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Seasonal variation in plasma thyroxine concentrations in juvenile alligators (*Alligators mississippiensis*) from three Florida Lakes

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Abstract

Circulating concentrations of thyroxine (T_4) vary seasonally in many vertebrates. This study examined the seasonal variation in plasma concentrations of T_4 in juvenile American alligators (*Alligator mississippiensis*) from three populations in central Florida, USA. One site, Lake Woodruff National Wildlife Refuge, is considered a reference site whereas the other two lakes, Lake Apopka and Orange Lake, are significantly impacted by human activity. Juvenile American alligators ranging from 75–150 cm in total length were hand-captured at night from November 2000–April 2002. Plasma thyroxine concentrations were analyzed using a radioimmunoassay (RIA) previously validated for alligator plasma. Juvenile American alligators display seasonal variation in circulating T_4 concentrations. Plasma T_4 concentrations decrease from August/September to November and then begin a slow rise until April, at which point they plateau. Sex of juveniles influenced plasma concentrations of T_4 in some months but did not appear to alter the pattern in seasonal variation. The pattern we observed in plasma T_4 concentrations is not directly related to an environmental factor such as ambient temperature but is similar to that seen in plasma sex steroid concentrations during the reproductive cycle of adult alligators. Although the pattern and plasma concentration of T_4 exhibits significant variation among the three lakes studied, the pattern in seasonal variation appears similar. Comparing the seasonal pattern in plasma T_4 with plasma concentrations of sex steroids (testosterone and estradiol-17β) or corticosterone could provide important information on the peripubescent life stage of the American alligator.

Keywords: Population variation; Thyroxine; Alligator; Contaminants; Lake Apopka; Lake Woodruff; Lake Orange; *Alligator mississippiensis*

1. Introduction

The thyroid hormones influence many aspects of reproduction, growth, differentiation, and metabolism in vertebrates. Metabolic effects of these thyroid hormones have been well documented (Lynn, 1970; Eales, 1985, 1988). Thermogenic action, such as positive and negative effects on carbohydrate, protein, and lipid metabolism, are among the actions of these hormones. Further, thyroid hormones increase synthesis of several mitochondrial respiratory proteins, such as cytochrome *c*, cytochrome oxidase, and succinoxidase (Norris, 1997). These compounds are necessary for the normal development of the nervous system and influence molting in amphibians, reptiles and birds as well as smoltification in many salmonids (Lynn, 1970; Norris, 1997; Shi, 2001).

Circulating concentrations of thyroxine (T_4) have been observed to fluctuate during the year in various species (Kar and Chandola-Saklani, 1985; Kuhn et al., 1985; Gancedo et al., 1997). For example, the frog *Rana ridibunda* has a plasma T_4 cycle which peaks during the months of February through April, T_4 plasma concentrations then drop and peaks again during October\November. The two peaks

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occur during periods of changing photoperiod and rainfall (Kuhn et al., 1985). A similar pattern in plasma concentrations of T_4 is found in a reptile, the Indian garden lizard, *Calotes versicolor*, from the same geographical region (Kar and Chandola-Saklani, 1985). The first peak is found prior to reproduction and the second prior to hibernation or a period of low metabolic activity. Decreased basal metabolic rate would be advantageous to animals during a period of hibernation or low caloric intake. Reduced food intake in mammals and fish has been shown to reduce thyroid hormone production (Eales, 1988; MacKenzie et al., 1998). Thyroxine concentration decreases prior to winter months and is lowest during hibernation in the Chinese cobra, *Naja naja* and the desert iguana, *Dipsosaurus dorsalis* (Bona-Gallo et al., 1980; John-Alder, 1984).

Although alligators in Florida do not exhibit true hibernation, they do endure a period of low caloric intake and inactivity during the winter months. Do alligators exhibit seasonal variation in circulating T_4 concentration similar to that observed in other vertebrates experiencing winter inactivity? Is an abiotic environmental factor, such as temperature correlated with plasma concentrations of T_4? For example, stress can influence thyroid hormone concentrations in humans, mice, birds, and fish (Bau and Parent, 2000; Davis et al., 2000; Kioukia et al., 2000; Morgan et al., 2000; Steinhardt and Thielscher, 2002; Coleman et al., 2003). Our laboratory has previously reported that contaminants can alter hormone concentrations in alligators and fish, including sex steroids and thyroid hormones (Crain, 1997; Guillette et al., 2000; Orlando et al., 2002; Toft et al., 2003). Thyroxine concentrations have been shown to be elevated in male juvenile alligators from a contaminated site when compared to reference juveniles (Crain et al., 1998). However, that study only examined animals for a single period in time. Would the pattern of plasma T_4 concentration found in alligators from a contaminated site mimic that found in alligators from reference sites or would it be different? Further, would the alterations, if present, be consistent throughout the year?

2. Materials and methods

2.1. Study sites

This study examined seasonal variation in plasma concentrations of T_4 in juvenile American alligators from three populations in central Florida, USA. One site, Lake Woodruff National Wildlife Refuge, is considered a reference site whereas the other two lakes, Lake Apopka and Orange Lake, are significantly impacted by human activity. Lake Woodruff (lat. 29°06′ N, long. 81°25′ W) is a relatively pristine environment with little modern agricultural activity in its watershed and little discharge of nutrient-ladened agricultural or storm water discharge. For example, alligators from this lake have lower concentrations of various organochlorine (OC) pesticides or their metabolites in their blood than Lake Apopka (Heinz et al., 1991; Guillette et al., 1999a,b). Animals from Orange Lake (lat. 29°26′ N, long. 82°11′ W) have similar low levels of OC pollutants as those from Lake Woodruff (Guillette et al., 1999a,b) but is eutrophic. The third population (Lake Apopka) is a historically contaminated site, receiving city effluent until 1970s as well as direct agricultural runoff until 1998 (Woodward et al., 1993; Guillette et al., 2000). Lake Apopka (lat. 28°40′ N, long. 81°38′ W) is the fourth largest lake in Florida and 1.5 miles downstream from an EPA Superfund site (EPA, 1994). Lake Apopka was directly connected *via* a freshwater stream to the site of a major pesticide spill of dicofol (composed of 15% DDT) and sulfuric acid in 1980 (EPA, unpublished report). Animals and eggs from this lake environment exhibit elevated concentrations of OCs and the lake is highly eutrophic relative to other areas (Heinz et al., 1991; Sengal and Pollman, 1991; Schelske and Brezonik, 1992; Guillette et al., 1999a,b).

2.2. Sample collection

Juvenile American alligators (*Alligator mississippiensis*) ranging from 75 to 150 cm in total length were hand captured at night during the hours (h) of 8 p.m.–1 a.m. The majority (80–90%) of the samples where collected between 9 and 11 p.m. Alligators of this size, range from 2–6 years of age (Milnes et al., 2002). A majority of juveniles collected were first time captures with a small percentage (approximately 10%) of recaptures. All animals captured conformed to the same size and age class. Approximately 30 alligators were collected each night with a minimum of 10 males and 10 females obtained from each lake. Collections occurred during the middle 2 weeks of each month and all samples where collected within a week of each other for all three sites. Samples from juvenile alligators living in Orange Lake were collected from November 2000 to April of 2002, except during March 2002. No collections of juvenile alligators where possible on Orange Lake during May and June of 2001 because of a drought that lowered water levels enough to prevent entry with boats. Blood samples were collected from juvenile alligators from Lake Woodruff between March 2001 and April 2002, except during March 2002. Finally, samples from the alligators living in Lake Apopka were collected between February 2001 and April 2002, except March 2002.

An immediate blood sample (within 3 min of capture) was obtained from the postcranial supravertebral blood vessel once the animals where secured. Approximately 10 mL of blood was taken from each animal (depending on size). Blood was collected in a heparinized Vacutainer® and stored on ice for 8–10 h until centrifugation at 1500 $\times g$ for 20 min. Plasma T_4 concentrations do not change in whole and clotted blood stored for 72 h at 4 °C or room temperature (22–26 °C) (Reimers et al., 1982). Plasma