

Regulation of Breeding Behavior: Do Energy-Demanding Periods Induce a Change in Prolactin or Corticosterone Baseline Levels in the Common Tern (*Sterna hirundo*)?

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ABSTRACT

Hormones are involved in reproductive decisions, linking environmental cues and body condition and adapting behavior. Mass loss is often accompanied by decreased prolactin and increased corticosterone concentrations, influencing incubation and brooding behavior and ultimately triggering nest desertion. Using blood-sucking bugs (*Dipetalogaster maxima*), we measured baseline prolactin, corticosterone, and ketone body values in incubating common terns (*Sterna hirundo*) between 2006 and 2009 during energy-demanding periods: 50 pairs were sampled hungry (after an incubation bout) and again fed (after foraging). In a second approach, we sampled 57 other pairs (experienced and inexperienced birds) three times over their individual breeding period, because reproduction, especially chick rearing, is a very energy-demanding process. In line with the common physiological pattern of fasting, we found significantly lower baseline prolactin values in hungry terns, which were negatively related to mass loss over the incubation bout, whereas corticosterone and ketone body levels were marginally increased. Compared to that in the incubation phase, the prolactin level dropped after hatching of chicks in inexperienced birds, perhaps indicating lower parental expenditure. Corticosterone, on the other hand, increased after hatching in males, probably linked to higher foraging activity, as males mainly deliver food during the first days. These energy-demanding periods clearly influenced hormones and ketone bodies, maybe reinforced by the low energy margin of this small seabird species, but energy reserves were not depleted to a level affecting behavior or reproductive success.

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Introduction

Within the annual cycle, there are different periods when birds face particularly energy-demanding phases. Besides migration and molt, the reproductive period is suggested to be the most constraining stage (Monaghan et al. 1998), especially the chick-rearing phase, when birds have to care not only for themselves but also for their young. This often leads to prolonged periods of fasting, mainly in capital breeders, but noncapital breeders also have to manage periods with less food, as incubation or brooding competes with foraging. Especially in small-bird species with a limited energy margin, even a short fasting period could lead to malnutrition (Pearson 1968) and ultimately to a change in behavior. But what are the regulating mechanisms for these hungry birds to still care for their young instead of refeeding? On the other hand, beneath constant incubation and guarding of the clutch, a high feeding performance and protection of young are crucial for achieving high fitness in birds. But how are these aspects of parental performance, and breeding behavior in general, regulated?

Hormones play an important role in breeding decisions of birds because they could act as mediators between individual body condition and environmental cues and adapt behavior to the actual circumstances (Sinervo and Svensson 1998; Ricklefs and Wikelski 2002). Very important in this context is corticosterone, the main avian glucocorticoid, which rises immediately after exposure to stress (Romero and Romero 2002; Romero and Reed 2005). It is suggested to be the main mediator of allostasis (achieving homeostasis through behavioral or physiological changes) in birds and to mobilize energy (McEwen and Wingfield 2003). Slightly elevated baseline levels increase locomotor activity (Astheimer et al. 1992; Angelier et al. 2007a) and enhance food intake (Koch et al. 2002). This is especially important under unfavorable conditions, when birds need energy to cope with the situation and regain homeostasis. A long-term elevation of corticosterone (the so-called stress level), on the other hand, which is reached during chronically stressful conditions, can interrupt any kind of reproductive behavior and instead support behavior linked to self-maintenance and survival (Wingfield et al. 1998). Baseline corticosterone is also involved in regulating parental care, as slightly elevated levels are linked to a higher feeding rate of young (Doody et al. 2008), a better growth rate for chicks (Bonier et al. 2009; Crossin et

al. 2012), and ultimately higher reproductive success (Bonier et al. 2011; Ouyang et al. 2011).

Parental care is dominantly controlled by prolactin, a pituitary hormone, which rises around egg laying and facilitates incubation (Buntin 1996) and brooding, feeding, or defending the young (Wang and Buntin 1999). Stimuli from eggs or chicks are often necessary to maintain high prolactin values (Buntin 1996). After hatching, prolactin concentration typically declines, although the temporal pattern is species dependent: the decrease is much faster in precocial birds than in altricial ones, where parents have to care for their young over a longer period (Safrieli 1971; Gratto-Trevor et al. 1990). Another parameter affecting prolactin concentration is body mass: if it deteriorates considerably, prolactin concentration decreases (Groscolas et al. 2008; Spee et al. 2010), and birds become less attentive to the brood (Wang and Buntin 1999). Long-term fasting with a severe drop in body mass is also linked to a rise in corticosterone to stress level (Groscolas et al. 2008; Spee et al. 2010) and is suggested to be involved in the redirection of behavior away from reproduction and toward self-maintenance and survival (Wingfield et al. 1998). However, a rise in corticosterone concentration alone is not enough to induce brood abandonment; only a concomitant decrease in prolactin is the trigger for such behavior (Spee et al. 2010). Mass loss and nutritional state are also linked to a change in blood metabolites: the level of β -hydroxy-butyrate, a ketone body, is one very useful parameter for assessing the nutritional state (Jenni-Eiermann and Jenni 1994; Hollmén et al. 2001; Artacho et al. 2007). During fasting, it is synthesized from free fatty acids and its concentration rises. Ketone bodies replace glucose, especially in the brain (Boismenu et al. 1992; Totzke et al. 1999), to immediately provide energy.

The common tern *Sterna hirundo* is a suitable model species to investigate the effect of energy constraints on baseline prolactin and corticosterone values and investigate their effects on breeding behavior, because common terns are small, long-lived seabirds with an energetically expensive foraging method and minor energy reserves (Pearson 1968). The low energy margin implies that comparably small mass losses might lead to a change in blood physiology (Seaman et al. 2005) and finally in behavior. Birds face periods of fasting due to food shortage or unpredictable weather conditions throughout the year. However, feeding is especially constrained during reproduction, when foraging competes with incubation duties so that individuals cannot avoid fasting. In common terns, this time/energy constraint becomes even more pronounced after hatching, when parents have to guard and feed their chicks. During this period, optimal parental behavior is crucial for high fitness, and prolactin and corticosterone values might be two parameters regulating and adapting parental behavior.

Against this background, we measured baseline prolactin and corticosterone in incubating common terns in two steps: (1) we sampled individual terns once in satiated condition (immediately after a foraging trip) and again after they had fasted 2–3 h on the nest during incubation (normally another incubation bout). We assumed prolactin to decrease during fast-

ing, as was shown by Criscuolo et al. (2006) and Spee et al. (2010). Baseline corticosterone, on the other hand, should increase with advancing mass loss (Kitaysky et al. 1999), as should ketone bodies (Totzke et al. 1999; Hollmén et al. 2001). (2) We sampled experienced and inexperienced common tern pairs three times during their individual breeding period, and we expected a clear hormonal change after hatching of chicks, as this is the most demanding period during reproduction (Wendeln and Becker 1996) and parents have different duties to fulfill. Corticosterone concentration should increase, especially in males, as they are mainly responsible for feeding the young during the first days, and prolactin is suggested to decrease slightly because of the changed parental duties (feeding instead of incubation) and stress for the parents. We assume that an increase in corticosterone and a decrease in prolactin concentration are more pronounced in inexperienced terns, as it is harder for them to cope with the demands of reproduction (Angelier et al. 2007b; Limmer and Becker 2007, 2010). The strength of our study results from the combination of detailed life-history data of sampled individuals with an effective method of noninvasive and repeated blood sampling under different conditions. Furthermore, we were able to take blood samples noninvasively from the same individual repeatedly during incubation and brooding.

Material and Methods

Study Population and Fieldwork

Our study was carried out in a common tern colony in northwestern Germany between 2006 and 2009. Since 1984, the terns have been using six artificial islands (11 m \times 5 m) in the lake Banter See as breeding sites. Each island is surrounded by a low concrete wall to prevent flooding of the breeding site and terrestrial predators from entering the colony. The walls are equipped with a total of 44 resting boxes, which are regularly used by the terns and are equipped with special antennas. These are necessary to identify adult and subadult common terns (see Wendeln and Becker 1996), as all fledglings have been individually marked with a passive microchip (TROVAN ID 100 transponder) since 1992. During the first 4 yr, 101 adults were caught on the nest and also fitted with such a transponder. The transponders have a unique alphanumeric code that allows lifelong identification of each bird in the colony. Nearly half of the resting boxes are also equipped with a scale (Sartorius TE6100, accuracy \pm 1 g), which records the weight of birds—in addition to the transponder code and time of day—regularly and remotely while they are sitting on the box.

Every other day the colony is checked, and new nests are marked, eggs are measured, laying and hatching dates are recorded, and newly hatched chicks are ringed (Wagener 1998). During incubation, each nest is equipped with a mobile antenna for 1 or 2 d to identify the pairmates and assign nest parameters and reproductive success to the individuals.

Blood Sampling

We used starved third-stage larvae of blood-sucking bugs (*Dipetalogaster maxima*) to take blood samples from incubating common terns. All intended clutches were equipped with a mobile antenna before sampling to identify the incubating bird. After the target bird was identified on the nest, the clutch was replaced with artificial eggs, one containing the bug (for further details, see Becker et al. 2006; Bauch et al. 2010; Riechert et al. 2012). After 20–30 min, the bugs had usually finished their meal, and the blood was extracted into a syringe. In one drop of blood we measured the ketone body level (to the nearest 0.1 mmol/L; Precision Xceed, MediSense). The common terns are used to researcher activities within the colony and immediately continued to incubate after an examiner passed the nest. Putting the bug egg into the nest did not alter baseline corticosterone levels (Arnold et al. 2008). During all sampling seasons, the bugs succeeded in taking enough blood in more than 80% of trials. This method was successfully validated for analyzing baseline prolactin (Riechert et al. 2012) and corticosterone (Arnold et al. 2008) in common terns as well as other physiological parameters in birds and mammals (Bauch et al. 2010; Voigt et al. 2003, 2004, 2005). Within 4–5 h after extraction, the blood was centrifuged, and the plasma was kept frozen until analysis in the lab.

Hormone Assay

Baseline concentrations of prolactin and corticosterone were determined at the Centre d'Etudes Biologiques de Chizé, France. The plasma level of prolactin was measured twice in each sample by a heterologous radioimmunoassay (RIA), as validated for the same species and same study site (Riechert et al. 2012). Prolactin concentrations of common terns resulted in a dose-dependent response curve parallel to that of standard chicken plasma (Riechert et al. 2012; personal communication, A. F. Parlow, UCLA Medical Center, Los Angeles). Baseline corticosterone level was analyzed in the remaining plasma (usually 25 μ L) via RIA, as described in Lormée et al. (2003). The minimal detectable corticosterone level was 0.4 ng/mL (lowest measurement: 1.26 ng/mL). Intra- and interassay variation for baseline prolactin and corticosterone was within a range of 6%–12% ($n = 4$ duplicates for intra-assay variation).

Sampling Schedule

Sampling in Hungry and Satiated States. During 2008 and 2009, a total of 50 pairs were sampled in hungry and satiated states (both samples were taken in the same individuals but in different incubation bouts). Therefore, we installed a scale combined with a mobile antenna under the respective nests for a few days. On the scale, which was hidden in a plastic box, we fixed a styrofoam block with a scrape for the nest (Becker and Frank 1990). Drain holes in the scrape pipe off the water through the plastic box into the ground. To ensure that we got the real mass of the incubating bird, the empty scale was tared

repeatedly during the day. The mean empty weight of the scale was automatically subtracted from the weight of a common tern by a computer program after the bird entered or before it left the scale.

The common terns accepted the distinct modification of the nest, and no bird stopped incubation. The scale was left under the nest for 2–5 d, until the hungry and satiated samples of both pairmates were taken. This time period also gave us the possibility to investigate the weight fluctuation in every individual and to define the individual body weight in the hungry and satiated states. For the satiated sample, we put the bug into the nest shortly after the bird had returned from a foraging trip and had released the partner from incubating. From that point it was an average of 23 min until the middle of the sampling process. For the hungry sample, we put the bug into the nest near the expected end of the incubation bout (on average, after 160 min of incubation; table 1). In common terns, both pairmates share incubation, and one foraging flight lasts 2–3 h on average (Frank and Becker 1992). During the incubation bouts, breeding birds lost about 14 g of body mass (table 1; see also Frank and Becker 1992 and Becker et al. 1997). Following our sampling schedule, individuals in the satiated state were sampled after an incubation interval significantly shorter than that during the hungry state ($Z = -6.154$, $P < 0.001$, $N = 50$; see table 1). Within the satiated state, the terns started their incubation bout with a body mass higher than that during the hungry sampling process ($t = -5.991$, $P < 0.001$, $N = 50$; see table 1), because we chose an incubation bout when they arrived quite heavy after foraging to ensure that they had fed adequately.

We tried to sample both pairmates, but in four cases we sampled only one member, as the other was an unknown bird that could not be detected by the electronic system (2008: two unknown males, one unknown female; 2009: one unknown female). Neither baseline prolactin and corticosterone nor ketone body levels between pairmates were significantly related within the hungry or satiated state ($-0.3 < r < 0.2$, $P > 0.2$ in every case, $N = 22$ or 23). We were not able to take both samples of every individual on the same day (often pairmates changed before we finished sampling), and in only four cases did we take both samples during the same incubation bout. In 35 cases we took both samples at the same day, in 10 cases we

Table 1: Difference in mass and incubation bout length within one individual common tern in hungry and satiated states

Variable	Satiated	Hungry	<i>P</i>	<i>N</i>
Mass at incubation start (g)	139.4 \pm 9.1	132.0 \pm 7.7	<.001	50
Mass at sampling start (g)	138.6 \pm 9.1	123.5 \pm 6.8	<.001	50
Incubation bout (min)	23 \pm 8	160 \pm 130	<.001	50

Note. Incubation bout refers to the time interval from the start of the incubation bout until the middle of sampling. *P* values are derived from a paired *t*-test; significant *P* values are in boldface. *N* denotes sample size.

took the satiated sample first and the hungry sample 1 or 2 d later, and in 5 cases we took the hungry sample first. However, the difference in hormone values between the hungry and satiated samples was not affected by the time (0–2 d) elapsed between taking the samples (prolactin: $F_{2,49} = 0.115$, $P = 0.861$; corticosterone: $F_{2,49} = 2.244$, $P = 0.118$).

Blood samples were taken throughout the whole day (5:37 a.m.–8:23 p.m.). However, sampling time in the hungry and satiated states did not differ within an individual (paired t -test: $t = -0.608$, $P = 0.546$, $N = 50$) but was positively correlated ($r = 0.269$, $P = 0.059$).

The birds were sampled 7–13 d after respective clutch completion. The day of sampling after clutch completion did not differ between the two samples (hungry vs. satiated: 11.0 ± 2.3 vs. 10.8 ± 2.3 , $t_{49} = 1.241$, $P = 0.220$), and in neither the hungry nor the satiated state did incubation duration until sampling affect baseline prolactin or corticosterone values (hungry: prolactin: $r = -0.239$, $P = 0.101$, $N = 49$; corticosterone: $r = 0.006$, $P = 0.966$, $N = 49$; satiated: prolactin: $r = 0.118$, $P = 0.439$, $N = 49$; corticosterone: $r = 0.125$, $P = 0.388$, $N = 50$).

Repeated Sampling during Incubation. Between 2006 and 2009, we sampled 57 breeding pairs repeatedly over the course of breeding. Blood samples were always taken in the early morning, between 5:00 and 10:00 a.m. However, prolactin values slightly but significantly increased with advancing time of day ($r_s = 0.130$, $P = 0.024$, $N = 304$), whereas baseline corticosterone levels decreased ($r_s = -0.142$, $P = 0.015$, $N = 292$). We sampled both pairmates three times over the course of breeding: during early incubation (2–6 d after clutch completion), in midincubation (9–14 d after clutch completion), and during early chick rearing (0–3 d after hatching of the first chick). During the late sampling phase, we took the chicks out of the nest and kept them warm to prevent the bug from sucking blood from the young. Within the first and second sampling periods, baseline prolactin and corticosterone levels were not related to the day of sampling after clutch completion (early phase: $r < 0.1$, $P > 0.4$, $N = 102$ for both hormones; midphase: $r < 0.2$, $P > 0.05$, $N = 108$ for both hormones). However, during the late sampling phase, prolactin concentration was negatively related to the number of days elapsed after hatching of young ($r = -0.253$, $P = 0.015$, $N = 92$), whereas baseline corticosterone level increased more the later a bird had been sampled after hatching of the first chick ($r = 0.217$, $P = 0.043$, $N = 87$).

We selected two groups of pairmates according to their age and breeding experience, because we know from former studies that body mass (Limmer and Becker 2007) and reproductive success (Nisbet et al. 2002; Ezard et al. 2007; Limmer and Becker 2010; Rebke et al. 2010) improve with age in common terns. The first group of sampled pairs contained inexperienced birds (3–6 yr old, 0–3 previous breeding attempts) and the second group experienced pairmates (10–20 yr old, 7–17 previous breeding attempts). No bird was sampled in different seasons. We mostly chose pairs in which both partners were marked

and of known experience; however, we sampled some pairs with one unknown pairmate (6 in 2006, 7 in 2007, 5 in 2008, 4 in 2009). Nevertheless, these pairs were included in the groups because it is known that common terns mate assortatively with partners of the same age (Ludwigs and Becker 2005; Ludwig and Becker 2008). Furthermore, within the pairs where both mates were known, age and breeding experience did not differ between pairmates (age: $t = 0.119$, $P = 0.907$, $N = 33$; experience: $t = -0.357$, $P = 0.723$, $N = 33$); the same was true for the total number of known pairs between 2006 and 2009 in the colony ($-0.9 < Z < -0.1$, $P > 0.3$ in every case, $64 < N < 99$).

Definitions and Statistical Analyses

We used a paired t -test to analyze the differences in mass at incubation start, mass at sampling start, mass loss before sampling, and length of an incubation bout, as well as baseline prolactin, corticosterone, and ketone body levels, between the hungry and satiated states. By Pearson correlation we related incubation bout length and mass loss in hungry and satiated birds. This test was also used to correlate incubation bout length, mass at incubation start, mass at sampling start, and mass loss with baseline prolactin, corticosterone, and ketone body levels in hungry and satiated terns. These three physiological parameters were also related to each other.

With a general linear model for repeated measurements we analyzed the change in prolactin, corticosterone, and ketone bodies (within-subject effects) within individuals in the hungry and satiated states. Sex and year were introduced as fixed factors and breeding experience (number of previous breeding attempts, 0–17) and time at sampling for hungry and satiated states as covariates. In addition, we included the interaction term between fixed factors. We used Bonferroni corrections to compare main effects, and we selected type III sum of squares.

To investigate the change in hormones over the course of incubation, we used a linear mixed-effects model (LME) with restricted maximum likelihood estimation, because it could handle repeated and unbalanced data (we were not able to analyze both hormones in all three samples of every individual because of the small amount of blood). Baseline prolactin or corticosterone was entered as the dependent variable, and sex, year, sampling phase (early and midincubation, early chick rearing), and breeding experience (inexperienced vs. experienced; see above), as well as all two-way interaction terms, were entered as fixed factors. Bird identity nested in nest number was used as a random effect to account for the fact that we sampled both pairmates three times during breeding. We used the random slope to model the random effect and the type III sum of squares, and main effects were compared by Bonferroni corrections. We used a stepwise backward approach to eliminate insignificant terms from the global model by considering their P values. In case of a significant interaction term, the main effects were left in the model even if they were not significant.

Via an unpaired t -test, we compared corticosterone values after hatching between males and females among experienced

and inexperienced individuals. Using a regression analysis, we investigated the change of corticosterone values over the incubation period in experienced and inexperienced terns separately. By Pearson correlation, we related prolactin and corticosterone values of both pairmates during the early, middle, and late incubation periods.

All statistical analyzes were two-tailed, and results were expressed as mean \pm 1 SD (graphs with mean \pm 1 SE). The level of significance was set to $P \leq 0.05$, and $0.05 < P < 0.1$ was defined as marginally significant. All analyses were performed with SPSS 18 or 20.

Results

Nutritional State

Body mass and change in hormones and ketone bodies. Individuals in the satiated state were sampled with a significantly higher body mass than those in the hungry state (table 1). From the start of incubation until the middle of sampling, satiated birds lost significantly more body mass per hour than hungry ones (table 2).

Prolactin level after feeding was significantly higher than that after fasting until the end of an incubation bout ($T = -2.596$; table 2). Ketone bodies and corticosterone level were marginally higher in hungry birds (ketone bodies: $T = 1.864$; corticosterone: $T = 1.746$; table 2). When birds were satiated, we found no correlation between incubation bout length, mass at sampling, or mass loss and prolactin, corticosterone, or ketone body values ($-0.243 < r < 0.002$, $P > 0.1$ in every case, $N = 49$ or 50). On the contrary, if terns had incubated for 2 or 3 h, prolactin level was significantly and positively linked to mass at sampling ($r = 0.356$, $P = 0.012$, $N = 49$). Neither corticosterone nor ketone body values were significantly correlated with the above-mentioned parameters ($-0.149 < r < 0.098$, $P > 0.4$ in every case, $N = 49$ or 50). In addition, prolactin, corticosterone, and ketone body levels were not significantly related to each other in either the hungry or the satiated state ($-0.1 < r < 0.2$, $P > 0.1$ in every case, $N = 48$ –50).

The model integrating other factors (sex, year, sampling time, and breeding experience) showed a significant influence of the birds' state (within-subject effect) on baseline prolactin level (table 3). Terns in the satiated state had higher prolactin values than those in the hungry state (see also fig. 1a). Sex and breeding experience, on the other hand, did not affect the change

in prolactin between the hungry and satiated states (table 3). However, prolactin value was influenced by year of sampling, as the level in 2009 was significantly higher than that in 2008 ($F_{1,48} = 3.231$, $P = 0.046$).

Baseline corticosterone was affected by nutritional state: we found slightly higher values in hungry birds (fig. 1b; table 3). In addition, baseline corticosterone was influenced by sampling time within the hungry sample, with higher values earlier in the day ($r = -0.323$, $P = 0.023$, $N = 49$), and by the interaction term sex \times year. Females sampled in 2009 had significantly higher corticosterone values in the hungry state than when satiated (7.0 ± 3.8 vs. 3.6 ± 0.9 ng/mL, $t = 2.673$, $P = 0.026$, $N = 10$), whereas females sampled in 2008 and males in both years had comparable values (females, 2008: 5.5 ± 1.7 vs. 5.8 ± 2.7 ng/mL, $t = -0.385$, $P = 0.706$, $N = 14$; males, 2008: 5.5 ± 1.6 vs. 4.4 ± 2.4 ng/mL, $t = 1.280$, $P = 0.225$, $N = 13$; males, 2009: 5.5 ± 2.2 vs. 5.5 ± 2.1 ng/mL, $t = -0.091$, $P = 0.937$, $N = 12$). In the case of ketone body level, the state turned out to be marginally significant, with slightly higher values in hungry birds (table 3; fig. 1c). All other analyzed parameters had no significant influence on the change in ketone body values from the hungry to the satiated state.

Hormonal Change over the Course of Incubation

The baseline concentration of prolactin changed significantly over the course of incubation (table 4): the prolactin level dropped significantly after hatching (post hoc tests: early vs. late: $P = 0.002$; middle vs. late: $P < 0.001$, fig. 2). The decrease was much more pronounced in inexperienced birds (fig. 2), which had lower prolactin values than experienced pairs (table 4). Baseline prolactin level was additionally influenced by the year of sampling (table 4). We found significantly lower values in 2006 than in 2007 (159 ± 44 vs. 188 ± 64 ng/mL, $P = 0.015$) and 2008 (159 ± 44 vs. 215 ± 44 ng/mL, $P < 0.001$). Furthermore, baseline prolactin was affected by the interactions experience \times year and experience \times phase (table 4).

In the case of baseline corticosterone, the LME revealed that males had higher values than females (table 4). This was most pronounced after hatching (fig. 3), when both inexperienced and experienced males had higher corticosterone levels than their female pairmates (inexperienced: $t = 2.917$, $P = 0.006$; experienced: $t = 2.565$, $P = 0.014$). The incubation phase had

Table 2: Difference in physiological parameters and mass loss within an individual common tern in hungry and satiated states

Variable	Satiated	Hungry	<i>P</i>	<i>N</i>
Mass loss (g/h)	7.1 \pm 4.4	4.8 \pm 3.3	.003	50
Prolactin (ng/mL)	215.3 \pm 62.2	186.7 \pm 50.8	.013	48
Corticosterone (ng/mL)	4.9 \pm 2.3	5.8 \pm 2.4	.087	49
Ketone bodies (mmol/L)	1.3 \pm .4	1.5 \pm .7	.068	50

Note. Mass loss per hour refers to the time interval from the start of an incubation bout until the middle of sampling. *P* values are derived from a paired *t*-test; significant *P* values are in boldface. *N* denotes sample size.

Table 3: Outcome of the general linear model for repeated measurements analyzing the change in prolactin, corticosterone, and ketone bodies within an individual common tern

Explanatory variable	Prolactin			Corticosterone			Ketone bodies		
	<i>F</i>	<i>df</i>	<i>P</i>	<i>F</i>	<i>df</i>	<i>P</i>	<i>F</i>	<i>df</i>	<i>P</i>
Sex	.038	1	.847	2.642	1	.112	.080	1	.779
Year	3.182	1	.082	.029	1	.866	1.987	1	.166
Breeding experience	.125	1	.725	1.595	1	.214	.353	1	.555
Nutritional state	6.975	1	.012	4.638	1	.037	2.645	1	.108
Sampling time (hungry)	1.933	1	.172	5.022	1	.030	1.309	1	.259
Sampling time (satiated)	.160	1	.692	.020	1	.889	.320	1	.575
Year × sex	.160	1	.691	8.426	1	.006	1.386	1	.246

Note. We present only the within-subject effects (influence of analyzed variables on the hormone/ketone body difference) in this table. Any significant between-subject effects (influence on the mean hormone/ketone body value) are mentioned in the text. Breeding experience is defined as the number of previous years of breeding, nutritional state refers to hungry or satiated, and sampling time refers to the time elapsed from the beginning of the incubation bout until the middle of sampling. Significant *P* values are in boldface.

no significant effect on the corticosterone concentration (table 4), but we found a significant interaction of sex × phase (table 4). Females showed quite stable values, whereas males exhibited increased values after hatching (fig. 3). Corticosterone concentration was also influenced by the year of sampling, but in the direction opposite that for prolactin (table 4): the mean concentration in 2006 was significantly higher than that in 2007 (7.4 ± 3.4 vs. 5.9 ± 3.1 ng/mL, $P = 0.031$).

The nested term nest(idcode) turned out to be not significant in both models ($P > 0.3$ in both cases). Prolactin values of pairmates were not significantly correlated during the early and middle sampling periods ($r < 0.2$, $P > 0.4$ in both cases, $N = 51$ and 46 , respectively). After hatching of chicks, however, we found a significant positive correlation of prolactin concentration between pairmates ($r = 0.439$, $P = 0.005$, $N = 39$). In the case of corticosterone, on the other hand, the values for pairmates were not significantly related to each other during any sampling period ($r < 0.3$, $P > 0.1$ in all cases, $N = 42$, 48 , and 37 in early, middle, and late phases, respectively).

Discussion

Within the annual cycle, the breeding period is suggested to be the most energy-demanding phase for birds (e.g., Monaghan et al. 1998). In common terns, both parents engage in incubation and rearing of young, and different requirements are demanded: the males have to provide the females with enough fish to enable them to produce eggs (Wendeln et al. 2000), which compose a considerable amount of a females' body mass and lower their own reserves (Bauch et al. 2010). During incubation, which is shared by both pairmates, they fast for 2–3 h (Becker and Frank 1990; Frank and Becker 1992), which affects body mass, and the switch to the catabolic state is reflected quickly in blood metabolites in such small birds (Jenni-Eiermann and Jenni 1994; Seaman et al. 2005). However, the most energy-constrained period is after hatching, when parents have to deliver enough food for two or three chicks. This high demand leads to malnutrition in parents and is reflected by a

severe drop in body mass (Wendeln and Becker 1996). We investigated hormones and ketone bodies in relation to the nutritional state (hungry vs. satiated) during incubation, as well as during the advancing breeding period (early and midincubation and early rearing period). We expected low prolactin and increased corticosterone and ketone bodies in hungry birds, especially after hatching of young, as these are probably the most energy-demanding phases.

Nutritional State

Common terns are quite small seabirds with minor energy reserves, and they work near their limit of energy expenditure during breeding (Pearson 1968); therefore, even comparatively small mass losses could alter their energy allocation and affect circulating prolactin or corticosterone values. We found a mean decline of 8.5 g in body mass over an incubation shift (2.5 h) until sampling, which is comparable to the results of Becker and Frank (1990) in common terns. The mean body mass difference in an individual tern between the hungry and satiated states at the beginning of sampling was 13.1 g, which is in accordance with an average food intake of 13.2–15.8 g by common terns during a feeding flight (Frank and Becker 1992).

Hormone Values. In line with our expectations, common terns had significantly higher baseline prolactin values after refeeding than after fasting for 2–3 h. Horses, too, had increased prolactin levels after feeding (Nadal et al. 1997), and Criscuolo et al. (2001) suggested that common eiders (*Somateria mollissima*) with a prolonged incubation period still exhibited high prolactin values due to refeeding. Accordingly, in black-browed albatrosses, baseline prolactin was negatively related to time spent fasting on the nest (Angelier et al. 2007b). High prolactin values are necessary for expressing parental behavior (Buntin 1996), and a decrease in prolactin concentration can lead to a disruption of incubation behavior (Wang and Buntin 1999) and ultimately trigger brood abandonment (Angelier et al. 2009);

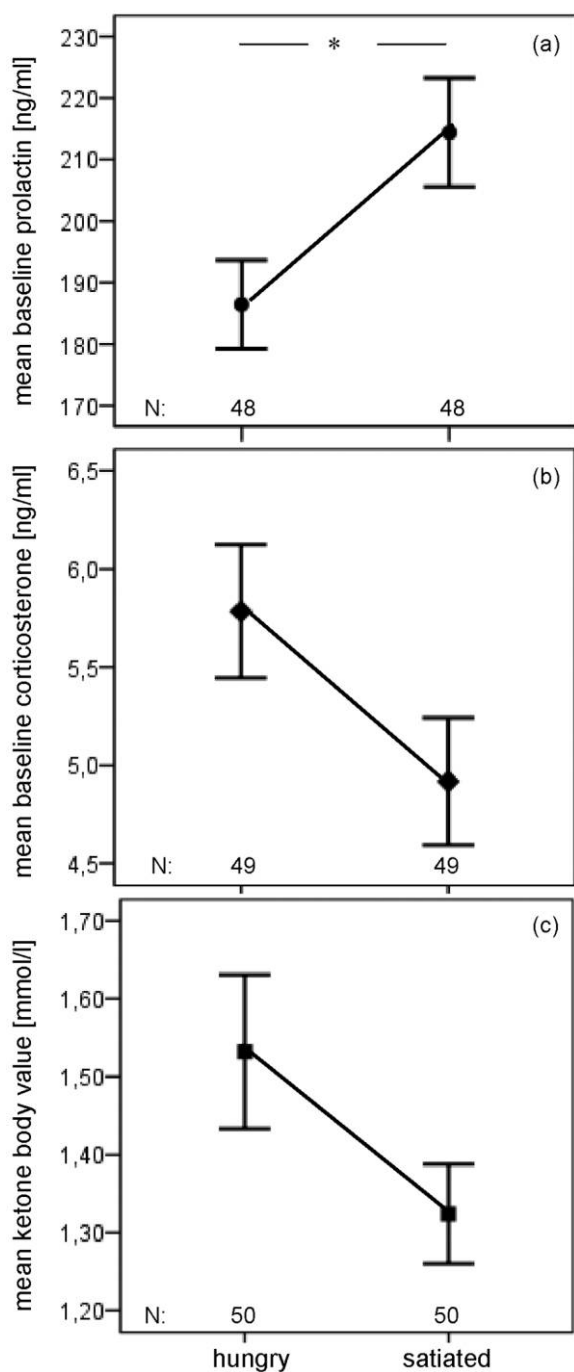


Figure 1. Mean (± 1 SE) levels of baseline prolactin (ng/mL; a), corticosterone (ng/mL; b), and ketone bodies (mmol/L; c) measured within an individual in hungry and satiated states. A significant difference (derived by a *t*-test) is marked by an asterisk. Sample size *N* is presented at the bottom of each panel.

Spee et al. 2010). However, even though prolactin significantly decreased, we are convinced that the levels in terns were high enough for sufficient parental care, supported by the fact that no bird abandoned its clutch after sampling. In line with this suggestion is the result from Riechert et al. (2013) that common

terns exhibited lower baseline prolactin values during re-nesting (another energy-demanding period) but with no effect on behavior or breeding success. These still-elevated prolactin levels, even after fasting, are involved in the regulation of behavior, because high levels are necessary to stay with the clutch or brood and provide parental care instead of leaving to refeed.

In satiated terns, baseline prolactin was not linked to incubation bout length, mass at sampling, or mass loss. In common eiders, too, Criscuolo et al. (2001) did not detect a relation between body mass and prolactin concentration at the beginning or end of incubation. But in contrast to common terns, common eiders are capital breeders, which rely completely on accumulated body reserves during incubation and can lose a large amount of reserves without threatening their reproduction or survival (Cherel et al. 1988). In Gould's petrels (*Pagodroma leucoptera*), O'Dwyer et al. (2006) did not detect a relation between body mass and prolactin value during the first incubation bout but found decreased prolactin values linked with higher body mass loss during the second bout, when petrels were more energy depleted. Accordingly, Criscuolo et al. (2003) detected a relationship between prolactin level and body mass only at hatching, when even common eiders were more energy constrained than during early incubation. Consistently, when terns had fasted for 2 or 3 h, the prolactin level was positively linked to mass at sampling. This confirmed that in terns, which are much smaller birds with a lower energy margin, comparatively small mass losses can affect blood physiology much earlier than in larger species. Common eiders, for example, can lose 30%–40% of their initial mass during incubation (Parker and Holm 1990), affecting prolactin values only at the end, when chicks hatched.

As expected, decreasing prolactin values in hungry common terns were accompanied by slightly but insignificantly increased baseline corticosterone values. Adélie penguins (*Pygoscelis adeliae*), too, had higher baseline corticosterone levels just before leaving the colony to feed after an incubation bout (Angelier et al. 2008). Moreover, herring gulls (*Larus argentatus*) showed increased corticosterone after some days of fasting (Totzke et

Table 4: Result of the linear mixed-effects model analyzing baseline prolactin and corticosterone over the course of breeding in common terns

Explanatory term	Prolactin			Corticosterone		
	<i>F</i>	<i>df</i>	<i>P</i>	<i>F</i>	<i>df</i>	<i>P</i>
Experience	6.643	1	.011			
Phase	13.787	2	<.001	.026	2	.974
Year	8.050	3	<.001	4.609	3	.004
Sex				8.979	1	.003
Experience \times phase	4.965	2	.008			
Experience \times year	3.872	3	.012			
Sex \times phase				3.695	2	.027

Note. "Experience" refers to inexperienced and experienced pairs and "phase" to early and midincubation and early chick rearing. Presented are all main effects and interaction terms of the last step of the model. Significant *P* values are in boldface. Pair identity was included as a random effect.

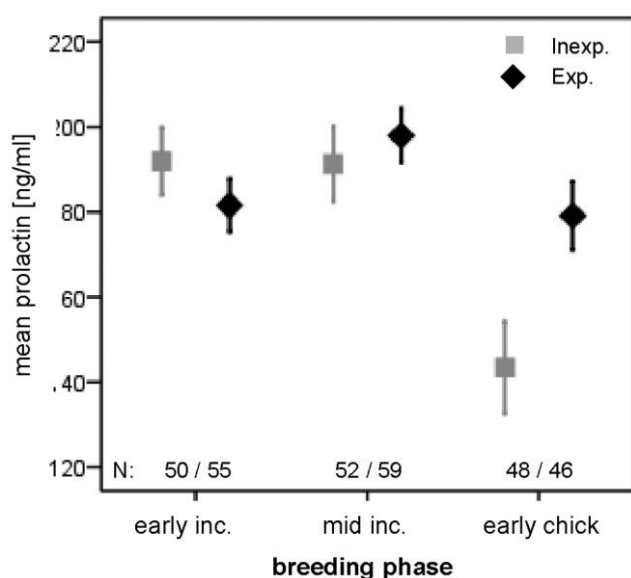


Figure 2. Mean (± 1 SE) baseline prolactin (ng/mL) measured within individual common tern pairs over the course of breeding (early incubation: 2–5 d after clutch completion; midincubation: 9–14 d after clutch completion; early chick rearing: 0–3 d after hatching). We sampled inexperienced (inexp.; gray squares, 0–3 previous breeding attempts) and experienced (exp.: black diamonds, 7–17 previous breeding attempts) birds. Sample size N is presented at the bottom of the figure.

al. 1999). A long-term elevation of corticosterone concentration up to stress level, on the other hand, can redirect energy allocation from parental duties to self-maintenance and survival (Wingfield et al. 1998), which become especially important under unfavorable conditions in long-lived species. However, as we did not find a relation between corticosterone concentration and body mass or mass loss, we suggest that the sampled terns were still in good condition after an incubation bout and were not energy constrained (see also Riechert et al. 2012).

In neither the hungry nor the satiated state did we find a correlation between baseline corticosterone and body mass at sampling or mass loss during the incubation bout. Accordingly, in Adélie penguins Angelier et al. (2008) did not detect a relation between body mass and corticosterone value before or after a foraging trip. The low baseline corticosterone level and the lack of a connection with body mass further support the suggestion that terns were still in good condition after fasting, without a need to regulate behavior by increased corticosterone values.

Ketone Body Level. Individual terns in the hungry state exhibited a mean increase of 15% in ketone bodies, compared to the same individuals in the satiated state. Accordingly, herring gulls also had elevated ketone body values after fasting (Totzke et al. 1999), as did garden warblers *Sylvia borin* (Jenni-Eiermann and Jenni 1994) and common eiders (Hollmén et al. 2001). However, the comparatively small increase measured in our study suggested that fasting had only just started in common

terns and that glucose had not yet been replaced by ketone bodies in the brain, in contrast to other species, which fasted for several days before this pronounced increase in ketone body concentration (Boismenu et al. 1992; Hollmén et al. 2001). This suggestion was further supported by the fact that we did not find a correlation between ketone bodies and body mass in hungry or satiated terns, in contrast to energy-constrained birds, which showed higher ketone body values with increasing mass loss (Jenni-Eiermann and Jenni 1994; Hollmén et al. 2001; Artacho et al. 2007).

Hormones over the Course of Incubation

Baseline prolactin is directly linked to the amount of incubation behavior and parental care. In species with biparental care, males and females do not differ in their prolactin level (Gratto Trevor et al. 1990; Silverin and Goldsmith 1990), but if only one parent incubates or cares for chicks, that bird clearly has higher baseline prolactin values (Gratto Trevor et al. 1990; Schmid et al. 2011). In common terns, both parents share incubation, and consequently we found no difference in prolactin values between males and females. Interestingly, prolactin concentration dropped significantly after hatching, but mainly in inexperienced individuals. Criscuolo et al. (2001) also found decreased prolactin values in common eiders after hatching. Nevertheless, the concentrations in both species were clearly above the prolactin level in nonbreeding individuals, whereas prolactin dropped completely back to prebreeding levels after hatching in mallards *Anas platyrhynchos* (Hall and Goldsmith

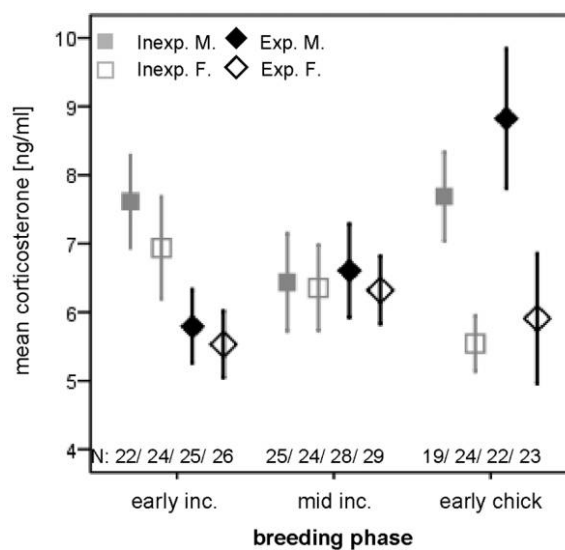


Figure 3. Mean (± 1 SE) baseline corticosterone (ng/mL) over the course of breeding (early incubation, midincubation, and early chick rearing) measured in inexperienced males (inexp. m.: filled gray squares), inexperienced females (inexp. f.: open gray squares), experienced males (exp. m.: filled black diamonds), and experienced females (exp. f.: open black diamonds). Sample size N is presented at the bottom of the figure.

1983) and turkey hens *Meleagris gallopavo* (Opel and Proudman 1980). But those species are precocial, whereas terns are semi-altricial and parents have to brood the chicks for the first 3 or 4 d, until they are thermally independent (Becker and Ludwigs 2004). In semipalmated sandpipers (*Calidris pusilla*), the chicks are brooded for at least a week (Safriel 1971) and prolactin values remained elevated (Gratto-Trevor et al. 1990). In the Eurasian hoopoe *Upupa epops*, females brood young chicks for several days, and accordingly, Schmid et al. (2011) detected gradually declining prolactin values after hatching. However, common terns brood their chicks for only a few days, and therefore we suggest that prolactin decreases soon after hatching, as confirmed by the negative relationship between prolactin concentration and the time elapsed from hatching until sampling. One could argue that prolactin starts to decrease earlier in males than in females, because they are mainly responsible for feeding the young during the first days after hatching while the mother stays with the chicks and because stimuli from eggs or chicks are necessary to maintain high prolactin levels (Buntin 1996). On the other hand, common terns care for their chicks over a long period even after their independence. Therefore, we suggest that elevated values in fathers are necessary to ensure that they return to their young after foraging. Furthermore, prolactin even enhances feeding in ring doves (*Streptopelia risoria*; Koch et al. 2002), and therefore a moderately elevated level after hatching could be important for a high success rate.

Inexperienced terns showed reduced prolactin concentrations during incubation (Riechert et al. 2012), which became most visible after hatching in this study. Perhaps a decreased prolactin level is linked to the lower body mass of young birds (Limmer and Becker 2007), as breeding is more stressful for these inexperienced individuals and they are poor foragers (Limmer and Becker 2009). Accordingly, in the hoopoe, baseline prolactin was positively related to body mass (Schmid et al. 2011). During the demanding chick phase, common terns provide much energy to their chicks, and inexperienced birds, as poorer foragers, fast more themselves, which leads to lower prolactin values comparable to the results of our fasting approach. Low prolactin could lead to a lower willingness to care for eggs or chicks and ultimately result in the poor breeding success of inexperienced breeders, which showed both reduced hatching (through shell cracking, egg chilling, and finally abandonment of the clutch) and reduced fledging success (Limmer and Becker 2010). In ring doves, inexperienced individuals were less attentive to the nest and did not defend their brood as much as experienced birds (Wang and Buntin 1999). However, in long-lived species this is an adaptive strategy, as young individuals should not spend too much energy on the brood in order not to endanger future reproduction or survival (Wingfield et al. 1998).

Baseline corticosterone was significantly higher in males than in females, especially after hatching. Accordingly, in ring doves, corticosterone is elevated during the first days after hatching, accompanied by high foraging effort and mass loss by parents (Lea et al. 1992; Koch et al. 2002). In contrast to our results, the increase in corticosterone was observed only in females and

not in males. In ring doves, both sexes perform parental care and feed the young, but Lea et al. (1992) could not explain why only females exhibited this increase. However, in common terns, the father is mainly responsible for feeding the young during the first days, while the mother stays with the chicks (Becker and Ludwigs 2004). Elevated corticosterone enhances food intake in ring doves (Koch et al. 2002), and together with the positive effect on foraging activity (Angelier et al. 2007a), supports fathers during the demanding chick-rearing phase. Furthermore, corticosterone implants increased the provisioning rate in kittiwakes *Rissa tridactyla* (Kitaysky et al. 2001), and in a year with low prey availability, common murre (*Uria aalge*) with higher corticosterone concentrations delivered more food to their young (Doody et al. 2008). Our study showed that experienced males that are known to have superior breeding success (Limmer and Becker 2010) had higher corticosterone values after hatching than inexperienced males. Combining these results could lead to the suggestion that one factor for the better success of older pairs is a higher feeding rate, supported by elevated baseline corticosterone.

In contrast to that in the late incubation stage, however, baseline corticosterone was elevated during the early incubation phase in inexperienced terns. We suggest that the courtship period is more demanding for inexperienced males, as they are inferior foragers (Limmer and Becker 2009), and that young females need more time to refill their body reserves, as they exhibit lower cholesterol concentrations than older birds after egg laying (Bauch et al. 2010). Following the “energy mobilization hypothesis” (Romero 2002), this could lead to slightly higher baseline corticosterone level in order to prepare the birds for increased foraging activity.

We found a clear year effect in both hormone values, as was found for prolactin concentration in Wilson’s phalaropes (Delehanty et al. 1997) and wandering albatrosses (*Diomedea exulans*; Angelier et al. 2006). Baseline corticosterone is also known to vary between years (Doody et al. 2008), mainly because of the relation with food availability. Common murre, for example, exhibited higher values in years with low food abundance (Kitaysky et al. 2007; Doody et al. 2008). We know from the work of Dänhardt and Becker (2011) that availability of herring and sprat, the main food of common terns breeding at Banter See, varies considerably between years and influences reproductive performance. Lower prolactin and higher corticosterone levels in 2006 suggest more demanding breeding conditions for terns, which is in accordance with low food abundance and low breeding success (Dänhardt and Becker 2011). Further studies are needed to investigate the link between hormone values, food abundance, and breeding success in common terns.

Conclusion

We showed that changes in baseline prolactin and corticosterone, as well as in ketone bodies, occurred during fasting in common terns but that these changes did not lead to lower parental care, because fasting bouts were too short and did not lead to a severe energy constraint in parents. Despite a reduced

prolactin level and body mass, parents did not leave the nest, as was found in longer-fasting species. Together with insignificantly higher corticosterone and ketone body levels, this indicates good body condition, even after fasting, of terns sampled in our study. After hatching, hormone values changed in accordance with different parental roles: males exhibited elevated baseline corticosterone levels that could support their increased foraging activity and high feeding rate of young, whereas females stayed with the chicks. Inexperienced pairs were able to maintain high prolactin during incubation but showed reduced prolactin values after hatching, when the most stressful period started. This lower prolactin value was probably accompanied by high body mass loss, as inexperienced terns struggle harder because of their lack of experience and lower foraging ability. Our work clearly underlines the involvement of baseline prolactin and corticosterone in regulating breeding behavior in birds.

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