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Reproductive physiology of free-living White Ibises (*Eudocimus albus*) in the Florida Everglades

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Abstract

We measured plasma concentrations of testosterone, estradiol, progesterone, and corticosterone; and recorded changes in gonad size, body condition, molt, and brood patch development of free-living adult White Ibises (*Eudocimus albus*) during the breeding season in the Florida Everglades. White Ibises are colonial breeding, long-legged wading birds that inhabit freshwater and estuarine wetlands. They have flexible breeding schedules (nest initiation dates can range from January to September) and onset of nesting is usually associated with increased prey availability caused by concentration of small fish in pools during periods of wetland drying. In this paper, we present the hormonal and physical characteristics of White Ibis reproductive physiology. We classified White Ibis breeding into five stages: pre-breeding, display, copulation/egg production, incubation, and chick rearing. White Ibises showed cyclic gonadal development which corresponded to reproductive stage. Male and female testosterone concentrations increased during the display stage and decreased during copulation, incubation, and chick rearing. Female estradiol concentrations were highest during display and chick rearing and male estradiol concentrations were lowest during copulation. Female progesterone concentrations increased during display and remained high throughout the breeding season. Female ibises had low corticosterone concentrations that increased during incubation and were highest during chick rearing, concomitant with lower body condition and flight muscle-mass scores. Male ibis progesterone and corticosterone concentrations did not show seasonal changes and were more variable than concentrations in female ibises at similar stages. Males and females had elevated body condition scores during the display stage, which were depleted by the onset of incubation. Increased energy stores during display may be used later for fasting in male birds that do not eat during the 10-day copulation/egg production stage, and for egg production in female birds. During incubation, male and female ibises developed brood patches. Ibises molted in all stages of reproduction, indicating that ibis molt and reproductive physiology may not inhibit each other as in most temperate bird species. White Ibises showed similar patterns in reproductive physiology to other monogamous, seasonally breeding bird species in which both sexes incubate and care for the young.

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1. Introduction

Though many reproductive endocrinology studies have been conducted on colonial waterbirds, most of these have focused on penguins (Cherel et al., 1994; Cockrem and Seddon, 1994; Lormée et al., 1999; McQueen et al., 1998; McQueen et al., 1999; and others) or other seabirds (Albatrosses: Hector, 1988; Hector

et al., 1985; Boobies and Tropicbirds: Lormée et al., 2000; Wingfield et al., 1999). There have been no studies on the reproductive physiology of free-living Ciconiiformes (herons, egrets, ibises, storks, and spoonbills), and studies of only two captive species (White Stork, *Ciconia ciconia*, Hall et al., 1987; White Ibis, *Threskiornis melanocephalus*, Ishii et al., 1994; Wingfield et al., 2000). Many wetlands utilized by wading birds are experiencing anthropogenic impacts in the form of hydrological alterations (Golladay et al., 1997; Light and Dineen, 1994; Lorenz et al., 2002; McIvor et al., 1994)

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and toxic contamination (Frederick et al., 2001; Sundlof et al., 1994). An understanding of wading bird reproductive physiology could provide a foundation for studies of endocrine disruption, breeding parameters, and energetics, all of which could be useful for the management of impacted wetlands. In addition, such information can provide a field-based endocrinological model for endangered birds such as the Japanese Crested Ibis (*Nipponia nippon*) (Wingfield et al., 2000).

Most wading birds are similar to other waterbirds studied to date in that they are often colonial breeders and both adults provide parental care. Wading birds, therefore, are likely to have analogous endocrinological processes associated with these reproductive strategies. For example, bi-parental care may mean that males and females are likely to have similar hormone concentrations and patterns that influence parental care behavior during the breeding season. Additionally, wading birds breed in wetlands where productivity fluctuates within and between years (i.e., dry and wet seasons, drought and flood years) and timing of reproduction can be extremely variable within and between years (Kushlan, 1975; Ogden, 1994).

White Ibises (*Eudocimus albus*) are one of the most abundant long-legged wading birds in southeastern North America (Kushlan and Bildstein, 1992). Ibises inhabit estuarine and freshwater wetlands and feed on small fish, crustaceans, and other aquatic invertebrates. Nest initiation can be highly variable, and is usually associated with the onset of favorable feeding conditions (Bildstein et al., 1990; Kushlan, 1976). In the Everglades, feeding conditions change as the result of hydrology and in most years ibises initiate nesting in February, the middle of the dry season. During south Florida's dry season (typically November–May) small fish are concentrated in drying pools of water and are more available to foraging ibises (Gawlik, 2002; Kushlan, 1979).

White Ibises are socially monogamous and both sexes care for the young through independence (Frederick, 1987). The breeding season can be divided into four stages: display, copulation and egg production, incubation, and chick rearing. Prior to courtship, ibis faces, bills, and legs change from pink to red in color (Heath, 2002). Female ibises also develop pronounced, red gular pouches. The display stage usually lasts 10 days (Kushlan and Bildstein, 1992), and copulation occurs during late display and continues during nest building and egg production. Nest building takes place during the end of display and the beginning of egg production. Female ibises construct a nest from sticks brought to them by the male. Over the course of approximately 10 days females will lay one egg every other day until they complete a clutch of 2–4 eggs (Kushlan and Bildstein, 1992). While females are producing eggs they make regular foraging trips away from the colony. Male ibises

guard and maintain the nest and guard the female from extra-pair copulations when she is at the nest (Frederick, 1987). During the time females are producing eggs, male ibises do not make regular foraging trips and for the most part, fast. Male and female ibises incubate the eggs beginning with the laying of the last egg. Incubation lasts about 3 weeks, and both sexes contribute to chick rearing by brooding and feeding the chicks. Chick rearing lasts approximately 6 weeks. A single adult cannot successfully rear chicks (Kushlan and Bildstein, 1992).

White Ibises are an interesting candidate for an endocrinological study because of their subtropical origin, flexible breeding period, colonial behavior, nest and mate defense, and bi-parental care. In this paper, we describe hormonal and body condition changes, molt activity, and brood patch development in free-living White Ibises during the breeding season. We predicted that male and female wading birds would have lower testosterone concentrations than most temperate species, comparable to terrestrial tropical birds that exist in seasonal environments with highly variable among-year productivity, and that both sexes would show similar changes in progesterone, a hormone associated with parental care (Davis et al., 1995; Fivizzani and Oring, 1986; Hector et al., 1985; Silver and Cooper, 1983).

2. Methods

2.1. Capturing ibises and classification of reproductive stage

In 1998, we trapped and sampled known nesting stage ibises at colonies in central Florida (Lake, Polk, and Orange, FL) using a cylinder wire-mesh trap (Frederick, 1986). From January through June of 1999, 2000, and 2001, we captured White Ibises in Everglades Water Conservation Areas (WCA's) 1, 3A, and 3B. In these years White Ibises initiated breeding in February and new initiations continued through June. In the Everglades, we lured ibises to a trap site with plastic white flamingos and captured them with either mist nets or a rocket net (Heath, 2002; Heath and Frederick, 2003). Traps were set by sunrise and we stopped trapping by 1000 h. We trapped during the early morning hours to avoid heat stress to the birds and control for diel variation in hormone concentrations.

To classify birds to breeding stage, we used a model based on the integument color changes of known stage birds (Heath, 2002). Male and female ibises had similar changes in integument color and a discriminant function model based on color changes correctly identified stage of reproduction 96.4% of the time (Heath, 2002). We also used other indications of reproductive stage. For example, we classified pre-breeding birds as those that

were captured before any nesting had begun in the Everglades area. We assigned females with large, pronounced gular pouches to the display stage (Kushlan and Bildstein, 1992). To identify birds that were laying eggs, we palpated female ibises' abdomens. In one case, we were able to follow a male back to his nest via radio-telemetry 25 days after he was captured. At his nest were three chicks less than 5 days old and we could backdate to calculate that he was captured during the time his mate was laying eggs (egg production stage).

2.2. Information collected from captured birds

Once birds were captured, we immediately collected a 3 ml blood sample from the jugular vein with a 22-gauge needle and 5 ml syringe (mean time from bird captured to completion of blood collection: 10.4 ± 0.7 min). Blood was transferred to a 5 ml heparinized Vacutainer and stored on ice until we returned to the laboratory (3–5 h after collection). We centrifuged the blood for 10 min at 2000 rpm and then separated the plasma from the cellular fraction. Plasma was stored at -20°C until analysis.

If we captured more than one bird at a time, we placed birds in cloth sacks until they could be sampled. While birds were being handled we placed a leather hood on their heads to cover their eyes. Birds typically responded to the hood by appearing to 'sleep' (i.e., drooped head, docile behavior). We marked each bird with a US Fish and Wildlife Service aluminum band placed on the leg above the tarsus joint. Some birds (females $N = 27$, males $N = 22$) were also marked with radio-transmitters attached with a Teflon figure-8 harness.

If a female did not have a palpable egg or if the bird was a male, we examined seasonal changes in gonad size using a laparoscopic procedure. Birds were anesthetized with isoflurane gas administered via a portable vaporizer (Ohmeda Portable Drawover Vaporizer, Abbott Laboratories, North Chicago, IL), and regulator (B & F Medical Oxygen Compressed Gas Regulator) with oxygen tank. A 50 cm rubber hose attached to a plastic cone (20 cm \times 7 cm) that covered the ibis bill and nasal openings delivered the anesthesia mixture of oxygen and isoflurane to the bird. Birds were exposed to high anesthesia concentrations (level 5) for 4–6 min until the bird appeared unresponsive to touch (3–4 min), after which we decreased the concentration (level 2–1) to maintain a surgical plane of anesthesia. To view the gonads, we made a small (5 mm) incision through the skin near the posterior rib on the left side of the bird, slid the incision over the musculature between the ribs, and made another incision into the abdominal cavity so that the two incisions would not overlap when the skin was slid back into place. We then inserted an otoscope (California Veterinary Supply, CA) to view the gonads. Testes or largest ovarian follicle length and width were

estimated using a scale (mm) on the otoscope and we described color and, for ovaries, presence of yolk. Later, we used equations for calculating volumes of ovoid sphere (testes) and spheres (ovary follicles) to estimate gonad size (Boswell et al., 1993; Wingfield et al., 1997). Once the exam was complete, we discontinued isoflurane treatment and sealed the external incision with veterinary quality super-glue. Ibises recovered quickly from the anesthesia, usually in less than 2 min. If we did not laparoscopy a bird, its gender was subjectively estimated from its bill and body size. Ibis males are larger than females (Kushlan, 1977) though there is some size overlap between the sexes. We later confirmed our subjective assessment using discriminant function analysis of body measurements of birds whose sex was positively identified by laparoscopy.

We measured body mass to the nearest 5 g (Pesola spring scale), straight and curved bill length, bill depth, wing chord, and tarsus length to the nearest mm. "Bill curved" was measured as the curved length from last facial feather to the bill tip. "Bill straight" was measured as the straight distance between the gape and the bill tip. We scored bill and leg colors by holding a paint swatch (Wal-Mart stores brand numbers 0071–1111) up to the body part and recording the color that most closely resembled the bill or leg (De Repentigny et al., 1997; Heath, 2002). We attempted to score colors in consistent light conditions. We visually scored subcutaneous furcular fat stores on a scale from 0 to 5 (0: no fat present–5: furcular area over full with fat; Brown, 1996; Wingfield et al., 1997) and palpated the pectoralis muscle to subjectively score pectoralis mass on a scale 1–5 (1: prominent keel, easy to feel ribs beneath thin muscle–5: hypertrophic muscle greater than flush with keel). We visually examined birds for body molt and brood patches. New feathers were in sheaths and obvious during examination of the bird's body, especially on the bird's back. We classified birds as molting if more than 5% of their body feathers were estimated to be in sheaths.

2.3. Hormone analyses

Testosterone (T), estradiol (E_2), progesterone (P), and corticosterone (B) concentrations were analyzed via radioimmunoassay. Dr. Tim Gross, at U.S.G.S. Agriculture Lab (Gainesville, FL), analyzed samples collected in 1998 and 1999 in one assay per hormone. We analyzed samples collected in 2000 and 2001 separately by radioimmunoassay (Guillette et al., 1997). Duplicate assay tubes were created for each plasma sample (100 μl for T, 150 μl for E_2 , and 50 μl for P and B). We then twice extracted steroids from plasma with ethyl ether, which removed the lipophilic fraction. Lipophilic and aqueous fractions were separated by freezing the aqueous portion in a methanol bath chilled to -25°C with dry ice. Ether

extracts were dried under filtered air in a warm (35 °C) water bath and then resuspended with 100 µl borate buffer. To reduce non-specific binding we added 100 µl bovine serum albumin (BSA). We aliquoted 200 µl of antibody (final dilutions: T = 1 : 36,000, E₂ = 1 : 55,000, P = 1 : 16,000, B = 1 : 70,000; Endocrine Sciences, Calabasas Hills, CA) and 100 µl of labeled hormone (Amersham International, Arlington Heights, IL) at 12,000 cpm per 100 µl into assay tubes. Assay tubes were vortexed and allowed to incubate 10 h at 4 °C.

To separate bound and free hormone, we added 500 µl of dextran charcoal solution and centrifuged the tubes for 30 min at 2500g. The supernatant (0.5 ml) was drawn with a re-pipette diluter (Lab Industries), and expelled into a sample vial with 5 ml scintillation cocktail. Samples were then counted on a scintillation counter (Beckman LS 5801).

We validated all steroid hormone assays by comparing the slopes of a plasma dilution curve and an internal standard curve to the assay standard curve slope. Plasma dilution curves consisted of different ratios of pooled ibis plasma to stripped plasma. For all hormones the ratios were 0:1, 2:8, 4:6, 6:4, 8:2, and 1:0 of pooled to stripped plasma. For testosterone and estradiol the internal standard curve consisted of 800, 400, 200, 100, 50, 25, 12.5, 6.25, 3.125, and 1.0625 µg of hormone standard added to 100 µl of stripped plasma. For progesterone and corticosterone we used 1000, 500, 250, 125, 62.5, 31.25, 15.625, 7.81, 3.9, and 1.95 µg of standard hormone. Plasma dilution curves and internal standard curves were assayed as described above. There were no significant differences among the slopes of the plasma dilution, internal standard curve, and assay standard curve for any of the four hormones (ANCOVA all P 's > 0.66). Intraassay (2000 and 2001 assays) and interassay (among all 3 assays: 1998 and 1999, 2000, and 2001) variances were $2.1 \pm 0.2\%$ and $12.5 \pm 3\%$ for T, $1.9 \pm 0.1\%$ and $13.0 \pm 3\%$ for E₂, $3.7 \pm 0.4\%$ and $5.6 \pm 3.0\%$ for P, and $3.16 \pm 0.3\%$ and $10.28 \pm 4.25\%$ for B, respectively. Sample values reported here have been adjusted for extraction efficiencies for each hormone (i.e., testosterone: 87%, estradiol: 84%, progesterone: 73%, and corticosterone: 92%). Minimum detectable concentrations were 24 pg/ml for T, 30 pg/ml for E₂, 42 pg/ml for P, and 44 pg/ml for B.

2.4. Statistical analyses

2.4.1. Handling effects

Many birds respond to capture and handling by increasing circulating corticosterone concentrations (Harvey et al., 1984). We examined the relationships among corticosterone, handling time, sex, and stage of reproduction. We found no significant effect of sex or reproductive stage (all P 's > 0.3) but there was a significant relationship between time from capture to blood sampling and B concentrations ($y = 517 + 1.64x$,

$r^2 = 0.18$, $P = 0.0001$). Subsequently, we used this equation to correct for the effects of time and used the corrected results for further analyses (Heath, 1997).

We also found a relationship between progesterone (P) concentrations and handling time ($y = 518 + 1.50x$, $r^2 = 0.12$, $P = 0.0002$). This relationship did not depend on the bird's gender or reproductive stage (all P 's > 0.6). We corrected for the effects of handling time on White Ibis progesterone concentrations using the above regression equation and used these corrected P concentrations for further analyses. Unfortunately, the relationship between P and handling time in birds remains poorly understood. The effect of stress on progesterone concentrations has been studied in mammals (Holzbauer and Newport, 1967; Holzbauer and Newport, 1969; Holzbauer et al., 1969; Macniven et al., 1992), but few studies of other vertebrates have examined this association (Guillette and Fox, 1985; also see Fowler et al., 1995).

2.4.2. Body condition

To evaluate changes in body condition, we calculated a condition index that corrected for size variation in females and males separately (Green, 2001). A principal component score created from tarsus length, bill depth, and wing length accounted for variation in size (Heath, 2002). We then regressed (model I) the PC1 score against body mass to obtain the residual for each individual. The relationship between the size score and body mass was linear (female $r_s = 0.45$, male $r_s = 0.45$). The residuals were considered a body condition 'score.' A negative score represented a lower mass/size ratio than expected (poor condition) and a positive score a greater mass/size ratio than expected (good condition).

2.4.3. Analyses

There were no significant differences in any morphological measurement among years (all MANOVA P 's > 0.05). Therefore, we pooled data collected in all years of this study (1998, 1999, 2000, and 2001). Before analysis, data were examined to make sure they fit the test assumptions (e.g., normality). The most common assumption violated was homoscedasticity. Log-transformed variables usually met this assumption (Sokal and Rohlf, 1995). All hormone parameters were log-transformed for analysis, but are graphically represented with untransformed values. If parametric requirements could not be met then the appropriate non-parametric test was used (Hollander and Wolfe, 1999). Descriptive statistics are reported as means \pm standard error. Statistical analyses were done on SAS software version 6.12 and 8.

3. Results

We captured 131 adult White Ibises (83 females and 48 males; 1998: $N = 15$, 1999: $N = 54$, 2000: $N = 40$,

2001: $N = 22$). We captured a similar number of females and males in the pre-breeding, incubation, and chick rearing stages, but we caught fewer males than females during display and copulation and egg production stages. During these stages males make only infrequent flights away from the colony and were difficult to catch at foraging areas.

3.1. Gender classification

Overall accuracy of the gender discriminant function model was 99% with 100% of males being correctly identified and 99.95% of females correctly identified. Bill straight length, bill depth, and tarsus length best discriminated between sexes (Heath, 2002). Bill curved length and wing length did not significantly contribute to the model.

3.2. Gonad changes

White Ibises responded well to anesthesia with isoflurane. The procedure of anesthetizing the bird, making an incision, examining the gonads, and resealing the skin took 16 min on average (± 0.7 min; $N = 45$) (Heath, 2002). We visually examined the gonad condition of 19 of the 21 female White Ibises in which we attempted to look at gonads. For two birds we were unable to see the ovaries because of a mass in the oviduct. Therefore, if we later felt the presence of an egg during a physical exam, we assumed the bird was producing an egg and did not attempt to perform a laparoscopy. Ovarian follicles were largest during egg production (Kruskal–Wallis $\chi^2 = 10.81$, $P = 0.0287$; Fig. 1a), and follicles began to accumulate yolk during the display stage. Ovary sizes seemed to correlate with increases in day length, though the brevity of egg production and the variation in timing of nesting attempts makes this relationship difficult to interpret (Fig. 2a). No plasma steroid concentrations correlated with ovary size (all P 's > 0.18).

We successfully viewed the testes of 16 of the 17 male ibises with laparoscopy. Testes were largest during display, egg production, and incubation (range: 113–873 mm³, $F_{4,11} = 10.79$, $P = 0.0008$; Fig. 1b). Enlarged testes were a yellowish-white color, but smaller testes (of pre-breeding and chick rearing birds) were bluish green. Although testes significantly changed in volume during the course of the breeding season, the relationship between testicular size and day length is difficult to interpret (Fig. 2b). Birds with large testes in late spring (e.g., May) may have been either late breeders or those attempting a second breeding effort. Testes are the primary site of testosterone synthesis during spermatogenesis and testicular size correlated with male ibis plasma testosterone concentrations ($r_s = 0.68$, $P = 0.0075$; Fig. 3). Plasma progesterone and estradiol concentrations did not correlate with testicular size (all P 's > 0.19).

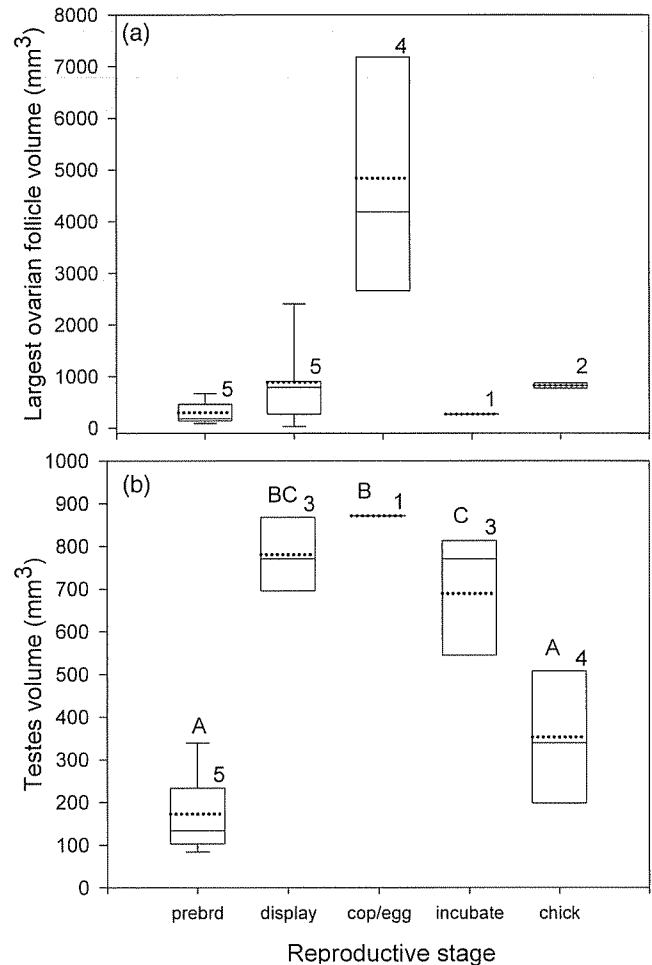


Fig. 1. White Ibis gonad changes during the course of the Everglades breeding season. (a) Female ovary volume changed significantly during the breeding seasons (Kruskal–Wallis $\chi^2 = 10.81$, $P = 0.0287$). (b) Male testes changed size during the breeding season ($F_{4,11} = 10.79$, $P = 0.0008$). Stages with different letters were significantly different. Box plots are 25–75% quartiles and bars mark 95–5% intervals. Medians are solid black line, means are dotted line. Sample sizes are noted above boxes.

We also examined the gonads of seven juvenile (3–18 months) birds to observe relative size and color. Juvenile (after-hatch year) female ovaries ($N = 2$) were small (76 mm³) and pale white. Hatch year and after-hatch year male testes were small (46 mm³ \pm 22; $N = 5$) and bluish green. Third year male (age identified by plumage; Kushlan and Bildstein, 1992) ibis testes were relatively small (25 mm³ \pm 0.7; $N = 2$) but yellowish-white, suggesting that third year birds could have a functional reproductive system.

3.3. Hormone changes

3.3.1. Testosterone

Female plasma testosterone concentrations were highest during the display stage and then decreased successively during egg production, incubation, and

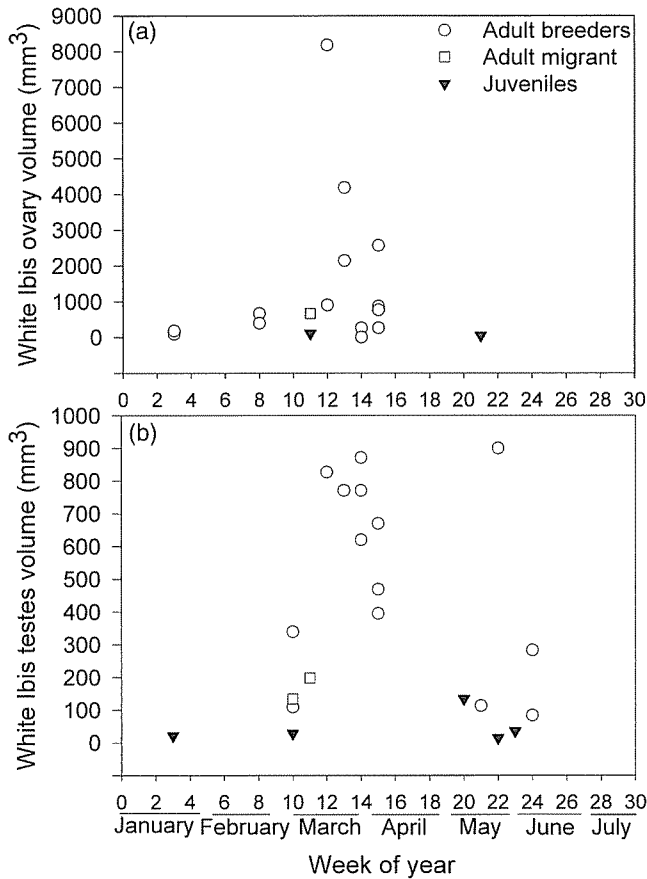


Fig. 2. The relationship between date and White Ibis (a) ovary and (b) testes volume changes. The relationship between gonad size and day-length was difficult to interpret given the brevity of egg production and the variation in timing of nesting attempts. Large gonads in early May probably represented late breeders or double brood efforts. Adult migrants showed gonad color similar to adult breeders. Juvenile gonads did not develop to volumes similar to adult gonads.

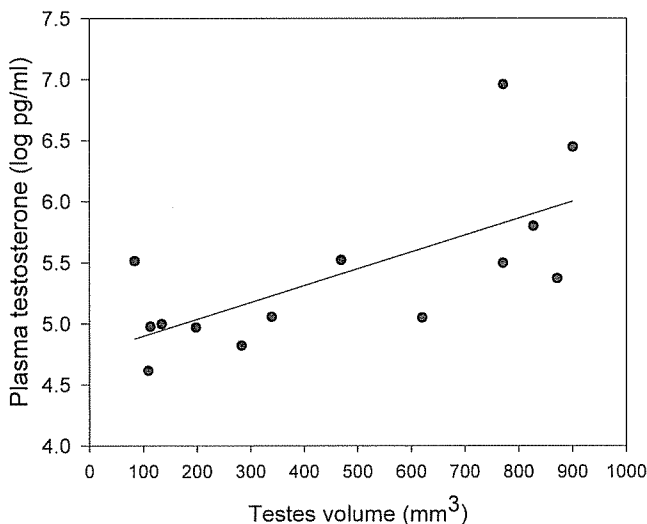


Fig. 3. The relationship between testes size and circulating testosterone concentrations (Pearson's $r_s = 0.68$, $P = 0.0075$) of male White Ibises breeding in the Everglades.

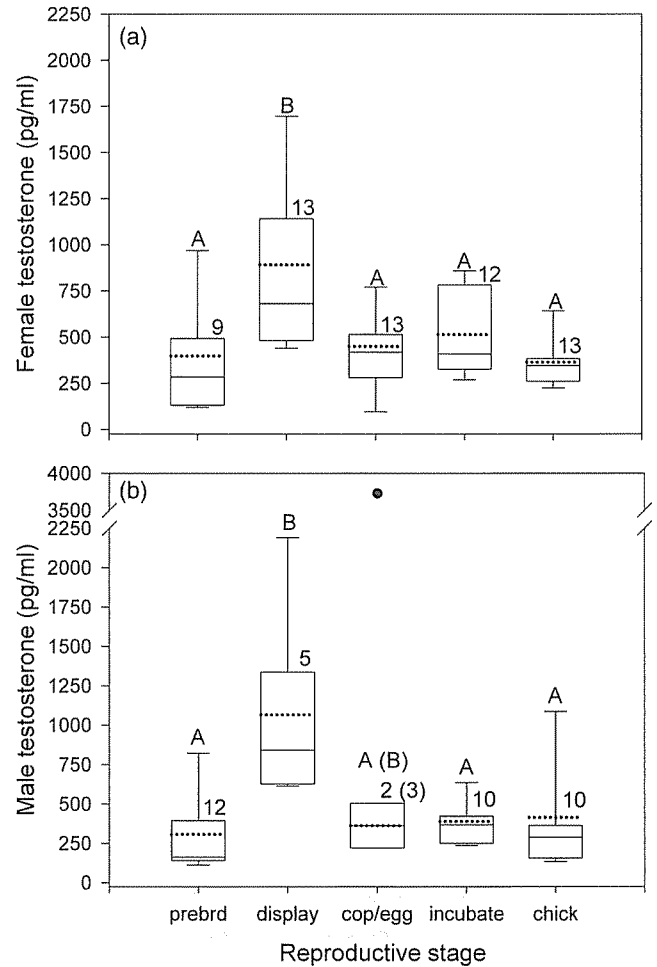


Fig. 4. Changes in White Ibis testosterone concentrations during the Everglades breeding season. (a) Female and (b) male White Ibis testosterone concentrations were highest during display and then decreased during later stages ($F_{4,52} = 4.92$, $P = 0.0019$; $F_{4,52} = 4.92$, $P = 0.0019$, respectively). Stages with different letters were significantly different. The circle symbol denotes an outlier during the copulate/egg stage. Numbers and letters in parentheses are results that include outlier. Box plots are 25–75% quartiles and bars mark 95–5% intervals. Medians are solid black line, means are dotted line. Sample sizes are noted above boxes.

chick rearing ($F_{4,52} = 4.92$, $P = 0.0019$; Fig. 4a). Male ibises showed a similar pattern of high T concentrations during the display stage ($F_{4,22} = 4.93$, $P = 0.0033$; Fig. 4b). These concentrations then decreased during the later breeding stages, with the exception of one outlier during the egg production stage that had one of the highest T concentrations recorded (3728 pg/ml).

3.3.2. Estradiol

Female ibises showed seasonal changes in plasma estradiol concentrations ($F_{4,60} = 2.94$, $P = 0.0274$; Fig. 5a). Estradiol concentrations showed no significant differences among pre-breeding, display, egg production, and incubation stages, though display birds tended to have higher E_2 concentrations than birds in other stages.

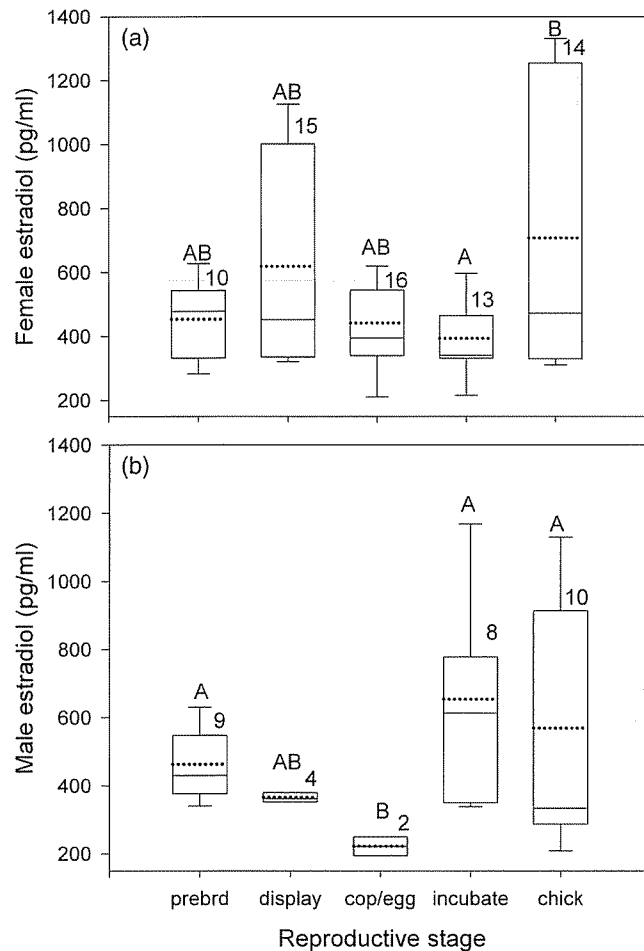


Fig. 5. Changes in White Ibis estradiol concentrations during the Everglades breeding season. (a) Female White Ibis estradiol concentrations were most variable in display and chick rearing stages and significantly increased from incubation to chick rearing ($F_{4,60} = 2.94$, $P = 0.0274$). (b) Male White Ibis estradiol concentrations were lowest in egg production stage and then significantly increased during incubation and chick rearing ($F_{4,25} = 2.86$, $P = 0.0441$). Stages with different letters were significantly different. Box plots are 25–75% quartiles and bars mark 95–5% intervals. Medians are solid black line, means are dotted line. Sample sizes are noted above boxes.

Estradiol concentrations increased from the incubation to chick rearing stage. Male ibises also showed seasonal changes in estradiol concentrations ($F_{4,25} = 2.86$, $P = 0.0441$; Fig. 5b). Estradiol concentrations were lowest during the egg production stage and then increased during incubation and chick rearing.

3.3.3. Progesterone

Pre-breeding female ibises had lower P concentrations than reproductive birds ($F_{4,50} = 2.91$, $P = 0.0307$; Fig. 6a). Plasma P concentrations increased significantly from the pre-breed to display stage and then were maintained at intermediate concentrations throughout egg production, incubation, and chick rearing. Male ibises showed no significant changes in plasma P concentrations during the breeding season ($F_{4,31} = 0.61$, $P = 0.6558$; Fig. 6b).

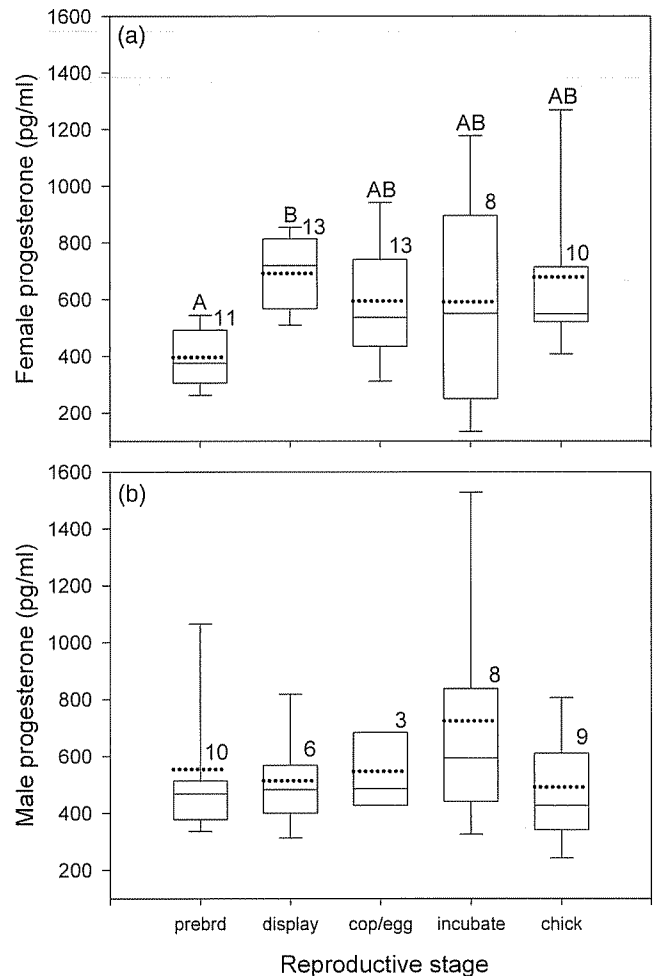


Fig. 6. Changes in White Ibis progesterone concentrations during the Everglades breeding season. (a) Female White Ibis progesterone concentrations increased significantly from pre-breed to display ($F_{4,50} = 2.91$, $P = 0.0307$). Stages with different letters were significantly different. (b) Male White Ibis progesterone concentrations showed no significant changes ($F_{4,31} = 0.61$, $P = 0.6558$) during the breeding season. Box plots are 25–75% quartiles and bars mark 95–5% intervals. Medians are solid black line, means are dotted line. Sample sizes are noted above boxes.

3.3.4. Corticosterone

Female plasma corticosterone concentrations changed during the breeding season ($F_{4,56} = 2.97$, $P = 0.0272$; Fig. 7a), with lowest B concentrations during pre-breed, display, and egg production. Corticosterone concentrations became more variable during incubation and were significantly higher than earlier stages during the chick stage. Male ibises showed no significant changes in plasma corticosterone concentrations during the breeding season ($F_{4,34} = 0.82$, $P = 0.5203$; Fig. 7b).

3.4. Morphological changes

Female ibis body condition scores changed significantly over the course of the breeding season ($F_{4,74} = 8.85$, $P = 0.0001$; Fig. 8a). Female body condi-

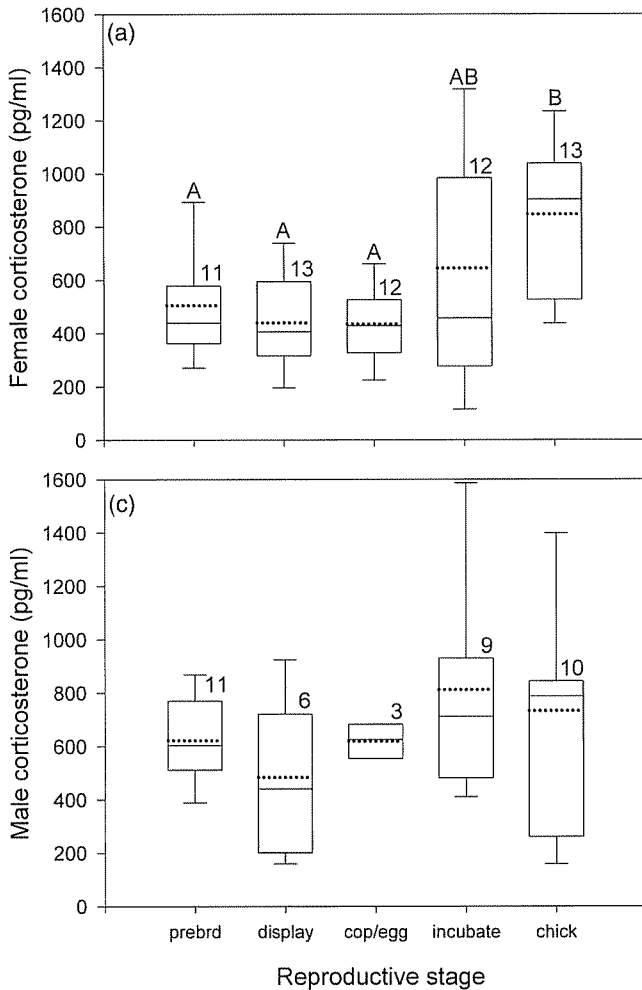


Fig. 7. Changes in White Ibis corticosterone concentrations during the Everglades breeding season. (a) Female White Ibis corticosterone concentrations increased significantly in later, chick breeding stages ($F_{4,56} = 2.97$, $P = 0.0272$). Stages with different letters were significantly different. (b) Male White Ibis corticosterone concentrations showed no significant changes ($F_{4,34} = 0.82$, $P = 0.5203$). Box plots are 25–75% quartiles and bars mark 95–5% intervals. Medians are solid black line, means are dotted line. Sample sizes are noted above boxes.

tion scores increased from the pre-breeding to display stage, and then decreased through the chick stage. Female pectoralis scores during chick rearing were significantly lower than during pre-breeding (Kruskal–Wallis $\chi^2 = 11.024$, $P = 0.0263$; Fig. 9a). Female fat scores did not show significant changes during the breeding season (Kruskal–Wallis $\chi^2 = 1.346$, $P = 0.8534$; Fig. 10a).

Male ibis body condition scores also changed significantly during the breeding season ($F_{4,40} = 2.98$, $P = 0.0305$; Fig. 8b). Male condition scores were highest early in the season and lower later in the season. However, within each stage, male birds tended to show more variation in condition scores than females (Fig. 8). Changes in male ibis fat and pectoralis scores were more distinct than female birds. Specifically, pectoralis scores

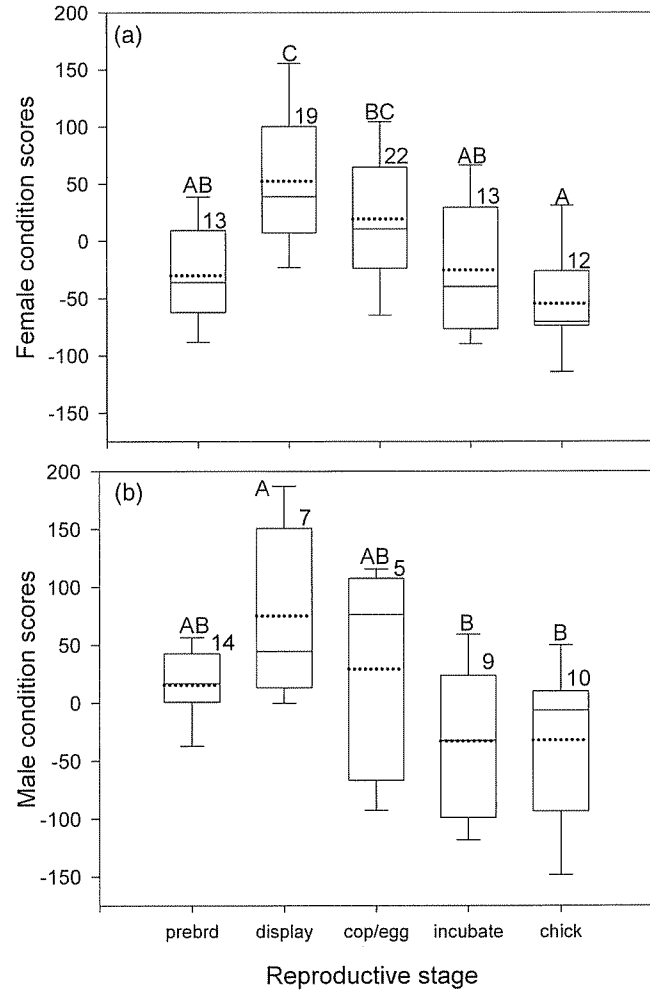


Fig. 8. Changes in White Ibis body condition scores during the Everglades breeding season. (a) Female White Ibis body condition scores were highest during the display stage and then decreased ($F_{4,74} = 8.85$, $P = 0.0001$). (b) Male White Ibis body condition scores changed significantly during the breeding season ($F_{4,40} = 2.98$, $P = 0.0305$). Body condition scores during incubation and chick rearing were lower than at display stage. Box plots are 25–75% quartiles and bars mark 95–5% intervals. Medians are solid black line, means are dotted line. Sample sizes are noted above boxes. Boxes with different letters were significantly different.

tended to decrease (Kruskal–Wallis $\chi^2 = 7.511$, $P = 0.1112$; Fig. 9b) and fat score significantly decreased (Kruskal–Wallis $\chi^2 = 10.231$, $P = 0.0367$; Fig. 10b) from the display stage to the egg production stage.

There were no significant differences between male and female molt patterns or brood patch development. Of birds captured during display ($N = 15$), 50% had begun to develop brood patches and by the egg production and incubation stages 90% of birds showed bare, vascularized brood patches ($N = 26$). During chick rearing, brood patches were less vascularized and birds tended to groom feathers over the bare area. Some birds captured in the chick brooding stages showed down growth in the brood patch region (12%, $N = 17$).

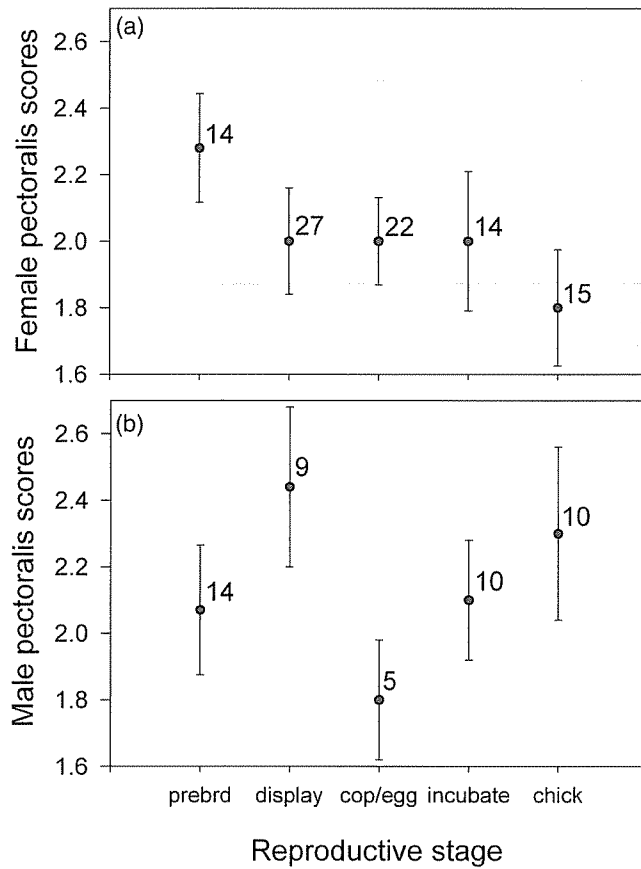


Fig. 9. The relationship between pectoralis score and stage of reproduction in (a) female and (b) male White Ibises in the Everglades. Females had lower scores in the chick rearing period than in pre-breeding stage (Kruskal–Wallis $\chi^2 = 11.024$, $P = 0.0263$). Males showed no significant patterns in pectoralis development (Kruskal–Wallis $\chi^2 = 7.511$, $P = 0.1112$). Sample sizes are to right of symbol.

Most White Ibises (85%; $N = 27$) molted greater than 5% of their body feathers during the pre-breeding stage. During the breeding season, fewer ibises molted their body feathers (5–20% of birds) than pre-breeding birds, but reproductive birds did show signs of molt in all stages. Of display birds, 33% ($N = 18$) had at least 5% of feathers in sheaths. In other stages: 16% of birds in egg production ($N = 19$), 14% of incubating birds ($N = 14$), and 15% of chick rearing birds ($N = 20$) were also molting.

4. Discussion

4.1. Endocrinology changes

White Ibis gonads showed a seasonal pattern, with testes and ovaries enlarging in early stages (display) and then decreasing in size during chick brooding. Two male birds captured and marked with radio-transmitters 2 weeks before they emigrated from the Everglades prior

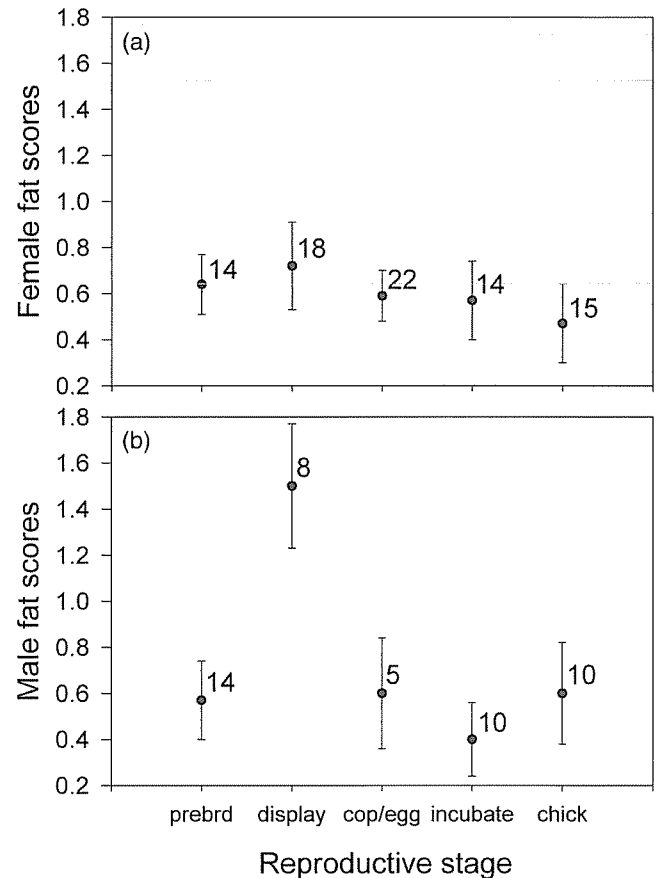


Fig. 10. The relationship between fat score and stage of reproduction in (a) female and (b) male White Ibises in the Everglades. Females showed no significant patterns in fat scores (Kruskal–Wallis $\chi^2 = 1.346$, $P = 0.8534$). Males had highest fat scores during the display stage (Kruskal–Wallis $\chi^2 = 10.231$, $P = 0.0367$). Sample sizes are to right of symbol.

to breeding had pale yellow testes similar to male ibises that stayed in the Everglades system and bred. If a yellow testes color represents a functional gamete and hormone producing testes then this would indicate that testes of migrating birds, in the absence of reproductive behavior and supplemental cues (e.g., nest site and mate availability; Wingfield et al., 1992), may be responding to predictive cues such as day-length to stimulate gonad activity. Given the temporal variability in White Ibis nest initiation date in the Everglades, other 'short-term' (or supplemental) cues probably most influence the timing of breeding (Wingfield et al., 1992). Ibises may be particularly receptive to favorable environmental conditions during the part of the year when day-length is increasing. Alternatively, one instance of a large number of White Ibises initiating nesting in September (Kushlan, 1976, nearly 6 months later than normal initiation) indicates that if environmental conditions were appropriate, ibises could develop functional gonads in the absence of increasing day-lengths. This seasonal pattern of gonadal development, mixed with the potential to

opportunistically reproduce has been described for other species with predictable environmental seasons but unpredictable food patterns (e.g., Red Crossbills, *Loxia curvirostra*; Hahn, 1998). Responses to photoperiod and subsequent gonadal development often depend on luteinizing hormone (LH) concentrations (Wingfield and Farner, 1993, although see Hau et al., 1998). A future study of the annual cycle of ibis gonad activity and LH concentrations, or perhaps GnRH production (MacDougall-Shackleton et al., 2001) could explain ibis sensitivity to photoperiod and annual cycles in gonad development.

Male testosterone concentrations were highest during the display stage and positively correlated with testicular size. Elevated plasma T concentrations during display and subsequent decreases during later stages are consistent with other studies that have found high T concentrations associated with spermatogenesis (Hirschenhauser et al., 1999; Wingfield and Farner, 1993), sexual display (Cherel et al., 1994; McQueen et al., 1999; Saint Jalme et al., 1996), and nest building (Mays et al., 1991; Schoech et al., 1991; Silver and Cooper, 1983). In addition, high androgen concentrations may stimulate lipid storage (Deviche, 1995) that could be important for subsequent fasting during nest and mate guarding. Male ibises fat scores were significantly higher during the display stage than any other time during reproduction.

Male White Ibises defend the nest site and guard females during nest building and egg laying. Aggressive interactions, such as nest defense are usually associated with high plasma T concentrations (Ketterson and Nolan, 1994) yet two males showed low testosterone concentrations shortly after the display stage. A decrease in testosterone following display occurs in males of other species that incubate and care for young (e.g., Mountain White-Crowned Sparrow, *Zonotrichia leucophrys oriantha*, Morton et al., 1990; Willow Ptarmigan, *Lagopus lagopus*, Hannon and Wingfield, 1990). Although circulating concentrations of T were low in the two post-display males, short agonistic interactions are common in ibis colonies and might cause rapid, but short-term increases in T concentrations that would facilitate aggressive behavior (Cockrem and Seddon, 1994; Morton et al., 1990). Ibis testes were not significantly smaller until chick rearing began and, upon stimulation, enlarged testes could rapidly release testosterone.

Female testosterone concentrations also increased during the display stage and then decreased during egg production and incubation. In female birds, testosterone may initiate display behavior (Hirschenhauser et al., 1999; Silver and Cooper, 1983), copulation (Hector et al., 1985), nest building (Mays et al., 1991), and bill and leg color changes (Heath, 2002). In addition, synergy between testosterone and estradiol may promote oviduct growth (Yu and Marquardt, 1973) and follicular development (Hirschenhauser et al., 1999).

The average ibis plasma T concentrations observed in this study were significantly lower than for most temperate bird species (Wingfield and Farner, 1993 for review), but were similar to other tropical species (Levin and Wingfield, 1992; Lormée et al., 2000) with the exception of Red-Footed Boobies (*Sula sula*, Lormée et al., 2000). Further, the ratio of male to female ibis androgen concentrations was close to 1:1. This is consistent with the hypothesis that in species with minimal sexual dimorphism and similar parental duties male to female androgen ratios will be close to 1:1, but in species with high sexual dimorphism and sex specific behaviors ratios may be as high as 20:1 (Wingfield and Farner, 1993).

Female estradiol concentrations tended to be high during display stage, consistent with other studies that show E₂ is elevated during periods of yolk formation and deposition (Wingfield and Farner, 1993). Estradiol concentrations also significantly increased from incubation to chick rearing. E₂ may facilitate parental behavior (Silver and Cooper, 1983), though only a few studies report similar increases during chick rearing (Cherel et al., 1994; Hector et al., 1985). Male estradiol concentrations were lowest during egg production and nest building, but then increased during incubation and chick rearing. Low estradiol concentrations during egg production and nest building may facilitate T action, without requiring high circulating T concentrations.

Female ibis progesterone concentrations increased during the display stage. This initial increase probably contributed to yolk deposition in the ovaries and the physiological preparations for egg production (Norris, 1997). Progesterone concentrations were more variable in subsequent stages and were not significantly different from pre-breeding or display birds. Male progesterone concentrations were highly variable throughout the breeding season and showed no significant differences by reproductive stage but did vary with bleeding time, suggesting an association with stress. Compared to the androgens and estradiol, the function of P in avian reproduction (i.e., behavior) remains poorly understood (Davis et al., 1995; Hirschenhauser et al., 1999). Some studies have reported no significant changes in plasma P concentrations during the breeding season (Cockrem and Seddon, 1994; Fivizzani and Oring, 1986; Hector et al., 1985). However, in other studies progesterone has been shown to stimulate incubation (Davis et al., 1995; Fivizzani and Oring, 1986; Silver and Cooper, 1983) and chick brooding (Hector et al., 1985). Alternatively, progesterone may act as a sex steroid inhibitor and reduce sexual behavior at the onset of parental care (Hector, 1988; Lormée et al., 2000; Schoech et al., 1991), and perhaps inhibit reproduction in juvenile and non-breeding adults (Hector et al., 1985; Schoech et al., 1991).

Though corticosterone is often associated with acute stressors and maintenance of physiological homeostasis

(Harvey et al., 1984), this hormone may also play a role in facilitating certain stages of reproduction by altering metabolism and fat deposition. In female ibises, corticosterone concentrations were low in early breeding stages and then increased through incubation and were highest in chick rearing. The increase in corticosterone during chick rearing coincided with significant decreases in body condition scores.

Corticosterone promotes feeding activity in hungry birds and stimulates gluconeogenesis in birds that are unable to feed (such as an incubating bird) (Astheimer et al., 1992; Gray et al., 1990). However, incubating Magellanic Penguins (*Spheniscus magellanicus*) that fast up to 31 days while incubating eggs show no increases in B concentrations (Hood et al., 1998). Male ibises fasting late in the display stage and during nest building and egg production also showed no significant increases in plasma B concentrations. Hood et al. (1998) suggested that fasting while breeding may be a normal occurrence in some species and therefore, not perceived (physiologically) as a stressor. Furthermore, birds may modulate B secretion during reproduction because of its potential to inhibit reproductive behavior and sex steroid activity (Wingfield, 1994; Wingfield et al., 1995). Fasting during nest and mate defense occurs early in the ibis reproductive cycle, an unfavorable time to inhibit reproductive behavior. Male fasting is a regular part of ibis reproduction, and males did not respond to fasting by increases in corticosterone. Consequently, reproductive activity was not interrupted by high B concentrations (or fasting; Cherel et al., 1994).

4.2. Morphology changes

Female White Ibis body condition scores increased during display and then subsequently decreased throughout reproduction. Male ibis condition scores showed a similar pattern to females, but the increase between pre-breeding and display was not significant. However, this may be an artifact of smaller male sample size and a decreased ability to detect differences. Other captive Ciconiiformes such as White Storks also show seasonal changes in mass (Hall et al., 1987). In addition, experiments with food-restricted captive Scarlet Ibises (*Eudocimus ruber*) suggest that increases in body mass can occur rapidly prior to initiation of breeding (e.g., 13–71 days) if birds were given food ad libitum (Babbitt, 2000). Mass gain associated with the onset of breeding in captive and free-living ibises was likely the result of increased food intake (Babbitt, 2000) and changes in metabolism. Increased pre-breeding energy stores may be important for fasting in male birds that do not eat during nest building and egg production, and important for egg production in female birds.

Female birds that use endogenous energy stores for egg production are sometimes referred to as 'capital breeders';

alternatively, birds that feed (use acquired energy) during egg production are 'income breeders' (Drent and Daan, 1980; Thomas, 1988). The body condition score changes of ibises suggest that they more closely fit the capital breeder paradigm. However, this study of free-living White Ibises suggests interesting sex specific differences. Male ibis fat scores increased from pre-breeding to display suggesting that males were storing energy. Males showed decreased fat scores and a non-significant tendency to lose pectoralis mass through the display and egg production stages. Therefore, endogenous fat and, in instances where fat stores may be depleted, protein (pectoralis) stores may be used during the egg production and nest building stages when male ibises fast. This use of endogenous stores would be consistent with the 'capital breeder' model. Female White Ibises also showed body condition score changes during the breeding season but because fat and pectoralis score changes were not consistent with body condition scores, the cause of these changes was less clear than in male ibises. Female ibis body condition score changes were consistent with 'capital breeder' patterns, but showed no significant sign of energy storage by increased fat stores or increased pectoralis scores. Some female mass changes may have been attributable to development of the ovary and oviduct, though it is unlikely that ovaries accounted for all body condition changes. In contrast to male ibises, female White Ibises forage throughout the breeding season. Thus, female ibises may not depend on endogenous energy stores for egg production. This would be consistent with our findings that females do not store fat or protein during the breeding season and consistent with the predictions of an 'income breeder.'

Studies of avian reproductive energetics often focus on female requirements because egg production is likely the most costly stage of reproduction per unit time (Drent and Daan, 1980; Meijer and Drent, 1999). In White Ibises, reproduction was also costly for males that fast during egg production and provide parental care. Future studies of reproductive physiology that compare male and female energy requirements could provide insight on sex-specific storage strategies.

Brood patches presumably function in heat transfer from the parent to eggs and, therefore, are associated with participation in parental care (Jones, 1969). In this species, male and female ibises incubate eggs (Heath, pers. obs.; Kushlan and Bildstein, 1992) and both sexes developed brood patches during incubation.

Adult ibises molted most of their body feathers during the pre-breeding stage. A pre-alternate molt may enhance plumage and provide more contrast for display coloration on ibis bill and legs. Ibises showed evidence of body molt in all stages of reproduction, including the display stage when males and females had high T concentrations. High testosterone concentrations inhibit molt in temperate species (Hahn et al., 1992; Hannon and Wingfield,

1990; Schlessner et al., 1985) and, likewise, hormonal correlates of molt (i.e., thyroid hormone) can inhibit reproduction (Wingfield and Farner, 1993). However, many tropical species molt during reproduction (Dittami and Gwinner, 1990; Foster, 1975; Payne, 1969; Poulin et al., 1992). The apparent lack of endocrinological conflict during molting and breeding in tropical species is poorly understood, though it may only be possible for both events to occur simultaneously in periods of high food resources (Foster, 1975; Poulin et al., 1992).

5. Conclusions

White Ibis reproductive physiology showed many characteristics similar to other seasonally breeding birds, including cyclic gonad development and hormonal changes. In addition, White Ibises present an interesting model of energy use because male mate guarding depends on the individual's ability to fast. Understanding the complex relationships among food availability, ability to gain mass, and nesting effort may show more specifically how ibises are able to respond quickly to favorable environmental conditions.

This is the first study to describe the reproductive physiology of a free-living Ciconiiform bird. Because this study was conducted with free-living birds, environmental contaminants, such as mercury, may have affected ibis hormone concentrations and patterns (Heath, 2002). Further research conducted with wading birds will lend interesting perspectives to the physiological patterns described here.

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