

Seasonal variation in plasma sex steroid concentrations in juvenile American alligators

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Abstract

Seasonal variation in plasma sex steroid concentrations is common in mature vertebrates, and is occasionally seen in juvenile animals. In this study, we examine the seasonal pattern of sex hormone concentration in juvenile American alligators (*Alligator mississippiensis*) and make a limited comparison of these seasonal patterns on two different lakes in Florida. Male juvenile alligators from a reference lake, Lake Woodruff, displayed temporal patterns in plasma testosterone (T) concentrations that appear to be seasonal. A similar pattern in plasma estradiol-17 β (E₂) was observed in juvenile females from Lake Woodruff. Males had significantly elevated T concentrations during the spring and late summer, whereas females had elevated E₂ in the spring and late summer and significantly depressed E₂ concentrations during the winter. A limited 4-month survey of animals from contaminated Lake Apopka found a lack of such seasonality. These results suggest that: (1) healthy wild populations of juvenile alligators have a prolonged peripubescent period that is marked by seasonal hormonal cycles, (2) juvenile alligators exposed to environmental contaminants can lack such seasonal cyclicity, and (3) future studies of juvenile alligators should incorporate such seasonality into the experimental design.

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1. Introduction

Seasonal patterns in sex steroids have been demonstrated for a wide variety of non-mammalian species including, fish (Callard et al., 1991; Orlando et al., 2003; Snelson et al., 1997), amphibians (Harvey et al., 1997; Licht et al., 1983; Zerani et al., 1991), and reptiles (Licht, 1984). Among the reptiles, clear annual cycles have been reported for squamates (Whittier, 1994), turtles (Callard et al., 1978), tuatara (Cree et al., 1992), and crocodilians (Guillette et al., 1997a; Lance, 1989). These cyclical patterns in reproductive activity and reproductive hormones have been assumed to imply sexual maturity. However, in species exhibiting long lives, individuals could require several years to reach sexual maturity, as occurs in many primates (Adams and

Steiner, 1988; Plant, 1988). During this transitional period in primates, gonadal steroidogenesis increases with concomitant increases in plasma sex steroid concentrations. Elevations in sex steroids are involved in many aspects of homeostasis that are only peripherally related to reproduction, including: growth, stress, and immune regulation (Norris, 1997).

Among crocodilians, seasonal cycles in sex steroids have been demonstrated in alligators and caimans. Our knowledge of most crocodilian species has been limited by the difficulty in maintaining captive breeding colonies of crocodilians in captivity and the deleterious effects of restraint on sex steroid concentrations and breeding success (Lance, 1989). The reproductive cycle for the American alligator (*Alligator mississippiensis*) was first published in late 1980s (Lance, 1989) when anatomical and histological data on adult male and female alligators from southern Louisiana were presented. The hormonal data indicated that reproductive activity was maximal during the late spring, a period when elevated steroid concentrations were observed. Guillette et al.

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(1997a) examined the reproductive cycle of adult female alligators in central Florida. This detailed endocrinological study demonstrated that females exhibit elevated ovarian steroidogenesis in September and October with associated hepatic vitellogenin synthesis. Ovarian and hepatic activity is suppressed during the winter months but resumes rapidly with significant increases in plasma estradiol-17 β (E₂) and vitellogenin in spring prior to ovulation in May or June.

As juvenile alligators grow and mature, it is not known how the plasma steroid concentrations increase to adult levels. For instance, hatchling alligators have plasma testosterone (T) concentrations of 20–30 pg/ml and E₂ concentrations of 10–30 pg/ml (Crain et al., 1997), whereas adult male alligators have plasma T concentrations of 2000–50,000 ng/ml (Lance, 1989) and adult female alligators have plasma E₂ concentrations of 100–900 pg/ml (Guillette et al., 1997a). The transition from hatchling to adult concentrations could either be rapid or slowly progressive, and could involve seasonal cycling or not. However, data from several different studies examining plasma T concentrations in juvenile alligators of similar size from several lakes in central Florida suggest that a seasonal pattern could exist in juvenile alligators (Guillette et al., 1999a; Milnes et al., 2002).

The ideal data to answer these questions would be those that tracked hormone concentrations of individual juvenile alligators from month to month over the entire juvenile period (10–15 years). It is unrealistic to expect to obtain these data in wild alligators, but observations can be made that begin to address the question of seasonal variation in sex steroids in juvenile alligators. Animals in the current study were sampled over a 12-month period, and mean concentrations of sex steroids from each sampling period were compared to investigate seasonal variations in sex steroids in juveniles.

Previous research has shown that neonatal and juvenile alligators from several contaminated lakes in central Florida have abnormal sex hormone concentrations when compared to well-controlled sampling studies. Much of the work on contaminant-induced endocrine alterations in alligators has focused on Lake Apopka, a lake with several point sources of contamination, including direct agricultural runoff, city effluent, and abandonment of the Tower Chemical Company pesticide plant in 1980. Following the pesticide plants closure, the primary chemicals of concern have been dicofol, DDT and its metabolites DDE and DDD, and heavy metals (Environmental Protection Agency, 1994). Alligators in Lake Apopka have serum contaminant concentrations that are three to four times those observed in alligators from Lake Woodruff (Guillette et al., 1999b). Many of the contaminants found at higher concentrations in the eggs and serum of Lake Apopka alligators show an affinity for the alligator estrogen re-

ceptor (Guillette et al., 2002; Vonier et al., 1996), and several have been shown to have endocrine disruptive abilities in alligator embryos (Crain et al., 1997; Matter et al., 1998). Male alligators from Lake Apopka have shown depressed T concentrations (Crain et al., 1998; Guillette et al., 1994, 1996) and elevated E₂ (Milnes et al., 2002) concentrations when compared to males from Lake Woodruff, whereas females from Lake Apopka have been shown to have elevated plasma E₂ (Guillette et al., 1994; Pickford et al., 2000) and depressed plasma T (Crain et al., 1998) compared to Woodruff females.

The published endocrine abnormalities associated with Lake Apopka alligators principally rely on data from hatchlings and juveniles. In fact, all of the wild alligators in previous studies that compare animals from Lakes Apopka and Woodruff (Guillette et al., 1997b, 1999a) were juveniles collected in early spring (March and April). In spring, adult alligators from Florida and Louisiana have elevated plasma concentrations of estrogens and testosterone (Guillette et al., 1997a; Lance, 1989). Thus, if juveniles have similar hormonal seasonality, the endocrine differences in populations may not be apparent year round.

To determine if juvenile alligators exhibited seasonality in sex-hormone concentrations, we examined male and female alligators from Lake Woodruff National Wildlife Refuge. Previous data demonstrated a consistent sexual dimorphism during the spring in testosterone and estradiol concentrations in juvenile alligators from Lake Woodruff National Wildlife Refuge (Guillette et al., 1997a,b, 1996, 1999a). We hypothesized that the sexual dimorphism in the sex steroids would be maintained throughout the year. Finally, juvenile alligators from Lake Woodruff NWR were examined relative to animals from Lake Apopka during the spring and summer months (April–July). As discussed above, we have found EDC-associated endocrine abnormalities in Lake Apopka alligators relative to the animals from Lake Woodruff. We hypothesized that lake differences would be maintained in the sex steroid concentrations during the months sampled.

2. Methods

2.1. Animals

Juvenile American alligators (*A. mississippiensis*) were collected from June 1997 to July 1998. All animals were captured by hand or noose from an airboat at night. The alligators ranged in size from 56 to 172 cm in total length. Air and surface water temperature was recorded each night (Fig. 1). Animals were collected from Lake Woodruff National Wildlife Refuge, throughout the study. Lake Woodruff was selected as the best site to test for normal seasonal differences in juvenile circulat-

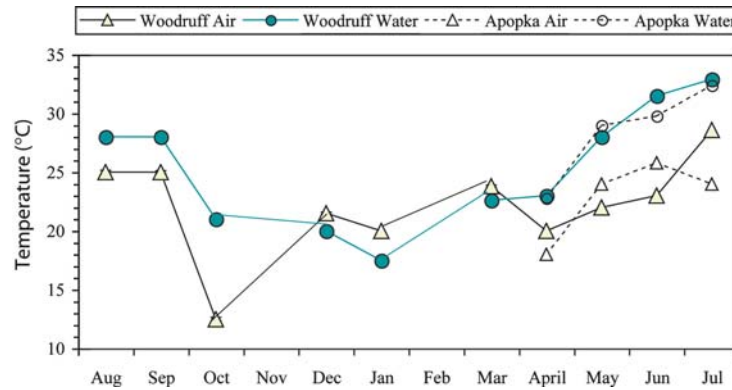


Fig. 1. Air and water temperature (August 1997–July 1998) for Lake Woodruff and Lake Apopka. Air and surface water temperature at the time animals were collected and for each month in the study.

ing sex steroids due to previous data that has established Lake Woodruff as a reference site and relatively unpolluted lake (Guillette et al., 1999b). Animals of similar size were also collected from Lake Apopka, a population of alligators with known high environmental contaminant burdens (Guillette et al., 1999b; Semenza et al., 1997), reproductive problems and endocrine abnormalities (Gunderson et al., 2001; Guillette et al., 1994, 2000; Woodward et al., 1993), during April, May, June, and July of 1998. These samples were used to compare sex steroids between lakes during several spring and summer months (an expected time of hormonal change and a time period during which many previous field studies have been performed). Abnormal endocrine parameters have been observed in juvenile alligators from Lake Apopka and have been hypothesized to be due to embryonic or neonatal exposure to endocrine disrupting contaminants (EDCs): sources of EDCs include city effluent, agricultural run off, and a major pesticide spill in 1980. The south end of Lake Apopka, Gourd Neck Spring (GNS), is the area of the lake with the closest proximity to the site of the 1980 pesticide spill and is also the area with the smaller phallus size in juvenile male alligators (Guillette et al., 1996). Due to these previously noted site-dependent differences, this study considered site differences within Lake Apopka—animals from GNS were considered separate from animals from the north end of Lake Apopka. In instances where GNS and North Apopka showed no significant difference for a particular variable, these two sites were combined and then compared with Lake Woodruff.

Following capture, animals were secured and tagged with a unique toe tag. Animals were either placed in cloth bags or immediately bled from the post-cranial sinus depending on other experimental constraints. Animals that were not immediately bled were removed from the cloth bags and bled within 2 h of capture. We have previously established that sex steroids do not change significantly within 2 h of capture using these methodologies (Guillette et al., 1997b). Blood samples

were taken with sterile syringes and stored on ice for 2–18 h in Vacutainer tubes containing sodium heparin. Sex, snout–vent length, and total length were recorded from each animal after blood was drawn. Sex was determined by manual palpation and extrusion of the phallus (Guillette et al., 1996). The blood samples were centrifuged at 1800g in a refrigerated centrifuge immediately upon removal from ice; and the resulting plasma was stored at -80°C until radioimmunoassays were performed.

2.2. Radioimmunoassay

Plasma was assayed for estradiol-17 β (E_2) and testosterone (T) using assays previously validated for juvenile alligators (Crain et al., 1997; Guillette et al., 1997b). All samples were analyzed in duplicate in a single assay. Plasma samples (150 μl for both E_2 and T) were extracted twice with 5 ml of ethyl ether to remove the lipophilic steroids. Extraction efficiency averaged 96% for T and 94% for E_2 . Intraassay variability was 3.5% for the estradiol-17 β assay and 3.6% for the testosterone assay. Any samples with coefficient of variation higher than 10% were not included in the analyses. Transformation of cpm to hormone values was done with a log-linear cubic spline standard curve generated by Microplate Manager 4.0 (Bio-Rad Laboratories, Hercules, CA).

2.3. Analysis

All analyses were performed using Statview 5.0 (SAS Institute, Cary, NC). Homoscedastic data were analyzed with one-factor analysis of variance (ANOVA). Heteroscedastic data were log transformed to achieve homoscedasticity. Data that remained heteroscedastic were analyzed with Kruskal–Wallis non-parametric analyses or the Mann–Whitney U comparison for paired variables such as sex or a direct comparison between two months.

3. Results

3.1. Juvenile alligators from Lake Woodruff

Juvenile female and male alligators were collected every month except November and February. We were prevented from collecting animals during those months because of weather or elevated water levels on the lake that prevented access to juvenile alligators. Air and water temperatures during collection are presented in Fig. 1.

The females displayed a mean snout–vent length (SVL) of 46 ± 1.1 cm, whereas males had a mean SVL of 47 ± 1.5 cm. Females were smaller than males in the month of May ($p = 0.048$) but there were no significant differences in the snout–vent length of alligators by sex for any other months. Within each sex, there was no difference in SVL of alligators by month (females $p = 0.11$; males $p = 0.60$).

Plasma estradiol-17 β (E_2) concentration of female alligators differed by month ($p = 0.02$; Fig. 2A), with female alligators in December and January having lower

plasma E_2 than females from all other months except October and March.

Previous studies have demonstrated a relationship between SVL and plasma testosterone (T) concentrations, such that animals smaller than 40 cm SVL had very low concentrations. We observed a similar phenomenon, as only males greater than or equal to 38 cm SVL had plasma T concentrations over 50 pg/ml (Fig. 3). Thus, only the larger juvenile males (>38 cm) were used in the monthly comparison of plasma T concentrations because animals smaller than 38 cm had consistently low, basal plasma T concentrations. Males greater than 38 cm SVL exhibited no correlation between SVL and plasma T when animals from all months were examined ($r^2 = 0.027$, $p = 0.138$). This would not be surprising if plasma T concentrations varied with month of capture. Juvenile male alligators from Lake Woodruff exhibit a pronounced seasonal pattern in plasma T concentrations when examined by monthly sampling ($p = 0.0004$; Fig. 2B). Males from December and January had the lowest concentrations with males from March exhibiting the greatest mean concentration. The March sample showed greater

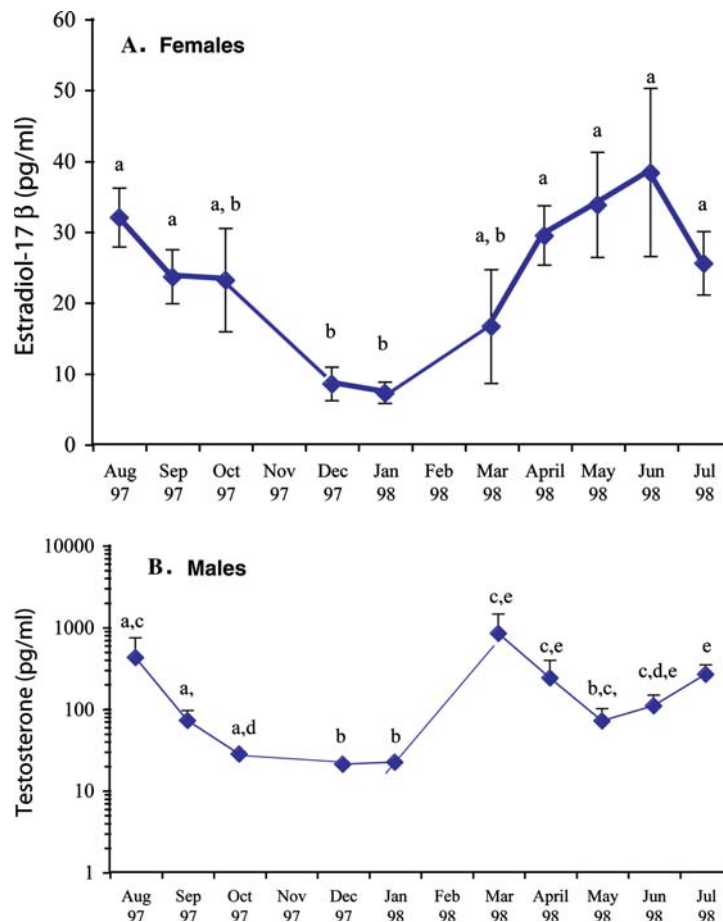


Fig. 2. Seasonal variation in plasma estradiol-17 β concentrations in juvenile female alligators (A) and plasma testosterone concentrations in juvenile male alligators (B) from Lake Woodruff. Shared superscript indicates lack of statistical difference. Each point represents the mean (± 1 SE) of 5–11 animals.

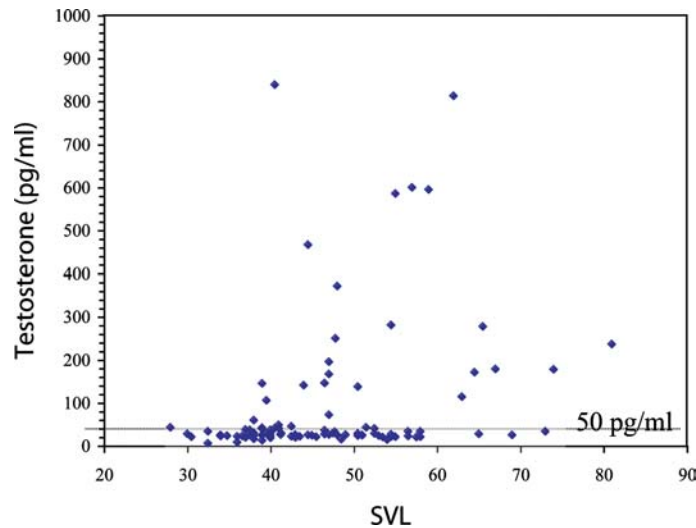


Fig. 3. Plasma testosterone (T) concentrations for individual male alligators from Lake Woodruff. Plasma T is plotted relative to snout–vent length and month is not considered; thus, some animals larger than 38 cm SVL have low plasma T concentrations. The dotted line represents 50 pg/ml, a plasma concentration not seen in any male below 38 cm snout–vent length. Some symbols represent more than one animal due to similar plasma concentrations.

variation as did samples from August and April, periods of apparent transition in the seasonal pattern.

3.2. Juvenile alligators from lake apopka

Plasma E_2 concentrations exhibited no relationship with the SVL of female alligators from the north end of Lake Apopka ($r^2 = 0.095$; $p = 0.143$), whereas females from the GNS area in the south exhibited a positive correlation with SVL ($r^2 = 0.321$; $p = 0.007$). Females from the north end of Lake Apopka had a higher plasma E_2 concentration in May than females in April ($p = 0.0242$) and July ($p = 0.0003$; Fig. 4A). North end females also had a higher plasma concentration of E_2 in June than females in July ($p = 0.01$). Females from GNS had a higher plasma E_2 concentration in April and May than females in June ($p = 0.01$ and $p = 0.0008$) and July ($p = 0.0261$ and $p = 0.001$). Plasma E_2 concentrations were greater in females from GNS than in the north end of Lake Apopka in April ($p = 0.0074$) and July ($p = 0.0040$) with no regional differences observed in May or June (Fig. 4A).

Plasma T concentrations were analyzed in male alligators greater than 38 cm SVL. Plasma T concentrations exhibited no relationship to the SVL of male alligators from either end of Lake Apopka (north, $r^2 = 0.011$; GNS, $r^2 = 0.049$). No significant variation in mean monthly concentrations of plasma T were observed (Fig. 4B).

3.3. Comparisons between lakes apopka and woodruff

No significant differences in plasma E_2 concentrations were found between female alligators from the two lakes during any month (Fig. 4A).

Male alligators from Lake Woodruff had higher plasma T concentrations in July than those observed in males from Lake Apopka ($p = 0.036$; Fig. 4B). No other month displayed a lake-associated difference in plasma T of male alligators. However, examination of Fig. 4B shows that plasma T concentrations in May in both lakes were similar, whereas plasma T concentrations in many males from Lake Woodruff in April were elevated; no significance in the comparisons were noted due to the large degree of variation in the recorded values.

4. Discussion

Female and male juvenile alligators from Lake Woodruff displayed temporal patterns in plasma estradiol-17 β (E_2) and testosterone (T) concentrations, respectively, that appear to be seasonal. In contrast to animals observed on Lake Woodruff, a smaller data set suggested that many of the juvenile alligators from Lake Apopka exhibited no spring to summer variation in plasma sex steroids as observed for animals obtained from Lake Woodruff. Males from Lake Apopka have depressed plasma T concentrations compared to those observed in males from Lake Apopka.

Our data indicate that male alligators begin to exhibit increasing gonadal androgen synthesis upon reaching 38 cm SVL. Thus, juvenile male alligators can be separated into two categories: (1) neonatal alligators below 38 cm SVL that have basal plasma T and (2) peripubescent alligators above 38 cm SVL that exhibit elevated plasma T. Not all males above 38 cm have elevated plasma testosterone concentrations, but it is not uncommon for individuals of a given population to begin

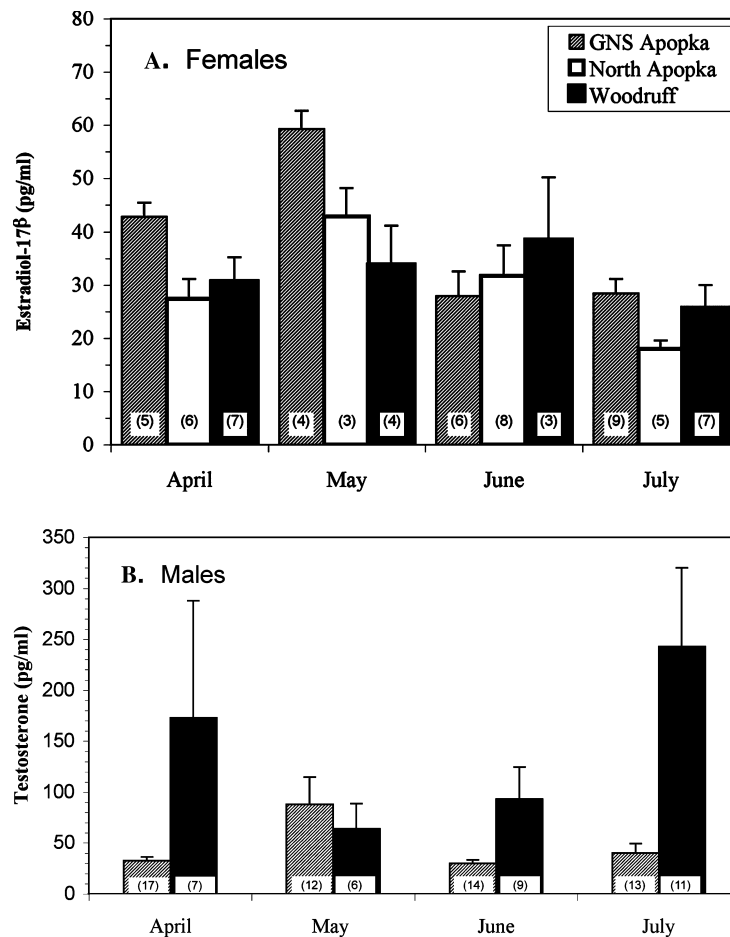


Fig. 4. Plasma estradiol-17 β concentrations in female juvenile alligators (A) or plasma testosterone (T) concentrations in male juvenile alligators greater than 38 cm SVL (B) from Lake Woodruff and Lake Apopka. Animals from Gourd Neck Spring (GNS) and North Apopka were separated in females because a regional difference was found in plasma E₂. Each bar represents the mean (\pm 1 SE). Shared superscript indicates lack of statistical difference. Sample size is indicated in parentheses.

puberty at differing ages or body sizes (Coutinho et al., 2001; Van Tienhoven, 1983). Sexual maturity in the American alligator is principally based on body size because growth rates can vary tremendously based on diet and ambient temperature. In wild populations, sexual maturity occurs when the animals are approximately 99–107 cm SVL or 12–15 years of age (Ferguson, 1985; Joanen and McNease, 1980), but the majority of males are not believed to reproduce until they are 140 cm SVL or more due to the aggression and territoriality associated with courtship and mating in this species (Joanen and McNease, 1980). Our data suggest that testicular activity in males begins years before the animals would be considered sexually mature. This is consistent with data suggesting that species showing a prolonged pubescent period exhibit plasma steroid profiles that reflect gradually increasing gonadal steroidogenesis (Lincoln, 1998; Plant, 1988).

Like males, juvenile female alligators from Lake Woodruff displayed a clear temporal pattern in plasma concentrations of E₂. Unlike males, there appeared to be

no obvious size influence on plasma estrogen concentrations. However, it should also be noted that plasma concentrations of E₂ never were higher than 100 pg/ml, approximately 1/7 to 1/15 the concentration of E₂ found in adult females prior to ovulation (Guillette et al., 1997a). Reproductively active female alligators exhibit a plasma E₂ cycle that begins in fall and culminates with ovulation in early June (Guillette et al., 1997a). Like the pattern presented by the juvenile females, plasma E₂ concentrations in adult females increase dramatically in the spring, with increasing water and air temperatures. At ovulation, plasma E₂ declines sharply in adults, but in juvenile females no decline in plasma concentrations occur until the onset of cooler weather in the fall. The function of plasma E₂ concentrations above basal levels in juvenile females is still poorly understood. It is assumed that rising levels of plasma sex steroids would initiate maturation and growth of reproductive organs, as differentiation and growth of the oviduct in alligators is clearly estrogen dependent (Forbes, 1940). Seasonal elevations in plasma E₂ concentrations are likely to have

an important role in the normal development of this structure as it does during puberty in other species (Adams and Steiner, 1988). Further, estrogens play important roles in the development of the skeleton and other organ systems in vertebrates and likely play a similar role in reptiles, including crocodilians.

While we support a seasonal explanation for the varying hormone concentrations, a non-seasonal explanation of the data must also be considered. The variation in sex steroid concentrations may relate to the interaction of juvenile alligators with environmental variables unrelated to season. For example, the animals could have responded to a change in density of conspecifics, a factor known to influence growth and corticosterone concentration in juvenile alligators (Elsey et al., 1990). Aggressive behavior in juvenile crocodilians under captive conditions has also been correlated to density as well as to diet (Morpurgo et al., 1993). However, change in alligator population densities are caused by water level fluctuations, and water levels were normal to higher than normal in the Ocklawaha and St. Johns River Drainage during 1997 and 1998 (St. Johns River Water Management District, unpublished data). Thus, we support a seasonal interpretation of the pattern of sex steroid concentrations observed in juvenile alligators in this study as the most logical interpretation, and this is supported by comparison of adult patterns.

The effect of size on the pattern of testosterone concentrations in juvenile males is almost identical to that reported previously for mature animals (Lance, 1989). Lance (1989) reported that adult males in Louisiana, USA displayed a significant rise in plasma T concentrations during April and May, with another elevation occurring in the fall months. We have observed that juvenile alligators from Lake Woodruff in central Florida, USA have elevated plasma T concentrations during March and April with a second elevation occurring in late summer. Given the strong photoperiodic and temperature influences on gonadal steroidogenesis reported for reptiles (Licht, 1984), it is not surprising that males from Florida might show a slight advance on the pattern compared to those at the more northern locality in Louisiana. Although the pattern of androgen production is similar, the plasma concentrations reported in the two studies vary greatly—juveniles display 1/10 to 1/100 adult concentrations—as would be expected. Why these juveniles are apparently responding to the same environmental signals as the adults is unknown. Furthermore, what roles these low, but non-basal levels of testosterone play in juvenile males are unknown at this time. Testosterone promotes anabolic responses in reptiles as in other vertebrates, and would contribute to growth and development of the reproductive system. It is clear that larger males have elevated plasma T concentrations and larger phallus size (this study and Guillette et al., 1996, 1997b). Developmental influences

on other reproductive organs as well as augmentation of somatic growth are likely effects.

Previous studies examining plasma sex steroid concentrations in alligators have noted a difference in plasma T concentrations when males from Lakes Apopka and Woodruff were compared (Crain et al., 1997; Guillette et al., 1996, 1997b). The earliest studies reported that yearling alligators hatched from eggs collected on these two lakes, and raised under identical conditions, displayed different plasma sex steroid concentrations, as well as altered gonadal steroidogenesis *in vitro* (Guillette et al., 1994, 1995a,b). These studies have been the basis for the hypothesis that the endocrine and reproductive abnormalities observed in the alligator population at Lake Apopka are due to embryonic exposure to EDCs (Guillette et al., 1995a,b). A recent study examining animals from Lakes Apopka and Woodruff, raised in captivity and treated with gonadotropin experimentally, support our hypothesis that organizational differences existed between animals from the different lakes (Edwards et al., unpublished data). That is, males from Lake Apopka exhibited a reduced response to gonadotropin stimulation when compared to the response of juvenile males from Lake Woodruff. Further, we observed that testicular tissue in large juvenile males (over 38 cm SVL) given superphysiological doses of gonadotropin *in vivo* produced adult-like concentrations of plasma T, whereas small juvenile males exhibited a greatly reduced response (Edwards et al., 2003). A re-evaluation of several juvenile studies also suggested that collection date could influence the magnitude of the differences reported between the lakes (Guillette et al., 1999b). Unfortunately, we could not obtain monthly data for juvenile alligators for Lake Apopka, due in part to the depleted juvenile population on this lake relative to that on Lake Woodruff. However, the data that are available confirm the hypothesis that date of capture does influence the concentration of sex steroid measured in juvenile alligator plasma. For example this study noted that, as reported previously, plasma T concentrations were statistically higher in males from Lake Woodruff NWR for some months, but were not elevated for other months. Interestingly, the lack of a difference is due, in part, to the large variation observed in plasma T concentrations found in animals from Lake Apopka. Altered variance in a population is an indicator of populations under stress and has been used to detect endocrine disruption in populations (Orlando and Guillette, 2001).

Importantly, males from Lake Apopka exhibited no monthly variation in plasma T during the period when samples were obtained, whereas males from Lake Woodruff did. This analysis was performed examining only males larger than 38 cm SVL, those showing elevated plasma T concentrations in the Lake Woodruff population. One explanation for these observations

suggests that animals from Lake Apopka are developmentally delayed and have not begun to exhibit the peripubital rise in plasma T concentrations as observed in animals from Lake Woodruff NWR. Embryonic and neonatal exposure to EDCs have been shown to alter the timing of puberty in rodents, and anti-androgenic contaminants have been shown to delay the onset of testicular steroidogenesis and puberty (Gray et al., 1996; Gray et al., 2002; Kelce et al., 1995). For example, the persistent metabolite of DDT, *p*, *p'*-DDE is a potent anti-androgen in rodents (Kelce et al., 1995). The juvenile alligators from Lake Apopka have nanograms per milliliter serum concentrations of *p*, *p'*-DDE that are four to five times that observed in juveniles from Lake Woodruff (Guillette et al., 1999b). Alternately, seasonal patterns in sex steroids in reptiles have been shown to be influenced by photoperiod, temperature, or nutrition (Licht, 1984), suggesting that the differences observed here could be due to non-synchronous patterns in the animals from the two lakes being compared. As we do not have complete data sets for both lakes, this hypothesis must remain a viable alternative. However, we detected no differences in air or lake water temperatures, and the lakes lie within 30 miles of one another, suggesting that photoperiod is unlikely to be a causal agent in the differences noted. Nutritional differences could influence growth and puberty as has been reported for numerous species (Foster, 1988; Ojeda and Urbanski, 1988; Plant, 1988; Van Tienhoven, 1983). However, a recent study found no difference in the apparent growth rates of animals from Lakes Woodruff and Apopka (Milnes et al., 2002) and we have never observed a difference in body condition (body size by mass indices) when juvenile animals from these lakes were compared (see Guillette et al., 2000).

In conclusion, juvenile alligators of both sexes display seasonal patterns in plasma T or E₂ concentrations. These patterns appear to change with seasonal temperature, rising in spring and declining during the autumn months. Puberty appears to be a multi-year phenomenon in alligators as in other species with long life spans, such as the great apes. The roles of seasonally changing sex steroids in peripubital juveniles need to be examined in more detail to clarify this potentially important developmental phenomenon. Additionally, this study has documented differences in sex steroid concentrations in individuals living in a reference and contaminated lake. These observations confirm previous studies, but for the first time we have shown that the magnitude of the difference between the lakes is due, in part, to seasonal variation in plasma sex steroid concentrations. Previous studies have been designed to minimize seasonal variation as all samples were collected on sequential nights or within a week of one another. Our current data suggest that this design is essential but further indicates that the month of sampling will also affect the outcome of

studies. We cannot conclude that the animals from Lake Apopka are developmentally delayed, but we saw no evidence of elevated plasma T concentrations in males. Further studies on the responsiveness of the reproductive system in males and females from Lake Apopka to endogenous and exogenous stimulators are warranted. Likewise, an effort should be made to look at more contaminated and non-contaminated areas to see if such patterns, or lack of patterns, in seasonal variation exist at these sites.

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