

## High levels of LH and testosterone in a tropical seabird with an elaborate courtship display

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### Abstract

For birds breeding in temperate areas, territoriality and courtship co-occur during spring and are mirrored by elevated plasma levels of luteinizing hormone (LH) and testosterone. By contrast, several tropical species show no major changes in LH and testosterone throughout the year and plasma levels are often low. This study describes changes in body condition, LH and testosterone levels throughout breeding in a tropical seabird, the magnificent frigatebird (*Fregata magnificens*). Although bi-parental care is the rule in frigatebirds, these seabirds are unique among seabirds in having a lek-like mating system where males perform an elaborate courtship display during which an extravagant sexual ornament (gular pouch) is inflated. Body condition peaked during the displaying stage in males and declined gradually in both sexes, being the lowest during chick-rearing. LH and testosterone titers were the highest in displaying males with a fully inflated gular pouch (means: 23.6 ng/ml for LH and 6.2 ng/ml for testosterone) and remained low (means: 3.8 ng/ml for LH and 0.3 ng/ml for testosterone) during incubation and chick-rearing in both sexes. Body condition was positively related to testosterone levels in displaying males. In male frigatebirds, high levels of testosterone are not associated with aggression but are probably required to display the inflated gular pouch and to perform an elaborate courtship display. Because males invest in incubation and chick-rearing, testosterone has to return to low levels, to permit parental care. This hormonal pattern differs greatly with that of the few tropical seabirds studied so far and is probably related to the unique reproductive strategy of frigatebirds.

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### 1. Introduction

For birds breeding in temperate areas or at high latitudes, where food is abundant for a short season, territorial and courtship behaviors generally co-occur during a relatively short period in spring. At the physiological level, luteinizing hormone (LH) is secreted by the pituitary gland as a response to the photoperiodic increase of

day length in spring (Follett et al., 1985). LH, in concert with follicle-stimulating hormone (FSH), orchestrates gonad growth and the release of sex steroid hormones such as testosterone. In a wide range of high latitude and temperate avian species, testosterone plays a key role in regulating territorial aggression and courtship (Wingfield et al., 1990). Accordingly, territorial and courtship behaviors are usually coupled with high concentrations of circulating LH and testosterone (Wingfield et al., 1987), which often decline during the period of parental care (Ball, 1991).

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In the tropics, environmental conditions are often viewed as rather constant with respect to photoperiod and climate, and since food is generally available year-round, one may expect tropical birds to breed year-round. Two general patterns characterize tropical breeding seasons: greater length of the breeding season and a greater variability among species (and individuals) in when breeding takes place (Stutchbury and Morton, 2001; Wikelski et al., 2000). In contrast with high latitude and temperate birds, studies conducted on a handful of tropical species have reported a dampened pattern of LH secretion, regardless of whether species are seasonal, opportunistic or aseasonal breeders (Degen et al., 1994; Hau et al., 2000; Levin and Wingfield, 1992; Lormée et al., 2000; McDonald et al., 2001; Wikelski et al., 2000). Concerning testosterone, tropical birds also break the rules established for temperate breeding birds (Stutchbury and Morton, 2001). In fact, many tropical species, even year-round territorial ones, show no major changes in testosterone throughout the year and plasma levels are often low (Dittami and Gwinner, 1985; Dittami, 1987; Levin and Wingfield, 1992; Lormée et al., 2000; McDonald et al., 2001; Wikelski et al., 2000; Wiley and Goldizen, 2003), or barely detectable (Hau et al., 2000; Lormée et al., 2000; Wikelski et al., 1999, 2000; Wingfield et al., 1991, 1999).

The few tropical species studied so far have been mainly territorial ones, because of the interest of studying the apparent paradox of defending a territory year-round with low plasma testosterone (Wikelski et al., 1999). This endocrine pattern may however apply to various mating systems. In some tropical polygynous lekking passerines, testosterone levels, although slightly more elevated than in tropical socially monogamous territorial species, hardly exceeded 1.5 ng/ml (Wikelski et al., 2003), much less than the 2–5.5 ng/ml generally measured in temperate species (review in Goymann et al., 2004; Stutchbury and Morton, 2001). Therefore, more work is clearly required on a wider range of tropical species, including non-passerines with different life-history traits and mating systems, to establish how broad this endocrine pattern is.

This paper reports changes in plasma levels of LH and testosterone throughout the breeding cycle in the magnificent frigatebird, *Fregata magnificens*, a long-lived tropical seabird. The courtship display of frigatebirds is unusual and is the most spectacular of any seabirds. Displaying males inflate and exhibit a red gular pouch while spreading out the wings and tipping their head back with the bill pointing skywards (Nelson, 1976). The gular pouch, a likely sexually selected trait, is used only by courting males in mate attraction (Dearborn and Ryan, 2002). These display are performed in groups at which females can assess many potential mates at once (Nelson, 1976). This courtship behavior resembles that of lekking birds in which aggregations of members of one

sex (nearly always males) are visited by the opposite sex solely for the purpose of mating (Höglund and Alatalo, 1995). The gular pouch shrinks and changed to skin color after the male has obtained a mate.

Frigatebirds are socially and genetically monogamous (Dearborn et al., 2001) but in contrast with most seabirds, the pair bond lasts only for the duration of the breeding attempt (one breeding attempt per year with no reneesting after failure in magnificent frigatebirds, Osorno, 1996).

Contrary to true lekking birds, bi-parental care is the rule in the 5 frigatebird species, and male and female incubate the single egg and feed the chick (Nelson, 1976).

From this and from what is known on tropical birds two opposite predictions can be made:

- (1) As tropical birds, frigatebirds may share the possibly general pattern of attenuated LH and low circulating plasma testosterone concentrations, as found in the few tropical seabirds studied so far (Lormée et al., 2000; Wingfield et al., 1999), a pattern possibly only weakly influenced by variations in life-history traits and mating systems (Goymann et al., 2004; Wikelski et al., 2003).
- (2) As male frigatebirds exhibit a lek-like mating system but have to provide parental care as soon as the egg is laid (Dearborn and Ryan, 2002; Osorno, 1996), plasma testosterone levels should be elevated during the displaying stage (Wingfield et al., 1990) whereas LH and testosterone levels should be kept low as soon as the egg is laid to permit parental care (Ketterson and Nolan, 1992; Wingfield et al., 1990).

## 2. Methods

### 2.1. Study area

Field work was carried out between 19 April and 5 May, 2002, and 3–14 March, 2003, on the Grand Connétable Nature Reserve (4°50'N, 51°56'W), an islet situated 18 km off French Guyana. In this area, magnificent frigatebirds (body mass:  $1299 \pm 17.3$  g,  $n = 50$  for males and  $1526 \pm 17.7$  g,  $n = 40$  for females) forage over neritic and coastal waters (Weimerskirch et al., 2003) and feed mostly on fishes (Dujardin and Tostain, 1990). The breeding population of magnificent Frigatebirds from the Grand Connétable Nature Reserve (650 active nests in March 2003) does not exhibit a clear seasonal breeding pattern, displaying, incubating, chick-rearing, and non-breeding birds being found altogether between November and July (Dujardin and Tostain, 1990). The incubation of the single egg lasts for 56 days and is shared by males and females (Osorno, 1999). In the magnificent frigatebird, the male will desert the chick after 19–160 days of chick-rearing, leaving the female to care

for the chick for the remaining 9–12 months (Osorno, 1999).

## 2.2. Blood sampling

In 2002 and 2003, blood samples were collected on 4 reproductive stages of birds: (1) displaying males exhibiting the red gular pouch; (2) males and females incubating an egg, (3) males and females rearing a 10–70 days old chick, and (4) non-breeding males and females. Displaying, incubating and chick-rearing birds were captured on their displaying site or on their nest by hand or with a noose on the end of a 5-m fishing pole. At the Grand Connétable, all frigatebird nests are constructed on the ground, which allows easy access to the birds (Dujardin and Tostain, 1990). Males display on the ground and if successful start nest-building on the same site. Non-breeding frigatebirds (birds still retaining some immature plumage) were captured by hand while roosting on rocky ledges. When sampling displaying males, we recorded if the gular pouch was fully inflated or partially deflated (Fig. 1). Females visiting displaying males were very difficult to catch and were not sampled. Because of the loose seasonal breeding pattern of the studied species, these four breeding stages could be sampled at the same time. Only one bird per nest was captured, and data obtained on males and females were considered as independent. Immediately after capture, a blood sample (maximum: 1 ml) was collected from the alar vein with a 1 ml heparinized syringe and a 25 gauge needle. To check for the possible effect of handling time on hormone levels (Gratto-Trevor et al., 1991), we measured the time elapsed between capture and the end of the blood sampling (range: 1–5 min). Samples were put into ice and centrifuged as soon as possible; afterward plasma was stored at  $-20^{\circ}\text{C}$  before hormone assays.

After blood sampling, birds were weighed to the nearest 2 g using a Pesola scale and their culmen was measured to the nearest 0.5 mm with a calliper. In both sexes adult body size, measured as culmen length, did not significantly correlate with adult body mass for both males ( $R=0.23$ ,  $P=0.09$ ,  $n=50$ ) and females ( $R=0.06$ ,  $P=0.72$ ,  $n=40$ ). Nevertheless, in order to control for any effect of structural body size on adult body mass, we used the residuals from the regressions between body mass and culmen length for males and females separately (Osorno, 1999).

## 2.3. Hormone assays

Plasma concentrations of LH and testosterone were determined by radioimmunoassays at the CEBC (see Lormée et al., 2000). For LH (sources: LH, Prf. Ishii and Wakabayashi, Wadesa University, Japan) pooled plasma samples produced dilution curves that paralleled the chicken LH standard curve (Fig. 2). Parallelism between the magnificent frigatebird and chicken curves indicates that the concentration-dependent binding dynamics of the frigatebird LH with the antibody is similar to that of the chicken LH with the antibody and that this heterologous RIA can be used to assess relative levels of plasma LH in the magnificent frigatebird. The lowest detectable quantities for LH, which were significantly different from zero at a 90% confidence level, were 1.7 ng/ml. The intra-assay coefficients of variation for LH and testosterone were 8.7 and 7%, respectively ( $n=3$  duplicates). Testosterone was extracted from a 50  $\mu\text{l}$  plasma sample in diethyl-ether and determined in duplicates in one single assay. The lowest detectable quantities for testosterone, which were significantly different from zero at a 90% confidence level, were 0.049 ng/ml. To check for

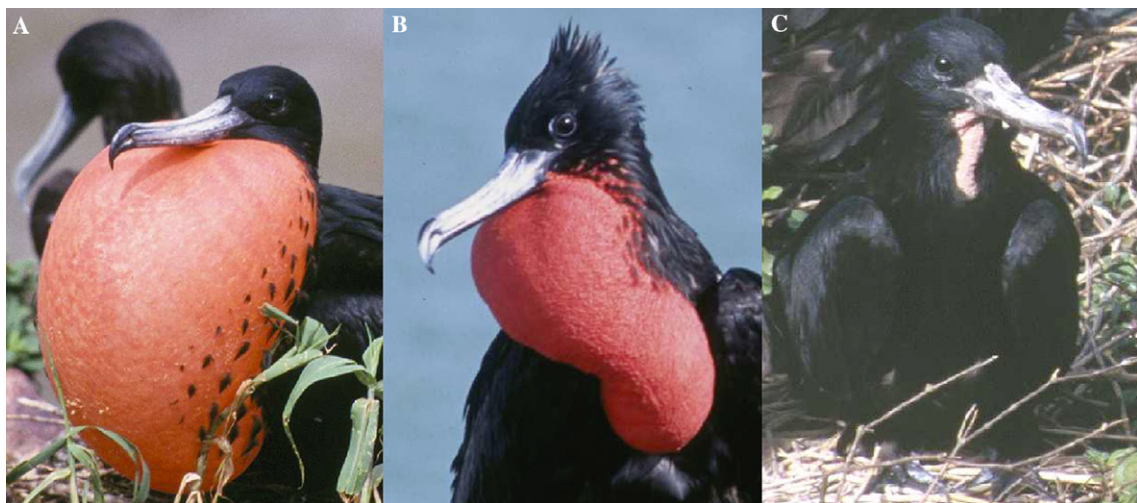


Fig. 1. Gular pouch aspects in male magnificent frigatebirds. (A) Displaying male with a fully inflated gular pouch; (B) displaying male with a partially deflated gular pouch; and (C) a chick-rearing male: the gular pouch has shrunk and changed to skin color after the male has obtained a mate.

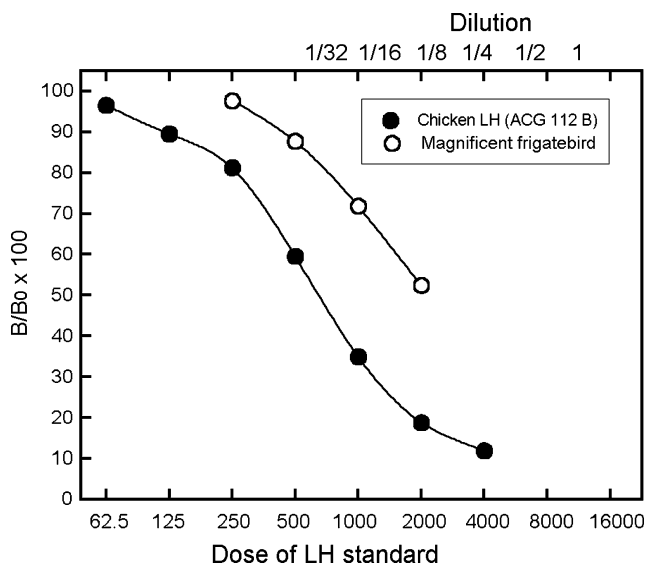


Fig. 2. Dilution curve for LH of chicken and magnificent frigatebirds. LH standard is expressed in picogram/tube.

cross-reactivity between testosterone and other androgens occurring in the plasma, concentrations of testosterone (T), androstenedione (A4), and dihydrotestosterone (DHT) were assayed in a random subsample of 10 plasmas. Steroid-specific antibodies were obtained from Dr. G. Picaper (non-commercial antibodies manufactured and provided by Médecine Nucléaire, CHU, 45900 La Source, France) for testosterone and from PARIS laboratories (60200-Compiègne, France) for A4 and DHT. Testosterone was the most common androgen (72%) followed by DHT (20.6%) and A4 (7.4%). There were high correlations among these three androgens (T–DHT:  $r = 0.95$ ,  $n = 10$ ,  $P < 0.001$ ; T–A4:  $r = 0.98$ ,  $n = 10$ ,  $P < 0.001$ ; A4–DHT:  $r = 0.938$ ,  $n = 10$ ,  $P < 0.001$ ). Cross reactivity was 35 and 1.88% between T and DHT and between T and A4, respectively. Given that testosterone was the androgen with the highest concentrations of the three and that the concentrations of three androgens were correlated, we are confident that results from our assay correctly reflects variations in testosterone among plasmas.

#### 2.4. Statistics

All breeding stages were sampled in 2002 and 2003. We ran General Linear Models (GLM) to study the effects of year, breeding stages, and body condition on hormone levels for males and females separately. Because birds were sampled between 07:30 and 23:00 h, we included time of the day as a factor in the GLM to control for possible diel variations in hormone levels. Date of sampling and handling time were also included as factor to control for possible effects on hormone levels. Comparisons of body condition and hormone levels between stages were conducted using post hoc Tukey

tests. LH and testosterone data were  $\log_{10}$  transformed to meet assumptions required by a statistical test according to Sokal and Rohlf (1981). Statistical analyses were performed using SYSTAT 7.0 (Wilkinson, 1997). All statistical tests are two-tailed, and probability levels  $< 0.05$  were considered significant. Values are presented as means  $\pm$  standard error.

### 3. Results

#### 3.1. Body condition

Time of the day (males:  $F_{1,40} = 0.37$ ,  $P = 0.55$ ; females:  $F_{1,32} = 3.34$ ,  $P = 0.08$ ) and date of sampling did not influence body condition (males:  $F_{1,40} = 0.05$ ,  $P = 0.83$ ; females:  $F_{1,32} = 0.36$ ,  $P = 0.55$ ). Body condition did not differ between years (males:  $F_{1,40} = 0.005$ ,  $P < 0.95$ ; females:  $F_{1,32} = 7.01$ ,  $P = 0.001$ ) but varied significantly in relation to breeding stages (males:  $F_{3,40} = 18.85$ ,  $P < 0.001$ ; females:  $F_{2,32} = 9.12$ ,  $P = 0.001$ ). There was no between-factor interaction (year \* breeding stages, males:  $F_{3,40} = 0.62$ ,  $P = 0.61$ ; females:  $F_{2,32} = 2.70$ ,  $P = 0.09$ ). Body condition peaked during the displaying stage in males and during incubation in females and declined gradually, being the lowest during chick-rearing (Fig. 3).

#### 3.2. LH and testosterone

In males, plasma LH titers were not influenced by handling time ( $F_{1,32} = 1.82$ ,  $P = 0.19$ ), date of sampling ( $F_{1,32} = 0.12$ ,  $P = 0.73$ ), time of the day ( $F_{1,32} = 0.70$ ,  $P = 0.41$ ), nor by body condition ( $F_{1,32} = 0.69$ ,  $P = 0.41$ ). Male plasma levels of LH did not differ between years ( $F_{1,32} = 0.21$ ,  $P = 0.65$ ) but varied significantly in relation to breeding stages ( $F_{3,32} = 13.33$ ,  $P < 0.001$ ). There was no between-factor interaction (year \* breeding stages:  $F_{3,32} = 0.80$ ,  $P = 0.50$ ). LH titers were the highest in displaying males (Tukey tests all,  $P < 0.004$ ) and remained low during incubation and chick-rearing, similar to that of non-breeding birds (Fig. 3). Among displaying males, LH titers (range: 4.10–37.41 ng/ml) were not influenced by body condition ( $F_{1,9} = 0.67$ ,  $P = 0.43$ ) but were significantly higher in birds with a fully inflated gular pouch ( $F_{1,9} = 7.22$ ,  $P = 0.007$ , Fig. 4).

In females, plasma LH levels were not influenced by handling time ( $F_{1,24} = 0.01$ ,  $P = 0.92$ ), date of sampling ( $F_{1,24} = 0.76$ ,  $P = 0.39$ ), time of the day ( $F_{1,24} = 0.43$ ,  $P = 0.52$ ) nor by body condition ( $F_{1,24} = 0.15$ ,  $P = 0.70$ ). Female plasma levels of LH did not differ between years ( $F_{1,24} = 0.20$ ,  $P = 0.66$ ) and showed no significant variation across breeding stages ( $F_{2,24} = 0.37$ ,  $P = 0.69$ , Fig. 3). There was no between-factor interaction (year \* breeding stages:  $F_{2,24} = 2.04$ ,  $P = 0.15$ ). On average, plasma levels of LH did not differ between males and females in incubating, chick-rearing, and non-breeding birds ( $F_{1,58} = 3.37$ ,  $P = 0.07$ ).



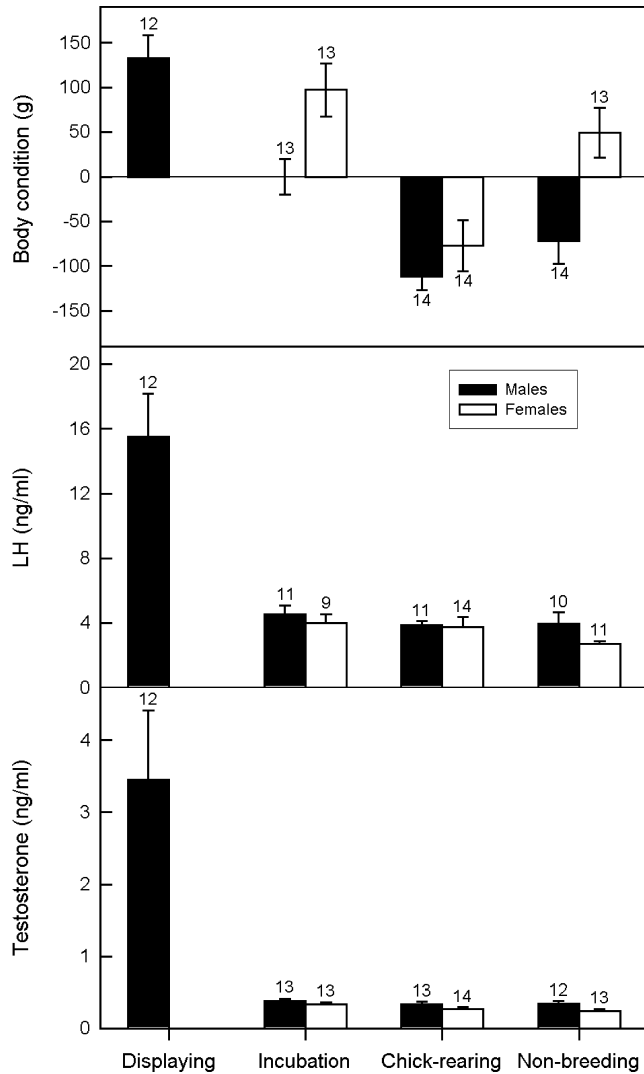


Fig. 3. Body condition (residuals), plasma levels of LH and testosterone in male and female magnificent frigatebirds in relation to reproductive stage. Shown are means  $\pm$  SE. Sample sizes are given above the bars.

In males, testosterone titers were not influenced by handling time ( $F_{1,38}=0.23$ ,  $P=0.63$ ), date of sampling ( $F_{1,38}=0.01$ ,  $P=0.92$ ) nor by time of the day ( $F_{1,38}=1.31$ ,  $P=0.26$ ). Male plasma levels of testosterone did not differ between years ( $F_{1,38}=0.03$ ,  $P=0.86$ ) but varied significantly in relation to breeding stages ( $F_{3,38}=17.39$ ,  $P<0.001$ ). There was no between-factor interaction (year \* breeding stages:  $F_{3,38}=2.40$ ,  $P=0.09$ ). Testosterone titers were the highest in displaying males (Tukey tests, all  $P<0.008$ ) and remained low during incubation and chick-rearing, similar to that of non-breeding ones (Fig. 3). Among displaying males, testosterone levels (range: 0.82–11.5 ng/ml) were significantly and positively influenced by body condition ( $F_{1,9}=4.07$ ,  $P=0.044$ ) and by gular pouch aspect ( $F_{1,9}=7.36$ ,  $P=0.007$ ): testosterone levels were significantly higher in birds with a fully inflated gular pouch (Fig. 4). Testos-

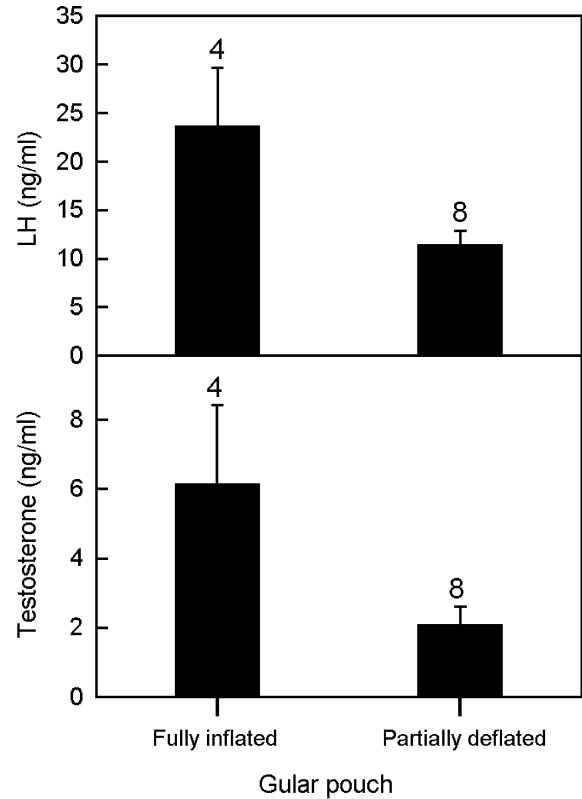


Fig. 4. Plasma levels of LH and testosterone in displaying male magnificent frigatebirds in relation to gular pouch aspect. Shown are means  $\pm$  se. Sample sizes are given above the bars.

terone and LH levels were positively correlated in displaying males ( $R=0.80$ ,  $P<0.002$ ,  $n=12$ ).

In females, testosterone titers were not influenced by handling time ( $F_{1,32}=0.11$ ,  $P=0.74$ ), date of sampling ( $F_{1,32}=1.80$ ,  $P=0.19$ ) nor by time of the day ( $F_{1,32}=2.83$ ,  $P=0.10$ ) but were positively and significantly influenced by body condition ( $F_{1,32}=6.66$ ,  $P=0.01$ ). Female testosterone levels did not differ between years ( $F_{1,32}=1.55$ ,  $P=0.22$ ), remained low and did not significantly vary across breeding stages ( $F_{2,32}=1.98$ ,  $P=0.15$ , Fig. 3). On average, male and female frigatebirds showed no difference in plasma levels of testosterone during incubation, chick-rearing and non-breeding stages ( $F_{1,70}=1.49$ ,  $P=0.23$ ).

## 4. Discussion

### 4.1. A tropical bird with an unusual endocrine pattern

In tropical bird species, low testosterone levels, involving low-amplitude cycles have been associated with a greater length of the breeding season (Goymann et al., 2004). The fact that tropical birds show plasma levels of testosterone that are 2–3 times less than those found in

temperate or high latitude ones (Goymann et al., 2004; Stutchbury and Morton, 2001) has been interpreted as a means to avoid the potential detrimental consequences of maintaining elevated testosterone over prolonged breeding seasons (Stutchbury and Morton, 2001; Wikelski et al., 2003). Sustaining continuously high levels of testosterone may depress the immune system and jeopardize adult survival (Folstad and Karter, 1992).

As tropical seabirds, frigatebirds are characterized by a very long breeding season, consequence of a slow chick growth, protracted period of parental cares, and poorly synchronized breeding (Ashmole, 1971; Dujardin and Tostain, 1990; Nelson, 1983). In male magnificent frigatebirds, however, LH and testosterone were high during the displaying stage and showed marked variations across the breeding cycle being 4 and 10 times higher in displaying males, respectively, than during incubation and chick-rearing during which levels were low and similar to those of non-breeding birds. Thus, contrary to our first prediction, in males of this tropical seabird, LH and testosterone secretions follow a pattern typical of temperate and high latitude seabirds where breeding occurs over a short season (Cherel et al., 1994; Groscolas et al., 1986; Hector et al., 1986; Mauget et al., 1994; Scanes et al., 1974; Williams, 1992). In our study, we did not sample females visiting displaying males and this puts some limit on our conclusions. In a study of female magnificent frigatebirds, Osorno (1996) reported slightly elevated testosterone levels (0.8–1 ng/ml, Osorno, 1996) prior to egg-laying; after what, levels returned to low values (0.3 ng/ml; Osorno, 1996; this study).

#### 4.2. LH, testosterone, and the reproductive strategy of frigatebirds

The pattern of testosterone secretion of magnificent frigatebirds resembles the pattern predicted for species where males interact aggressively for part of the breeding season, but where the frequency of aggression abates once the parental phase is under way (the “Challenge Hypothesis,” Wingfield et al., 1990). In frigatebirds, operational sex ratio is often male-biased (Dearborn et al., 2001) and therefore there is a potential for intense competition between males for the access to females. However, the “Challenge Hypothesis” (Wingfield et al., 1990) cannot be invoked for frigatebirds since displaying males show virtually no aggression toward each other, tolerating even physical contact during display (Dearborn and Ryan, 2002; Nelson, 1976). Furthermore, the territory (restricted to the nest) is only established once the pair has been formed (Nelson, 1976). Even then, birds show almost no aggressive behavior.

Contrary to temperate species, a decoupling of aggression and testosterone has been established in several tropical species (Levin and Wingfield, 1992; Wing-

field and Lewis, 1993; Wiley and Goldizen, 2003, but see Wikelski et al., 1999) with only behaviors involved in courtship being under the control of testosterone (McDonald et al., 2001; Wiley and Goldizen, 2003). Frigatebirds depart even further from the temperate birds’ model by showing almost no aggression and territorial behaviors while having high testosterone levels (up to 11.5 ng/ml), which are probably mainly devoted to a complex and elaborate courtship. In several bird species males raise unfeathered areas of the head and neck such as combs, wattles, lappets or esophageal pouches that are used in mate attraction (Ligon, 1999). These brightly colored, fleshy ornaments that can be inflated and deflated rapidly in some pheasants and grouses are often directly controlled by testosterone (Ligon, 1999). It is therefore possible that in male frigatebirds, high levels of testosterone are required to display the inflated gular pouch. During the displaying stage, males alternate intense gular pouch displays with resting periods during which the gular pouch is more or less deflated but never returns to the totally deflated and skin-colored aspect found in non-displaying males (Chastel et al. unpublished data). Even during these resting periods, displaying males show testosterone levels that are still six times higher than during the parental and non-breeding stages when the gular pouch has shrunk and changed to skin color. This supports the idea that this fleshy, highly vascularized sexual ornament is likely to be controlled by testosterone. The fact that males with a fully inflated gular pouch show the highest testosterone titers also suggests that the intensity of the gular pouch displays mirror testosterone levels.

In our study, plasma levels of LH paralleled those of testosterone. Although a causal relationship between testosterone and sexual ornamentations has been established (e.g., comb of male red junglefowl *Gallus gallus*; Zuk et al., 1995), a potential causal role for LH is less clear. As LH promotes testosterone secretion (Follett et al., 1985), it is probable that the high LH levels found in displaying males reflect mainly a sustained secretion of testosterone. The red inflatable gular pouch is only part of an elaborate courtship display, which includes erecting the iridescent ruff feathers, extending and shaking the wings, and vocalizing (Dearborn and Ryan, 2002; Nelson, 1976). During display, male magnificent frigatebirds produce a characteristic drumming sound with their bill and the inflated gular pouch may function as a resonance chamber that could be used by females as a cue to assess gular pouch size (Madsen et al., 2004). It is well known that in many temperate and in some tropical passerines testosterone regulates song (Hau et al., 2000; Wingfield et al., 1987). In the magnificent frigatebirds, however, vocalizations (drumming) are only performed in the presence of a visiting female (Madsen et al., 2004). We sampled all but one male with a fully inflated gular pouch when they were passively (not vocalizing) attending the

lekking area while having elevated testosterone levels. This suggests that the correlation of testosterone with the gular pouch is not a side effect of vocalizations but that high testosterone levels are actually associated with the inflation of the gular pouch. However, the male with the highest testosterone concentration (11.5 ng/ml) was actively displaying in front of a female when captured. It is therefore possible that the highest testosterone levels are necessary to express the whole sequence of complex and elaborate courtship behaviors of male frigatebirds.

In our study, body condition was positively related to testosterone levels in displaying males, suggesting that courtship display might be condition-dependent. Body condition was the highest in displaying males and declined sharply thereafter. Because male frigatebirds spend a significant part of their time performing courtship displays (Dearborn and Ryan, 2002), this probably reduces foraging opportunities and leads body to deteriorate. Males may have to accumulate a certain amount of body reserves to sustain a potentially energy-demanding courtship display while fasting at the colony.

Male testosterone levels were low during incubation and chick-rearing, and similar to those of females and non-breeding birds. Male magnificent frigatebirds invest heavily in incubation and in a variable fraction of the chick-rearing (Osorno, 1999). Because the high testosterone levels required for mate attraction are likely to be incompatible with parental behavior (Ketterson and Nolan, 1992), male LH and testosterone have probably to return to low levels as soon as the egg is laid.

In other tropical true lekking species, like manakins (Pipridae), males attend the arena and display over a relatively prolonged season. In these birds, low testosterone levels (Wikelski et al., 2003) coupled with many testosterone receptors in specialized motoneurons (Schultz and Schlinger, 1999) may permit elaborate acrobatic courtship displays while avoiding the costs of elevated testosterone. For male frigatebirds, the period of displaying and hence high levels of testosterone is relatively short (depending on success, males may display for 1–15 days in a given season), compared to the period of parental cares (several months) when testosterone are kept at very low levels. Male frigatebirds may therefore afford to raise temporarily high testosterone levels. It is not yet clear whether the gular pouch, although a necessary component of courtship, is in itself the basis of female choice (Dearborn and Ryan, 2002). However, according to the “Handicap Hypothesis” of sexual selection (Folstad and Karter, 1992), it is possible to imagine that variations in the gular pouch or in the intensity of the courtship display convey to female frigatebirds information about the intrinsic quality of a given male through its ability to secrete and hold elevated testosterone levels (Zuk et al., 1995). Future studies should focus on the implication of testosterone on sexual selection in frigatebirds and on the

potential trade-offs between testosterone secretion, immunocompetence and sexual ornamentations (Verhulst et al., 1999).

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### References

- Ashmole, N.P., 1971. Seabird ecology and the marine environment. In: Farner, D.S., King, J.R. (Eds.), *Avian Biology*, vol. 1. Academic Press, New York., pp. 224–286.
- Ball, G.F., 1991. Endocrine mechanisms and the evolution of avian parental care. In: *Acta XX Congressus Internationalis Ornithologici*, pp. 984–992.
- Cherel, Y., Mauget, R., Lacroix, A., Gilles, J., 1994. Seasonal and fasting-related changes in circulating gonadal steroids and prolactin in king penguins, *Aptenodytes patagonicus*. *Physiol. Biochem. Zool.* 67, 1154–1173.
- Dearborn, D.C., Anders, A.D., Parker, P.G., 2001. Sexual dimorphism, extra pair fertilizations, and operational sex ratio in great frigatebirds (*Fregata minor*). *Behav. Ecol.* 12, 746–752.
- Dearborn, D.C., Ryan, M.J., 2002. A test of the Darwin–Fisher theory for the evolution of male secondary sexual traits in monogamous birds. *J. Evol. Ecol.* 15, 307–313.
- Degen, A.A., Weil, S., Rosenstrauch, A., Kam, M., Dawson, A., 1994. Seasonal plasma levels of luteinizing and steroid hormones in male and female domestic ostriches (*Struthio camelus*). *Gen. Comp. Endocrinol.* 93, 21–27.
- Dittami, J.P., 1987. A comparison of breeding and moult cycles and life histories in two tropical starling species: the Blue-Eared Glossy Starling, *Lamprotornis chalybaeus*, and Ruppell's Long-tailed Glossy Starling, *L. purpuropterus*. *Ibis* 129, 69–85.
- Dittami, J.P., Gwinner, E., 1985. Annual cycles in the African stonechat *Saxicola torquata axillaris* and their relationship to environmental factors. *J. Zool. Lond.* 207, 357–370.
- Dujardin, J.L., Tostain, O., 1990. Les oiseaux de mer nicheurs de Guyane Française. *Alauda* 58, 107–134.

- Folstad, I., Karter, A.J., 1992. Parasites, bright males and the immuno-competence handicap. *Am. Nat.* 139, 603–622.
- Follett, B.S., Foster, R.G., Nicholls, T.J., 1985. Photoperiodism in birds. In: Ciba Foundation Symposium 117. Photoperiodism, Melatonin and the Pineal, Pitman, London, UK, pp. 93–105.
- Goymann, W., Moore, I.T., Scheuerlein, A., Hirschenhauser, K., Grafen, A., Wingfield, J.C., 2004. Testosterone in tropical birds: effects of environmental and social factors. *Am. Nat.* 164, 327–334.
- Gratto-Trevor, C.L., Oring, L.W., Fivizzani, A.J., 1991. Effects of blood sampling stress on hormone levels in the semipalmated sandpiper. *J. Field Ornithol.* 62, 19–27.
- Groscolas, R., Jallageas, M., Goldsmith, A., Assenmacher, I., 1986. The endocrine control of reproduction and molt in male and female emperor (*Aptenodytes forsteri*) and Adélie (*Pygoscelis adeliae*) penguins. I. Annual changes in plasma levels of gonadal steroids and LH. *Gen. Comp. Endocrinol.* 62, 43–53.
- Hau, M., Wikelski, M., Soma, K.K., Wingfield, J.C., 2000. Testosterone and year-round territorial aggression in a tropical bird. *Gen. Comp. Endocrinol.* 117, 20–33.
- Hector, J.A.L., Follett, B.K., Prince, P.A., 1986. Reproductive endocrinology of the black-browed albatross *Diomedea melanophris* and the grey-headed albatross *D. chrysostris*. *J. Zool. Lond.* 208, 237–253.
- Höglund, J., Alatalo, R.V., 1995. *Leks*. Princeton University Press, New Jersey.
- Ketterson, E.D., Nolan Jr., V., 1992. Hormones and life histories: an integrative approach. *Am. Nat.* 140S, 33–62.
- Levin, R.N., Wingfield, J.C., 1992. The hormonal control of territorial aggression in tropical birds. *Orn. Scand.* 23, 284–291.
- Ligon, J.D., 1999. *The Evolution of Avian Breeding Systems*. Oxford University Press, Oxford.
- Lormée, H., Jouventin, P., Lacroix, A., Lallemand, J., Chastel, O., 2000. Reproductive endocrinology of tropical seabirds: sex-specific patterns in LH, steroids and prolactin secretion in relation to parental care. *Gen. Comp. Endocrinol.* 117, 413–426.
- McDonald, P.G., Astheimer, L.B., Buttemer, W.A., 2001. Gonadal and hormonal phenology in free-living male rufous whistlers, *Pachycephala rufiventris* (Passeriformes: Pachycephalidae). *Aust. J. Zool.* 651–662.
- Madsen, V., Balsby, T.J.S., Dabelsteen, T., Osorno, J.L., 2004. Bimodal signaling of a sexually selected trait: gular pouch drumming in the Magnificent frigatebird. *Condor* 106, 156–160.
- Mauget, R., Jouventin, P., Lacroix, A., Ishii, S., 1994. Plasma LH and steroid hormones in king penguin (*Aptenodytes patagonicus*) during the onset of the breeding cycle. *Gen. Comp. Endocrinol.* 93, 36–43.
- Nelson, J.B., 1976. The breeding biology of frigatebirds: a comparative review. *Living Birds* 14, 113–155.
- Nelson, J.B., 1983. Contrasts in breeding strategies between some tropical and temperate marine pelecaniformes. *Stud. Avian Biol.* 8, 95–114.
- Osorno, J.L., 1996. Evolution of breeding behavior in the Magnificent Frigatebird: copulatory pattern and parental investment. Unpublished PhD dissertation, University of Florida, Gainesville, FL, USA.
- Osorno, J.L., 1999. Offspring desertion in the Magnificent Frigatebird: are males facing a trade-off between current and future reproduction?. *J. Avian Biol.* 30, 335–341.
- Scanes, C.G., Cheeseman, P., Phillips, J.G., Follett, B.K., 1974. Seasonal and age variation of circulating immunoreactive luteinizing hormone in captive Herring gulls, *Larus argentatus*. *J. Zool. Lond.* 174, 369–375.
- Schultz, D., Schlinger, B.A., 1999. Widespread accumulation of <sup>3</sup>H-testosterone in the spinal cord of a wild bird with an elaborate courtship display. *Proc. Natl. Acad. Sci. USA* 96, 10432–10436.
- Sokal, R.R., Rohlf, F.J., 1981. *Biometry*. W.H. Freeman, San Francisco.
- Stutchbury, B.J.M., Morton, E.S., 2001. *Behavioral Ecology of Tropical Birds*. Academic Press, San Diego, CA.
- Verhulst, S., Dieleman, S.J., Parmentier, H.K., 1999. A tradeoff between immunocompetence and sexual ornamentation in domestic fowl. *Proc. Natl. Acad. Sci. USA* 96, 4478–4481.
- Weimerskirch, H., Chastel, O., Barbraud, C., Tostain, O., 2003. Frigatebirds ride high on thermals. *Nature* 421, 333–334.
- Wikelski, M., Hau, M., Wingfield, J.C., 1999. Social instability increases plasma testosterone in a year-round territorial neotropical bird. *Proc. R. Soc. Lond. B* 266, 1–6.
- Wikelski, M., Hau, M., Wingfield, J.C., 2000. Seasonality of reproduction in a neotropical rain forest bird. *Ecology* 81, 2458–2472.
- Wikelski, M., Hau, M., Robinson, W.D., Wingfield, J.C., 2003. Reproductive seasonality of seven neotropical passerines species. *Condor* 105, 683–695.
- Wiley, C.J., Goldizen, A.W., 2003. Testosterone correlated with courtship but not aggression in the tropical buff-banded rail, *Gallirallus philippensis*. *Horm. Behav.* 43, 554–560.
- Wilkinson, L., 1997. *Systat 7.0*, New Statistics. SPSS, Chicago.
- Williams, T.D., 1992. Reproductive endocrinology of macaroni (*Eudyptes chrysolophus*) and gentoo (*Pygoscelis papua*) penguins. *Gen. Comp. Endocrinol.* 85, 230–240.
- Wingfield, J.C., Ball, G.F., Dufty, A.M., Hegner, R.E., Ramenofsky, M., 1987. Testosterone and aggression in birds. *Am. Sci.* 75, 602–608.
- Wingfield, J.C., Hegner, R.E., Dufty Jr., A.M., Ball, G.F., 1990. The “challenge hypothesis”: theoretical implications for patterns of testosterone secretion, mating systems, and breeding strategies. *Am. Nat.* 136, 829–946.
- Wingfield, J.C., Hegner, R.E., Lewis, D., 1991. Circulating levels of luteinizing hormone and steroid hormones in relation to social status in the cooperatively breeding white-browed sparrow weaver, *Plocepasser mahali*. *J. Zool. Lond.* 225, 43–58.
- Wingfield, J.C., Lewis, D.M., 1993. Hormonal and behavioural responses to stimulated territorial aggression in the cooperatively breeding white-browed sparrow weaver, *Plocepasser mahali*. *Anim. Behav.* 45, 1–11.
- Wingfield, J.C., Ramos-Fernandez, G., Nuñez-de la Mora, A., Drummond, H., 1999. The effects of an “El Niño” southern oscillation event on reproduction in male and female blue-footed boobies, *Sula nebouxi*. *Gen. Comp. Endocrinol.* 114, 163–172.
- Zuk, M., Johnsen, T.S., Maclarty, T., 1995. Endocrine-immune interactions, ornaments and mate choice in red junglefowl. *Proc. R. Soc. Lond. B* 260, 205–210.