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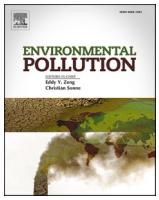

Seasonal changes in parental hormone levels and their association with mercury contamination in a tropical seabird

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ABSTRACT

Parental investment is a life-history trait whose expression is tightly regulated by endocrine mechanisms. Mercury (Hg) contamination poses a significant threat to wildlife physiology in tropical ecosystems, where naturally high concentrations have been exacerbated by anthropogenic activities. Although evidence remains limited, Hg may disrupt secretion of the pituitary hormone prolactin, a key regulator in the expression of avian parental care. In this study, we first evaluated whether prolactin secretion experienced a seasonal pattern across different stages of the breeding cycle in magnificent frigatebirds (*Fregata magnificens*) breeding in French Guiana. We then investigated whether Hg exposure was associated with prolactin secretion at any of these stages. Prolactin levels followed the expected seasonal pattern for altricial species, peaking during incubation, and declining sharply during chick-rearing in both sexes. Similarly low baseline prolactin levels were observed in displaying males and non-breeding adults. Prolactin levels did not differ between sexes. Despite high Hg concentrations in frigatebirds, Hg was not negatively associated with prolactin levels at any breeding stage. Our findings suggest that prolactin dynamics are closely linked to parental investment in breeding magnificent frigatebirds, but Hg contamination does not appear to impair prolactin at observed exposure levels. Further research is needed to determine whether Hg affects parental hormones in more sensitive altricial species, particularly during the critical, energetically demanding chick-rearing period.

1. Introduction

Parental care is regulated by endocrine mechanisms that allow vertebrates to adjust reproductive investment in response to environmental stressors and energetic constraints (Angelier and Chastel, 2009). Although multiple endocrine axes govern reproductive decisions and parental behaviour (Lynn, 2016), the pituitary hormone prolactin is considered essential for avian parental care (Smiley, 2019). This includes behaviour such as incubation (Lea et al., 1986), brooding, nestling provisioning, and even alloparenting (Buntin et al., 1991; Lormée et al., 1999; Smiley and Adkins-Regan, 2018). Hence, prolactin concentrations are regarded as a good endocrine proxy for parental investment and, together with stress hormones, help determine resource allocation between self-maintenance and parental care (Angelier and Chastel, 2009).

In birds, prolactin secretion follows a predictable seasonal trajectory, peaking during incubation, and declining from chick-rearing onwards. This decline is more abrupt in precocial species in which fledglings directly fend for themselves (e.g. Goldsmith and Williams, 1980). Conversely, prolactin levels remain elevated during chick-rearing in altricial birds, whose offspring require greater parental investment (Goldsmith, 1982). In predictable breeders with short, well-timed breeding seasons, endogenous prolactin cycles are likely driven by photoperiod (Dawson et al., 2001; Dawson and Goldsmith, 1982). However, in opportunistic breeders, such as many tropical species lacking a fixed breeding period, other environmental variables such as

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rainfall or food availability (Serag El Din et al., 2025; Vieira et al., 2025) may play a more prominent role. In both cases, prolactin secretion may be regulated through a negative feedback loop involving gonadotropin hormones. According to this mechanism, prolactin secretion would be stimulated by elevated circulating luteinizing hormone (LH) and other sex steroids that promote mating behaviour (Dawson and Goldsmith, 1982). Following pair formation and copulation, rising prolactin concentrations may inhibit gonadotropins, inducing gonadal regression and redirecting energy resources toward parental care (Dawson et al., 2001; Hall, 1986). Additionally, tactile and visual stimuli from nests, eggs, or chicks can trigger prolactin secretion (Lea et al., 1986). However, stimulus-driven mechanisms likely play a smaller role in pelagic seabirds, which spend extended periods foraging at sea and experience infrequent contact with eggs or chicks (Garcia et al., 1996; Lormée et al., 2000).

Environmental contaminants pose a significant threat to wildlife health (Kumar, 2023). When these compounds interfere with endocrine function, they are classified as endocrine disruptors (La Merrill et al., 2020). Mercury (Hg) is among the most pervasive contaminants of ecological concern owing to its endocrine-disrupting properties (Tan et al., 2009). Even though anthropogenic emissions of Hg have declined in developed countries since 1990 and in China since 2010, emissions continue to rise in many developing regions due to activities such as artisanal and small-scale gold mining (ASGM) in the tropical belt (Qiu et al., 2025). When inorganic Hg is converted to methylmercury through microbial processes, this toxic and bioavailable form of Hg can be assimilated and enters food webs (Chételat et al., 2020). As a result, Hg biomagnifies into upper trophic levels (Córdoba-Tovar et al., 2022; Lavoie et al., 2013), increasing top predators' susceptibility to toxicological and endocrine-disrupting effects (Scheuhammer et al., 2015; Whitney and Cristol, 2018).

Recent efforts have highlighted evidence of Hg effects on different avian hormones (Hernandez-Gonzalez et al., 2025), with prolactin identified as a potential target. This is consistent with known Hg neurotoxicity (Branco et al., 2021), given that prolactin is secreted by the anterior pituitary gland. However, evidence for an association between Hg exposure and prolactin levels in wild birds remains limited and inconsistent. Negative associations have been reported in some species (Smith et al., 2023; Tartu et al., 2016, 2015), whereas other studies found no detectable relationship (Blévin et al., 2020; Gilmour et al., 2019). Importantly, with one exception (Tartu et al., 2016), all investigations have focused on incubation, when prolactin reaches maximum levels. Yet chick-rearing may be equally or even more energetically demanding (Tulp et al., 2009), representing a susceptible stage when Hg disruption may affect parental commitment to chick provisioning (Buntin et al., 1991; Farrar et al., 2022).

Magnificent frigatebirds (*Fregata magnificens*) provide an ideal model for investigating seasonal prolactin dynamics in tropical birds. Because frigatebirds encounter relatively stable environmental conditions, this long-lived seabird can breed year-round and exhibits several peaks (or seasons) within the year (Diamond, 1973; Kushlan, 2009). Thus, prolactin cycles are unlikely to be regulated by photoperiod. Interestingly, reproductive hormones in frigatebirds follow patterns similar to those observed in temperate birds (i.e. pronounced LH and testosterone peaks during courtship and display, followed by strong declines during subsequent parental stages, Chastel et al., 2005). We therefore hypothesised that frigatebirds also exhibit a traditional seasonal pattern of prolactin secretion, with pronounced increases during incubation, consistent with the gonadotropin-inhibiting hypothesis (Dawson et al., 2001; Dawson and Goldsmith, 1982). Because frigatebirds provide bi-parental care, with both males and females incubating a single egg and feeding one altricial chick, prolactin levels are expected to remain elevated during chick-rearing. Intriguingly, magnificent frigatebirds represents the only frigatebird species with highly variable male commitment to chick-rearing (19-160 days) compared to females (9 to 12 months) (Osorno, 1999). Although reasons behind this behavioural variability remain unclear, physiological mechanisms may influence males' decisions to maintain investment in current reproduction or desert earlier to prioritize self-maintenance and/or even re-engage in mating with another partner (Osorno, 1999). Prolactin arises as a suitable biomarker to explain these differences because it can be modulated in response to stress to adjust parental investment depending on resource availability (Angelier and Chastel, 2009). We hypothesised that deserting males experience steeper declines in prolactin levels during chick-rearing than males that continue providing care (Chastel and Lormée, 2002).

As top predators in marine ecosystems, magnificent frigatebirds provide an excellent system to investigate endocrine-disrupting effects of Hg. Adult frigatebirds are exposed to high Hg concentrations (Sebastiano et al., 2016), and disruption of endocrine pathways has been suggested as an underlying mechanism explaining important conservation threats (Sebastiano et al., 2022). For instance, in the South American colony on Grand Connétable Island (French Guiana), it has been hypothesised that Hg-induced prolactin disruption in adults may result in suboptimal parental effort, increasing nestling susceptibility to infectious disease outbreaks (Sebastiano et al., 2022). Given potential dimorphism in prolactin strategies between sexes, it is crucial to understand whether males and females experience differential susceptibility to Hg due to mechanisms such as reduced Hg burden in females post egg-laying (Ackerman et al., 2020, 2016; Robinson et al., 2012).

The aims of this study were (i) to characterise seasonal variation in prolactin levels across major breeding stages (display, incubation, chick-rearing, and non-breeding) in magnificent frigatebirds and ii) to determine whether Hg contamination was associated with reduced prolactin levels in adult males and females at any of these stages. To address these questions, we used a cross-sectional dataset collected in 2002 and 2003. We expected prolactin levels to rise from display to incubation and remain elevated during chick-rearing to sustain the high parental care. We anticipated higher variability in prolactin levels amongst chick-rearing males compared to females, consistent with their variable commitment to chick provisioning. Finally, we hypothesised that Hg exposure would be negatively associated with prolactin levels, with the strongest relationship occurring during chick-rearing, when maintaining elevated prolactin levels is critical for sustaining parental investment but represents a trade-off against self-preservation due to the accumulated physiological toll of breeding. To further assess susceptibility to Hg during chick-rearing, we combined data collected in 2002-2003 with data from 20 frigatebird breeding pairs collected in 2022 during two different breeding peaks, in which both males and females from each pair were sampled. This additional approach aimed to account for potential correlations between prolactin levels of pair members, given that the magnitude of prolactin secretion can be related to the strength of parental bonds (Bebbington et al., 2024).

2. Materials and methods

Further details about fieldwork and laboratory analyses are provided in the Supplementary Materials.

2.1. Fieldwork and sample collection

Fieldwork was conducted in a magnificent frigatebird colony on Grand Connétable Island (4°49′30N; 51°56′00W). For the cross-sectional study on seasonal changes, we sampled birds following Chastel et al. (2005). Briefly, during two reproductive seasons (May 2002 and March 2003), we captured adult birds at 4 different reproductive stages: (1) displaying males exhibiting the red gular pouch, (2) males and females incubating an egg, (3) males and females rearing a 10–70 day-old chick and (4) non-breeding adults (still retaining some signs of immature plumage to make sure they were not successful breeders). In all instances, we captured only one bird per nest (i.e. males and females were always independent from each other). Given that frigatebirds do not show a synchronized breeding pattern, we found individuals at all

breeding stages during the same sampling period. Immediately after capture, we collected a 1 mL blood sample by venipuncture from the alar vein using a heparinized 25G needle. Blood samples were kept in a cooler with ice packs and centrifuged as soon as possible in the field to separate plasma from red blood cells (hereafter, RBCs). We stored plasma samples at −20 °C at Centre d'Etudes Biologiques de Chizé (CEBC) for prolactin assay, and RBCs at −20 °C at laboratory Littoral, Environnement et Sociétés (LIENSs) for Hg quantification. After blood sampling, we measured body mass (g) and culmen length (mm) of each bird to calculate a body condition index (following Peig and Green, 2009).

To further focus on chick-rearing and take advantage of two different breeding peaks occurring each year on Grand Connétable island, we captured 20 breeding pairs during June 2022 (n = 5 pairs) and November 2022 (n = 15 pairs). Adult birds were distracted using a green headlamp at night and captured directly at the nest by hand. Immediately after capture, we collected and processed 2 mL blood samples as previously described. We captured both male and female from each nest on different days. After blood collection, body mass (g) and skull length (mm) were recorded to calculate a body condition index (as described above, but using skull length instead of culmen length as the linear measurement to adjust body mass based on allometric scaling, Peig and Green, 2009)

2.2. Hg analyses

We freeze-dried and homogenized RBC samples to quantify total Hg concentration (hereafter Hg) using an Advanced Mercury Analyzer spectrophotometer (Altec AMA 254) as described in Bustamante et al. (2006). We analysed the samples in duplicate (1–2 mg each) and considered sample quantification valid when the relative standard deviation (RSD) between duplicate measurements was <10%. We used average values for further statistical analyses. We performed all Hg analyses following a rigorous quality control program, including quantification of the certified reference material lobster hepatopancreas TORT-3 (National Research Council of Canada, CNRC, certified value = 0.292 ± 0.022 μg/g dry weight, dw). Our results agreed with certified values (mean = 0.301 ± 0.003 μg/g dw), with an average recovery of 103.2 ± 1.2%. The detection limit of the AMA was 0.2 ng.

2.3. Prolactin analyses

We quantified plasma concentrations of prolactin by heterologous radioimmunoassay, following the modified protocol described in Cherel et al. (1994) and after validation for frigatebird samples. We measured all plasma samples in duplicate in a single assay, and levels are expressed relative to the chicken prolactin standard (AFP-4444B). The intra-assay coefficient of variation, calculated by measuring a bird control sample in 12 replicates distributed at the beginning and end of the assay, was 6.37%. The lowest detectable concentration was 0.62 ng/mL.

2.4. Statistical analyses

All statistical analyses were carried out in R version 4.4.0 (R Core Team, 2024).

i) Differences in Hg concentrations among breeding seasons

We first investigated whether Hg concentrations differed among breeding seasons and between sexes. To this end, we fitted a Generalised Linear Mixed Model (GLMM) with breeding season (four levels: May 2002, March 2003, June 2022, and November 2022), sex (two levels: males and females) and their interaction as explanatory variables, and Hg concentration as response variable, using *glmmTMB* from the *glmmTMB* package (Brooks et al., 2017). Given that for June 2022 and November 2022 both male and female from the same nest were sampled, we included pair ID as a random effect to account for potential similarities in Hg concentrations within pairs. Given the distribution of the response variable (positive, skewed and heteroscedastic), we fitted the model using a gamma distribution with a log link function, which better modelled the mean-variance relationship than a Gaussian distribution. Subsequently, we tested significance of explanatory variables using a Type III likelihood ratio test implemented in *Anova* from the *car* package (Fox et al., 2001), and we generated the estimated mean and standard deviation of Hg concentrations for each breeding season and sex using the *emmeans* package (Lenth, 2025). We finally carried out post-hoc pairwise comparisons (Tukey test correcting for multiple testing), by using *pairs* from the *emmeans* package (Lenth, 2025).

ii) Differences in Hg concentrations among breeding stages

We investigated whether Hg concentrations varied amongst breeding stages. To this end, we fitted a Generalised Linear Model (GLM) with breeding stage (four levels: display, incubation, chick-rearing, and non-breeding) and its interaction with breeding season (two levels: May 2002 and March 2003) as explanatory variables. Sex was also included to account for biological variation. To address this question, we excluded data from June 2022 and November 2022, for which only chick-rearing birds were sampled. We evaluated the significance of breeding stage by using a Type III likelihood ratio test and we generated post-hoc pairwise comparisons as described above.

iii) Prolactin levels in relation to sex and breeding stages

Given that females were not sampled during display, we fitted 3 GLMs: one for males, one for females, and one testing for sex-specific differences in prolactin levels among breeding stages. The main explanatory variable in both male and female models was breeding stage, which included four levels in males (display, incubation, chick-rearing, and non-breeding), and three levels in females (incubation, chick-rearing, and non-breeding). The overall model investigated the interaction between breeding stage (three levels: incubation, chick-rearing, and non-breeding) and sex. All models included three additional explanatory variables: (1) breeding season (two levels: May 2002 and March 2003), to account for potential inter-seasonal differences that could impact physiology such as annual differences in food availability; (2) hours since midnight (continuous covariate) to account for potential circadian fluctuations in prolactin secretion (Proudman, 1991), given that birds were randomly sampled throughout day and night; and (3) scaled mass index (SMI, continuous covariate) to account for the possibility that birds in poorer body condition might exhibit lower prolactin levels irrespective of reproductive stage. SMI was calculated following Peig and Green (2009), using culmen length as the linear measurement to standardise body mass while accounting for allometric scaling. All three models were fitted with *glm* (R Core Team, 2024) using a gamma distribution with a log link function, which provided the best fit for positively skewed, heteroscedastic prolactin data. We ran Type III likelihood ratio tests using the *Anova* function from the *car* package (Fox et al., 2001) to evaluate the significance of different explanatory variables. Subsequently, we carried out post-hoc Tukey tests with a multiple-testing correction, using *pairs* from the *emmeans* package (Lenth, 2025).

iv) Association between Hg concentrations and prolactin levels at different breeding stages

Given the strong seasonal variation in prolactin levels and the cross-sectional design of our study, we investigated correlational evidence for potential endocrine-disrupting effects of Hg separately for each breeding stage. To this end, we fitted four GLMs (one per breeding stage) following the approach described before, including Hg, sex, and their interaction, breeding season (two levels: May 2002 and March 2003),

hours since midnight, and SMI as explanatory variables. Hg*sex was included to test for differential susceptibility to Hg and/or differences in Hg burden between sexes (it was not included in the display model). Given that in frigatebirds, prolactin secretion can only be elicited following successful mating, we did not use calendar date as a predictor of prolactin levels. All models were fitted using *glm* (R Core Team, 2024) with a gamma distribution and a log link function, and model outputs were investigated using Type III likelihood ratio tests reported by *Anova* from the *car* package (Fox et al., 2001).

In the chick-rearing model, to account for potential physiological dependence between pair members, we first fitted a GLMM using 2022 data, with pair ID as a random effect (*lmer* function from *lme4* package, Bates et al., 2015). However, estimated random-effect variance (calculated with *VarCorr*) was effectively 0, suggesting overparameterization of the model (confirmed with *isSingular* function). Hence, we pooled all data and fitted the same GLM used for other breeding stages (four levels for breeding season: May 2002, March 2003, June 2022, and November 2022). For both 2022 breeding seasons, SMI was calculated using skull length instead of culmen length. More importantly, the chick-rearing model also included chick age at sampling as a continuous covariate to account for expected declines in prolactin levels from brooding (when adults were attending recently hatched nestlings) to later chick-rearing stages (attending older thermally independent chicks). However, after visually exploring data (Fig. 1S) and testing for collinearity amongst explanatory variables using variance inflation factors (*vif*), we removed breeding season because it was highly correlated (*vif* > 5) with chick age at sampling (i.e. most parents attending chicks younger than 20 days-old belonged to 2022 breeding seasons).

3. Results

i) Differences in Hg concentrations among breeding seasons

Hg concentrations differed significantly among breeding seasons (χ^2_3 = 58.6, p = 1.2 × 10^{-12}), and the association between sex and Hg concentrations depended on breeding season (χ^2_3 = 14.2, p = 2.6 × 10^{-3}, Fig. 1, Table 1S). In both sexes, Hg concentrations were highest in May 2002 and were generally lower during subsequent sampling periods. In females, Hg concentrations remained relatively stable among subsequent sampling periods, whereas in males they reached their lowest levels in November 2022. Sex differences were generally absent, except in November 2022, when females exhibited higher Hg concentrations than males (Fig. 1, Table 2S).

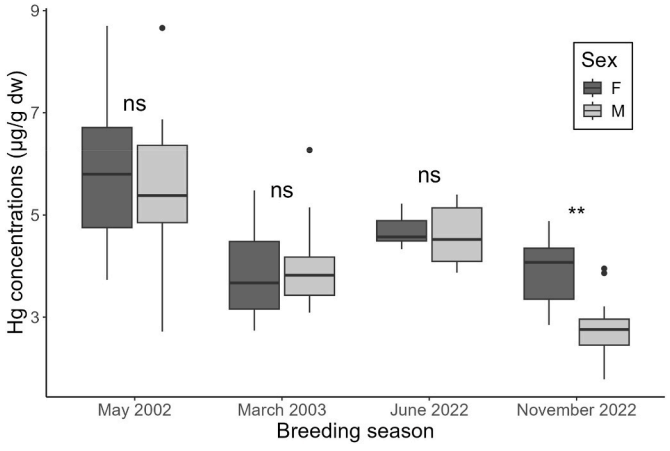

Fig. 1. Blood Hg concentrations (µg/g dw) in adult magnificent frigatebirds from Grand Connétable sampled in 4 different breeding seasons. Males (M) are represented in light grey and females (F) are represented in dark grey. Sample sizes for each breeding season are: May 2002: n = 25 males and 25 females; March 2003: n = 20 males and 14 females; June 2022: n = 5 males and 5 females; November 2022: n = 15 males and 15 females. Boxes represent the median and interquartile range (IQR), and whiskers represent the largest and smallest values within 1.5 × IQR. The significance of the pairwise comparisons between sexes within the same breeding season have been indicated over the boxes (ns, non-significant, **p < 0.001).

Table 1
Model outputs of the Generalised Linear Models (GLM) investigating differences in prolactin levels among breeding stages in male and female magnificent frigatebirds breeding in French Guiana. Significant variables have been highlighted in bold.

Response: prolactin	Explanatory variables	X^2	Df	P
Males	Breeding stage	64.8	3	**5.5 x 10^{-14}**
n = 44	Breeding season	0.5	1	0.50
	Hours since midnight	0.0	1	0.97
	SMI	0.6	1	0.44
Females	Breeding stage	59.5	2	**1.2 x 10^{-13}**
n = 39	Breeding season	0.1	1	0.77
	Hours since midnight	0.2	1	0.67
	SMI	1.4	1	0.23
Adults (males + females)	Breeding stage	65.3	2	**6.6 x 10^{-15}**
n = 71	Sex	1.0	1	0.32
	Breeding season	0.0	1	0.96
	Hours since midnight	0.1	1	0.73
	SMI	0.7	1	0.40
	Breeding stage * Sex	2.5	2	0.28

ii) Differences in Hg concentrations among breeding stages

Breeding stage was significantly associated with Hg concentrations (χ^2_3 = 14.5, p = 2.3 × 10^{-3}), but this relationship was influenced by a near-significant interaction with breeding season (χ^2_3 = 7.6, p = 0.06, Table 3S). After accounting for breeding season, pairwise comparisons among breeding stages within each season were not significant (Fig. 2S, Table 4S). Sex was not significantly associated with Hg concentrations (χ^2_1 = 2.2, p = 0.13).

iii) Prolactin levels in relation to sex and breeding stages

Prolactin levels varied significantly with breeding stage in both sexes

Table 2
Model outputs of the Generalised Linear Models (GLM) investigating the association between Hg contamination and prolactin levels in magnificent frigatebirds. Significant variables have been highlighted in bold.

Breeding Stage	Explanatory variables	X^2	Df	P
Display	Hg	1.4	1	0.23
n = 12	Breeding season	0.3	1	0.58
	Hours since midnight	0.0	1	0.88
	SMI	2.1	1	0.15
Incubating	Hg	0.3	1	0.59
n = 24	Sex	0.1	1	0.82
	Hg:Sex	0.7	1	0.42
	Breeding season	1.4	1	0.25
	Hours since midnight	0.5	1	0.46
	SMI	5.9	1	**0.02**
Chick-rearing	Hg	0.0	1	1.00
n = 66	Sex	0.5	1	0.50
	Hg:Sex	0.0	1	0.84
	Hours since midnight	0.1	1	0.72
	SMI	0.0	1	0.95
	Chick age at sampling	5.8	1	**0.02**
Non-breeding	Hg	0.1	1	0.79
n = 21	**Sex**	4.4	1	**0.04**
	Hg:Sex	2.5	1	0.11
	Breeding season	0.0	1	0.88
	Hours since midnight	0.5	1	0.47
	SMI	1.7	1	0.19

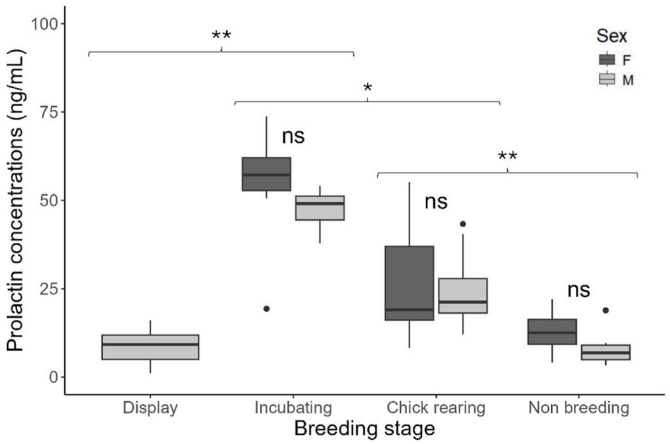

Fig. 2. Seasonal changes in prolactin levels (ng/mL) across the breeding season in adult magnificent frigatebirds. Males (M) and females (F) are represented in light grey and dark grey respectively. Sample sizes for each period: display: n = 12 males; incubating: n = 11 males and 13 females; chick-rearing: n = 13 males and 13 females; non-breeding: n = 8 males and 13 females. Boxes represent the median and interquartile range (IQR), and whiskers represent the largest and smallest values within 1.5 × IQR. Data are cross-sectional (birds were sampled a single time on a single breeding stage) and include two different breeding seasons (2002 and 2003). Pairwise comparisons between sexes within the same breeding stage were not significant (ns) and significant differences between consecutive breeding stages have been indicated over the boxes (**p < 0.001, *p < 0.05).

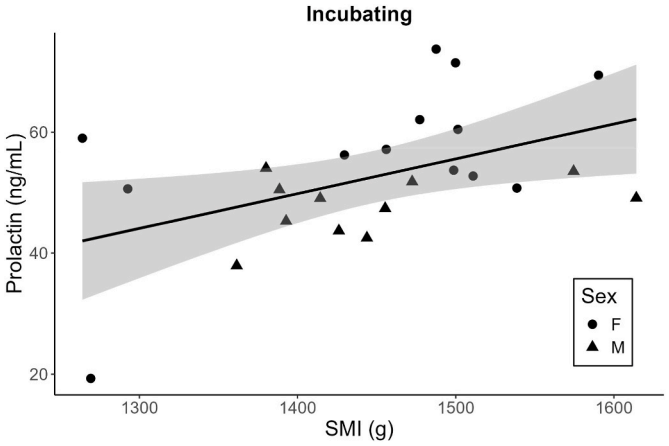

Fig. 3. Association between body condition index, SMI (Scaled Mass Index), and prolactin levels (ng/mL) in incubating magnificent frigatebirds (n = 24) sampled in French Guiana during the breeding seasons of May 2002 and March 2003. Data from males (n = 11, ▲) and females (n = 13, ●) have been pooled. The solid line shows the fitted linear regression, and the shaded area represents the 95% confidence interval of the fitted mean. SMI was calculated following Peig and Green (2009).

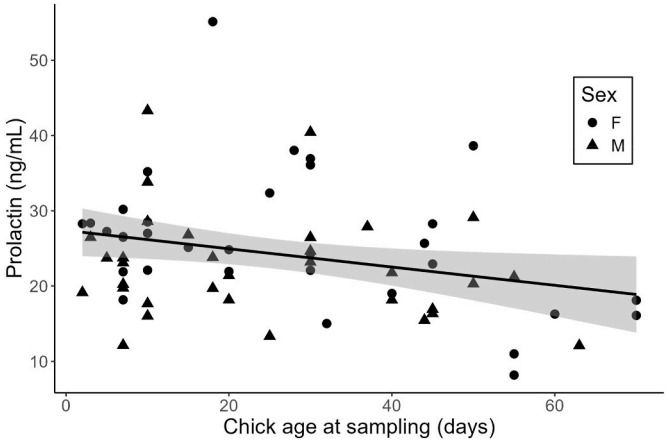

Fig. 4. Association between chick age at sampling (in days) and prolactin levels (ng/mL) in chick-rearing magnificent frigatebirds (n = 66) sampled in French Guiana during 4 different breeding seasons (May 2002, March 2003, June 2022 and November 2022). Data from males (n = 33, ▲) and females (n = 33, ●) have been pooled. The solid line shows the fitted linear regression, and the shaded area represents the 95% confidence interval of the fitted mean.

(Table 1), showing a consistent pattern. The highest prolactin levels were observed during incubation (experiencing almost a 5-fold increase compared to displaying in males), and they were followed by an abrupt decline to reach intermediate levels during chick-rearing (i.e. about half of the incubating levels, Fig. 2). Both displaying and non-breeding individuals had the lowest prolactin levels (which represented almost a third of the chick-rearing concentrations, Fig. 2, Tables 5S–8S). There were no sex differences in prolactin levels, and neither breeding season, hours since midnight, nor SMI were associated with prolactin levels (Table 1).

iv) Associations between Hg concentrations and prolactin levels at different breeding stages

Hg concentrations were not associated with prolactin levels at any breeding stage (Table 2, Figs. 3S–6S). However, prolactin levels were positively associated with body condition during incubation (m_{SMI} = 0.001, $t_{1,17} = 2.5$, p = 0.02, Fig. 3) and negatively associated with chick age during chick-rearing (i.e. parents attending older chicks had lower prolactin levels, $m_{chick\text{-}age} = -0.006$, $t_{1,59} = -2.5$, p = 0.01, Fig. 4). In non-breeding birds, prolactin levels differed between sexes, with males exhibiting lower levels than females (Fig. 7S).

4. Discussion

Our results revealed a clear seasonal pattern of prolactin secretion in magnificent frigatebirds, consistent with our expectations based on the species' altricial, biparental care reproductive strategy (Osorno-Cepeda, 1996), and documented seasonal patterns in other reproductive hormones (Chastel et al., 2005). Despite elevated Hg concentrations reported in frigatebirds from this colony, we found no evidence that Hg disrupted prolactin secretion at any breeding stage.

i) Differences in Hg concentrations among breeding seasons

The average Hg concentrations reported in birds in May 2002 were high, but similar to those documented a decade later in the same colony (Sebastiano et al., 2016). More surprisingly, we expected Hg concentrations to increase, consistent with documented trends in both tropical (Cusset et al., 2023) and non-tropical (Evers et al., 2024) seabirds and ongoing ASGM activities (Hammond et al., 2007; Sebastiano et al., 2022; Tudesque et al., 2012). Contrary to our expectations, we documented an apparent decline in Hg concentrations between the early 2000s and the early 2020s, with the lowest Hg concentrations ever recorded in this colony in November 2022. An overall decrease in Hg contamination is unlikely because Hg emissions are still rising in the Global South (Qiu et al., 2025). However, due to the complexity of Hg biogeochemical cycling, greater Hg inputs into coastal waters do not necessarily translate into increased biological exposure, given that inorganic Hg must first be converted into methylmercury through microbial methylation (Wang et al., 2022). Lag periods between Hg methylation and its incorporation into food webs (Wang et al., 2019) could explain the absence of an increasing trend. Furthermore, because foraging is the main source of Hg exposure in frigatebirds, food-web

structure, prey availability and diet composition are likely key drivers of temporal variation in Hg concentrations. Rising water temperatures induce selective pressures that affect the diversity and composition of fish communities in French Guiana (Joncour et al., 2020; Vallée et al., 2018), with species reaching their upper thermal limits (Vallée et al., 2018) being replaced by others whose Hg concentrations may differ from those of prey consumed by frigatebirds in the early 2000s. More evidently, the collapse of shrimp fisheries in French Guiana eliminated abundant discards, which used to provide a reliable food resource during the breeding period (Joaquim et al., 2007; Martinet and Blanchard, 2009). These discards included many benthic species (Joaquim et al., 2007), potentially more reflective of local Hg contamination. The lack of discards may force frigatebirds to forage offshore more frequently, thereby shifting diet composition toward prey with different Hg profiles.

Finally, given that the lowest Hg concentrations were recorded in birds captured in November, outside of the typical breeding season (that spans from March to July), environmental processes may also affect the presence, density and levels of exposure of different prey during different times of the year, contributing to the documented pattern. For instance, Hg concentrations in sediments and coastal waters around Guiana are higher during the rainy season (Tagliarolo and Rousseau, 2022). Further work is needed to reconstruct how changes in frigatebirds' trophic niche over time influence Hg exposure and to understand how rapidly Hg inputs become bioavailable in this region.

ii) Differences in Hg concentrations among breeding stages

Shifts in prey preference may also explain the apparent decrease in Hg concentrations across breeding stages. Frigatebirds show high foraging flexibility and unpredictable space use compared to temperate and cold-adapted seabirds (Oppel et al., 2017). This is particularly true during the breeding period, when they increase opportunistic foraging on fisheries' discards (Joaquim et al., 2007) and engage more often in kleptoparasitism around the colony (Osorno et al., 1992; Sebastiano, 2024). Frigatebirds' foraging strategy usually includes 1) long offshore trips to feed on schooling pelagic fish and 2) a coastal feeding strategy, with short trips targeting surface species on the continental shelf (Austin et al., 2019). During display, individuals might prioritize local feeding to maximize time spent at display grounds, increasing exposure to more heavily contaminated coastal prey. During incubation, they may engage in both feeding strategies, thereby reducing overall Hg exposure. Eventually, chick-rearing males prioritize offshore foraging trips, whereas females, which are more invested in parental duties, alternate offshore trips with coastal feeding, and engage more often in kleptoparasitism (Austin et al., 2019; Osorno et al., 1992), increasing local fish ingestion. Overall, given substantial variation in Hg exposure arising from complex foraging strategies, frigatebirds might be less suitable bioindicators of local Hg contamination along the Guianan coast (they can feed as far as 1000 km away from the colony, Zaluski et al., 2019) than more resident coastal seabirds. Taken together, our findings emphasize the importance of long-term monitoring to disentangle contaminant trends from ecological processes (Fort et al., 2016; Tartu et al., 2022; Wang et al., 2019).

iii) Prolactin levels in relation to sex and breeding stages

The pattern of prolactin secretion in frigatebirds supports the role of this pituitary hormone as an endocrine mechanism regulating avian parental care (Smiley, 2019). Displaying male magnificent frigatebirds had low prolactin levels, a pattern which was likely the same in mate-searching females, as reported in many other seabirds (Lormée et al., 2000). Non-breeding adults with immature features exhibited similarly low, detectable prolactin levels. These findings support prolactin's predominantly reproductive function. Prolactin is a pleiotropic hormone which continually fluctuates along the annual cycle (Stewart and Marshall, 2022) rather than being secreted exclusively during reproduction. Baseline prolactin levels are likely essential for other background physiological functions (Stewart and Marshall, 2022), whereas higher concentrations beyond a biological threshold are necessary to trigger parental effects (Boos et al., 2007). This pattern is observed in other species, in which baseline prolactin levels are also detectable in immature birds (Williams and Sharp, 1993).

After mating, prolactin concentrations increase dramatically in frigatebirds, peaking during incubation as shown in other tropical (Lormée et al., 2000) and non-tropical (Hall, 1986; Riou et al., 2010) seabird species with similar reproductive strategies. Even though we cannot determine whether endogenous signalling (Garcia et al., 1996; Lormée et al., 1999), external stimuli (Dawson and Goldsmith, 1985) or a combination of both trigger prolactin secretion in frigatebirds, previous results support a gonadotropin-induced negative-feedback mechanism of regulation (Chastel et al., 2005). Following this mechanism, high LH and testosterone levels during display (Chastel et al., 2005) may induce prolactin secretion in the hypothalamus and anterior pituitary. Photoperiod alone is unlikely to be the primary cue synchronizing endocrine cycles in frigatebirds, given the irregular reproductive timing in this species (with multiple breeding peaks within the same year, Diamond, 1973; Kushlan, 2009), and the limited annual variation in daylight at tropical latitudes.

Regardless of triggering mechanism, maintaining elevated prolactin levels is probably the main physiological process underlying incubating behaviour (Vleck et al., 2000). Prolactin levels drive the impulse to remain at the nest during prolonged fasting, and to return to the nest to replace the breeding partner after long periods foraging offshore without any contact with the egg (Garcia et al., 1996; Lormée et al., 1999; Weimerskirch et al., 2010). The significant positive association between body condition and prolactin concentrations during incubation supports the hypothesis that physiological condition influences parental investment (O'Dwyer et al., 2006). Assuming that parental investment is modulated through prolactin secretion, birds in better body conditions may express higher prolactin levels (Mohring et al., 2024; O'Dwyer et al., 2006; Riechert et al., 2012). As documented in other species, these elevated prolactin levels are likely associated with stronger commitment to incubation, manifested though behaviours such as egg turning (Blévin et al., 2020) or incubation constancy (Hope et al., 2020), which may eventually translate into higher breeding success (Mohring et al., 2024; O'Dwyer et al., 2006).

Once eggs hatch, prolactin levels generally decrease in seabirds, as observed in our results, reflecting gradual declines in the need for parental investment as chicks approach fledging (Hall, 1986; Hector and Goldsmith, 1985; Lormée et al., 2000; Riou et al., 2010). However, we found no support for our hypothesis that males would exhibit greater variability in prolactin levels, driving their variable commitment to chick-rearing (Osorno, 1999). It is possible that our study design was insufficient to address this question because 1) we lacked information on which males ultimately deserted their chicks and 2) chick ages spanned a broad range (10-70 days old), pooling together parents that were brooding (i.e. attending recently hatched non-thermally independent chicks around 10-20 days old) and parents that were rearing (i.e., attending homeothermic chicks around 70 days old). Indeed, we found a significant negative association between chick age and prolactin levels, supporting the hypothesis that prolactin levels decline from brooding to rearing, as shown in other species (Hall, 1986; Hector and Goldsmith, 1985; Lormée et al., 2000; Riou et al., 2010). Because males may desert chicks as young as 19 days old (Osorno, 1999), unaccounted-for age-related variation may have obscured biologically meaningful differences associated with desertion probability. Hence, a more targeted study design would be required to evaluate whether prolactin influences early chick desertion in male frigatebirds (e.g. by comparing prolactin levels exclusively amongst brooding males and monitoring eventual chick abandonment).

An unexpected finding was the absence of significant sex differences in prolactin levels. Nonetheless, female frigatebirds generally exhibited

higher prolactin levels across breeding stages (a pattern which was significant in the non-breeding model). Although females of both tropical (Lormée et al., 2000) and temperate species (Riou et al., 2010) often exhibit higher prolactin levels, this sexual dimorphism is not universal (e.g. Garcia et al., 1996; Hector and Goldsmith, 1985). Its absence in frigatebirds is particularly puzzling, given that both partners do not equally share parental duties (McCully et al., 2022). We expected females not only to exhibit higher prolactin levels, but also to show a less pronounced decline during chick-rearing to maintain greater parental investment without males' contribution during one of the longest fledgling periods of any bird (Osorno-Cepeda, 1996). It is possible that the lack of statistical significance between sexes resulted largely from high variability within chick-rearing females. This variability was not influenced by the prolactin levels of males within their breeding pair. Further research is needed to understand prolactin's role during the critical chick-rearing period, but high variability in both sexes suggests that this hormone plays a major role in regulating parental investment in frigatebirds.

iv) Associations between Hg concentrations and prolactin levels at different breeding stages

Although prolactin has been proposed as one of the most sensitive targets of Hg-induced endocrine disruption (Hernandez-Gonzalez et al., 2025), our results provided no evidence of prolactin disruption in magnificent frigatebirds at any breeding stage. In contrast, in male black-legged kittiwakes (*Rissa tridactyla*), Hg was negatively associated with prolactin levels both during incubation and chick-rearing (Tartu et al., 2016). Similar findings were reported in incubating male snow petrels (*Pagodroma nivea*) in relation to stress-induced prolactin (Tartu et al., 2015). In both cases, these associations were attributed to hypothetical disruption of dopaminergic pathways in the hypothalamus and subsequent inhibition of prolactin release (Tartu et al., 2016, 2015). Interestingly Hg levels in these species were 2-3 times lower than those reported in frigatebirds. Frigatebirds may be naturally more resilient than polar species to high Hg burdens because of evolutionary adaptations associated with long-term exposure in tropical environments. Hg is a relatively new threat in remote Arctic and Antarctic ecosystems (Beal et al., 2015; Dietz et al., 2009; Kirk et al., 2012), where concentrations have increased by an order of magnitude over just a few hundred years (Dietz et al., 2009). However, the Amazonian soils of the Guianan Shield are naturally enriched in Hg (Kroonenberg et al., 2022), acting as important reservoirs from which deposited Hg is mobilised into aquatic ecosystems (Laperche et al., 2014). Although ASGM and deforestation increase Hg emissions and mobilisation (Boudou et al., 2005; Guedron et al., 2011), historically elevated background Hg concentrations from natural processes may have favoured evolution of adaptations that allow frigatebirds to tolerate higher Hg burdens. Indeed, even magnificent frigatebird populations outside of the Guianan Shield are naturally exposed to high Hg concentrations (e.g. California, Castillo-Guerrero et al., 2025; and Barbuda, Sebastiano et al. unpublished data) possibly because of their pelagic foraging ecology (Sebastiano et al., 2017). Frigatebirds may possess physiological mechanisms to mitigate Hg toxicity such as upregulation of Hg demethylation pathways (e.g. Hg sequestration into selenium compounds, El Hanafi et al., 2022; Manceau et al., 2021), which may protect them against endocrine disruption. A similar explanation was proposed by Gilmour et al. (2019) to account for the absence of Hg effects on prolactin levels in two seabirds foraging on Hg-rich mesopelagic prey.

Finally, it is worth discussing that, beyond biological tolerance, other less likely factors could explain absence of Hg effects. For instance, our capacity to detect significant associations may be limited by insufficient sample sizes. Similarly, narrow ranges of Hg concentrations or prolactin levels may hinder detection of biologically meaningful associations. Finally, if prolactin disruption was caused by long-term Hg exposure rather than short-term exposure, a temporal mismatch caused by matrix of analysis may prevent detection of biological associations (Hernandez-Gonzalez et al., 2025). Furthermore, we could only examine associations between Hg contamination and prolactin levels within individual breeding stages because birds were sampled only once to avoid causing nest desertion. Consequently, we could not determine whether Hg alters the magnitude of seasonal prolactin fluctuations.

5. Conclusions

Our results demonstrated that different stages of the breeding cycle in magnificent frigatebirds are characterised by changes in prolactin secretion, supporting a central role for this pituitary hormone in regulating parental investment. Although we could not test our hypothesis that variation in prolactin levels during chick-rearing determines differential nest desertion by males, we suggest that further research using a more targeted design is needed. Overall, we found no evidence that Hg disrupts prolactin secretion despite elevated Hg concentrations in this species, which contrasts with the disrupting associations reported in other seabirds at lower exposure levels. These findings are important because it has been hypothesised that Hg-induced reductions in prolactin may reduce parental investment and indirectly increase nestling susceptibility to infectious disease outbreaks in this colony (Sebastiano et al., 2022). Nonetheless, further research on Hg effects on this pituitary hormone is needed, particularly during the often-overlooked chick-rearing period. Studies investigating avian taxa potentially more sensitive to Hg contamination than predatory seabirds (Heinz et al., 2009; Jackson et al., 2015) are particularly encouraged. Finally, although Hg concentrations in this colony have not increased dramatically over the last 20 years, our findings highlight the importance of accounting for trophic ecology when disentangling effects of prey availability and prey choice from local toxicological processes.

CRediT authorship contribution statement

Miguel Hernández-González: Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis, Data curation, Conceptualization. **Marcel Eens:** Writing – review & editing. **Olivier Chastel:** Writing – review & editing, Investigation. **Charline Parenteau:** Validation, Investigation. **Geoffrey Monchaux-Lefèvre:** Investigation. **Paco Bustamante:** Writing – review & editing, Investigation. **David Costantini:** Writing – review & editing, Conceptualization. **Manrico Sebastiano:** Writing – review & editing, Validation, Investigation, Conceptualization.

Data accessibility

Data will be made available on request.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2026.128780.

Data availability

Data will be made available on request.

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