SEASONAL VARIATION IN THYROXINE LEVELS IN THE AMERICAN ALLIGATOR (*Alligator mississippiensis*)

A Thesis

by

JARED LOUIS CRAWFORD

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

December 2003

Major Subject: Zoology

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Approved as to style and content by:

Duncan S. MacKenzie (Chair of Committee)	Timothy P. Scott (Member)
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ABSTRACT

Seasonal Variation in Thyroxine Levels in the American Alligator (*Alligator mississippiensis*). (December 2003)

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Male and female alligators ranging from 58 cm to 361 cm in length (N=1054) were captured and their blood was sampled between 9 A.M. and midnight from June 2000 – June 2002 at the Rockefeller Wildlife Refuge in Grand Chenier, LA. Animals were captured in a variety of habitats including marsh, open water, canals, and ponds. Total thyroid hormones were measured by radioimmunoassay. Four criteria were used to evaluate changes in hormone levels: sex, total length, season, and time of day. Thyroxine (T_4) varied over a wide range from 0.5 to 57 ng/mL. Triiodothyronine (T_3) levels were below the sensitivity of the assay (<0.6ng/mL). A distinct seasonal peak in T_4 was observed between December and April, peaking in March with highest mean T_4 levels observed in the spring. Mean T_4 declined significantly from the spring to an intermediate summer mean and declined significantly again in the winter. No T_4 differences between the sexes were observed. Mean T_4 was highest in juveniles, significantly lower in subadults, and significantly lower still in adults. Highest levels were found between 5 P. M. and midnight. With values up to 57 ng/ml in the spring, alligators exhibit T_4 levels among the highest recorded for reptiles.

DEDICATION

To my loving wife, Suzanne; my family Jeff, Sylvia, and Jake; Ben, Marcus, Tyler, and my many other friends for all of their love, support, and encouragement.

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INTRODUCTION

The American alligator, *Alligator mississippiensis*, is a predatory reptile inhabiting wetlands in the southeastern United States. Valued for their skins and meat, alligators were hunted to the brink of extinction in the early 1900's. Due to intense conservation efforts begun in the 1970's, they have made a strong recovery. Populations of wild alligators are now healthy enough to support annual harvests in most southern states. Alligators have also become a lucrative farming and ranching commodity (McIlhenny, 1935; Dixon, 1987).

Recently alligators have come under scrutiny as being affected by anthropogenic endocrine disrupting chemicals (EDC's) in freshwater habitats. Bioaccumulation of environmental toxins should have the greatest influence on predators atop the food chain. The American alligator may, therefore, be a useful organism in which to study the potential for environmental endocrine disruption. Studies in Florida indicate that differentiation of reproductive organs during development, as well as reproductive and thyroid endocrine pathways, may be disrupted as a result of environmental contaminants (Crain et al, 1998). Among the anomalies reported in alligators from impacted areas are defective genitalia (Guillette et al, 1999), abnormal thyroid follicular cell morphology (Hewitt et al, 2002), and aberrant circulating levels of thyroid hormone (Crain et al, 1998). Circulating thyroxine in alligators has been suggested as a parameter used to assess the extent of anthropogenic impact on wetland habitat (Crain et al, 1998, Gunderson et al, 2002). The controlled nature of these studies dictates sampling animals

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This thesis follows the style and format of *General and Comparative Endocrinology*.

representing only one life history stage during limited times of day and only in certain times of year potentially missing an ontogenic or acute temporal changes in thyroid function. There is little information available on the basal thyroid function in wild alligators that might serve as the basis for environmental endocrine disruption studies. This study was therefore undertaken to characterize baseline seasonal variation in thyroid hormone levels in wild alligators.

The intent of this study is to establish a range of normal thyroid hormone levels for the American alligators, *Alligator mississippiensis*, by characterizing seasonal cycles of thyroid hormones in normal populations. This will be achieved by:

1. Measuring thyroid hormone levels in alligators collected in the wild at Rockefeller Wildlife Refuge
2. Evaluating these samples to determine whether thyroid hormone levels vary by age, sex, or season

This study will provide seasonal references of thyroxine (T_4) values among male and female alligators ranging in size from 53 cm (hatchling) to 361 cm (adult) from a pristine environment. The ability to collect animals over the course of multiple years is very important in determining the repetition of seasonal cycles. This study will produce the largest set of thyroid hormone levels, taken over the longest period of time, comprising one of the most diverse sampling of a single reptilian species.

This study shall serve as reference to which other wild alligator thyroid research may be compared. Additionally it will provide an index of normal values in order to more effectively assess the effects of anthropogenic contaminants. Currently, no such index reference exists (Barnett, et al, 1998).

Thyroid hormones and their functions

The gross morphology of the thyroid gland varies throughout vertebrates. In mammals, for example, the thyroid gland has two lobes connected by an isthmus, whereas reptiles and elasmobranch fishes have monolobular thyroid glands (McNabb, 1992). Birds and amphibians posses bilateral thyroid glands and teleost fish thyroid tissue consists of diffuse follicles scattered throughout the pharyngeal region. Regardless of this diversity in gland morphology, the structure and synthesis of the primary thyroid hormones, triiodothyronine (T_3) and thyroxine (T_4) appear to be identical in all species studied to date. The thyroid gland concentrates iodide from the blood and synthesizes a high molecular weight glycoprotein, thyroglobulin, stored within the thyroid follicles. Within follicular cells, tyrosine residues on thyroglobulin are iodinated by thyroid peroxidase, then coupled to form T_3 and T_4. Free T_4 is released into the bloodstream after lysosomal degradation of thyroglobulin. In mammals, T_4 is drawn into the bloodstream from the thyroid gland by the presence of high affinity binding proteins that bind and transport T_4 in circulation. At target cells, outer-ring deiodinase cleaves an iodine atom from thyroxine's outer ring, converting it to T_3 (McNabb, 1992). Most actions of thyroid hormones are mediated through the binding of T_3 to nuclear receptors that modulate gene activity. For this reason, it is believed that T_4 is a prohormone and T_3 is the biologically active molecule (McNabb, 1992).

The thyroid hormone system allows for multiple levels of regulation. In the mammalian hypothalamus, thyrotropin releasing hormone (TRH) stimulates the release of thyrotropin (TSH) from the pituitary. Thyrotropin causes the thyroid to secrete T_4 into the bloodstream. Feedback inhibition exists between T_4 and TSH so that the pituitary will halt release of TSH when a plasma T_4 threshold is reached. Peripherally, the conversion of T_4 to T_3 is regulated by the expression of deiodinase at the target tissue. Expression of deiodinase can be modulated by many environmental factors, including developmental state, diet, and ambient temperature (McNabb, 1992).

Thyroid function in reptiles

Little is currently known about reptilian thyroid physiology aside from turtles. Turtles possess hypothalamic peptides that modulate thyrotropic activity of the pituitary (Denver and Licht, 1989). Thyrotropin has been purified from turtle pituitaries (MacKenzie et al, 1981) and has been demonstrated to stimulate thyroid hormone secretion (Licht, et al, 1989). These studies indicate that components of the mammalian model previously described exist in turtles, and probably occur in other reptilian species as well. Most studies that have been undertaken on other reptilian species have been either extirpation/replacement or seasonal cycle studies. These few studies suggest that the reptilian thyroid plays a regulatory role in a variety of fundamental physiological processes and, consequently, is seasonally activated.

Extirpation/replacement studies provide the bulk of experimental demonstration of thyroid hormones' regulation of physiological processes in reptiles. Thyroidectomized chequered water snakes, *Natrix piscator*, show decreased red blood cell counts that are restored with administration of l-thyroxine, thus implicating T_4 in hemopoiesis (Thapliyal and Kaur, 1976). In rat snakes, *Ptyas korros*, thyroidectomy resulted in an increased skin shedding frequency. The conclusion is that thyroid secretions target epidermal cells to inhibit proliferation (Chiu et al, 1983). The opposite phenomenon was observed in lizards, *Calotes versicolor*, when thyroidectomy decreased shedding frequency that was restored by T_4 injections. Thyroxine also caused the sham operated lizards to increase oxygen consumption and gonadal weight. Based on increased shedding frequency and decreased oxygen consumption with T_4 and iopanic acid (IOP inhibits peripheral conversion of T_4 to T_3) the study concluded that T_4 and T_3 have differential effects and that not all of the physiological actions of thyroid hormones are elicited via T_4 conversion to T_3 (Chandola-Saklani and Kar, 1990).

In juvenile *Trachemys scripta*, thyroidectomy resulted in stunted growth relative to controls. Administration of T_4 restored normal growth rates in the thyroidectomized animals (Denver and Licht, 1991). Other researchers have found thyroid hormones to exert direct control over several metabolically important enzymes, including glucose-6-phosphatase, lactate dehydrogenase, acid and alkaline phosphatase, glutamic oxaloacetic transaminase, and glutamic pyruvic transaminase. Thyroidectomized lizards (*C. versicolor* and *Sceloporus undulatus*) had decreased activity in several of these enzymes. Injections of T_4 or T_3 restored enzymatic activity revealing the role of thyroid hormones in activating the intermediary metabolism of lizards (Jacob and Oommen, 1990 and John-Alder, 1990). Henry John-Alder demonstrated T_4 as promoter of aerobic capacity in lizards, *S. undulatus*. Thyroidectomy resulted in slower overall growth rates relative to sham operated lizards and administration of T_4 increased basal metabolic rate (John-Alder, 1983 and 1984). John-Alder also demonstrated that captive lizards lacked the response to T_4 administration of wild lizards and suggested laboratory conditions are not sufficient to replicate the thyroid physiology of wild reptiles (John-Alder and Joos, 1991).

The results of these studies illustrate the diversity of physiological processes associated with thyroid hormones. Despite this diversity, an underlying theme becomes apparent. Thyroid hormones generally serve to promote anabolic processes either by directly increasing metabolism or supporting that capacity via aerobic enhancement. Although experimental reptilian thyroid research has provided valuable information, limitations are inherent. As Henry John-Alder noted, the effects of exogenous thyroxine administration to wild lizards were far greater than those in captive lizards. This important observation indicates that the results of laboratory studies may be valuable in elucidating physiological roles of thyroxine, but may not provide an accurate representation of natural physiological conditions. The resulting conclusion is that invasive field based studies are required in order to gain a more accurate picture of natural thyroid function in reptiles (John-Alder and Joos, 1991).

Many reptilian thyroid studies consist of examining the seasonal cycles of circulating T_4 of wild animals. Physiological functions of T_4 may be suggested by correlating plasma levels with discrete behavioral or physiological events such as hibernation, feeding, migration, or breeding. Seasonal studies have revealed diverse patterns of plasma thyroid hormones in different reptilian species. The male Indian garden lizard, *Calotes versicolor*, shows two discrete peaks in plasma T_4 immediately before and after hibernation (Kar and Chandola-Saklani, 1985). Dual peaks in T_4 were also found in the male Aspic viper, *Vipera aspis*, immediately preceding primary and secondary periods of mating activity (Naulleau et al, 1987). Desert tortoises, *Gopherus agassizii*, also show seasonality in their plasma T_4 levels (Kohel et al, 2001). Males have a biphasic seasonal pattern with the peaks occurring before and after hibernation. Females have a single peak that corresponds with the first male peak in early spring prior to hibernation. The Indian cobra, *Naja naja*, exhibited a single peak in T_4 during the spring (Bona-Gallo et al, 1980). Painted turtles, *Chrysemys picta*, show a single seasonal peak in plasma T_4 in early spring after emergence from hibernation and after mating activity (Licht et al, 1985).

These results demonstrate that circulating T_4 exhibits specific seasonal fluctuations and suggest several things. The obvious seasonal cycle demonstrates that the reptilian thyroid gland is dynamically activated and deactivated. When correlated with seasonal events, authors have suggested that T_4 production is associated with increased locomotor activity, ambient temperature, nutrient assimilation, or reproductive activity. These correlations lead to a general suggestion that the thyroid axis may be activated during times of increased metabolic activity, supporting the conclusions of the laboratory experiments described above. Thus far, however, field studies in reptiles have considered only adult animals whose availability is seasonally restricted. Additionally, these studies normally measure hormones during a single season and conclude it is representative of a typical annual cycle. Annual repetition of hormone cycles in subsequent years is necessary to confirm seasonal changes and unambiguously establish correlations with other physiological processes. Some species, such as Desert tortoises display annual thyroid hormone profiles that appear to be gender and species specific (Kohel, et al, 2001). Comparisons that utilize sex, season, age as variables and co variables may prove to be invaluable in elucidating their respective influences on thyroid hormone cyclicity.

Crocodilian thyroid function

Of the few studies that exist on crocodilian thyroid physiology, two general themes emerge. The first involves comparisons between wild and captive raised alligator thyroid hormone levels. Researchers found higher titers of plasma T_4 and T_3 in captive-raised versus wild alligators (Barnett et al, 1998; 1999). When moved to captivity, thyroid hormones of wild animals were elevated to mirror those of captive-raised animals. This was attributed to the regular feeding regimen in captivity. In addition to lower thyroid hormone levels, relative to captive-raised animals the wild alligators were anemic, had less body fat, and weighed less per unit length. The authors suggested that thyroid hormone levels may serve as an indicator of starvation in wild alligators (Barnett et al, 1998). However, the authors did not provide any reference to a normal range of thyroid hormone levels for wild alligators. No such reference exits and thus it is not possible to discern what constitutes high or low thyroid hormone levels. The findings in these studies are useful, however, in illustrating the plasticity of the crocodilian thyroid system and its responsiveness to nutrient intake.

The second perspective from which crocodilian thyroid physiology has been studied involves assessing the extent to which anthropogenic environmental contaminants impact alligator thyroid function. Point source pollution and agricultural runoff have contaminated bodies of water in Florida. Studies to evaluate the effect of environmental endocrine disruption on local wildlife populations have focused on comparisons between alligators from contaminated and pristine habitats. Animals collected from contaminated sites demonstrated elevated plasma T_4 relative to pristine-site animals (Crain et al, 1998; Gunderson et al, 2002; Hewitt et al 2002). Histological analysis of thyroid tissue from pristine, intermediate, and contaminated sites demonstrated that contaminated animals had significantly less colloid, and greater epithelial cell width relative to the pristine controls (Hewitt et al, 2002). No gender differences were observed in circulating T_4.

These studies suggest that aspects of the thyroid axis may be disrupted by anthropogenic environmental contaminants but are difficult to interpret without a broader understanding of basic thyroid biology in alligators. As with the previous study, it suggests that thyroid function may be used as an indicator of health in wild alligator populations. However, it is anticipated from comparative studies that the regulation of circulating thyroid hormones in alligators is a dynamic process resulting from interactions between feedback-regulated hormone secretion, blood protein transport, peripheral deiodination, and multicompartmental hormone metabolism. Therefore much more data from wild animals are required to define normality.

One of the highest concentrations of America alligators along the Gulf Coast is at Rockefeller Wildlife Refuge in South Louisiana (Lance, 1989). An ongoing collaboration between the refuge and the San Diego Zoo to examine reproductive and stress physiology provides an opportunity to examine the seasonal pattern of thyroid hormones in alligators, comprising all sizes and both sexes, sampled throughout day during all months of multiple years. The Rockefeller staff is extremely cooperative and knowledgeable, has experience in handling the animals, and is well equipped for a large-scale alligator blood sampling operation. The alligator population in the refuge has ample numbers of all sizes and both sexes to support such a study.

Studies conducted at the Rockefeller Wildlife Refuge outline the natural history of alligators with emergence from torpor occurring in late February and early March (Joanen and McNease, 1975). After emergence, both male and female alligators begin foraging immediately. In late April, as males continue bellowing and fighting, females abandon last year's hatchlings and travel from their inland habitat to meet the males in the deeper open water where copulation occurs (Joanen and McNease, 1975; Coulson and Hernandez, 1983; Lance 1989). In May, adult reproductive females ovulate while adult males bellow and compete for mating dominance (Joanen and McNease, 1975; Lance, 1989). Copulation occurs in May and afterwards reproductive females move into interior shallow marshes to begin nest construction, feeding rarely, if ever, while males and non-reproductive females resume foraging until temperatures decline in October. Egg deposition occurs in June and these females remain in the interior habitat until the next breeding season (Coulson and Hernandez, 1983; Joanen and McNease, 1979; Lance 1989). During incubation (June – August) females aggressively guard their nest sites, foraging only occasionally, while males and non-reproductive females remain in deeper open water habitat and continue foraging (McIlhenny, 1935; Joanen and McNease, 1975; Lance 1989). Torpor usually begins in October and during this time, alligators may appear briefly on warm days but do not consume food until the spring (McIlhenny, 1935; Coulson and Hernandez, 1983; Lance, 1989). Many alligators construct elaborate dens in which they spend the winter (October – January).

Eggs begin hatching in late August/early September and the hatchlings generally remain near their mother until the following spring. Hatchlings may spend the winter with their mother in her den (McIlhenny, 1935; Joanen and McNease, 1975). In the wild, hatchling alligators grow at an average rate of approximately 30 cm per year up to six years in females and ten years in males. This average growth rate declines drastically after these thresholds (McIlhenny, 1935; Chabreck and Joanen, 1979). The diet of alligators up to 90 cm in length consists of small aquatic invertebrates almost exclusively. After the animal reaches 90 cm, alligators incorporate small vertebrates into their diet as they begin to explore more open water habitat and at 180 cm begin consuming mammals and larger prey (McIlhenny, 1935; Coulson and Hernandez 1983). The Rockefeller alligators thus provide an excellent opportunity for characterization of the scope of thyroid hormone levels in a wild population.

The ongoing collaboration between Rockefeller Wildlife Refuge and the San Diego Zoo provided an opportunity to examine alligator seasonal thyroid hormone profiles. Samples for the thyroid hormone analysis were collected as part of a larger study of adrenal and reproductive steroids. The objectives of the steroid study are to characterize the seasonal patterns of circulating testosterone, estradiol, and corticosterone in Rockefeller alligators, with a major focus on the subadult size class. As a result of this focus, sampling was concentrated on subadult animals.

METHODS

Alligator sampling

A wild population of American alligators living on Rockefeller Wildlife Refuge in Grand Chenier, Louisiana was utilized for this study. Habitat on the refuge consists of open, brackish water marsh and canals enclosed by levees and floodgates. Alligators are free to roam the refuge and may be found in either marsh or canal/pond habitat. The refuge is a self-sustaining ecosystem in which the alligators feed on naturally occurring forage such as invertebrates, amphibians, reptiles, and small mammals. Alligators were captured during all hours of the day and night from both shore and airboat. At night, animals were located by spotlight, noosed with a steel snare, and quickly restrained once aboard the airboat. During daylight captures, animals were hooked with a treble hook attached to a steel leader on heavy fishing tackle and reeled to shore, then noosed with a steel snare. Measurements taken were: sex, total length, time of capture, and date of capture. All blood samples (10ml) were taken from the post cranial sinus with a heparinized 18 ga needle on a 10 ml syringe. All alligators were captured, measured, sexed, and tagged, blood sampled and released within 5 to 10 minutes. Blood samples were kept on ice until returned to the laboratory (approx. 1-2 hrs.). After centrifugation and the removal of plasma, samples were frozen, and stored at -4°C at the refuge. Rockefeller Wildlife Refuge staff performed all alligator handling and sampling. Blood was collected continually throughout two consecutive years to characterize seasonal changes in hormone levels. During the winter months, alligators may emerge briefly to bask, but do not feed (McIlhenny, 1935). Thus, during the winter, some alligators were able to be captured and sampled. To examine the possible daily cycling of thyroid hormones, blood samples were taken throughout the day and night from animals of differing ages.

Administrative staff at the refuge recorded data collected from each animal in a Microsoft Access database. This data comprised sex, length, time of capture, date of capture, tag numbers, and serial sample numbers assigned at the time of capture. This database was emailed from the refuge to our laboratory where the data were transferred into a Microsoft Excel spreadsheet, and T_4 data was added. Steroid hormone data from the same blood samples (analyzed by Dr. Valentine Lance, Center for the Reproduction of Endangered Species, San Diego Zoological Society) were also received in Excel format and added to the spreadsheet.

Blood analysis

Total T_3 and T_4 were determined in blood samples by radioimmunoassay (RIA). Validations of the RIA were performed to establish parallelism of alligator blood to standards, recovery of exogenous hormone from blood, and intra- and inter-assay variability.

Radioimmunoassay of T_4 was run according to the protocol outlined by Leiner et al (2000). A standard curve was prepared with 50 ul of native T_4 diluted serially in duplicate at the following doses: 250 pg, 125 pg, 62.5 pg, 31.25 pg, 15.6 pg, and 7.8 pg per tube. The alligator plasma was analyzed in duplicate 12 µl aliquots along with 38 µl RIA buffer (15.46 g barbital: 0.5 g EDTA; 0.1 g thimersol: 1.0 g bovine gamma globulins: 900 mL ddH_2O, pH 8.6). Each assay contained at least two dilutions of three quality control standard plasma pool samples taken from redfish (RDP5), a pregnant human (KSC1), and randomly sampled wild alligators (RGP1; 30 samples representing a variety of animal sizes of both sexes taken by Dr. Ruth Elsey at the Rockefeller Wildlife Refuge on 6/25/01 and 6/29/01; tag #'s 1203-1227, 1272-1277). After the samples were delivered into numbered test tubes, 150 µl of radiolabelled T_4, (1250 µCi/µg, Perkin-Elmer/NEN, Billerica, MA) diluted with RIA buffer to ~25,000 counts per minute/150 ul, was added. Fifty µl of rabbit anti-T_4 (ICN lot #0010, Irvine, CA) at a dilution of 1:2358 was added to the tubes and incubated at 37ºC for 90 minutes, then at 23°C for 8-10 hours. After incubation, a secondary antibody (goat anti-rabbit-gamma gobulin) was diluted 1:20 in 5% aqueous polyethyleneglycol (PEG) and added to the tubes in 0.5 ml aliquots. Incubation with the secondary antibody was for 60 min at 4ºC. The tubes were removed from the refrigerator and centrifuged at 3200 rpm for 15 minutes to precipitate the bound primary antibody/T_4 complex. The supernatant and unbound T_4 was discarded as the tubes were allowed to drain upside down for 30 min. After draining, the tubes were loaded into a gamma counter (LKB Riagamma Quatro) and counted for 60 sec/tube.

Radioimmunoassay for T_3 was run the same as for T_4, but with the following modifications: the primary antibody used in the T_3 assay was a rabbit anti –T_3 (ICN lot #2669E, Irvine, CA) diluted 1:12000. Radiolabelled T_3 (1250 µCi/µg, Perkin-Elmer/NEN, Billerica, MA) was diluted and added to the tubes similarly to the protocol outlined in the T_4 assay.

RIAMENU software, (a gift from Dr. Paul Licht, University of California, Berkeley) was used to calculate hormone concentrations in ng/ml from raw gamma counts per minute using a four parameter logistic function. RIAMENU was also used to determine parallelism between standards and alligator plasma.

Validation of RIA for *Alligator mississippiensis*

Validation of the RIA for alligator plasma involved two primary tests: one for parallelism and one for recovery. A third test quantified intra- and inter-assay variability. The parallelism test determines whether T_4 contained in alligator plasma dilutes similarly to a purified T_4 standard. Four pools of alligator plasma (M1RGP, M2RGP, F1RGP, and F2RGP: random males and females sampled from January to June (1) and from July to December (2) were diluted serially with RIA buffer to 1:2, 1:4, 1:8, 1:16, and 1:32. T_3 was not tested due to low plasma concentration in wild alligator plasma. A standard 5ng/ml T_4 solution dissolved in RIA buffer was similarly diluted. Parallelism of the diluted standard to the plasma was analyzed using the parallelism test in RIAMENU.

The recovery test determined if any substances present in alligator plasma hinder accurate T_4 measurement by RIA. The RGP1 pool of alligator plasma was divided into two 0.250 ml aliquots. One aliquot was treated with 0.010 ml of a 500 ng/ml T_4 solution in methanol, (containing 5 ng T_4, yielding a final concentration in the plasma of 20 ng/ml) while the other was treated with 0.010 ml of methanol as a control. Radioimmunoassay was used to determine the percentage of native T_4 that could be detected in both.

The intra-assay variability test analyzes the reliability of the RIA to precisely measure T_4 in a sample. The RGP1 pool of alligator plasma was run repeatedly, at 12 µl volumes, within one assay. The inter-assay variability test analyzes the reliability of an assay to precisely measure the same sample in multiple assays. A 12 µl aliquot from the RGP1 pool of wild alligator plasma was run in each assay in this study (n=12). Both intra- and inter-assay variability test results were analyzed by calculating coefficients of variation (CV) using Microsoft Excel by the method of Rodbard (1974).

Data analysis

Animals were grouped for analysis by sex, size class, season and time of day. Size class assessment was based on total body length in 90 cm intervals (0- 90 cm = juveniles, 91-180 cm = subadults, and >180 cm = adults). This distinction is based on a marked change in habitat niche that occurs at 90 cm total body length and reproductive maturity occurring at approximately 180 cm (Fitzgerald, 1988). For seasonal analysis, samples were clustered by date: Spring (February-May), Summer (June-September), and Winter (October-January). This method of clustering is based on 30-year mean temperatures recorded by the National Oceanic and Atmospheric Administration (NOAA) at Lake Charles, LA; a site approximately 60 mi. northwest of refuge headquarters (Figure 1). The four warmest months (June-Sept) were classified as “summer”. The following four month period (Oct-Jan) saw declining temperatures, and was classified as “winter”. The four month period following winter and prior to summer (Feb-May) displayed increasing temperatures and was classified “spring”. To evaluate the potential for changes in T_4 based on time of day, data were clustered together in 2 hour intervals. One-way ANOVA’s and Tukey’s *ad hoc* comparisons were used to evaluate the possibility of significant differences between groups (sex, size class, season, and time of day). A three-way ANOVA was used to determine if there were any significant interactions between sex, length, and season on T_4. Pearson’s correlations were also used to identify significant relationships between body size, T_4, testosterone,

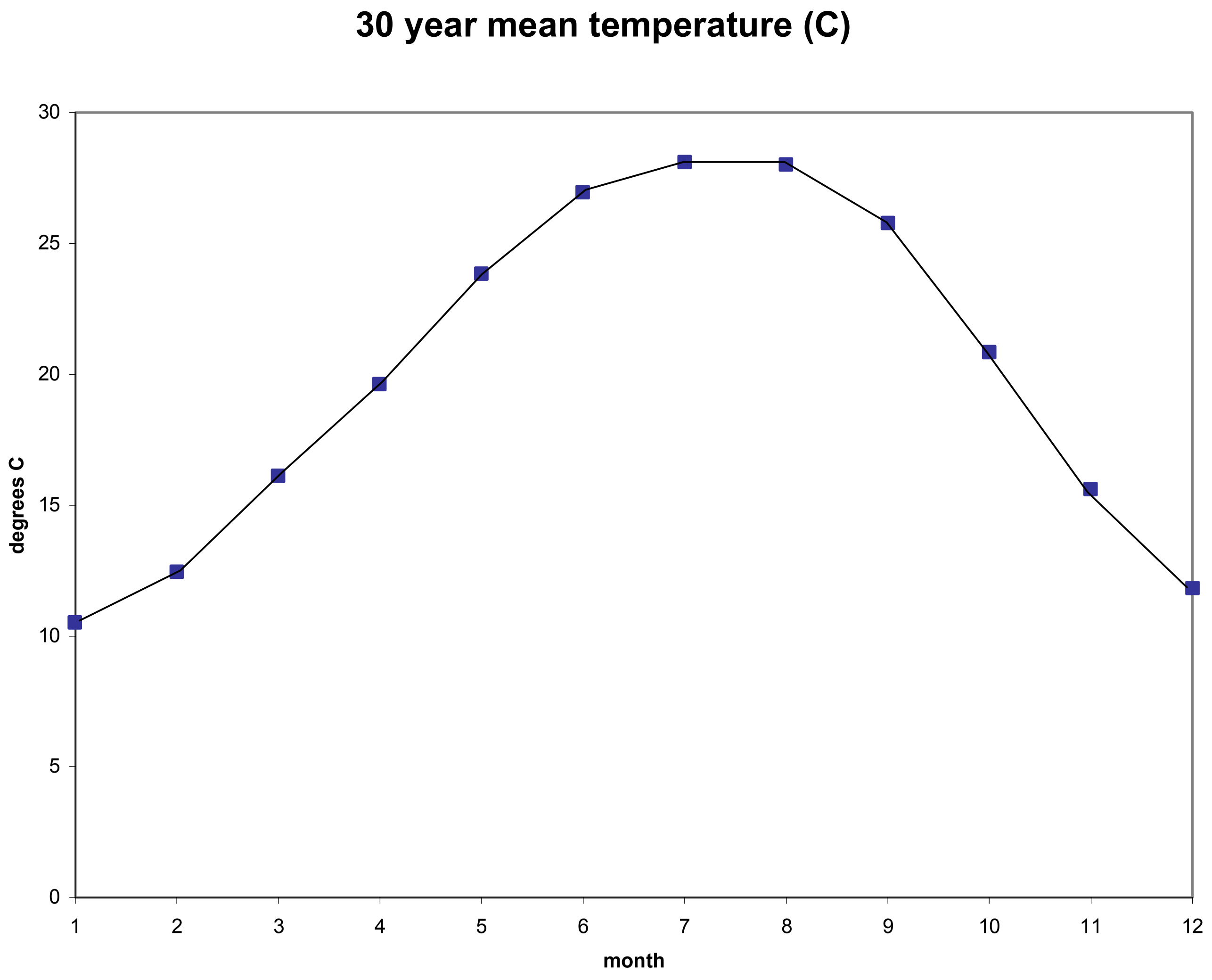

Figure 1. 30 year mean temperature data recorded at a National Oceanic & Atmospheric Administration station in Lake Charles, LA.

estradiol, and corticosterone. All statistical analyses were performed using the SPSS statistical package (SPSS Inc., Chicago, Illinois).

RESULTS

Animals

The data set for this study comprised 2293 animals (1147 males and 1146 females) ranging in length from 53 cm to 361 cm (Table 1). Juvenile alligators (n=173) were primarily male, (67%) and were also the least common size collected. The subadult Age group was largest (n=1716) with an equal distribution of males and females. Adults (n=404) were primarily female (57%). Most samples were collected in the spring (1027) with roughly equal numbers in the winter (618) and summer (648). Overall, an equal number of males and females were sampled. Significant differences in length were observed between sexes within size groups, with females consistently being significantly larger than males in each size class until adulthood. No differences in length were observed among seasons.

RIA validation

Figure 2 shows the comparison of serial dilutions of pooled alligator plasma samples to the standard curves from 8 assays. The RIAMENU parallelism test for T_4 did not detect significant differences between the slopes of alligator plasma dilution curves and serially-diluted T_4 standard. The results of the recovery test yielded a 101.3% recovery of T_4 added into the wild alligator plasma pool (buffer before treatment = 0.7 ng/ml, buffer after treatment = 20.2 ng/ml; RGP1 before treatment = 4.9 ng/ml, RGP1 after treatment = 24.6 ng/ml). Results of the intra and inter-assay variability tests of the plasma pool showed a 6.5% intra-assay coefficient of variation and 17.3% inter-

Table 1. Thyroxine levels in American alligators

Group	*N*	*Mean Length (cm) (Range)*	*Mean T_4 (ng/ml) ± standard error*	*T4 Range (ng/ml)*
Sex				
Male	1147	140.9^{A} (53.0-361.0)	8.7^{B} ± 0.2	0.7-58.0
Female	1146	148.5^{B} (61.0-255.0)	8.0^{A} ± 0.2	0.2-47.7
Season				
Winter	338	144.7^{A} (57.0-361.0)	4.5^{A} ± 0.1	0.2-25.7
Spring	493	142.9^{A} (53.0-356.0)	11.8^{C} ± 0.3	1.3-58.0
Summer	316	147.5^{A} (58.0-335.0)	8.4^{B} ± 0.2	0.7-24.1
Sizeclass				
Juvenile	173	79.3^{A} (53.0-90.0)	10.5^{C} ± 0.5	1.6-33.6
Subadult	1716	135.0^{B} (91.0-180.0)	8.6^{B} ± 0.1	0.8-58.0
Adult	404	214.0^{C} (182.0-361.0)	6.4^{A} ± 0.2	0.2-29.4
Juvenile				
Male	116	74.4^{A} (53.0-90.0)	9.9^{A} ± 0.1	1.6-33.6
Female	57	81.0^{A} (61.0-90.0)	11.8^{A} ± 0.1	1.7-27.4
Subadult				
Male	857	132.2^{A} (91.0-180.0)	8.9^{B} ± 0.3	1.3-58.0
Female	859	137.7^{B} (91.0-180.0)	8.2^{A} ± 0.2	0.8-47.7
Adult				
Male	174	225.3^{B} (182.0-361.0)	6.8^{A} ± 0.2	0.7-29.4
Female	230	205.5^{A} (182.0-225.0)	6.1^{A} ± 0.2	0.2-19.2
Total	2293	144.7 (53.0-361.0)	8.3 ± 0.1	0.2-58.0

Values sharing the same superscript do not differ significantly within the same group (P=0.05)

assay coefficient of variation. The sensitivity of the T_4 assay was determined by the RIAMENU program to be 0.75 ng/ml. As only 0.1% of the T_4 samples were below this limit, all T_4 samples were included in data analyses. The sensitivity of the T_3 assay was 0.6 ng/ml. As 63% of the 51 T_3 determinations were below this value, it was decided that the T_3 RIA was not adequately sensitive for alligator plasma.

Sex

Whether analyzed by size class or season (Tables 1 and 2), few significant differences were found between sexes. Furthermore, Figures 3 and 4 further show no apparent differences in seasonal changes in T_4 between sexes. The only significant differences were observed in subadults, and then only in spring (male – 12.1, females – 10.9) and summer (males – 8.5, females – 7.3).

Season

Table 1 shows significant differences in mean T_4 between the three seasons with the spring highest, summer significantly lower than spring, and winter significantly lower than summer. In Figures 3 and 4, a rapid rise in T_4 was observed during February of year one (2001) leading to the highest levels of the study observed in March. Following this spring peak, levels declined to an intermediate summer plateau, then fell again to the lowest levels in the winter. During the second year of our study (2002), the spring increase did not return to the same levels as year 1 (2001), although a spring increase was still evident.

Size class

Although subadults showed maximum T_4, juveniles had the highest overall mean (Table 1). Mean value for subadults was significantly lower than juveniles and the adult

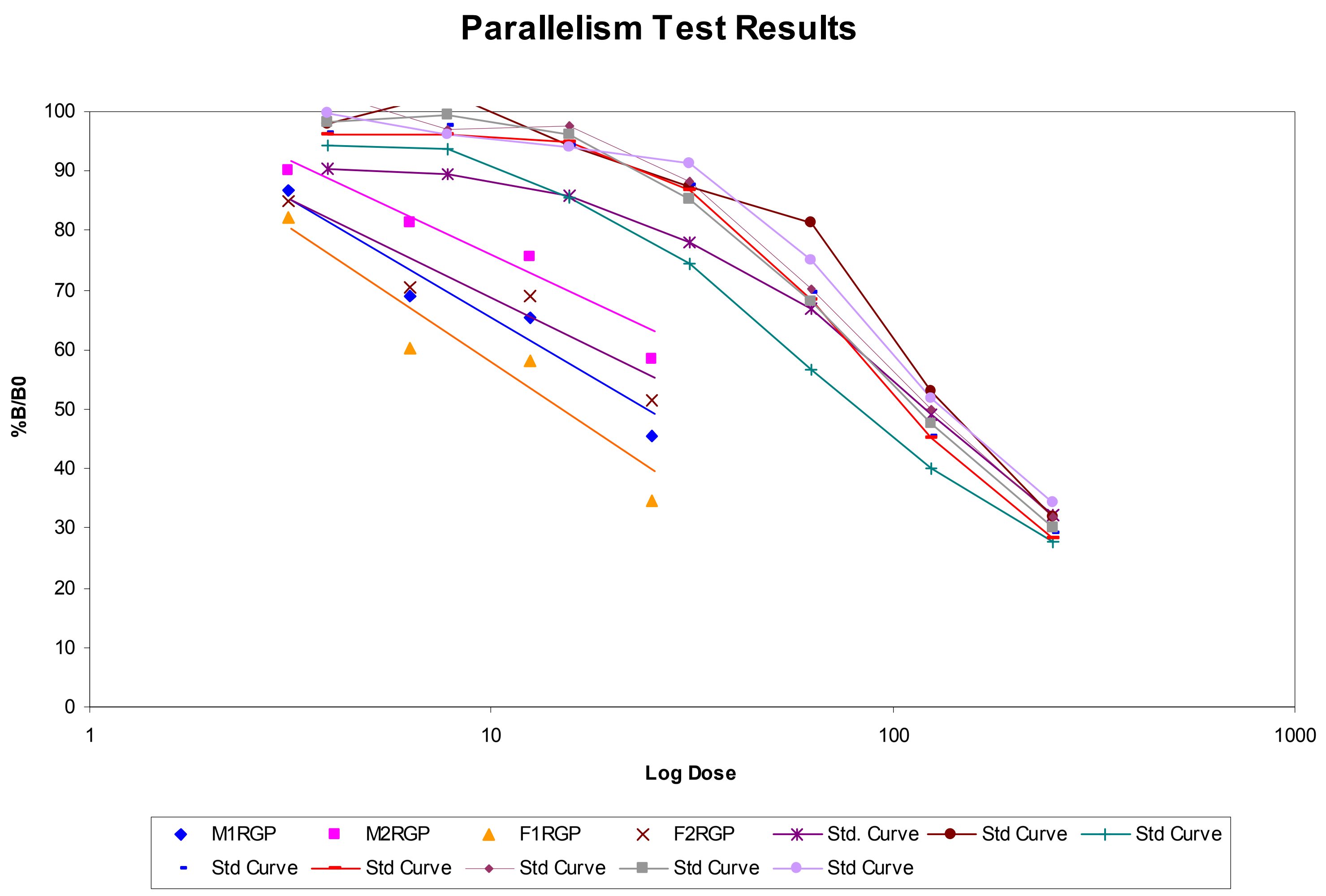

Figure 2. Results from the T_4 parallelism test. Wild alligator thyroxine values diluted parallel with serially diluted native T_4. Standard curve values are in pg and plasma values are in ng/ml. M1RGP and M2RGP are pools from males sampled during the first and second half of the year. F1RGP and F2RGP are pools from females sampled during the first and second half of the year.

Table 2. Thyroxine values* by season and size class

Group	*Males*	*Females*
Juvenile		
Winter	$4.5^{A,1}$ ± 0.3, (40)	$4.2^{A,1}$ ± 0.4, (13)
Spring	$13.1^{B,1}$ ± 0.8, (55)	$14.9^{B,1}$ ± 1.0, (28)
Summer	$11.9^{B,1}$ ± 1.2, (21)	$12.6^{B,1}$ ± 1.2, (16)
Subadult		
Winter	$4.6^{A,1}$ ± 0.2, (244)	$4.3^{A,1}$ ± 0.1, (207)
Spring	$12.1^{C,2}$ ± 0.4, (366)	$10.9^{C,1}$ ± 0.3, (393)
Summer	$8.5^{B,2}$ ± 0.3, (247)	$7.3^{B,1}$ ± 0.2, (259)
Adult		
Winter	$3.8^{A,1}$ ± 0.3, (54)	$4.5^{A,1}$ ± 0.3, (60)
Spring	$9.1^{B,1}$ ± 0.7, (72)	$6.9^{B,1}$ ± 0.3, (113)
Summer	$6.7^{A,B,1}$ ± 0.4, (48)	$6.1^{A,B,1}$ ± 0.4, (57)
Total	8.7 ± 0.2 (1147)	8.0 ± 0.1 (1146)

* Mean T_4 (ng/ml) ± standard error, (N)

Values sharing the same superscript letter do not differ significantly within the same group (P=0.05)

Values sharing the same superscript number do not differ significantly between sexes, within the same treatment (P=0.05)

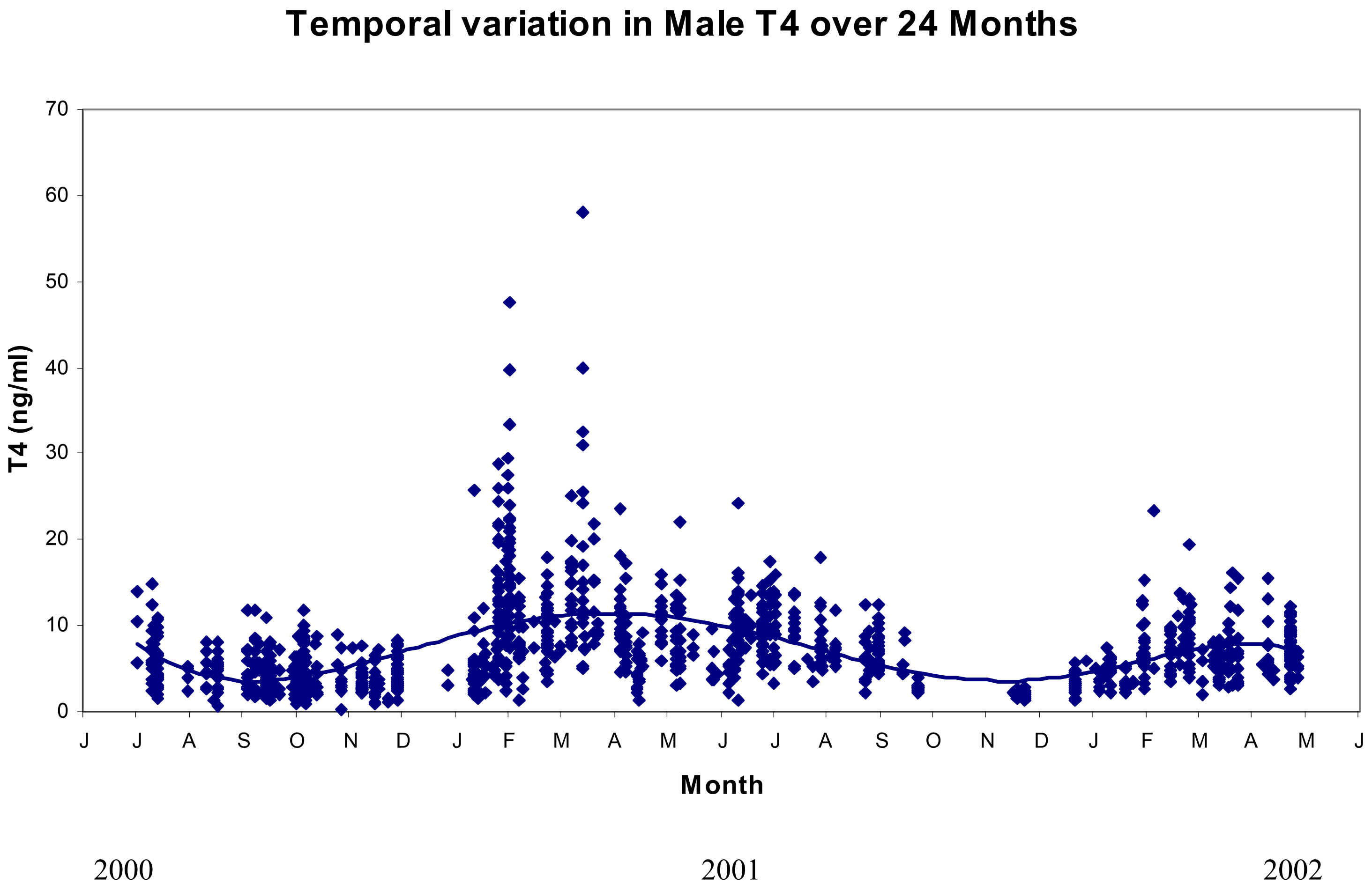

Figure 3. Thyroxine values for male alligators collected between June 2000 and June 2002 with a polynomial trendline indicating seasonal changes in mean T_4.

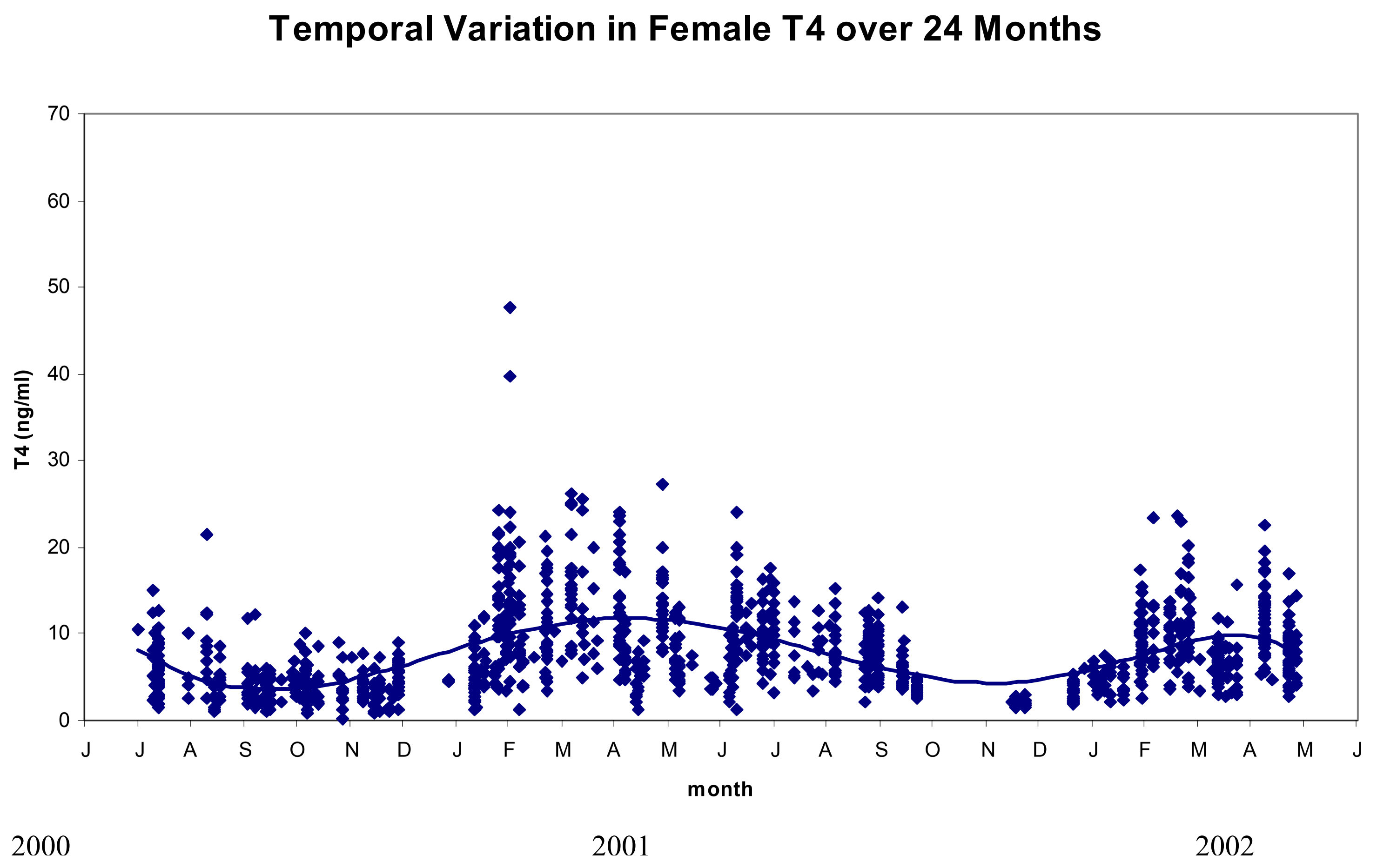

Figure 4. **Thyroxine values for female alligators collected between June 2000 and June 2002 with a polynomial trendline indicating seasonal changes in mean T_4.**

value was significantly lower than subadults (Table 1). Figures 5 – 7 also illustrate that highest T_4 (ranging up to 58.0) were found in subadults. Juveniles and adults displayed less variability in seasonal distribution than subadults.

Time of day

Daily T_4 was sorted by sex and analyzed in two-hour clusters. No consistent significant sex differences were found over the 24-hour sampling period (Table 3). Although highest values were found after 6 p.m. (Figures 8, 9, 10), no consistent relationship was observed between T_4 and time of day. To examine the possibility of a seasonal effect of time of day on T_4, male and female data were combined and then divided seasonally and analyzed in 2-hour blocks as above (Table 4). In both spring and summer, values in the evening were significantly higher than values in the morning. Additionally, significantly higher levels were found in the evening in spring animals versus summer and winter animals.

Correlations

Pearson Correlation Coefficients were calculated by sex for the following variables: length, thyroxine, testosterone, estradiol, and corticosterone. Most correlations were not significant (Tables 5 and 6). However, there was a significant negative correlation of T_4 with length and a significant positive correlation of T_4 with estradiol in both male and female alligators. Length was significantly positively correlated with testosterone in males and estradiol in females (Tables 5 and 6).

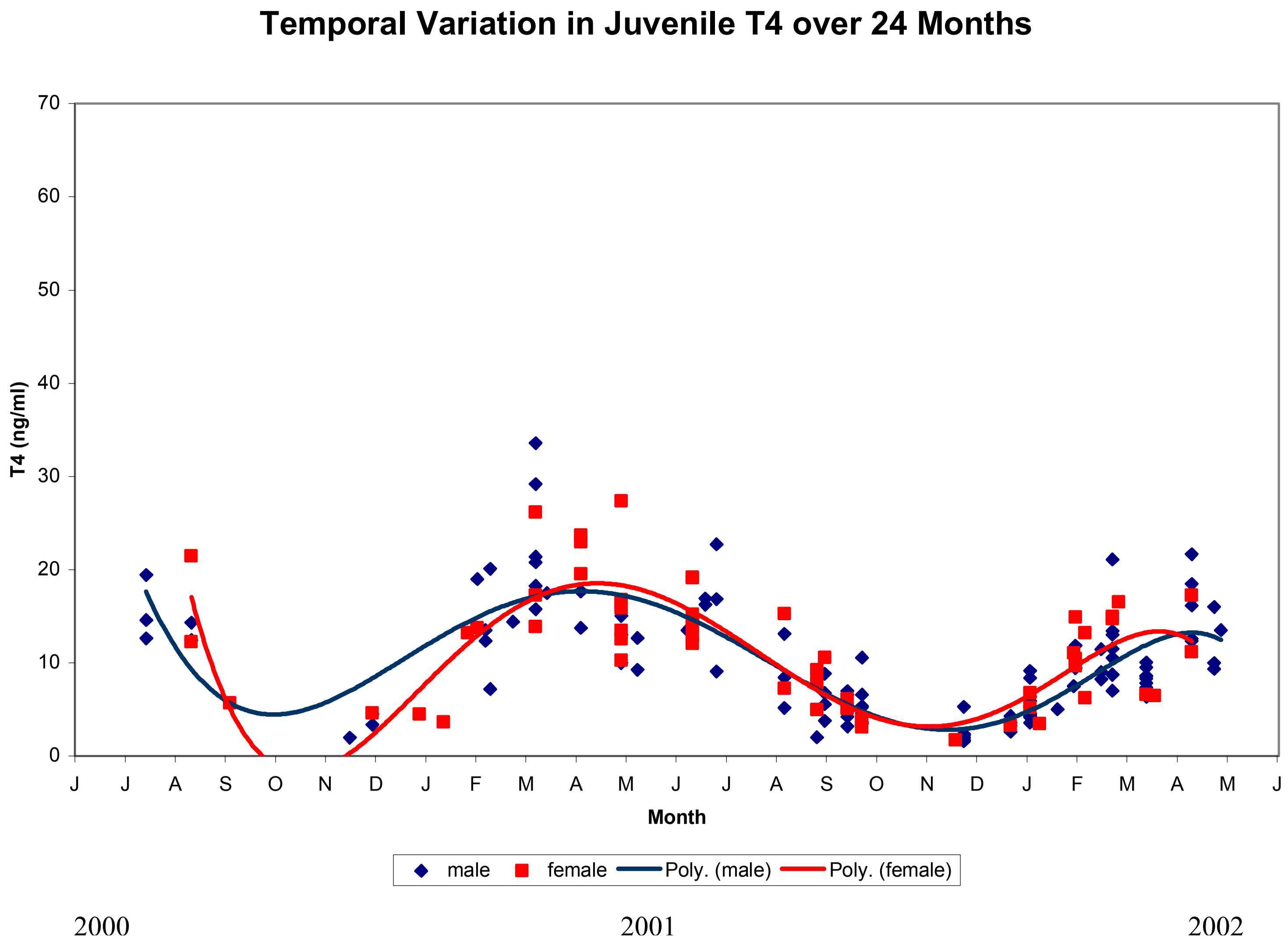

Figure 5. Thyroxine values for juvenile alligators collected between June 2000 and June 2002 with polynomial trendlines indicating seasonal changes in mean T_4.

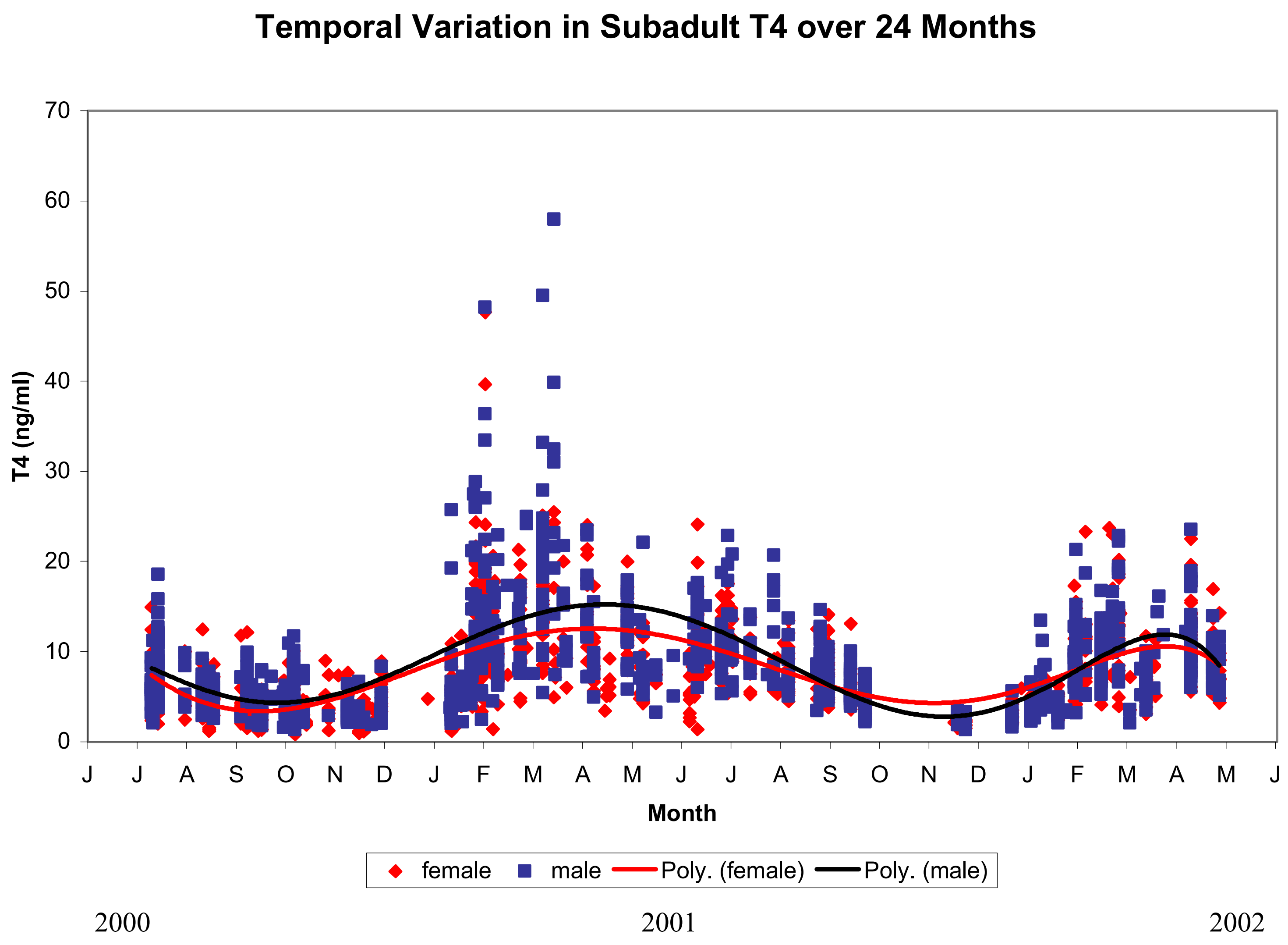

Figure 6. Thyroxine values for subadult alligators collected between June 2000 and June 2002 with polynomial trendlines indicating seasonal changes in mean T_4.

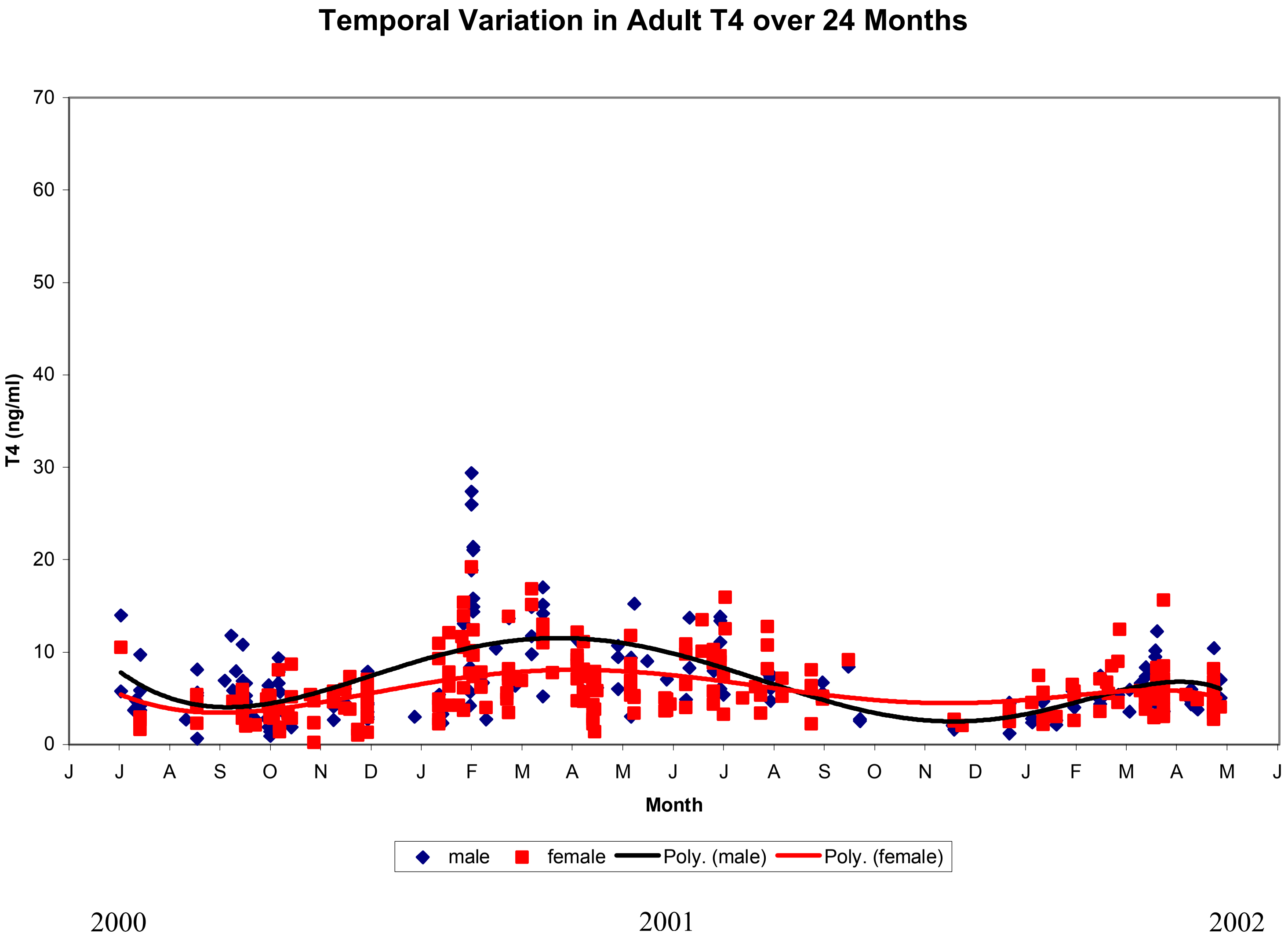

Figure 7. **Thyroxine values for adult alligators collected between June 2000 and June 2002 with polynomial trendlines indicating seasonal changes in mean T_4.**

Table 3. Hourly thyroxine values by sex

Time	*Male*	*Female*
0:00-2:00	$5.1^{*,1}$ ± 0.8, (8)	$5.4^{*,1}$ ± 1.0, (4)
2:00-4:00	ND	ND
4:00-6:00	ND	ND
6:00-8:00	$5.4^{*,1}$ ± 1.7, (3)	$5.2^{*,1}$ ± 0.9, (7)
8:00-10:00	$6.1^{*,A,1}$ ± 0.4, (47)	$5.5^{*,A,1}$ ± 0.4, (55)
10:00-12:00	$6.4^{*,A,C,1}$ ± 0.8, (45)	$4.9^{*,A,1}$ ± 0.4, (51)
12:00-14:00	$8.3^{*,1}$ ± 0.7, (73)	$7.4^{*,1}$ ± 0.7, (70)
14:00-16:00	$8.0^{*,1}$ ± 0.7, (71)	$6.8^{*,A,C,D,1}$ ± 0.5, (66)
16:00-18:00	10.8^{*} ± 6.5, (2)	ND
18:00-20:00	$8.3^{*,2}$ ± 0.4, (297)	$7.7^{*,C,D,1}$ ± 0.3, (256)
20:00-22:00	$9.2^{*,B,C,1}$ ± 0.3, (473)	$8.8^{*,B,1}$ ± 0.2, (491)
22:00-0:00	$10.1^{*,B,1}$ ± 0.5, (131)	$8.7^{*,B,D,1}$ ± 0.4, (146)
Total	8.7 ± 0.2, (1147)	8.0 ± 0.1, (1146)

ND – not determined due to insufficient samples available (n<2)

*Values sharing an asterisk do not differ significantly between consecutive times within sex (P=0.05)

^{A,B,C}Values sharing the same superscript do not differ significantly within sex (P=0.05)

^{1,2}Values sharing the same superscript do not differ significantly between sexes at the same time (P=0.05)

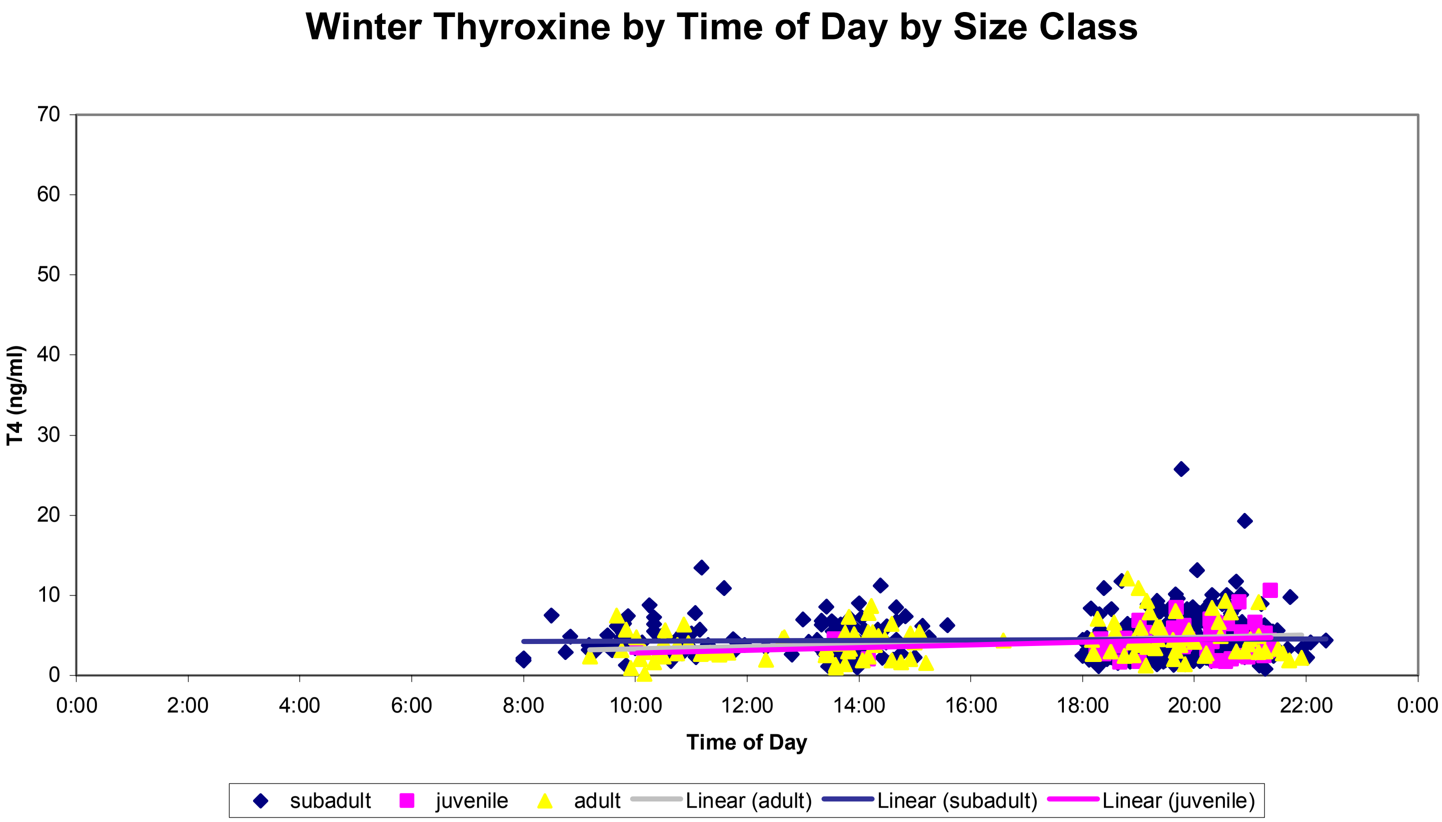

Figure 8. Diurnal distribution of thyroxine from alligators collected October through January with linear trendlines indicating diurnal trajectories in mean T_4.

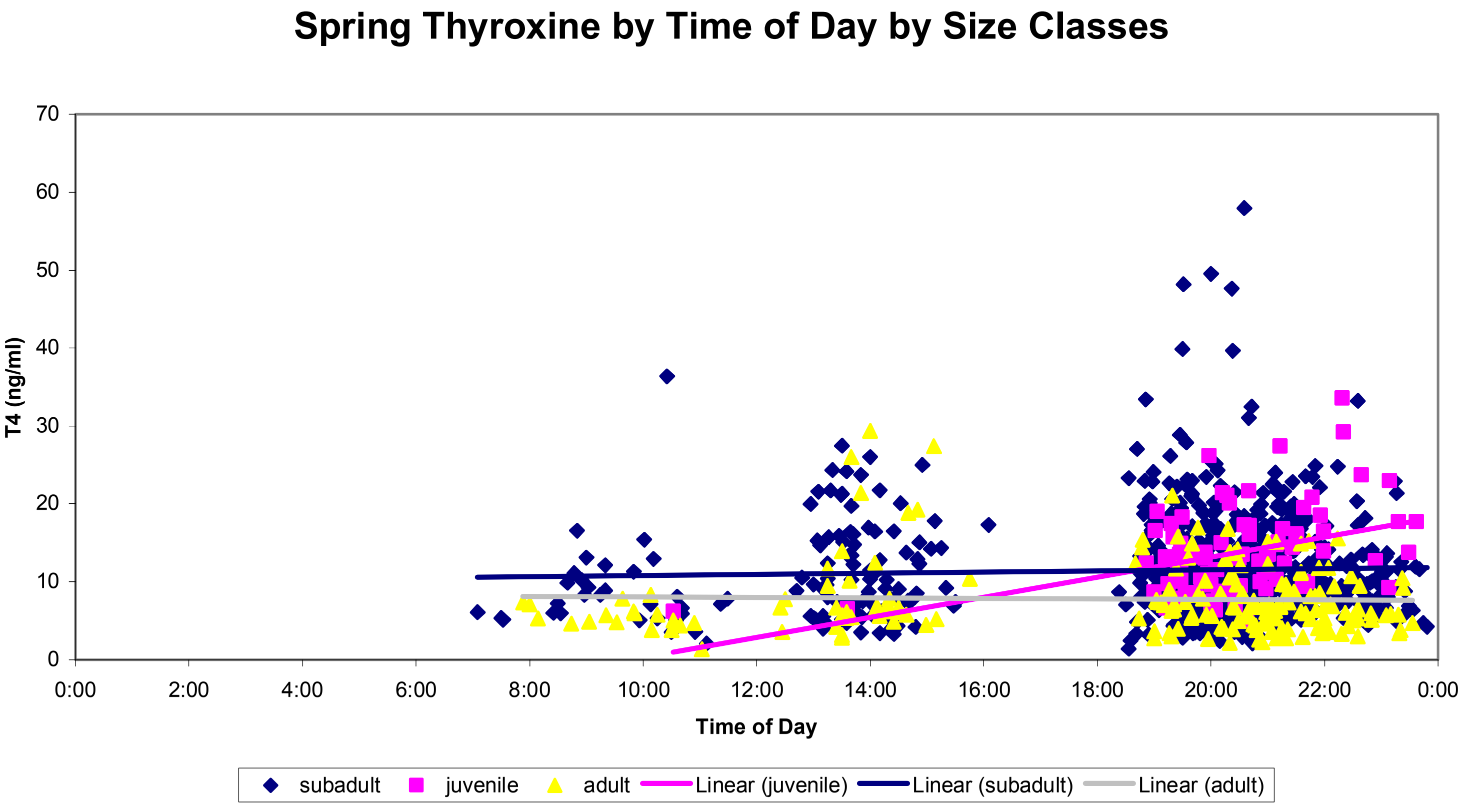

Figure 9. Diurnal distribution of thyroxine values for alligators collected between February and May with linear trendlines indicating diurnal trajectories in mean T_4.

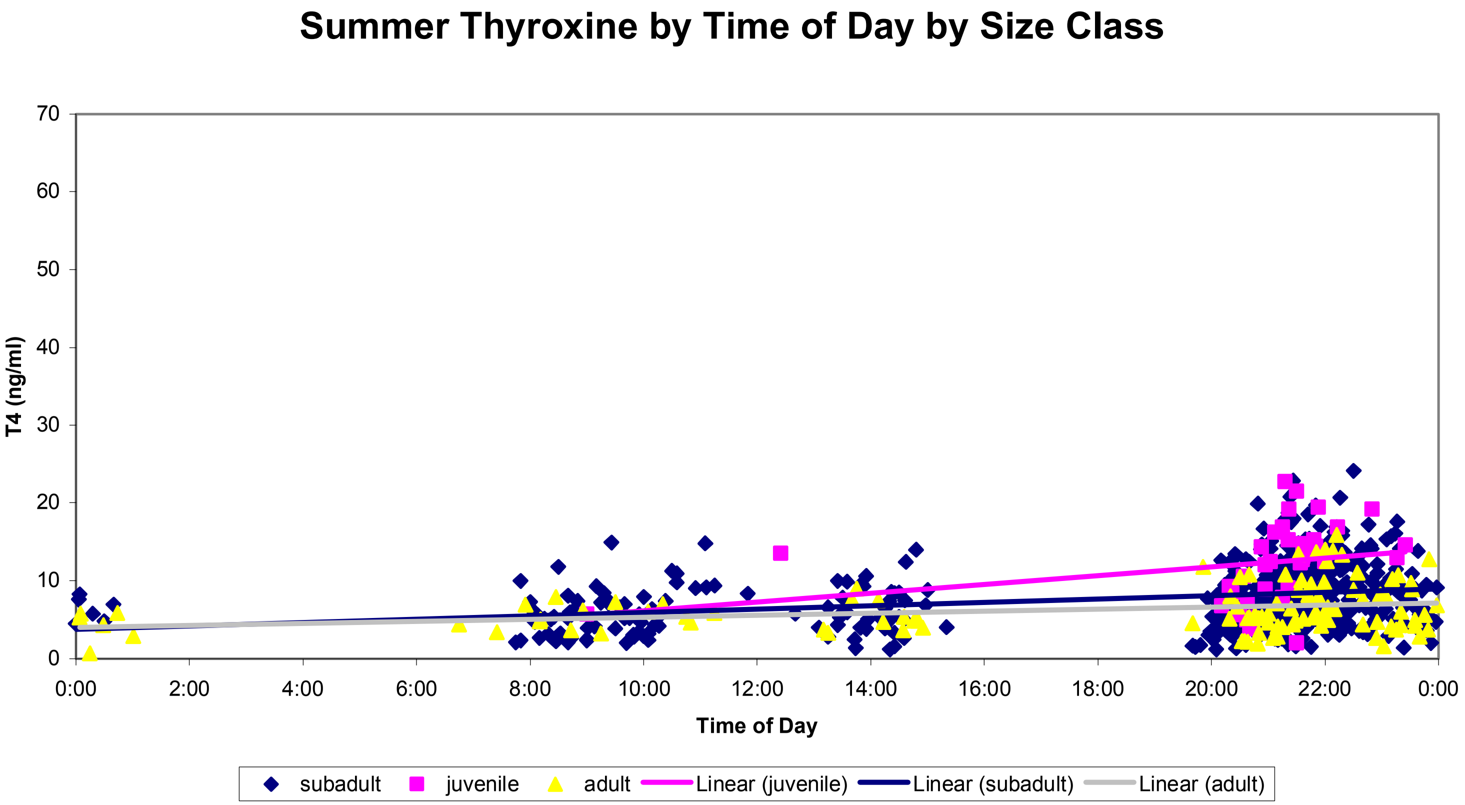

Figure 10. Diurnal distribution of thyroxine values for alligators collected between June and September with linear trendlines indicating diurnal trajectories in mean T_4.

Table 4. Seasonal comparisons of hourly thyroxine values

Time	Winter	Spring	Summer
0:00-2:00	ND	ND	5.2* ± 0.6, (12)
2:00-4:00	ND	ND	ND
4:00-6:00	ND	ND	ND
6:00-8:00	ND	6.0^{*,1} ± 0.5, (4)	4.8^{*,1} ± 1.3 (6)
8:00-10:00	4.2^{*,1} ± 0.3, (26)	8.1^{*,2} ± 0.6, (24)	5.4^{*,A,C,1} ± 0.4, (49)
10:00-12:00	4.2^{*,1} ± 0.3, (51)	7.4^{*,2} ± 1.5, (23)	7.1^{*,2} ± 0.7, (22)
12:00-14:00	3.9^{*,1} ± 0.3, (50)	11.2^{*,3} ± 0.8, (69)	6.3^{*,2} ± 0.6, (24)
14:00-16:00	4.6^{*,1} ± 0.3, (51)	10.8^{*,2} ± 0.8, (57)	5.9^{*,A,2} ± 0.5, (29)
16:00-18:00	ND	ND	ND
18:00-20:00	4.3^{*,1} ± 0.2, (255)	11.4^{*,2} ± 0.4, (290)	4.4^{*,A,B,C,1,2} ± 1.3 (8)
20:00-22:00	4.8^{*,1} ± 0.2, (178)	11.2^{*,3} ± 0.4, (443)	8.4^{*,B,C,2} ± 0.2, (343)
22:00-0:00	2.7^{*,1,2} ± 0.5, (6)	10.3^{*,2} ± 0.5, (116)	8.9^{*,B,1} ± 0.4, (155)
Total	**4.4 ± 0.1 (617)**	**11.0 ± 0.2 (1026)**	**7.9 ± 0.2 (648)**

ND – not determined due to insufficient samples available (n<2)

*Values sharing an asterisk do not differ significantly between consecutive times within season (P=0.05)

^{A,B,C}Values sharing the same superscript do not differ significantly within season (P=0.05)

^{1,2}Values sharing the same superscript do not differ significantly among seasons at the same time (P=0.05)

Table 5. Correlations between Female hormone levels and body length.

	Length	*T_4*	*Estradiol*	*Testosterone*	*Corticosterone*
T_4	**-0.262**	1			
Sig	**0.000**		-----	-----	-----
N	**1146**				
Estradiol	**0.250**	**0.222**	1		
Sig	**0.000**	**0.002**		-----	-----
N	**195**	**195**			
Testosterone	0.014	0.079	-0.061	1	
Sig	0.742	0.062	0.503		-----
N	551	551	122		
Corticosterone	-0.071	-0.075	**-0.195**	-0.013	1
Sig	0.089	0.070	**0.007**	0.764	
N	581	581	**188**	506	

Table 6. Correlations between Male hormone levels and body length.

	Length	*T_4*	*Estradiol*	*Testosterone*	*Corticosterone*
T_4	**-0.179**	1			
Sig	**0.000**		-----	-----	-----
N	**1147**	1147			
Estradiol	**-0.464**	**0.627**	1		
Sig	**0.017**	**0.001**		-----	-----
N	**26**	**26**	26		
Testosterone	**0.330**	0.063	-0.069	1	
Sig	**0.000**	0.122	0.778		-----
N	**606**	606	19	606	
Corticosterone	**0.158**	**-0.102**	0.087	0.060	1
Sig	**0.000**	**0.016**	0.672	0.173	
N	**556**	**556**	26	526	556

SUMMARY AND CONCLUSIONS

The thyroid hormones measured in our study are similar to levels (0-40 ng/ml) previously reported for other alligators (Barnett et al., 1998, 1999; Crain et al., 1998; Gunderson et al., 2002; Hewitt et al., 2002). Ninety-nine percent of alligators sampled at Rockefeller Wildlife Refuge had T_4 values within this range. During the first year of our study, a rapid increase in subadult T_4 occurred between January and February resulted in the highest observed values in the entire data set. Titers in February – March of the first year (30-60 ng/ml) were the highest reported for any non-emydid reptile. Understanding the factors that cause high T_4 levels will ultimately lead to a more thorough knowledge of the regulation of reptilian thyroid function. There have been several proposed factors that may contribute to the regulation of the reptilian thyroid system. As mentioned in the introduction, reproductive activity, nutrition, temperature, and locomotor activity have been suggested to influence thyroid function in other reptiles and may also regulate that of the Rockefeller alligator population. Additionally, our findings suggest a relationship between body size and thyroid function.

In most other reptilian thyroid hormone studies, only one size class of animals has been examined, generally adults. The constraint presented in this type of study is that the potential effects of the different natural histories of each size class on thyroid function are missed. Our study included juveniles, subadults, and adults, with significantly higher mean T_4 observed in juveniles than in subadults. Similarly, subadults exhibited significantly higher T_4 than adults. The significant negative correlation we found between length and T_4 was also found by Crain et al. (1998) at Bella Glade in Florida, a relatively pristine alligator habitat, and by Gunderson et al. (2002) in a few, but not all groups of alligators at varied sites in Florida. Denver and Licht (1991) showed that thyroidectomized turtles ceased somatic growth and that T_4 treatment restored growth, suggesting that somatic growth is promoted by thyroid hormones. The authors suggested a two tiered mechanism involving the direct actions of thyroid hormones in promoting skeletal and muscular growth as well as indirect actions via recruitment of other endocrine pathways, such as the growth hormone axis. Juvenile and subadult alligators grow at approximately 30 cm in length per year in the wild. At 180 cm in females and 300 cm in males this growth rate begins to slow as alligators approach adulthood (McIlhenny, 1935; Chabreck and Joanen, 1979). The elevation of thyroid hormones in juvenile alligators my thus reflect a role in supporting rapid growth. Alternatively, it may instead reflect differences in diet. Adult alligators, especially reproductive females, spend a large portion of the year engaged in reproductive activities that may diminish feeding, whereas juveniles and subadults may be focused on feeding from February to October. The reduced feeding may contribute to the lower observed plasma T_4 titers in adults and further, may account for the gender difference in adult growth rates. Additionally, the observed difference between juveniles and subadults may reflect different diets (McIlhenny, 1935).

Nutritional status has been suggested to positively influence T_4 secretion in reptiles. In mammals and fish, reduced feeding results in reduced thyroid hormone production (Eales, 1988; MacKenzie et al., 1998). Barnett et al. (1999) demonstrated in alligators that increased feeding in captivity led to a significant increase in circulating thyroid hormones. Studies in tortoises concluded that seasonal changes in T_4 reflect seasonal changes in feeding activity (Kohel et al, 2001). Kohel et al (2001) further demonstrated that increased feeding can promote thyroid activity by refeeding tortoises that had been food restricted and measuring a resultant rise in T_4. In many vertebrates, nutrient intake has been linked to stimulation of hypothalamic peptides such as neuropeptide Y (NPY), capable of promoting secretion of pituitary hormones, including TSH (reviewed in MacKenzie et al, 1998). Our data from the Rockefeller alligators also suggest that seasonal feeding may activate T_4 secretion, as highest levels are observed during spring and summer, seasons of most intense feeding activity (McIlhenny, 1935). In our study juveniles had significantly higher mean T_4 than adults although the range in adults was greater than that of juveniles. Adult alligators are episodic feeders and are capable of consuming much larger prey items than juveniles. As a result, adults may go for longer periods of time between meals, whereas juveniles cannot (McIlhenny, 1935). The episodic feeding in adults may contribute to their observed low plasma T_4, while the relatively high mean T_4 in juveniles may be reflective of a continuous feeding pattern. In contrast, subadults represent a change in feeding behavior in the transition from juvenile to adulthood (McIlhenny, 1935; Fitzgerald, 1988). Remaining at a relatively high growth rate (~30 cm/yr), subadults begin consuming larger prey items (larger fish, amphibians, other reptiles, etc) that possess substantially more thyroid tissue than juvenile prey (invertebrates, small fish). As suggested by Eales (1997), ingested thyroid tissue may contribute to the circulating levels of T_4 as thyroid hormones are absorbed from the gut. The higher percentage of thyroid tissue consumed by the subadults combined with continual feeding may account for the highest observed individual values as well as the greatest amount of variability in plasma T_4.

Several studies have proposed that reproductive condition influences T_4 secretion. Many reptilian seasonal studies reveal reciprocating levels of T_4 and reproductive steroid hormones (Bona-Gallo et al., 1980; Kar and Chandola-Saklani, 1985; Licht et al., 1985; Naulleau et al., 1987; Kohel et al., 2001), suggesting that reproduction inhibits T_4 secretion. Additionally, Jallageas et al. (1978a, b, 1979) found a consistent reciprocal relationship between T_4 and reproductive steroids in birds. Our adult alligators displayed highest seasonal T_4 immediately prior to the breeding season. Thyroxine declined as testosterone and estradiol peaked (Lance, 1989), supporting the reciprocal relationship. However, no gender difference was observed in T_4 in most contrasts made between sexes in adults or juveniles, as was also observed by Gunderson et al. (2002) and Hewitt et al. (2002) in Florida alligators. This is surprising given the differences in the annual reproductive cycles of adult male and female alligators. While females are almost constantly participating in some physiological or behavioral event associated with reproduction (vitellogenesis, ovulation, copulation, nest building, oviposition, nest guarding), males primarily participate during the spring in preparation for mating, along with spermatogenesis in the winter (Lance, 1989). After mating, males resume foraging while females complete the remainder of their reproductive tasks. Estradiol in females and testosterone in both males and females reach highest plasma titers at the peak of the breeding season then rapidly decline. Males, however show a secondary prespermatogenic peak in testosterone in October, just prior to torpor, while estradiol in females remains basal throughout the remainder of the year (Lance, 1989). With very gender specific natural histories and steroid hormone profiles as these, one might expect to see a greater gender difference in seasonal T_4 if reproductive steroids directly influence thyroid function. A similar gender specific seasonal steroid cycle in gopher tortoises was reflected in a gender specific T_4 cycle, suggesting a closer relationship between thyroid function and reproductive condition in tortoises (Kohel et al., 2001). Additional support for a lack of reproductive influence on thyroid function comes from the observation of no difference in the annual pattern of T_4 between adult and juvenile alligators. However, Lance and Elsey reported (2002) that juvenile male alligators exhibit circulating testosterone in a seasonal pattern similar to that of adult males, but at a much lower magnitude. The one reproductive similarity among adult males, adult females, and juvenile males is thus a transient elevation of testosterone in April and May, suggesting that the February/March peak in thyroxine may reflect a metabolic activation that precedes testosterone production. The lack of consistent seasonal differences between adult males and females and the similarity between adult and juvenile seasonal T_4 cycles suggest, however, that reproduction is not a primary factor influencing circulating T_4 in alligators.

Corticosterone is the primary glucocorticoid released from the alligator adrenal in response to chronic stress (e.g. overcrowding, noise, exposure to inappropriate light, temperature, humidity, nutrition, salinity, or pH) (Lance, 1994). Corticosterone has been shown to inhibit growth in alligators in a dose dependant manner (Elsey et al., 1990; Morici et al., 1997). Elsey et al. (1990) demonstrated that higher stocking densities of alligators resulted in higher mean titers of corticosterone as well as decreased mean growth rates. Interestingly, within each group, a gradient existed so that dominant animals had lower corticosterone and higher growth rates relative to their peers. In environmentally controlled chambers containing identical stocking densities, groups of alligators treated with subcutaneous corticosterone implants displayed a dose dependant reduction in growth rate (Morici et al., 1997). The results of these studies indicate that the physiological response to stress in alligators is a significant reduction in somatic growth capacity. It is currently unknown, however whether or not thyroid hormones are involved in this inhibition. The only significant correlation we found between corticosterone and T_4 was in males, with a very low Pearson coefficient. However, as these were free-living animals, corticosterone levels were generally lower than those found in confined or crowded alligators. The relationship between adrenal function and circulating thyroid hormone levels may be more clearly observed in confined or corticosterone-treated alligators.

It has been proposed that temperature may play a role in the regulation of the reptilian thyroid axis. Several seasonal studies show a T_4 peak immediately following emergence from dormancy as temperatures begin to increase in the spring (Bona-Gallo et al., 1980; Kar and Chandola-Saklani, 1985; Kohel et al., 2001; Licht et al., 1985; Naulleau et al., 1987). Licht et al. (1989) discovered that turtle pituitaries in culture respond to TRH stimulation maximally at higher temperatures suggesting that elevated temperatures increase the sensitivity of the thyroid axis to stimulation. In a study done at Rockefeller Wildlife Refuge, it was reported that decreasing temperature is the environmental cue that signals the beginning of torpor, and that during induced torpor, alligators do not feed. In animals held in environmentally controlled chambers at constant high temperature, torpor did not occur and the animal’s metabolism remained elevated (Coulson and Hernandez, 1983), suggesting that temperature is a primary factor in signaling the reduced metabolism associated with torpor. Elevated temperatures were also associated with increased growth rates in captive alligators (Joanen and McNease, 1971). In our study, during the winter months, the lowest temperatures (NOAA, 2003) coincided with the lowest T_4 of the year. The highest T_4 peak was late in February as temperatures began increasing from the winter. The increase in T_4 associated with warming temperatures in February is consistent with a stimulatory effect of temperature on alligator thyroid function. However, during the summer months, when temperatures were highest, T_4 declined from the spring peak. If temperature directly stimulates the hypothalamo-pituitary-thyroid (HPT) axis by enhancing T_4 production or transport, then we would expect highest T_4 in the summer when body temperatures should be highest. Although alligators thermoregulate behaviorally by basking and swimming and may use this technique to maintain lower body temperatures (McIlhenny, 1935), studies in Florida indicate that body temperatures of alligators increase from the spring through the summer (Percival et al, 2002). Because we lack data on the actual body temperature of our alligators in the summer we are unable to determine if the decline in T_4 is associated with thermoregulation at a lower body temperature. Alternatively, alligators may possess physiological mechanisms for catabolizing T_4 in peripheral tissues to reduce circulating levels. Finnison and Eales, (1999) and Plohman, et al (2002) have suggested that fish may activate enzymes that catabolize T_4 in order to diminish peripheral stimulation. If a similar system existed in alligators, circulating T_4 would be reduced during the summer in the face of high temperatures.

Locomotor activity has been implicated in stimulating T_4 secretion. Several seasonal reptilian studies show seasonal T_4 elevations immediately following emergence from winter dormancy (Bona-Gallo et al., 1980; Kar and Chandola-Saklani, 1985; Kohel et al., 2001; Licht et al., 1985; Naulleau et al., 1987) that may be associated with increased foraging, combat, or searching for mates. John-Alder (1983, 1984, 1990, 1991) correlated increased T_4 with increased aerobic capacity and endurance in lizards. The conclusion from these studies is that T_4 promotes or supports aerobic metabolism in reptiles. Our alligators display a spring peak immediately following emergence. Telemetric studies at Rockefeller show that alligators are very active at this time with females migrating to the breeding grounds and males bellowing and fighting (Joanen and McNease, 1970, 1972). Additionally, it was observed that juveniles and subadults were generally more active over a greater range of environmental conditions than adult cohabitants (McNease and Joanen, 1974). This might account for higher T_4 in juveniles and subadults. Based on this consistency in seasonal pattern among reptilian studies and our knowledge of alligator behavior at this time, locomotor activity may be a factor that positively influences thyroid function in alligators.

In mammals, 90% of thyroid gland secretion is T_4, which is deiodinated at target tissues into T_3 (McNabb, 1992). After deiodination, T_3 binds to nuclear receptors where it modulates transcription. In our wild alligator samples T_3 was generally nondetectable (<0.6 ng/ml). In other wild reptilian thyroid hormone studies, T_3 has been nondetectable although when retained in captivity wild alligators begin to circulate detectable levels of T_3 (Barnett et al., 1999). This significant increase in T_3 was attributed to increased feeding. In the wild, nutrient availability is sporadic and may result in the increased physiological utilization of T_3. In captivity, nutrients are virtually unlimited and thus T_3 production may exceed its utilization. Denver and Licht (1988) report that T_3 is more effective than T_4 in inhibiting pituitary secretion of thyrotropin *in vitro* and Shepherdley et al. (2002) discovered outer ring deiodinase in peripheral tissues of crocodiles. Because of the apparent capacity of crocodilians to generate T_3 and its physiological importance in many vertebrate species, nondetectable amounts in the wild do not exclude T_3 as a relevant hormone in reptilian metabolic physiology. Eales suggested that the relative abundance of circulating T_3 in fish may indicate that peripheral regulation of deiodinase expression is more important than the central control along the HPT axis in this vertebrate group. In humans and other homeothermic vertebrates, the primary regulatory agents of the thyroid axis are located in the central nervous system, resulting in low to non-detectable levels of circulating T_3. Reptiles represent an interesting phylogenetic position between fish and mammals. Wild reptiles, including alligators, are similar to mammals in that T_3 levels are generally non-detectable indicating that central regulation along the HPT axis may be the primary agent responsible for control of the thyroid hormone system.

This study provides the largest thyroxine data set yet recorded for wild alligators, comprising all size classes, sampled during every month, and during all times of day for two consecutive years. This sample shows a distinct T_4 cycle that, with one exception, is closely replicated in the second year. In year 1, T_4 levels above 25 ng/ml were noted in twenty-four animals in February. Similar levels were not observed in year 2. The reason that this spring peak does not repeat in year 2 with the same amplitude is unknown. It does not appear to be due to inconsistencies in the T_4 assay, as our assay has been validated for alligator plasma and contained internal standards that showed acceptable low interassay variability. Alternatively, it is possible that environmental conditions were different during the second year (e.g. temperature, cloud cover, rainfall, salinity, disturbance, food availability etc.). Perhaps the elevated animals in year 1 had recently fed, or may have had more opportunity to raise body temperature. A similar phenomenon was observed by Gunderson et al. (2002), who reported significant differences in mean T_4 in Florida alligator populations between two consecutive years. This underscores the importance of examining overall patterns of circulating hormones over longer periods rather than comparing absolute values between studies conducted at discrete times (Gunderson et al., 2002). As previously described, the thyroid axis is regulated at multiple levels and seasonal changes in total T_4 levels may not reflect seasonality in hormone activation of target tissues. To gain further insight into the regulation of the reptilian thyroid system, future studies should investigate seasonal changes in proteins critical to thyroid hormone production and delivery to target tissues, such as deiodinase, TSH, and blood binding proteins. The complexity of control of the thyroid axis and its ability to compensate for disruption requires that studies of thyroid hormone levels over brief periods must be interpreted with caution.

Seasonal changes in thyroid function of alligators have received recent attention because of the possibility of environmental endocrine disruption. Several studies from Florida have proposed that alligator thyroid hormones may serve as a useful tool for evaluating environmental health. Crain et al. (1998), Hewitt et al. (2002), and Gunderson et al. (2002) have measured thyroid hormones in alligators from habitats ranging from pristine to heavily contaminated, and have suggested that thyroid hormones or thyroid cell histology may reflect alterations in habitat quality. As with our study, they found no gender difference in circulating T_4 (Crain et al., 1998; Gunderson et al., 2002; and Hewitt et al., 2002), and a negative relationship between length and T_4 (Crain et al., 1998; Hewitt et al., 2002). Although mean total T_4 did not consistently reflect environmental contamination with organochlorides, it was suggested that variance in T_4 may be a reliable indicator of contaminant exposure (Gunderson et al., 2002). However, these studies described only juvenile alligators collected at night in April-May. In our study, we observed ranges in T_4 which were much greater than those observed in the Florida studies. Developmental, daily or seasonal changes in thyroid function may thus contribute to variability in plasma hormone concentrations. Our study provides a comprehensive seasonal thyroxine profile in normal animals from a pristine habitat as well as identifying periods of maximum levels (e.g. February-April) during which the thyroid axis may be most sensitive to disruption. The seasonal changes and results from our study may thus serve as a platform from which to launch future studies that examine more closely components of both basic and disrupted alligator thyroid physiology.

The seasonally high plasma T_4 titers (40-60 ng/ml) illustrate the dynamic nature of the American alligator's thyroid system. The ability of an animal to regulate its thyroid hormone levels over such a broad range endows it with the potential to become a new reptilian model system for thyroid physiology. Additionally, the availability of wild animals of all sizes, potential for obtaining multiple, substantial blood samples, ease of captive maintenance, and accessibility to eggs make the American alligator a prime model species for studying reptilian thyroid physiology. The results of this study not only serves as a seasonal T_4 reference upon which future lab studies may be based, but suggests that the American alligator has great potential for the examination of the relationships between thyroid function and growth, reproduction, and temperature in a reptilian species.

REFERENCES

Abercrombie, C.L., S.R. Howarter, H.F. Percival, K.G. Rice, and C.R. Morea (2002). "Everglades Alligator Thermoregulation: Unanswered Questions," Proceedings of the 16th Working Meeting of the Crocodile Specialist Group. Gainesville, Florida, 131-140.

Barnett, J. D., P. T. Cardeilhac, B. Barr, W. Wolff, O. L. Bass, and D. L. Fleming (1998). "Utilization of thyroid hormone levels to determine starvation in alligators from the Everglades National Park," Proceedings of the International Association of Aquatic Animal Medicine **29**, 52-56.

Barnett, J. D., P. T. Cardeilhac, B. Barr, W. Wolff, O. L. Bass (1999). "Differences between captive-raised and wild-caught Everglades National Park alligators in serum chemistry values, serum protein electrophoresis, thyroid hormone levels, and complete blood counts," Proceedings of the International Association of Aquatic Animal Medicine **30**, 136-140.

Bona-Gallo, A., P. Licht, D. S. MacKenzie, and B. Lofts (1980). Annual cycles in levels of pituitary and plasma gonadotropin, gonadal steroids, and thyroid activity in the Chinese cobra (*Naja naja*). *General and Comparative Endocrinology* **42**, 477-493.

Cardeilhac, P.T., D.L. Winternitz, J.D. Barnett, O.L. Bass, and W.R. Wolff (1999). "The American Alligator as a sentinel for the environmental status of southern wetlands," Proceedings of the International Association of Aquatic Animal Medicine **30**, 69-70.

Chabreck, R.H. and T. Joanen (1979). Growth rates of American alligators in Louisiana. *Herpetologica*, **35**(1): 51-57.

Chandola-Saklani, A. and A. Kar (1990). Evidence for the role of thyroxine as a hormone in the physiology of a lizard. *General and Comparative Endocrinology* **78**, 173-179.

Chiu, K. W., M. S. Leung, and P. F. A. Maderson (1983). Thyroid and skin-shedding in the rat snake (*Ptyas korros*). *Journal of Experimental Zoology* **225**, 407-410.

Coulson, R.A. and T. Hernandez (1983). "Alligator Metabolism," p. 182. Pergamon Press, Elmsford, NY.

Crain, D. A., L. J. Guillette Jr., D. B. Pickford, H. F. Percival, and A. R. Woodward (1998). Sex-steroid and thyroid hormone concentrations in juvenile alligators (*Alligator mississippiensis*) from contaminated and reference lakes in Florida, USA. *Environmental Toxicology and Chemistry* **17** (3) 446-452.

Denver, R.J. and P. Licht (1989). Neuropeptides influencing *in vitro* pituitary hormone secretion in hatchling turtles. *Journal of Experimental Biology* **251**, 306-315.

Denver, R.J. and P. Licht (1991). Dependence of body growth on thyroid activity in turtles. *Journal of Experimental Biology* **258**, 48-59.

Dixon, J.R (1987). “Amphibians and Reptiles of Texas,” p. 434. Texas A&M University Press, College Station, TX.

Eales, J.G (1997). “Iodine Metabolism and Thyroid-Related Functions in Organisms Lacking Thyroid Follicles: Are Thyroid Hormones also Vitamins?” Proceedings of the Society for Experimental Biology and Medicine, **214**, 302-317.

Elsey, R. M., T. Joanen, L. McNease, and V. Lance (1990). Growth rate and plasma Corticosterone levels in juvenile alligators maintained at different stocking densities. *Journal of Experimental Zoology* **255**, 30-36.

Finnison, K.W. and J.G. Eales (1999). Effect of T_3 treatment and food ration on hepatic deiodination and conjugation of thyroid hormones in Rainbow trout, *Oncorhynchus mykiss*. *General and Comparative Endocrinology* **115**, 379-386.

Fitzgerald, L.A. (1988). “Dietary patterns of *Caiman crocodilus* in the Venezuelan Llanos,” M.S. thesis, University of New Mexico, Albuquerque, NM.

Guillette, L.J., Woodward A.R., Crain D.A., Pickford D.B., Rooney A.A., and H.F. Percival (1999). Plasma steroid concentrations and male phallus size in juvenile alligators from seven Florida lakes. *General and Comparative Endocrinology* **116** (3), 356-372.

Gunderson, M. P., D. S. Bermudez, T. A. Bryan, D. A. Crain, S. Degala, T. M. Edwards, S. A.E. Kools, M. R. Milnes, and L. J. Guillette, Jr. (2002). Temporal and spatial variation in plasma thyroxine (T_4) concentrations in juvenile alligators collected from Lake Okeechobee and the northern Everglades, Florida, USA. *Environmental Toxicology and Chemistry* **21** (5), 914-921.

Hewitt, E.A., D. A. Crain, M. P. Gunderson, and L. J. Guillette Jr. (2002). Thyroid status in juvenile alligators (*Alligator mississippiensis*) from contaminated and reference sites on Lake Okeechobee, Florida, USA. *Chemosphere* **47**, 1129-1135.

Hulbert, A. J. (2000). Thyroid hormones and their effects: A new perspective. *Biological Review* **75**, 519-631.

Jacob, V. and O. V. Oommen (1990). Intermediary metabolism in a lizard, *Calotes versicolor*: Role of thyroid hormones. *General and Comparative Endocrinology* **77**, 324-336.

Jallegeas M., B.K. Follet, and I. Assenmacher (1978a). Thyroid-gonadal interactions during the postnuptial phase of the sexual cycle in male ducks. *General and Comparative Endocrinology* **34**, 68-69.

Jallegeas M., A. Tamisier, and I. Assenmacher (1978b). A comparative study of the annual cycles in sexual and thyroid function in male Peking ducks (*Anas platyrhyncos*) and teal (*Anas creca*). *General and Comparative Endocrinology* **36**, 201-210.

Jallegeas M. and I. Assenmacher (1979). Further evidence for reciprocal interactions between the annual sexual and thyroid cycles in male Peking ducks. *General and Comparative Endocrinology* **37**, 44-51.

Joanen, T. and L. McNease (1970). "A Telemetric Study of Nesting Female Alligators on Rockefeller Refuge, Louisiana." Proceedings of the Southeast Association of Game and Fish Commissioners. **24**, 175-193.

Joanen, T. and L. McNease (1971). "Propagation of the American Alligator in Captivity." Proceedings of the Annual Conference of the Southeastern Association of Game and Fish Commissioners. **25**, 106-116.

Joanen, T. and L. McNease (1972). "A Telemetric Study of Adult Male Alligators on Rockefeller Refuge, Louisiana." Proceedings of the Southeast Association of Game and Fish Commissioners. **26**, 252-275.

Joanen, T. and L. McNease (1975). "Notes on the Reproductive Biology and Captive Propagation of the American Alligator." Proceedings of the Southeast Association of Game and Fish Commissioners. **29**, 407-415.

Joanen, T. and L. McNease (1979). "Time of Egg Deposition for the American Alligator." Proceedings of the Annual Conference of the Southeast Association of Fish & Wildlife Agencies **33**, 15-19.

John-Alder, H (1983). Effects of thyroxine supplementation on metabolic rate and aerobic capacity in a lizard. *American Physiological Society* R659-R666.

John-Alder, H (1984). Reduced aerobic capacity and locomotory endurance in thyroid-deficient lizards. *Journal of Experimental Biology*. **109**, 175-189.

John-Alder, H (1990). Effects of thyroxine on standard metabolic rate and selected intermediary metabolic enzymes in field-active lizards, *Sceloporus undulatus. Physiological Zoology*. **63**, 600-614.

John-Alder, H. and B. Joos (1991). Interactive effects of thyroxine and experimental location on running endurance, tissue masses, and enzyme activities in captive versus field-active lizards (*Sceloporus undulatus*). *General and Comparative Endocrinology* **81**, 120-132.

Joos, B. and H. B. John-Alder (1990). Effects of thyroxine on standard and total metabolic rates in the lizard, *Sceloporus undulatus*. *Physiological Zoology*. **63** (5), 873-885.

Kar, A., and A. Chandola-Saklani (1985). Circulating thyroid hormone concentrations in relation to seasonal events in the male Indian garden lizard, *Calotes versicolor*. *General and Comparative Endocrinology* **60**, 14-19.

Kohel, K. A., D. S. MacKenzie, D. A. Rostal, J. S. Grumbles, and V. A. Lance (2001). Seasonality in plasma thyroxine in the desert tortoise, *Gopherus agassizii*. *General and Comparative Endocrinology* **121**, 214-222.

Lance, V. A. (1989). Reproductive cycles of the American alligator. *American Zoologist* **29**, 999-1018.

Lance, V. A. (1994). Life in the slow lane: Hormones, stress, and the immune system in reptiles. In: Perspectives in Comparative Endocrinology. *National Research Council of Toronto Canada*, 529-534.

Lance, V. and R. Elsey (2002). “Sexual Maturity in Male American Alligators: What Can Plasma Testosterone Tell Us?” Proceedings of the 16th Working Meeting of the Crocodile Specialist Group. Gainesville, Florida, 152.

Leiner, K. A., G. S. Han, and D. S. MacKenzie (2000). The Effects of Photoperiod and Feeding on the Diurnal Rhythm of Circulating Thyroid Hormones in the Red Drum, *Sciaenops ocellatus*. *General and Comparative Endocrinology* **120**, 88-98.

Licht, P., G. L. Breitenbach, and J. D. Congdon (1985). Seasonal cycles in testicular activity, gonadotropin and thyroxine in the painted turtle, *Chrysemys picta*, under natural conditions. *General and Comparative Endocrinology* **59**, 130-139.

Licht, P., R.J. Denver, and S. Pavgi (1989). Temperature dependence of *in vitro* pituitary, testis, and thyroid secretion in a turtle, *Pseudemys scripta*. *General and Comparative Endocrinology* **76** (2), 274-285.

MacKenzie D.S., P. Licht, and H. Papkoff (1981). Purification of thyrotropin from the pituitaries of two turtles: The green sea turtle and the snapping turtle. *General and Comparative Endocrinology* **45**(1), 39-48.

MacKenzie, D.S., C. M. VanPutte, and K. A. Leiner (1998). Nutrient regulation of endocrine function in fish. *Aquaculture* **161**, 3-25.

McIlhenny, E.A. (1935). “The Alligator’s Life History,” p. 117. Christopher Publishing House, Boston, MA.

McNabb, F.M.A. (1992). “Thyroid Hormones,” p. 283. Prentice Hall International, Englewood Cliffs, NJ.

McNease, L. and T. Joanen (1974). “A Study on Immature Alligators on Rockefeller Refuge, Louisiana.” Proceedings of the Southeast Association of Game and Fish Commissioners. **28**, 482-500.

Morici, L. A., R. M. Elsey, and V. A. Lance (1997). Effects of Long-Term Corticosterone Implants on Growth and Immune Function in Juvenile Alligators, *Alligator mississippiensis*. *Journal of Experimental Zoology* **279**, 156-162.

Naulleau, G., F. Fleury, and J. Boissin (1987). Annual cycles in plasma testosterone and thyroxine in the male aspic viper *Vipera aspis* L., (Reptilia, Viperidae), in relation to the sexual cycle and hibernation. *General and Comparative Endocrinology* **65**, 254-263.

Plohman, J. C., T. A. Dick, and J. G. Eales (2002). Thyroid of Lake Sturgeon, *Acipenser fulvescens*. *General and Comparative Endocrinology* **125**, 56-66.

Rodbard, D. (1974). Statistical quality control and routine data processing for radioimmunoassays and immunoradiometric assays. *Clinical Chemistry* **20/10**, 1255-1270.

Shepherdley, C. A., S. J. Richardson, B. K. Evans, E. R. Kuhn, and V. M. Darras (2002). Characterization of outer ring iodothyronine deiodinases in tissues of the saltwater crocodile (*Crocodylus porosus*). *General and Comparative Endocrinology* **125**, 387-398.

Thapliyal, J. P., and R. J. Kaur (1976). Effect of thyroidectomy, l-thyroixine and temperature on hemopoiesis in the chequered water snake, *Natrix piscator*. *General and Comparative Endocrinology* **30**, 182-188.

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