatory physiology of fish, amphibians, and reptiles.