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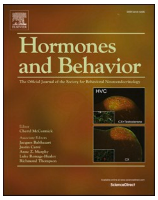

Prolactin is associated with proximity to incubating partner rather than parental care in black-headed gulls

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ABSTRACT

The peptide hormone prolactin plays an important role in the expression of parental care behaviours across bird and mammal taxa. While a great deal is known about how plasma prolactin concentrations vary across the reproductive cycle, the few studies that investigate how prolactin relates to individual-level variation in parental care have reported mixed results. We argue that, since parental care is also affected by social interactions and environmental constraints, prolactin may better reflect behaviours that are indirectly related to parenting than the absolute level of care that is eventually expressed. In this study, we tested for associations between plasma prolactin and the expression of both parental care and proximity to the partner in incubating black-headed gulls, *Chroicocephalus ridibundus*. Baseline prolactin levels increased with calendar date but were unrelated to incubation behaviours. However, parents who showed a weaker decrease in prolactin to an acute stressor spent more time in close proximity to their incubating partner while not on the nest themselves, suggesting that individual variation in stress-induced prolactin changes reflect differences in parents' tendency to be closely associated with their partner and the joint nesting attempt. Baseline and stress-induced levels of the stress hormone corticosterone were unrelated to both prolactin levels and parental behaviours, suggesting that this hormone is not a strong moderator of parental care in black-headed gulls. One potential explanation for the link between prolactin dynamics and partner proximity is that prolactin reflects parental motivation to provide parental care or retain contact with the breeding partner, but further work is needed to directly test this hypothesis.

1. Introduction

Parental care is widespread throughout the animal kingdom and plays a crucial role in determining reproductive success in many taxa. The strong link between parental care and fitness is an important topic of study for evolutionary biologists, especially since substantial variation in parental care exists not only between species (e.g. Gross, 2005; Cockburn, 2006; Wong et al., 2013), but also within individuals of the same species (e.g. Houston and Davies, 1985; Westneat et al., 2011; Saltzman et al., 2017). In an attempt to understand the proximate mechanisms underpinning variation in parental care, the peptide hormone prolactin has been a key point of focus (Smiley, 2019). Prolactin is present in all vertebrates (Kawauchi and Sower, 2006) and, among a variety of other functions, regulates the expression of parental behaviours (reviewed in Forsyth and Wallis, 2002; Angelier and Chastel, 2009; Smiley, 2019). Especially among avian species, where parental care is prevalent and highly variable (Cockburn, 2006), the link between prolactin and breeding behaviour, life history decisions and reproductive success has attracted decades of research attention from endocrinologists and behavioural ecologists alike (Angelier and Chastel, 2009; Angelier et al., 2016).

Circulating plasma levels of prolactin vary seasonally in bird species and the sharp increase typically seen during incubation and nestling-rearing (e.g. Lormée et al., 2000; Chastel et al., 2005; Riechert et al., 2014; Wojczulanis-Jakubas et al., 2018) is a strong indication of the role of prolactin in regulating parenting behaviours. Prolactin also tends to decrease in response to environmental stressors (reviewed in Angelier et al., 2016) and the magnitude of this decrease is usually smaller when parents are actively providing care (Chastel et al., 2005; Riou et al., 2010; Angelier et al., 2009*a*, 2013, but see Krause et al., 2015). This observation has led to the suggestion that the extent of prolactin decrease under stress is inversely related to the value parents place in

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current reproduction; in other words, parents who maintain high levels of prolactin under stress are less inclined to temporarily redirect resources away from parental care and towards their own survival (Angelier and Chastel, 2009). However, both our understanding of how prolactin is linked to individual variation in parental care and the extent to which stress-induced changes in this hormone can tell us about parental care strategies are still highly limited.

Our current lack of in-depth understanding can be linked to several factors. First, while many studies have quantified variation in baseline and stress-related changes in prolactin across the breeding cycle at the population level (reviewed in Angelier and Chastel, 2009; Angelier et al., 2016; Smiley, 2019), only a small number have examined the relationship between naturally-occurring levels of prolactin and individual investment in parental care (Schoech et al., 1996; Duckworth et al., 2003; Ouyang et al., 2011; Smiley and Adkins-Regan, 2016; Wang et al., 2020). Second, we are aware of only two studies that tested the relationship between the magnitude of stress-induced change in prolactin and individual parental care behaviour (Angelier et al., 2007; Hope et al., 2020). These studies both report a positive association between changes in prolactin in response to stress and parental care, but in order to properly test the hypothesis that stress-induced prolactin levels are indicative of parental motivation, we require more information about individual-level variation in parental responses to stress. Lastly, in the few studies that were performed at the individual level, correlations between baseline prolactin and parental care are mixed. During incubation, some studies find a positive relationship (Wang et al., 2020) and others do not (Riechert and Becker, 2017). Similarly, the relationship between prolactin and chick provisioning rate is sometimes positive (Smiley and Adkins-Regan, 2016), while other studies show neutral (Khan et al., 2001; Ouyang et al., 2011) or mixed (Schoech et al., 1996) results. The picture remains unclear even in studies across a variety of taxa where prolactin has been experimentally manipulated: while elevation or suppression of prolactin often produces the expected increase or decrease in parental care, respectively in birds (e.g. Wang and Buntin, 1999; Angelier et al., 2009*b*; Smiley and Adkins-Regan, 2018) and fish (Páll et al., 2004), many experimental studies fail to find any effect of prolactin manipulation in mammals (Almond et al., 2006), fish (Bender et al., 2008) and birds (Crossin et al., 2012).

We argue that the lack of clear direction in the relationship between prolactin and parental care arises from the fact that expressed parenting behaviours are an emergent property of both the physical and social environment; the amount of care a parent is selected (and thus intrinsically motivated) to provide may differ from the amount of care that is eventually expressed because of moderation through processes like sexual conflict (Royle et al., 2002; Harrison et al., 2009) or variation in resource availability (Ruffino et al., 2014). As such, individual levels of prolactin might be more strongly linked to indirect behaviours that are related to parental care than to the absolute level of care provided. Indeed, accumulating evidence from several penguin species suggests that prolactin might increase parental tendency to remain close to the nest rather than directly induce parental care behaviours (Vleck et al., 2000; Angelier et al., 2007; Smiley, 2019); the tendency to remain close to or return faster to the nest after disturbance has also been linked to prolactin in other species (Pedersen, 1989; Angelier et al., 2013). Nest proximity, or the tendency for non-caring parents to remain close to the reproductive attempt, may therefore be a very useful measure of indirect parental behaviour which should be explored alongside parental care itself.

Here, we explored the link between plasma hormones and the expression of both parental care and proximity to the breeding partner during incubation. We measured baseline and stress-induced changes in prolactin and also in corticosterone; since stress-induced increases in corticosterone are thought to facilitate the redirection of resources away from reproduction and towards short-term survival (Wingfield and Sapolsky, 2003), this hormone is important to include when investigating the link between prolactin and parental care (Angelier et al., 2007). We studied a captive, semi-natural colony of black-headed gulls, *Chroicocephalus ridibundus*. In this species, both parents contribute equally to all aspects of parental care (Van Rhijn and Groothuis, 1985) but individual investment in care is highly variable (Bebbington and Groothuis, 2023). In addition, there is a great deal of variation in the behaviour of non-incubating parents: while some individuals leave the nesting area completely when their partner is incubating, others remain in the nest vicinity (*pers. obs.*). We interpret the tendency to remain close to the incubating partner as an indirect measure of parenting-related behaviour. Importantly, the expression of this behaviour is likely to be independent of the behaviour of the partner; as such, it has greater potential to be associated with intrinsic hormone levels than absolute incubation effort, which is at least partly determined by the behaviour of the breeding partner.

We first explored how environmental factors and individual properties contribute to variation in both baseline levels of prolactin and corticosterone, and how these hormones change in response to environmental stress. We then tested the prediction that individuals investment in incubation behaviour is positively related to baseline and stress-induced prolactin levels and negatively related to corticosterone levels. Lastly, we tested whether a parent's tendency to remain close to the nest when their partner was incubating is greater among parents with higher prolactin levels and lower corticosterone levels.

2. Methods

2.1. Study system and housing

We studied a captive population of 114 black-headed gulls (*Chroicocephalus ridibundus*) kept in semi-natural conditions at the University of Groningen, the Netherlands, in 2021. Both sexes of this species contribute to all aspects of parental care and pairs breed largely synchronously in large, dense colonies that enable easy observation of behaviour and reproductive output (Bebbington and Groothuis, 2023). The study colony is housed in a large aviary (10x35m) with two pools for swimming, natural light and temperature exposure, and shelters and protective platforms to facilitate nest building during the breeding season. Birds receive *ad. lib.* pelleted trout food (E-3P Stlla, Trouw Nutrition Nederland) and are also supplemented with cat food three times a week (Huismerk 3-mix kattenbrok, Arie Blok). The long-term study population is derived from wild black-headed gulls that were introduced periodically into the colony as eggs or nestlings (most recently in 2010) in order to maintain genetic diversity and wild-type behaviour. All individuals are fitted with 4–5 colour rings that allows them to be uniquely identified, and the sex of all birds in the colony has been determined by amplification and identification of the CHD-W gene, which is only present on the female sex chromosome (following Griffiths et al., 1998).

2.2. Breeding data

During the 2021 breeding season (April–June), the colony was intensely observed to determine the identity of breeding pairs at each of the 34 nests where focal individuals (sampled for hormone analysis) were breeders. At 29 of these nests, we recorded incubation on day 0–5 after clutch completion by using small cameras (GoPro Hero 5), which were set up near the nests and programmed to take a photo of a focal nest every two minutes from 08:00 until dusk (around 20:00). We were able to confirm which of the two parents was incubating based on their colour rings, the colour rings of the nearby partner, or occasionally using size and plumage differences (see Bebbington and Groothuis, 2023 for details). We calculated individual incubation effort as the number of photos where a focal bird was observed on the nest as a proportion of all photos. Observations revealed that non-incubating partners were almost always either in close vicinity of the nest or completely outside the nesting area of the aviary. To quantify this in a standardised way that controlled for varying distances between the camera and focal nest, we calculated partner proximity as the number of photos where a non-incubating focal bird was within 3 body-lengths of its partner on the nest, as a proportion of all photos where that partner was incubating.

2.3. *Blood sampling and hormone assays*

Birds were caught at the nest between 09:00 and 14:00 on one of 8 catching days during the second half of the incubation period (total length = 19–23 days). All individuals were caught between day 13 and 18 of incubation, with the exception of one bird who was caught on day 8 of incubation (data from this individual gave no indication of bias and were therefore retained in the analyses). One week prior to catching at a given nest, a wire cage with the bottom and front sides completely open was placed directly over the nest to allow parents to move freely on and off the nest. Three days before catching, free-swinging bars were added to the front side of the cage. Birds readily adapted to the presence of the cage and swinging bars during the acclimatisation period and incubated as normal. On the day of catching, the bars were fixed to allow only an inward and not an outward movement (allowing a parent to enter the cage and sit on the nest, but not to leave). A maximum of five focal cages were closed at any time and we performed multiple rounds of catching on each day; since observers entering the aviary caused the whole colony to temporarily leave the nesting area, we recorded the round in which a bird was caught to account for any accumulating effects of repeated disturbance on hormone levels. After observers entered the colony to close target cages, all parents returned to the breeding area to resume incubation. Once target parents had entered their cages and begun to incubate, we waited on average 15 min (range 5–30 min) to ensure that the birds were settled on the nest and incubating properly before entering the aviary to remove them from the cages. An initial blood sample of ca. 150μl was taken from the wing of each bird via puncture of the brachial vein as soon as possible after observers entered the aviary (average = 3:20 min, range = 1:00–7:10). The birds were then kept in a cloth bag until 30 min after capture, when a second blood sample was taken to measure stress-induced hormone levels. After blood sampling was complete, the birds were released back into the aviary.

Prolactin and corticosterone concentrations were assayed by radioimmunoassay (RIA) at the Centre d'Etudes Biologiques de Chizé-UMR7372. The prolactin assay was a direct heterologous assay as described in Lormée et al. (2003), and was performed using chicken prolactin and antibody against chicken prolactin (supplied by Dr. A. F. Parlow, Harbor-UCLA Medical Center, Torrance, CA, USA). A dose–response curve of pooled black-headed gull plasma samples paralleled that for chicken, indicating that the assay could be used to measure relative plasma PRL in black-headed gulls. All samples were run in one assay, in duplicate, with 25 μl of plasma per well. The intra-assay variation was 14.99 % and the limit of detection was 0.99 ng/ml. Samples were re-assayed when the intra-assay coefficient of variation (CV) was great than 12 %, but due to limited plasma volume for repeated assays, the CV was nonetheless higher than average for our laboratory; this seems to be due to variation in the quality of the final tracer used for labelling.

Corticosterone was first extracted from plasma (10 to 50 μl) with 3 ml of diethyl-ether. Following Lormée et al. (2003), the dried extract was re-dissolved in phosphate 0.01 M pH 7.4 buffer and corticosterone was then assayed in duplicate, in 3 runs. One hundred μl of extract was incubated with H3-corticosterone (Perkin Elmer, US) and a rabbit antiserum against corticosterone (Sigma Aldrich, US). Intra- and inter-assay variations were respectively 7.88 % and 14.90 %. Corticosterone lowest detectable concentration was 0.28 ng/ml.

2.4. *Statistical analysis*

To determine whether baseline hormone concentrations were influenced by our sampling regime, we constructed two separate linear models with baseline prolactin and corticosterone as two dependent variables, respectively. We included sex, nest age (since clutch completion), catch round, sampling date (days since 30th May 2021, when the first birds were caught), time that an individual had spent incubating inside the cage before it was removed and time between capture and first blood sampling as predictors. Baseline corticosterone was also included in the prolactin model (and vice-versa) to test for correlations between the two hormones. To test for the presence of stress-induced changes in both hormones (hereafter: prolactin stress response and corticosterone stress response), we constructed two separate linear mixed models with prolactin and corticosterone concentrations as dependent variables, respectively, and sample type (baseline or 30 min) as a predictor. Individual identity was included as a random effect (intercept only) to account for repeated sampling. Lastly, we explored variation in the magnitude of the stress response in prolactin and corticosterone (separate dependent variables: 30-min concentration – baseline concentration of prolactin and corticosterone, respectively). Since measures of changing concentration between two time points are susceptible to regression to the mean effects (Yudkin and Stratton, 1996), we re-calculated stress response values with a correction for regression to the mean as described in Verhulst et al. (2013) to use as a dependent variable. We tested whether these corrected stress response measures for prolactin and corticosterone were related to the baseline hormone concentration, sampling date, nest age and sex. We tested for a correlation between the stress responses of the two hormones by including the corticosterone stress response in the model of prolactin, and vice-versa.

To determine the relationship between hormone dynamics and incubation behaviour, we modelled incubation effort and proportion of time spent in proximity to the incubating partner as separate dependent variables with a beta distribution using the package glmmTMB (Brooks et al., 2017). In all models, we included individual sex, baseline prolactin and corticosterone, as well as stress-response values for the two hormones, as predictors. Pair identity was included as a random intercept to control for possible breed pair effects on parental care. Since all incubation observations were conducted at similar timepoints in the breeding cycle (0–5 days after clutch completion), we did not control for variation in nest age. We tested for interactions between sex and baseline prolactin, and sex and prolactin stress response. In order to avoid over-fitting, we did not test for interactions between sex and corticosterone levels. Individuals may respond differently to the stress protocol and this could affect the extent to which stress-induced prolactin and parental care are linked. To test whether the relationship between prolactin and parental care varies with the degree of individual stress, we tested for an interaction between the prolactin and corticosterone stress responses. For each response variable (incubation effort and proximity to partner), we fitted the model with and without these interaction terms and used conditional Akaike Information Criteria (AICc) values to compare them with a model containing only fixed effects.

All analyses were conducted in R version 4.2.0 (R Core Team, 2022). We used base functions and the “lme4” package (Bates et al., 2015) to construct models and used the “performance” (Lüdecke et al., 2021) and “DHARMa” (Hartig, 2022) packages to assess collinearity and model fit, respectively. We found no evidence of influential data points in any of our models (Cook's distance <0.5 in all cases). Some variables were on very different scales; to enable interpretation all predictors were rescaled using the “arm” package (Gelman and Su, 2021). We present full models with all main effects retained (Burnham and Anderson, 2002; Whittingham et al., 2006).

3. Results

3.1. *Baseline and stress-induced levels of prolactin and corticosterone*

Baseline prolactin concentrations did not vary with nest age

Table 1
Effect of sampling protocol, sex and concurrently measured hormones on baseline concentrations of prolactin and corticosterone in breeding black headed gulls. Significant relationships are indicated in bold font.

Hormone	Predictor	β ± SE	*t*	*P*
Baseline prolactin concentration ($N = 64$)	**Sampling date**	**25.10 ± 10.91**	**2.30**	**0.02**
	Catch round	−8.88 ± 10.24	−0.87	0.39
	Nest age	10.19 ± 10.07	−1.01	0.32
	Minutes in cage	6.24 ± 10.79	0.58	0.57
	Time until first bleed	−5.10 ± 11.53	−0.44	0.66
	Sex (versus female)	−2.29 ± 9.42	−0.24	0.81
	Baseline corticosterone	1.59 ± 9.92	0.16	0.87
Baseline corticosterone concentration ($N = 57$)	Sampling date	−1.10 ± 2.74	−0.40	0.69
	Catch round	−1.30 ± 2.46	−0.53	0.60
	Nest age	1.37 ± 2.42	0.56	0.58
	Minutes in cage	−4.77 ± 2.50	−1.91	0.06
	Time until first bleed	2.78 ± 2.73	1.02	0.31
	Sex (versus female)	0.31 ± 2.25	0.16	0.87
	Baseline prolactin	0.31 ± 2.24	0.14	0.89

(Table 1), but were higher among birds sampled on later dates (Fig. 1, Table 1). There was no effect of catch round, the amount of time individuals spent on the nest prior to catching or time between catching and sampling on baseline prolactin levels (Table 1). Baseline prolactin also did not differ between sexes or with baseline levels of corticosterone (Table 1).

Baseline concentrations of corticosterone increased with time between catching and blood sampling (β ± SE = 5.34 ± 2.46, $P = 0.03$), although this pattern was driven by a single sample which was obtained >7 min after capture. In order to ensure that our baseline corticosterone measures accurately reflect concentrations of this hormone in the absence of stress, we removed the sample taken after 7 min from all analyses. After this removal, 85 % of baseline samples were obtained within 4 min of capture and there was no effect of bleeding time on baseline corticosterone concentration. There was also no effect of sampling date, catch round, nest age, time spent on the nest before capture, sex or baseline prolactin levels on baseline corticosterone (Table 1).

Prolactin levels were significantly lower in samples taken 30 min after capture (β ± SE = −12.84 ± 4.33, $P < 0.01$) (Fig. 2a) and corticosterone levels significantly increased between the first and second sample capture (β ± SE = 47.86 ± 2.61, $P < 0.01$) (Fig. 2b). There was no effect of baseline prolactin levels (corrected for regression to the mean), sex, sampling date or nest age on the magnitude of the prolactin stress response, and there was no correlation between the stress responses of prolactin and corticosterone (Table 2). The magnitude of the corticosterone stress response was similarly unrelated to baseline (corrected) corticosterone levels, and also did not vary with sex, sampling date or nest age (Table 2).

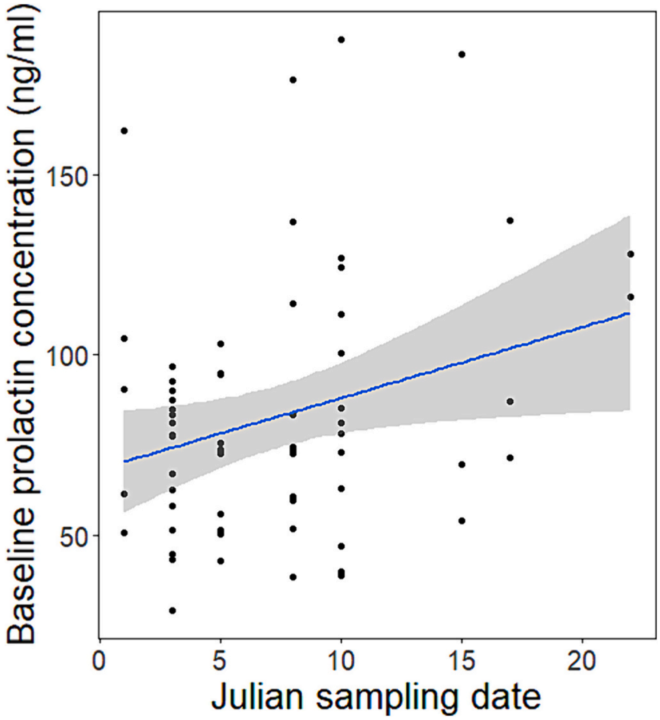

Fig. 1. Relationship between baseline concentration of plasma prolactin and sampling date (since 1st April) in breeding black headed gulls. Dots represent individual data points, line and shading represent linear model fit and 95 % confidence intervals, respectively. $N = 63$ individuals.

3.2. *Associations between hormones and behaviour during incubation*

There was no effect of either baseline concentrations or stress-induced changes in either prolactin or corticosterone on the proportion of time parents spent incubating (Table 3). Incubation effort was similar between males and females (Table 3). There were no sex-dependent effects on either baseline prolactin or the prolactin stress response on incubation and no interaction between prolactin and corticosterone stress responses. Compared to a model with only main effects, ΔAICc values for the models with sex interaction terms were > 2 (AICcs: main effects only = −67.49; sex * baseline prolactin = −64.67, sex * prolactin stress response = −64.64). The model including the, prolactin stress response * corticosterone stress response was within 2AICcs of the main effects model (ΔAICc = 0.85) but this interaction was not significant (Table 3).

However, parents who had a smaller reduction in prolactin levels in response to acute stress spent more time in close proximity to their incubating partner (Fig. 3, Table 3). Proximity was not related to either baseline prolactin or baseline corticosterone concentrations (Table 3). Males spend more time in proximity to their partner on the nest than females (Table 3), but there was no interaction between sex and either baseline prolactin or the prolactin stress response on the proportion of time spent in proximity to the incubating partner (Table 3). Compared to a main effects only model, ΔAICc values for the models with interaction terms were all >2 (AICcs: main effects only = −29.39; sex * baseline prolactin = −27.32, sex * prolactin stress response = −26.625, prolactin stress response * corticosterone stress response = −26.58).

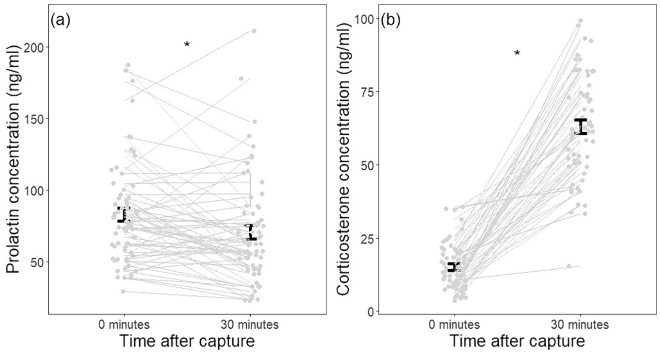

Fig. 2. Stress-related changes in (a) prolactin and (b) corticosterone in breeding black headed gulls. Grey dots represent raw values and lines connect samples of the same individual. Black dots and bars represent mean and standard errors, respectively, asterisk denotes significant difference at $P < 0.05$. $N = 64$ and 57 individuals for prolactin and corticosterone, respectively.

Table 2
Effect of factors predicted to influence the magnitude of stress-induced changes in prolactin and corticosterone in breeding black headed gulls.

Hormone	Predictor	β ± SE	*t*	*P*
Prolactin stress response (*N* = 63)	Baseline prolactin	3.64 ± 9.59	0.38	0.71
	Sex (versus female)	−11.99 ± 9.23	−1.30	0.20
	Sampling date	4.52 ± 10.04	0.45	0.66
	Nest age	−4.37 ± 9.08	−0.48	0.72
	Corticosterone stress response	3.39 ± 9.21	0.37	0.72
Corticosterone stress response (*N* = 53)	Baseline corticosterone	0.14 ± 5.37	0.03	0.98
	Sex (versus female)	8.51 ± 5.48	1.55	0.13
	Sampling date	−2.51 ± 5.68	−0.44	0.66
	Nest age	0.34 ± 5.45	0.06	0.95
	Prolactin stress response	0.32 ± 5.40	0.06	0.96

Table 3
Relationships between incubation behaviour and prolactin and corticosterone concentrations in black-headed gulls.

Parental behaviour	Predictor	β ± SE	*z*	*P*
Percentage time spent incubating (*N* = *54*)	Baseline prolactin concentration	−0.10 ± 0.13	−0.79	0.43
	Baseline corticosterone concentration	0.08 ± 0.13	0.63	0.12
	Prolactin stress response	<−0.01 ± 0.13	0.06	0.95
	Corticosterone stress response	0.08 ± 0.13	0.57	0.57
	Sex (versus female)	0.18 ± 0.12	1.56	0.12
	Prolactin * corticosterone stress response	−0.50 ± 0.33	−1.54	0.12
Proportion of time spent near incubating partner (*N* = 54)	Baseline prolactin concentration	0.21 ± 0.24	0.85	0.40
	Baseline corticosterone concentration	0.22 ± 0.23	0.95	0.34
	Prolactin stress response	**0.50 ± 0.23**	**2.19**	**0.03**
	Corticosterone stress response	0.31 ± 0.25	1.25	0.21
	Sex (versus female)	**0.48 ± 0.23**	**2.45**	**0.01**

4. Discussion

In this study, we found no evidence to support the prediction that prolactin and corticosterone levels were correlated with individual investment in incubation behaviour. However, parents with larger reductions in prolactin were less often in close proximity to their incubating partner. While the effect of prolactin stress response on partner proximity was relatively small, it suggests that individual variation in prolactin is more closely linked to a parent's indirect parenting-related behaviours during reproduction than to absolute parental effort.

The proportion of time that black-headed gull parents spent incubating is highly variable (Bebbington and Groothuis, 2023). However, neither baseline prolactin nor the magnitude of the prolactin stress response was associated with individual incubation effort. While such an association has been reported in some bird species (Hope et al., 2020; Wang et al., 2020), this is far from ubiquitous (e.g. Pedersen, 1989; Riechert and Becker, 2017). It is important to note that data on incubation behaviours were collected approximately 10 days before hormone levels were measured, and this may explain why no relationship between incubation and prolactin was found. However, in other seabird species with similar reproductive mode to black-headed gulls (extended biparental care, semi-precocial offspring) prolactin levels are maintained at a consistently high level throughout the incubation period (Riou et al., 2010; Wojczulanis-Jakubas et al., 2015); prolactin levels at the time of catching were therefore unlikely to have been vastly different from those when birds were observed incubating. Moreover, the fact that we do find an effect of prolactin on an incubation-related behaviour (proximity to incubating partner) suggests that it would have also been possible to detect an effect on incubation itself, if it was present. Given that incubation effort is known to vary with pair bond duration in our study population (Bebbington and Groothuis, 2023), and that parental care more generally is affected by the behaviour and quality of the breeding partner (Burley, 1977; Lessells and McNamara, 2012; Adams et al., 2015), we suspect that the relationship between circulating prolactin levels and parental care itself could be obscured by social influences that impact the absolute level of care that is expressed. The effect of social circumstances on parental care are likely to vary between species depending on factors such as the mating system (Burley, 1977); in ring doves *Streptopelia risorii,* presence of the breeding partner is a key precursor of parental behaviour (Lehrman and Brody, 1961) and even helps maintain prolactin levels (Ramsey et al. 1985). Such variation may explain why a clear link between prolactin and individual parental care can be found in some species, but not others. With increasing availability of data on this link in more species, it would be very useful to test whether interspecific differences in breeding biology can indeed explain variation in the extent to which prolactin levels relate to individual variation in parental care behaviour.

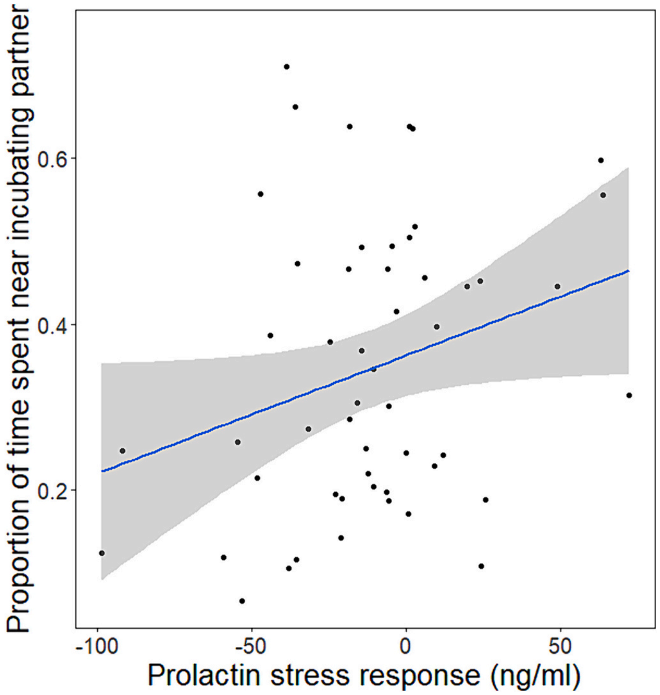

Fig. 3. Relationship between the magnitude of stress-induced decrease in prolactin and the proportion of time black-headed gulls spent near their partner when the partner was incubating. Dots represent individual data points, line and shading represent linear model fit and 95 % confidence intervals, respectively. *N* = 54 individuals.

In black-headed gulls at least, our results suggest that prolactin is more closely linked to indirect parenting behaviour than to absolute care itself. The extent to which parents decrease the production of prolactin under acute stress has been suggested to reflect the trade-off between investment in current reproduction and individual survival (Angelier and Chastel, 2009; Smiley, 2019). Although this would require further testing, our finding that parents who remained close to the nest when not performing incubation duties had smaller stress-induced reductions in prolactin tentatively supports this hypothesis; parents who highly value their current reproductive attempt are likely to remain close to the nest and this appears to be reflected in prolactin dynamics. Although never explicitly tested, there is some indirect support for this interpretation: female willow ptarmigans *Lagopus lagopus* who received prolactin supplementation did not increase their incubation effort, but were more inclined to stay near their offspring when exposed to a stressor (Pedersen, 1989). In black-legged kittiwakes *Rissa tridactyla*, parents with experimentally reduced prolactin levels took longer to return to their nest after disturbance, which is also indicative of a role for prolactin in parental motivation rather than care itself (Angelier et al., 2013). What is less clear is exactly why some parents might be more driven to remain by the nest than others. One intriguing possibility is that nest and partner proximity is related to the strength of the pair bond; perhaps individuals in high quality partnerships are more likely to remain close to their partner, and potentially also have higher prolactin. Due to the fact that parental intention to provide to care is perhaps more difficult to quantify than absolute parental effort, few studies have explicitly measured it; we suggest that this is important not only for understanding variation in the link between hormones and behaviour, but also more broadly in order to understand how selective forces like sexual conflict over care and sexual selection might influence the evolution of parental behaviour.

Male and female black-headed gulls both contribute to all aspects of parental care (Van Rhijn and Groothuis, 1985); as such, the lack of sex differences in prolactin in the current study are unsurprising and align with similar results in other seabirds (e.g. Lormée et al., 2000). Interestingly, there was a sex difference in the amount of time non-incubating parents spent near the nest: males were much more often in close proximity to their incubating partner than females. Given that the colony has ad libitum access to food, this sex difference is unlikely to result from sex differences in foraging requirements. Extra-pair paternity is very low in the species as a whole and we have never observed successful extra-pair copulations in the captive colony (Van Rhijn and Groothuis, 1985; *pers. obs.*), which also excludes mate-guarding as a likely explanation. Particularly in combination with the finding that frequent proximity to an incubating partner is linked to small prolactin stress responses, we suggest that males may simply be more inclined than females to remain close to the nest, perhaps because males perform a greater amount of territorial defence (Bebbington and Groothuis, 2023). However, an alternative explanation is that males require visual exposure to their incubating partner in order to stimulate incubation behaviour, as seems to be the case in ring doves *Streptopelia risoria* (Silver et al., 1973). We suggest that more work is needed to quantify and unravel the social conditions that influence the extent of parental nest proximity while not providing parental care.

In a similar way to prolactin and potentially for the same reason, the empirical relationship between corticosterone and parental care varies between species (Crossin et al., 2012; Villavicencio et al., 2014; de Bruijn et al., 2020). Despite considerable individual variation in both baseline corticosterone and the magnitude of the stress response, we found no evidence that baseline levels or stress-induced changes in this hormone were correlated with prolactin or that corticosterone affected incubation behaviour in black-headed gulls. Since elevated corticosterone levels compromise the ability of parents to provide care (Wingfield et al., 1995) and stress-induced increases in this hormone reflect relative investment in current reproduction versus individual maintenance in a similar manner to prolactin (Wingfield and Sapolsky, 2003; Angelier and Chastel, 2009), it is perhaps surprising that corticosterone was unrelated to partner proximity in the current study. The lack of association between the two hormones supports the general consensus that prolactin and corticosterone are not mechanistically linked (Angelier and Chastel, 2009), although more study of how corticosterone is related to parental motivation to care would certainly be warranted.

5. Conclusion

The results of this study contribute to our limited knowledge about how naturally-circulating levels of plasma prolactin relate to individual parental effort in care-giving vertebrates. The finding that parents who remain close to their incubating partner have smaller stress-induced reductions in prolactin suggests that the prolactin stress response might be used to measure variation in how strongly parents value current reproduction over self-maintenance and future reproduction, but this requires more research. In addition, our results can help explain why the link between hormones and absolute parental care is so mixed. Endocrinological measures of the intrinsic precursors to behaviour are potentially of great use in studying various aspects of reproductive biology such as sexual conflict and the evolution of breeding systems.

Ethics statement

Data were collected from captive black-headed gulls under a permit issued by the Dutch Competence Agency (AVD1050020209348). Ethical approval for the work was given by the Animal Welfare Body of the University of Groningen.

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CRediT authorship contribution statement

Kat Bebbington: Writing – review & editing, Writing – original draft, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Charline Parenteau:** Writing – review & editing, Resources, Methodology. **Olivier Chastel:** Writing – review & editing, Resources, Methodology. **Ton G.G. Groothuis:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

None.

Data availability

Data will be made available on request.

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