

# Reproductive Endocrinology of Tropical Seabirds: Sex-Specific Patterns in LH, Steroids, and Prolactin Secretion in Relation to Parental Care

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Plasma levels of luteinizing hormone, prolactin, testosterone, and progesterone were measured throughout breeding in masked boobies, red-footed boobies, and red-tailed tropicbirds at Europa and Tromelin Islands (Indian Ocean). LH secretion showed a dampened pattern in the three species, particularly in tropicbirds. Such specific differences may be related to the less elaborate courtship displays in tropicbirds. Testosterone levels were very low throughout breeding in all three species, particularly in boobies. Low testosterone values in boobies may be related to their year-round attendance at the colony. Prolactin secretion increased from the prelaying period until the incubation and brooding periods and declined thereafter in boobies but stayed relatively unchanged throughout the breeding cycle in tropicbirds. The relatively constant prolactin secretion in the more pelagic tropicbirds might allow them to undertake parental care despite long absences at sea. Boobies perform postfledging care with basal prolactin levels. For all species, females always have higher prolactin levels than males. This hormonal dimorphism, being more pronounced in boobies, may be associated with differences in parental care between mates. © 2000 Academic Press

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For seabirds breeding at high latitudes, where food is abundant for a short season, the annual cycle typically follows a precise schedule, and breeding is highly synchronized. On the other hand, in tropical waters, seasonal fluctuations in the food supply are often slight and prey availability is fairly uniform for most of the year. Consequently, tropical seabirds show a wide range of breeding regimes to fit with local conditions, resulting in poorly synchronized breeding (Ashmole, 1971). Such differences between high latitude and tropical seabirds raises the question of the physiological, regulatory processes underlying breeding.

Only one study has been conducted so far on the reproductive endocrinology of tropical seabirds (Wingfield *et al.*, 1999). Studies conducted on tropical terrestrial birds during breeding have shown a dampened pattern of LH secretion, regardless of whether species are seasonal, opportunistic, or aseasonal breeders (Degen *et al.*, 1994; Levin and Wingfield, 1992). Do tropical seabirds share the same dampened pattern of LH secretion? With regard to testosterone, do tropical seabirds show the same low testosterone values found in many terrestrial tropical species (Dittami and Gwinner, 1990; Wingfield *et al.*, 1991; Levin and Wingfield, 1992)? Some tropical seabird species attend the breeding colony all year round, even when not engaged in breeding activities (Nelson, 1978). It is of interest to investigate if, as in terrestrial tropical birds, the changes in testosterone

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levels within a breeding cycle are small or even absent in species that hold year-round territories (Dittami and Gwinner, 1985; Dittami, 1987; Wingfield *et al.*, 1990).

A second point concerns the hormonal basis of parental care in tropical seabirds. Seabirds breeding at high latitudes have a chick rearing period which coincides with the maximum local food availability, allowing rapid fattening of the chick, before the arrival of winter. In tropical areas, the weak productivity of "blue waters" has led to low chick growth rates and thus a protracted chick rearing period. Instead of fueling their chick with vast amounts of fat, numerous species have evolved some degree of postfledging care, sometimes for months (Nelson, 1981; Burger, 1980). Tropical seabirds offer an opportunity for the study of the relationship between hormone levels and the maintenance of parental behavior over a long period and particularly the hormonal basis of the postfledging care.

Prolactin secretion in males and females is broadly correlated with their respective contribution to parental duties throughout the breeding cycle (Ball, 1991; Oring *et al.*, 1988). In most cases, the high-latitude seabirds studied hitherto are consistent with this finding since, when they share roughly equal parental duties, they showed similar prolactin levels during breeding (gentoo penguin *Pygoscelis papua*, Williams and Sharp, 1993; black-browed and gray-headed albatrosses *Diomedea melanophris* and *D. chrysostoma*, Hector and Goldsmith, 1985; king penguin *Aptenodytes patagonicus*, Garcia *et al.*, 1996). However, studies on macaroni penguins *Eudyptes chrysolophus* (Williams and Sharp, 1993), wandering albatross *Diomedea exulans* (Hector *et al.*, 1986b; Weimerskirch, 1995), and emperor penguins *Aptenodytes forsteri* (Lormée *et al.*, 1999) present conflicting results, because prolactin levels in each sex did not exactly reflect their respective investment in parental duties. Thus, there is as yet no clear understanding of how diversity in seabird parental care strategies is associated with underlying physiological mechanisms.

Tropical seabirds and especially pelecyaniformes exhibit a large range in sexual size dimorphism (Nelson, 1978, 1983), from monomorphic (tropicbird) to marked reversed sexual dimorphism (females being larger than males: boobies and frigatebirds). This sexual dimorphism is associated with differences in foraging behavior and nest attendance during breeding (Nel-

son, 1978; Gilardi, 1992; Anderson and Ricklefs, 1992; Gilardi, 1994). Does such a difference in size dimorphism and breeding ecology lead to differences in prolactin levels between sexes?

The present paper reports changes in plasma levels of LH, testosterone, progesterone, and prolactin throughout the breeding cycle in male and female masked booby, *Sula dactylatra*; red-footed booby, *Sula sula*; and red-tailed tropicbird, *Phaeton rubricauda*. The prediction was that such tropical seabirds with a very protracted breeding season should exhibit an attenuated pattern of secretion of LH and steroids in comparison with high-latitude breeding species. The study examines prolactin secretory variations throughout the different breeding stages (prelaying, incubation, brooding, shading, rearing, and postfledging care). Finally the study addresses three species ranging from monomorphic (red-tailed tropicbird) to highly dimorphic (masked and red-footed boobies). The fact that three closely related species are compared tends to reduce phylogenetic considerations inherent in many other comparative studies. As sexual size dimorphism has implications for parental behavior, particularly foraging behavior, it should be expected that in the more dimorphic booby species compared with the monomorphic tropicbird, there would be more accentuated sexual differences in hormones underlying parental behavior.

## MATERIALS AND METHODS

### *Study Area and Birds*

The study was carried out on Europa Island (22°20' S, 40°22' E) and Tromelin Island (15°33' S, 54°31' E), in the Western Indian Ocean. Europa Island is located in the Mozambic Channel whereas Tromelin Island lies northwest of Madagascar. The study was conducted during one stay on Tromelin Island, from mid-November to mid-December 1997, and three stays on Europa Island from April 1997 to October 1998. The breeding population of masked boobies from Tromelin Island (200–250 pairs) exhibits a loosely seasonal breeding pattern (Fig. 1), most eggs being laid between June and November (Le Corre, 1996). Masked boobies lay two eggs on the ground which are incubated for approximately 45 days but only one chick is fledged. In

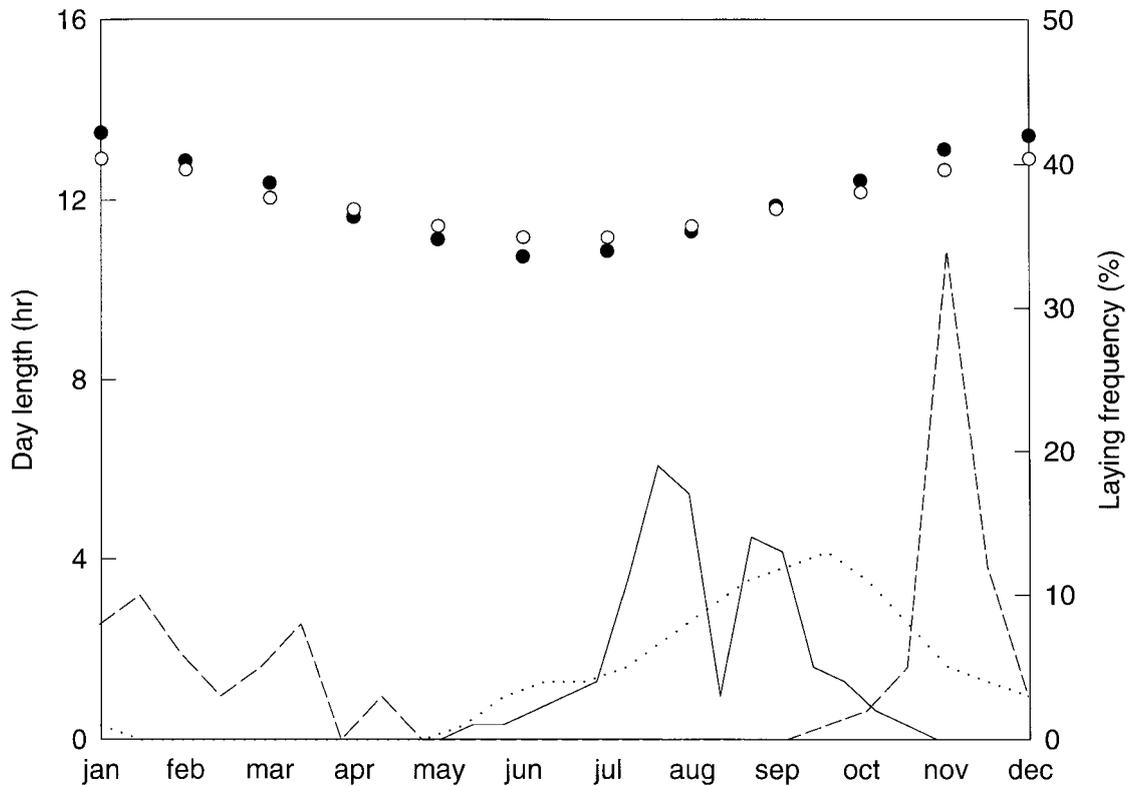


FIG. 1. Annual variation in day length on Europa Island (black dot) and Tromelin Island (white dot). Laying frequencies of masked boobies (dotted line), red-footed boobies (solid line), and red-tailed tropicbirds (broken line) are given below (red-footed booby and tropicbird, after Le Corre unpublished data; masked booby, after Le Corre, 1996).

this species the chick is fed for 120 days but postfledging care can extend the chick rearing period to 156 days (Nelson, 1978). Large colonies of red-footed boobies and red-tailed tropicbirds breed on Europa Island (respectively both 3000 to 4000 pairs); red-footed boobies breed during the austral winter from July to December, whereas tropicbirds breed in summer, from November to April (Fig. 1; see Le Corre and Jouventin, 1997). Red-footed boobies nest in trees while tropicbirds nest on the ground and both species lay a single egg which is incubated for 45 days. Chicks fledge at approximately 100 and 85 days, respectively. Red-footed boobies also perform postfledging care for about 1 month. Adult tropicbirds do not perform postfledging care (Nelson, 1978; Fleet, 1974; M. Le Corre, unpublished data).

### Blood Sampling

Birds were captured on the nest by hand or with a landing net and ringed with a metal ring (masked

booby,  $n = 77$ ; red-footed booby,  $n = 152$ ; red-tailed tropicbird,  $n = 116$ ). Within each pair, one adult was dyed with picric acid marks on crown and belly, allowing further identification from distance and thereby reducing disturbance at the nest. Blood samples were collected from birds throughout the breeding period which was divided into seven reproductive stages: (1) the prelaying stage, which includes the copulation and nest building periods; (2) the first half of the incubation; (3) second half of the incubation (for masked boobies, these two breeding stages were pooled together because of the small sample sizes); (4) brooding of the non-thermally independent chick; (5) shading of the nearly homeothermic chick; (6) rearing of the large, thermally independent chick until it fledges (before the first flight); and (7) the postfledging care period (feeding of the flying chick). In this later stage, adults were sampled just after they fed the chick. Birds were sampled only once in a given reproductive stage. Because of the loosely seasonal breeding pattern of the

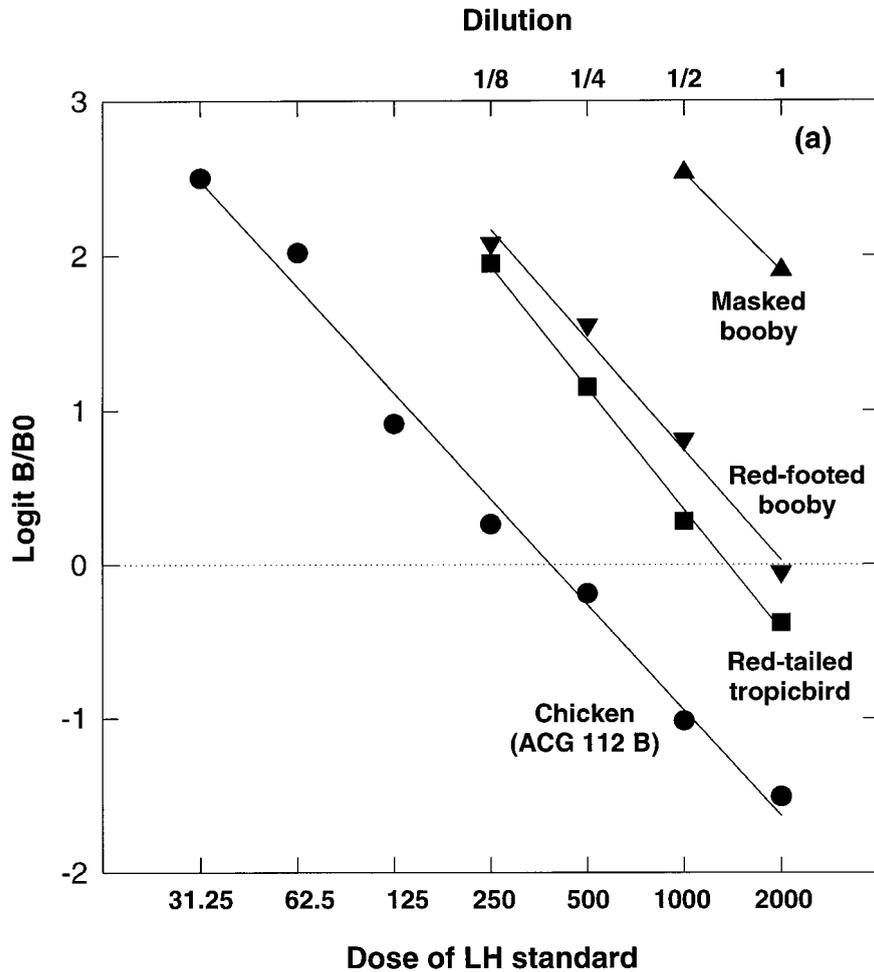


FIG. 2. Dose-response curves for LH (a) and prolactin (b) of chicken, masked booby, red-footed booby, and red-tailed tropicbird plasma. LH and prolactin standards are expressed in pg/tube.

species studied, all breeding stages could be sampled at roughly the same time. Blood samples were taken between 0730 and 1730 h, by collecting a maximum of 2 ml of blood (range 0.3–2 ml) from the tibial vein into heparinized tubes. Samples were put on ice and centrifuged as soon as possible; afterward plasma (for assays) and red cells (for sexing) were stored at  $-20^{\circ}\text{C}$ .

### Sexing

Boobies were sexed using voice (notes are higher pitched and more metallic in males, Nelson, 1978; H. Lormée *et al.*, unpublished data) and bill coloration (masked booby, Nelson, 1978). Additionally some birds were sexed using behavioral observation (laying, copu-

lation). Since tropicbirds do not show any obvious sexual dimorphism, the birds were sexed using a molecular method (Fridolfsson and Ellegren, 1999). The results were first validated on birds of known sex, using behavioral observations during copulation (tropicbirds, two females and one male; red-footed booby, one male and one female). To perform molecular sexing, DNA was prepared from red cells: DNA extraction was performed by adding 500  $\mu\text{l}$  PLB to 5  $\mu\text{l}$  of blood. After centrifugation, the pellet was again washed in 1 ml PLB. After removing the supernatant, the pellet was incubated for 1 h at  $58^{\circ}\text{C}$  with proteinase K (0.01% in PEB buffer). PCR reactions were performed in 20- $\mu\text{l}$  vol on a Perkin-Elmer 9600 thermal cycler using 0.5 U AmpliTaq polymerase, 200  $\mu\text{M}$  dNTPs, 10 mM Tris-

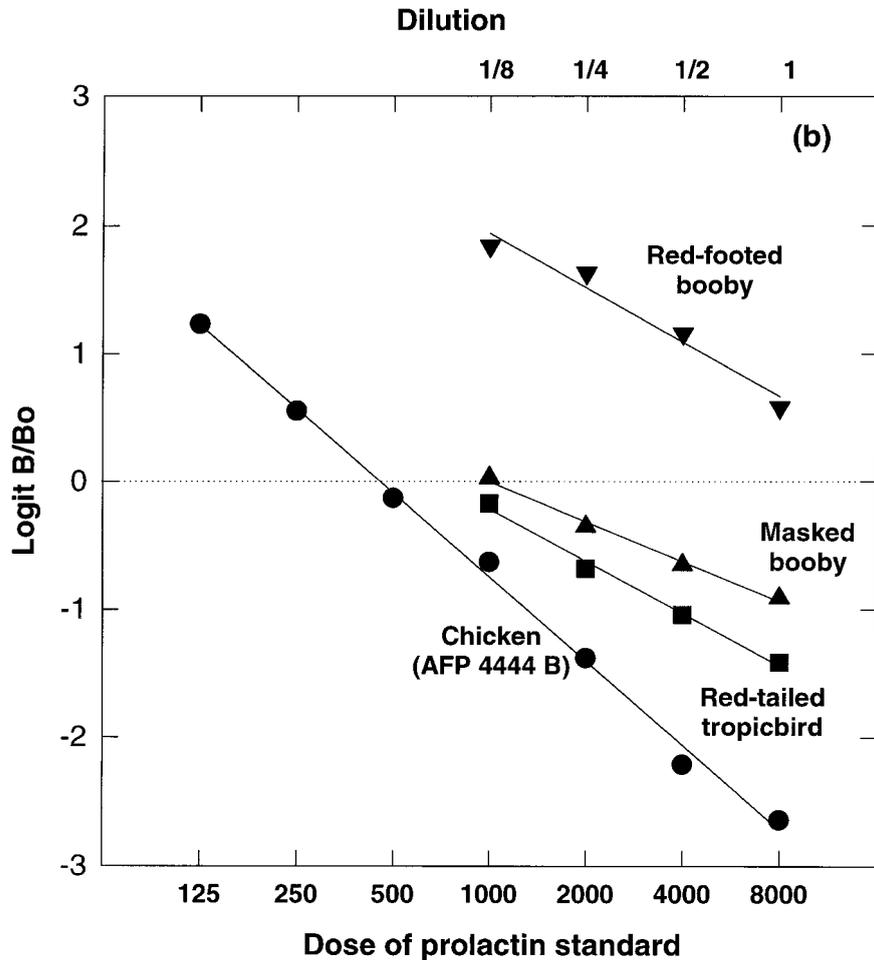


FIG. 2 Continued

HCl, pH 8.3, 50 mM KCl, 2 mM MgCl<sub>2</sub>, and 2 pmol of primers 2550F (5'-GTTACTGATTCGTCTACGAGA-3') and 2718R (5'-ATTGAAATGATCCAGTGCTTG-3'). The thermal profile comprised an initial denaturing step of 94°C for 4 min. Then 25–35 additional cycles were ran at an annealing temperature of 50°C. Denaturation was at 94°C for 30 s, annealing was for 30 s, and extension was at 72°C for 40 s. A final extension step of 5 min was added after the last cycle. PCR products were separated in 1% agarose gels, run in standard TBE buffer for 1 h, and visualized by ethidium bromide staining.

### Hormone Assays

Plasma concentrations of prolactin, LH, testosterone, and progesterone were determined by radioimmu-

noassays at the CEBC (Cherel *et al.*, 1994; Mauget *et al.*, 1994). For LH and prolactin (sources: LH, Professor Ishii and Professor Wakabayashi, Wadesa University, Japan; prolactin, Dr. Parlow, NHPP Harbor-UCLA Medical Center, Los Angeles, CA), pooled plasma samples of each species produced dose-response curves that paralleled the chicken LH and prolactin standard curves (Figs. 2a and 2b), their slopes being not significantly different (LH, chicken 2.39, red-footed booby 2.37, masked booby 2.09, red-tailed tropicbird 2.61; prolactin, chicken 2.1, red-footed booby 1.41, masked booby 1.19, red-tailed tropicbird 1.48). The intraassay coefficients of variation for LH and prolactin were 9.7 and 7.8%, respectively. The interassay coefficient of variation for LH ( $n = 3$ ) was 16.5%. Only two assays were performed for prolactin, with a variation of 8.3%. The lowest detectable quantities for testosterone and

progesterone, which were significantly different from zero at a 90% confidence level, were 3.9 and 7.8 pg/tube, respectively. This corresponds to 0.039 and 0.078 ng/ml.

### Statistical Analysis

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. For LH and prolactin, a two-way ANOVA was used to study the effects of sex and breeding stages on hormone values, followed by post hoc Tukey test. For LH we pooled values of the rearing and postfledging stage to increase the sample size and make a stronger analysis. Despite  $\log_{10}$  transformation, prolactin data sets in red-footed boobies and LH in masked boobies and red-tailed tropicbirds were not normally distributed. Such a nonnormal distribution in hormonal values is commonly encountered when describing reproductive hormone patterns. However,  $F$  test is remarkably robust to deviations from normality (see Lindman, 1974). Furthermore, the effects detected in the two-way ANOVA were also confirmed using non-parametric tests.

## RESULTS

### LH Changes Throughout the Breeding Cycle

Plasmatic LH varied significantly during the breeding cycle in both sulids species (masked,  $F = 13.88$ ,  $P < 0.001$ ; red-footed,  $F = 8.007$ ,  $P < 0.001$ ; Figs. 3a and 4a), being the highest during prelaying and decreasing significantly when birds began incubation. The decline was more pronounced in red-footed boobies (threefold decrease) than in masked boobies (around twofold). From this period until the end of the breeding cycle, LH values remained low. Adults performing postfledging care displayed low LH values similar to those found when rearing a large, thermally independent chick fledge. In contrast, plasma LH in tropicbirds were relatively unchanged throughout breeding ( $F = 3.27$ ,  $P = 0.075$ ; Fig. 5a) and plasma levels of LH encountered during the parental phase

(incubation and chick rearing) were very similar to those during the prelaying period.

### Comparison of LH Levels between Sexes

Male masked boobies had slightly higher LH values than females during the breeding cycle ( $F = 4.84$ ,  $P = 0.033$ ; Fig. 3a). There was a similar pattern in red-footed boobies but the difference was not statistically significant (Fig. 4a). In both boobies, males and females performing postfledging care also had similar LH values. Females tropicbirds tended to show higher LH plasma titers than males at every breeding stage ( $P = 0.075$ ), particularly during the brooding stage (Fig. 5a).

### Steroid Changes throughout the Breeding Cycle

In both boobies, most testosterone values were below the level of detection except during the prelaying period (Figs. 3b and 4b). In tropicbirds, the highest testosterone values were found during the prelaying period. From this period onward, testosterone levels progressively decreased until the chick rearing period (Fig. 5b). In contrast to boobies, testosterone levels remained detectable during the incubation and brooding periods.

Progesterone secretion did not show any clear pattern in the two sulid species and were close to undetectability throughout most of the breeding cycle (Figs. 3c and 4c). In tropicbirds (Fig. 5c), progesterone titers were highest during the prelaying and the first half of incubation periods and returned to basal levels during the second half of the incubation period.

### Comparison of Steroid Levels between Sexes

There were no significant sexual differences in the three species, whatever the breeding stage, excepted for testosterone in both booby species (Figs. 3b and 4b), in which males tended to show higher testosterone titers than females during the prelaying period.

### Prolactin Changes throughout the Breeding Cycle

Plasma levels of prolactin varied significantly over the different phases of the breeding cycle (masked

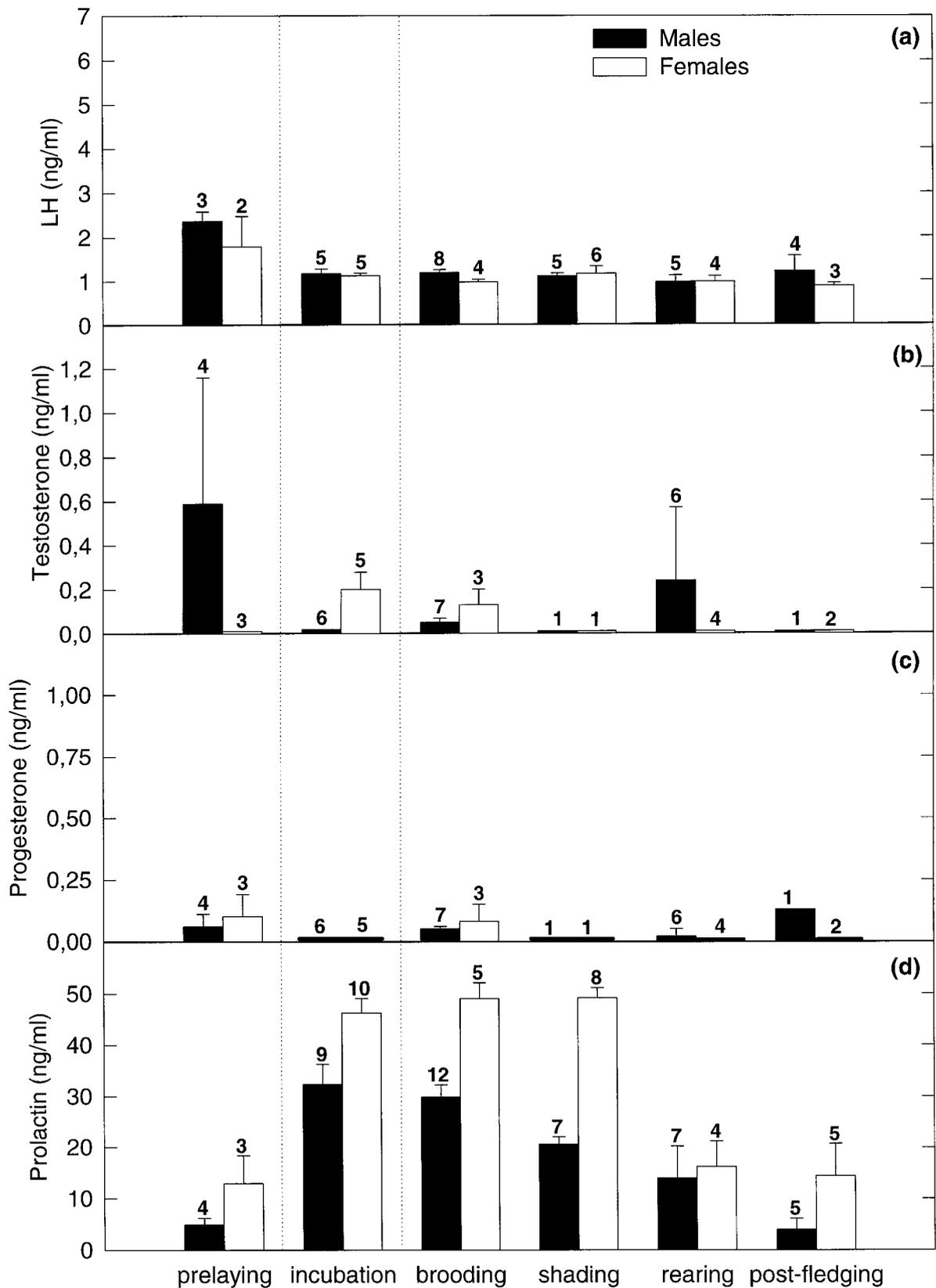


FIG. 3. Variation throughout the breeding cycle in mean plasma levels ( $\pm$ SE) in masked booby for (a) LH, (b) testosterone, (c) progesterone, and (d) prolactin. Values are presented as means  $\pm$  SE. Samples sizes are given above the bars.

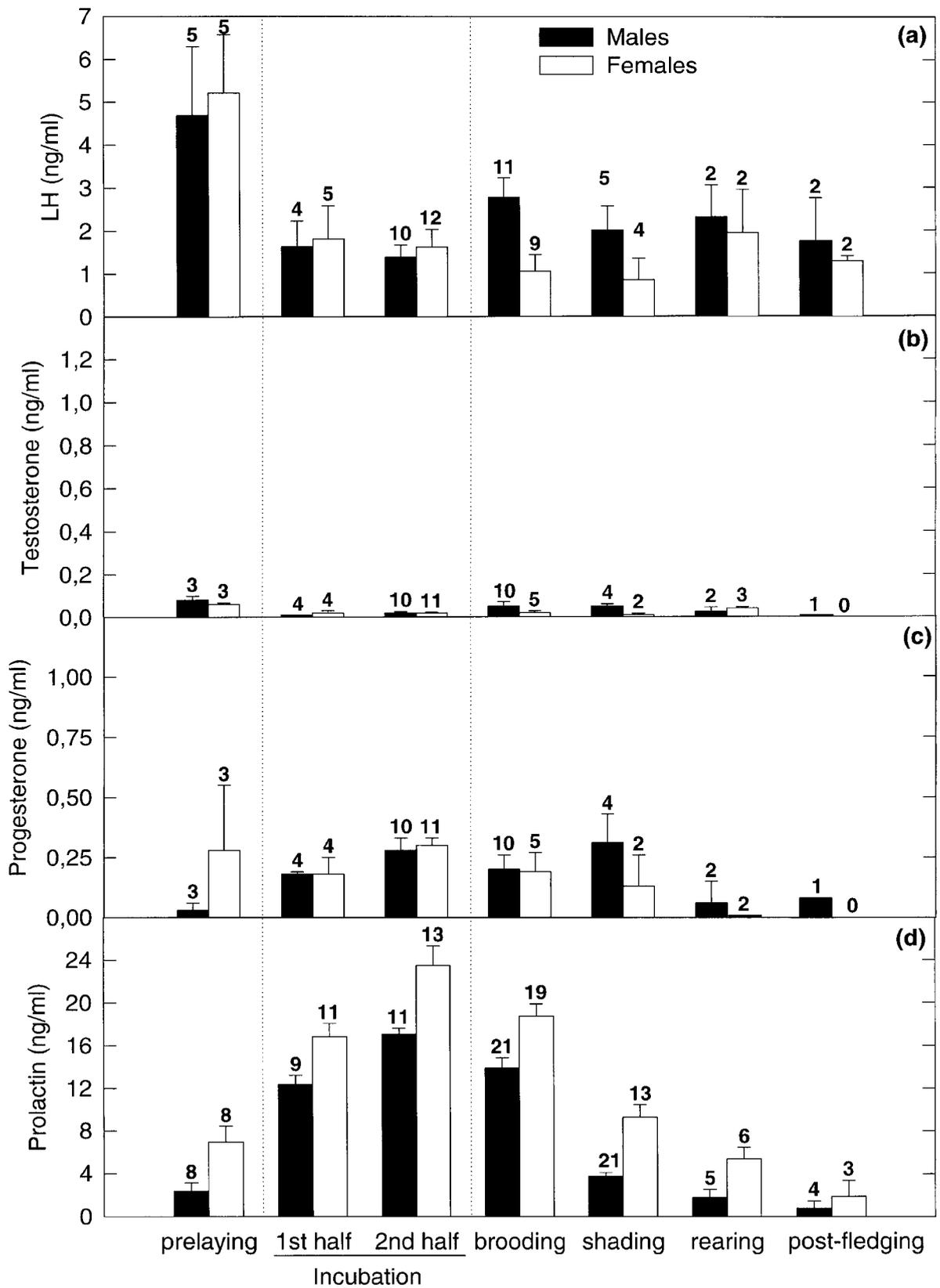


FIG. 4. Variation throughout the breeding cycle in mean plasma levels ( $\pm$ SE) in red-footed booby for (a) LH, (b) testosterone, (c) progesterone, and (d) prolactin. Values are presented as means  $\pm$  SE. Samples sizes are given above the bars.

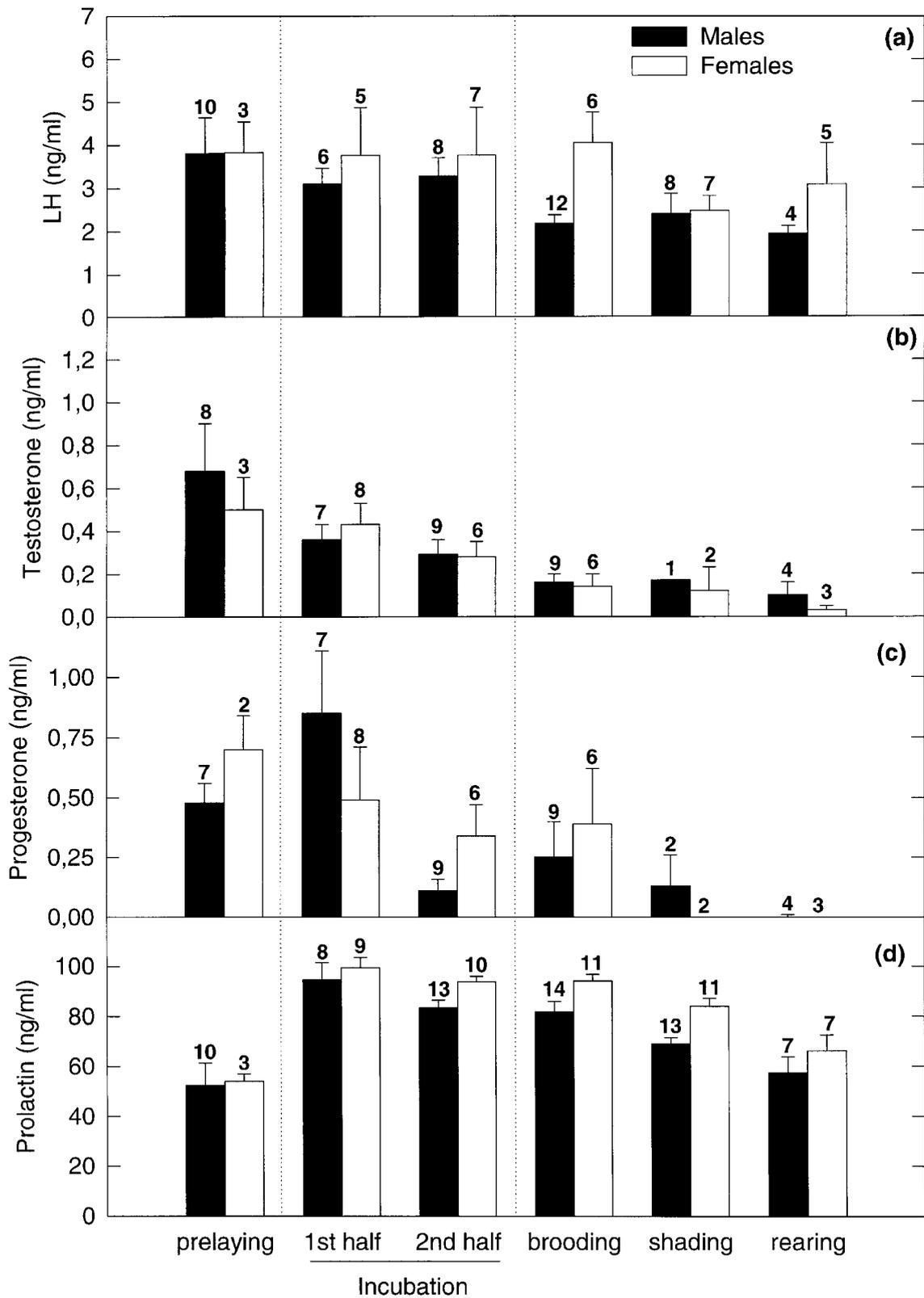


FIG. 5. Variation throughout the breeding cycle in mean plasma levels ( $\pm$ SE) in red-tailed tropicbird for (a) LH, (b) testosterone, (c) progesterone, and (d) prolactin. Values are presented as means  $\pm$  SE. Samples sizes are given above the bars.

booby,  $F = 35.19$ ,  $P < 0.001$ ; red-footed booby,  $F = 65.42$ ,  $P < 0.001$ ; tropicbird,  $F = 18.19$ ,  $P < 0.001$ ). However, such variations were less pronounced in tropicbirds (Fig. 5d). In boobies, prolactin titers increased from the prelaying stage until the end of incubation ( $P < 0.001$ ) and were maintained over the brooding period. Thereafter prolactin secretion declined from the end of the brooding period until the end of the rearing period (Figs. 3d and 4d). In both sulids, birds performing postfledging care had lower prolactin values than when rearing the large, thermally emancipated chick (masked boobies,  $F = 20.37$ ,  $P < 0.001$ ; red-footed boobies,  $F = 6.30$ ,  $P = 0.022$ ). In tropicbirds, prolactin values increased from the prelaying stage to the first half of incubation ( $P = 0.001$ ) and were unchanged until the end of the brooding period. Thereafter prolactin secretion declined slightly until the end of the chick rearing period (Fig. 5d).

### Comparison of Prolactin Levels between Sexes

Females always had higher prolactin levels than males in all the species (masked booby,  $F = 50.84$ ,  $P < 0.001$ ; red-footed booby,  $F = 34.86$ ,  $P < 0.001$ ; tropicbird,  $F = 18.69$ ,  $P = 0.004$ ). This hormonal dimorphism persisted throughout the whole breeding cycle in red-footed boobies (Fig. 4d). In masked boobies (Fig. 3d), there were greater levels in females from the incubation period to the shading period, except during the chick rearing period ( $P = 0.26$ ). From hatching until the shading period, prolactin secretion declined in males ( $P = 0.053$ ) but not in females. When performing postfledging care, female masked boobies had higher prolactin levels than males ( $F = 9.73$ ,  $P = 0.017$ ). There was no such difference in red-footed boobies. Sexual differences in prolactin levels were significantly maintained in tropicbirds only from the second half of incubation to the shading period (Fig. 5d).

## DISCUSSION

### LH Secretion and Breeding Initiation

As in high-latitude seabirds, tropical seabirds show typical high LH values during the sexual phase of the breeding cycle, that is, nest building and copulation. In

red-footed boobies, most of the birds engage in prelaying activities as photoperiod decreases, whereas most of the tropicbirds start breeding as day length increases. Thus it is possible that LH secretion is associated with a decreasing (red-footed booby) or increasing (tropicbird) photoperiod. However, in these two species, breeding pairs can be found totally outside their respective season. Furthermore, in masked boobies, breeding occurs during both increasing and decreasing day length. It may be that the variation in photoperiod is not a primary factor in the initiation of LH secretion, although we cannot preclude that LH levels might differ between early and late breeders. Indeed, at Europa Island, breeding of red-footed boobies is known to rely on peculiar sea-surface temperatures and the occurrence of tuna schools (M. Le Corre, unpublished data).

LH secretion in the three tropical seabirds studied within the breeding cycle was relatively low (red-footed booby, increase of about three- to fivefold from minimum to maximum levels; masked booby, about twofold; tropicbird, less than twofold). This is in contrast with high-latitude breeders like penguins in which plasma LH values are five- to sevenfold higher during the sexual phase of the breeding cycle than during the rest of the breeding cycle (Groscolas *et al.*, 1986; Williams, 1992; Mauget *et al.*, 1994), eightfold in herring gull *Larus argentatus* (Scanes *et al.*, 1974), fivefold in black-browed and gray-headed albatrosses (Hector *et al.*, 1986a), and three- to fourfold in Cape cormorant *Phalacrocorax capensis* (but with large interindividual variations; Berry *et al.*, 1979). Lower variations are the exception in some high-latitude breeding seabirds (around twofold in male wandering albatrosses, Hector *et al.*, 1986b; about twofold in Magellanic penguin, Fowler *et al.*, 1994). The attenuated LH variation in tropical seabirds may be a consequence of their relatively unseasonal breeding, as in ostriches, *Struthio camelus*, breeding at low latitudes (Degen *et al.*, 1994).

### LH and Breeding Behavior

LH secretion peaks during courtship and nest-building periods (Sharp *et al.*, 1998; Ball, 1991). In the present study, tropicbirds exhibited similar LH values from prelaying until the end of incubation. The LH decline after the prelaying period is much more marked

in the two boobies. Such a difference may be the result that, in contrast to boobies, tropicbirds do not build a nest and do not perform extensive and elaborate courtship displays (Nelson, 1983).

### **Testosterone and Breeding Behavior**

Average testosterone levels were low and often close to being undetectable in the two boobies and to a lesser extent in tropicbirds. Such a finding agrees with previous studies on tropical terrestrial species (Dittami and Gwinner, 1990; Levin and Wingfield, 1992) and results found in blue-footed boobies (Wingfield *et al.*, 1999), which showed that, as a rule, tropical birds have lower testosterone titers during breeding than temperate species. Nevertheless, the highest testosterone and LH titers within the breeding cycle were found at the same period (prelaying period) which concurs with the general pattern found in temperate species (Ball, 1991).

Testosterone values during the prelaying period were higher in tropicbirds than in the two sulid species. Furthermore, in both boobies, testosterone titers were virtually undetectable after the prelaying period. In contrast, in tropicbirds, testosterone progressively decreased from the prelaying to the rearing period, but with plasma levels staying detectable during the incubation and brooding periods. The results on boobies are consistent with data obtained on terrestrial tropical species holding year-round territories and showing few or no obvious changes in testosterone secretion (Dittami and Gwinner, 1985; Dittami, 1987; Levin and Wingfield, 1992; see also the "Challenge Hypothesis" in Wingfield *et al.*, 1990). Indeed, contrary to tropicbirds which only hold a territory for 6 months, masked and red-footed boobies attend the colony all year round, even outside the reproductive season (Nelson, 1978). Blue-footed boobies, which stay in the vicinity of the breeding colonies all the year long, also display testosterone values which decline to basal levels from the egg laying phase to the parental phase (Wingfield *et al.*, 1999).

Within the three species studied, female plasma testosterone levels are often similar to those of males and vary in a similar way. Elevated testosterone concentrations have been reported in female Western gull, *Larus occidentalis*, which shares the defense of the territory with the male (Wingfield *et al.*, 1982), as also

in females gray-headed and black-browed albatrosses (Hector *et al.*, 1986a). Hall (1986) reported that testosterone levels were similar in male and female Cape gannet, *Sula capensis*, before laying. Wingfield *et al.* (1999) also found similar testosterone values in male and female blue-footed boobies. For both boobies and tropicbirds, the male is more responsible for the establishment of the territory, occupying the nest site first, and, in the case of boobies, often bringing materials to the female which constructs the nest (Nelson, 1978; Fleet, 1974). By the time the egg is laid, both mates defend the nest against intruders or conspecifics; it is thus not surprising that females and males have relatively similar testosterone levels during this period. In monogamous birds, it has been suggested that there was a positive relationship between the absolute dimorphism index (mean of the sum scores from body size, plumage, and territorial aggression) and the ratio of circulating testosterone in males versus females (Wingfield, 1994). Data obtained from the less dimorphic species, the red-tailed tropicbird, fit such a relationship since testosterone ratio, during the prelaying, is close to 1. However, when considering data from red-footed, masked (this study), and blue-footed boobies (Wingfield *et al.*, 1999), the relationship becomes less evident. In boobies, females are larger than males (around 20%), blue-footed boobies being the most dimorphic (Nelson, 1979). However, testosterone ratio during the prelaying period is the highest in masked, the lowest in red-footed, and intermediate in blue-footed boobies.

### **Progesterone and Breeding Behavior**

In both boobies, progesterone titers were close to undetectability throughout breeding and show no obvious secretory patterns. The sustained elevation of progesterone in the first part of incubation in red-tailed tropicbirds may suggest a role for progesterone in association with prolactin, in the transition from sexual to parental behavior (Silver, 1990).

### **Prolactin Secretion and Parental Care**

In all three species, there were elevated prolactin titers during incubation and the brooding period. Among seabirds, elevated prolactin values are typically found during the incubation and when brooding

the non-thermally independent chick (Hector and Goldsmith, 1985; Hall, 1986; Williams, 1992; Garcia *et al.*, 1996; Lormée *et al.*, 1999). Prolactin titers decline after brooding in the two boobies and adults rearing a large, thermally independent chick have prolactin values that are similar or lower to those during the prelaying period. Such dissociation between parental care and prolactin secretion has been reported for several seabird species (Williams and Sharp, 1993; Hector and Goldsmith, 1985). However, in the red-tailed tropicbird, prolactin secretion stays relatively unchanged from incubation to the end of the rearing period. In this latter species, since the brooding period is rather short, a marked decline of the prolactin secretion would be expected when the chick is thermally independent. It seems therefore that prolactin secretion does not depend exclusively on the type of parental care performed. An alternative way to investigate the relationship between the pattern of prolactin secretion and the parental behavior in seabirds could be to consider how pelagic these species are in their foraging behavior. Recent studies on pelagic seabirds such as king and emperor penguins have shown that prolactin secretion was maintained relatively unchanged throughout the breeding cycle (Cherel *et al.*, 1994; Jouventin and Mauget, 1996; Garcia *et al.*, 1996; Lormée *et al.*, 1999). The authors suggested that "a prolactin secretion, independent of external stimuli, might have evolved in pelagic seabirds to maintain parental care despite long absences from the breeding colony." During the rearing of the large, thermally independent chick, red-footed boobies and masked boobies perform foraging trips rarely exceeding 24 h (Nelson, 1978; H. Lormée, unpublished data). In contrast to inshore foraging boobies, tropicbirds are oceanic foragers and spend up to 3 days at sea during the rearing of the thermally independent chick (Fleet, 1974). This study supports the idea that the relative constancy of prolactin secretion over the reproductive cycle in tropicbirds may have evolved to reconcile the maintenance of the parental care despite long absences at sea and therefore the prolonged absence of stimuli from eggs or chicks.

The present results show that postfledging care in red-footed and masked boobies does not rely on elevated prolactin levels. Parental care after fledging is performed as prolactin is still declining and even

lower than during the rearing of the thermally independent chick. It is probable that the vigorous begging behavior of the fledging chick at this time, that is, wing beating, head jobbing, and sometimes aggressively beating the adult's bill (Nelson, 1978), is sufficient to obtain food from parents despite very low prolactin levels.

### ***Sexual Dimorphism in Prolactin Secretion***

In the present study, females had higher prolactin levels than males during the breeding cycle in all three species. Interestingly, this hormonal dimorphism is particularly marked in the boobies which also show the most pronounced sexual size dimorphism (females being about 20% larger than males). It is of note that in the Cape gannet, where males and females show no obvious size dimorphism, prolactin levels are similar between sexes throughout breeding (Hall, 1986). However, in the monomorphic tropicbirds, prolactin titers are always slightly but constantly higher in females than in males. In the case of red-footed boobies, this hormonal sexual dimorphism is exhibited when breeding since nonbreeding males and females have similar prolactin values (H. Lormée, unpublished data). Does such a hormonal dimorphism correspond to differences in parental care performed by males and females (Ball, 1991; Oring *et al.*, 1988)? Some studies on tropical boobies have given insights into sexual differences in nest attendance and foraging behavior which support this hypothesis (longer stays at sea in female masked and brown boobies *Sula leucogaster*, Anderson and Ricklefs, 1992; Gilardi, 1992; and longer brooding spells in female blue-footed booby, *Sula nebouxii*, Guerra and Drummond, 1995). Furthermore, at Europa Island, females of red-tailed tropicbirds and red-footed boobies spend more time on the nest than males when brooding the chick (H. Lormée, unpublished data). Similarly, it has been suggested in emperor penguins that, due to longer absences at sea than males (up to 2 months), females might need higher prolactin levels to successfully achieve parental behavior (Lormée *et al.*, 1999). A similar relationship could arise in these tropical seabirds: as females tend to spend more time foraging at sea, they may need more sustained prolactin levels to maintain appropriate parental care. To confirm such a conclusion, more precise data are needed on the respective parental contribution of

red-footed boobies and tropicbirds during the post-hatching period.

In conclusion, the present study provides new data on the endocrinology of reproduction of tropical seabirds. LH and steroid secretions throughout the breeding cycle were relatively attenuated, and testosterone levels were lower in the species attending the colony all year round (boobies) than in more seasonal breeding species (tropicbirds). However, the role of proximal factors such as photoperiod and food availability, in a tropical marine environment, on the onset of LH secretion in seabirds must be investigated further. Because field work was concentrated into some months, we did not attempt to describe the seasonal variation of LH and steroids over the year. Thus, we cannot exclude that these hormonal levels in a given reproductive stage may differ between early and late breeders, due to a seasonal influence. For prolactin, results obtained on the most pelagic species, the red-tailed tropicbird, concur with the hypothesis that prolactin secretion has evolved in pelagic seabirds to maintain parental care despite long absences at sea (Garcia *et al.*, 1996; Lormée *et al.*, 1999). Finally the sexual dimorphism in the prolactin secretion paralleled the sexual size dimorphism and possibly sexual differences in the parental care performed. This offers large opportunities, in a group (Pelecaniformes) exhibiting a wide range of parental care strategies, to explore the relationship between hormones and parental care. Experimental investigations within dimorphic and monomorphic tropical seabirds species, using manipulations of the parental behavior and looking for the hormonal responses, could allow a better understanding of how hormones, particularly prolactin, affect parental behavior in seabirds.

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