

# Is the additional effort of renesting linked to a hormonal change in the common tern?

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**Abstract** The production of and care for a replacement clutch can bear costs in terms of future reproduction or survival. However, renesting is quite common among seabirds and can contribute considerably to individual fitness. Prolactin and corticosterone are two hormones involved in the mediation of breeding behavior and, as they are linked to body condition or effort, it is of interest if these hormone values change during a second demanding breeding phase within a year. We compared baseline prolactin and corticosterone between the first and the renesting attempt in common terns (*Sterna hirundo*) on individual level. Therefore, in addition to control birds, 37 breeders were sampled during incubation of their first and their replacement clutch in 2008 and 2009. Blood samples were taken non-invasively by blood-sucking bugs. Prolactin level was lower during the renesting period, especially in birds which abandoned their clutch afterwards, whereas corticosterone did not change. Excluding the deserting birds, the reduced prolactin level was not linked to minor success, but could be related to seasonal processes. The control group of late laying common terns showed comparably low prolactin values, but increased corticosterone

concentrations. Renesting individuals exhibited higher prolactin during incubation of their first clutch than non-renesting birds, probably indicating their higher quality. The fact that terns still have relatively high prolactin and low corticosterone values during renesting might confirm their higher quality and suggests that they are able to meet the costs of a second demanding breeding period without being considerably stressed.

**Keywords** Baseline level · Corticosterone · Prolactin · Replacement clutch · Season · *Sterna hirundo*

## Introduction

If birds lose their clutch quite early in the season, pair mates have to decide whether to start a renesting attempt or skip reproduction until the next breeding season. This decision is especially important in species that normally produce only one clutch per season and spend a high level of parental care, as do most seabirds (Spear et al. 1986; Furness and Monaghan 1987). In many monogamous species, the initiation of a replacement clutch bears costs for both sexes: females have to produce additional eggs (Monaghan et al. 1998; Bauch et al. 2010), while males have to engage in a second demanding courtship period (Wendeln et al. 2000). Based on life history theory, many studies suggest a trade-off between current investment in the brood and future survival and reproductive opportunities (reviewed in Lindén and Møller 1989; Stearns 1992). Therefore, the production of a replacement clutch and a second demanding breeding cycle could have negative consequences on fitness and life span of parents. Nevertheless, renesting is quite common in several seabirds (Hipfner et al. 1999; Wendeln et al. 2000; McFarlane

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Tranquilla et al. 2003; Gasparini et al. 2006) and can increase the fitness of an individual considerably (Becker and Zhang 2011).

In most species, the probability to relay declines with the date of loss of the first clutch (Hipfner et al. 1999) due to decreasing food availability (“food supply hypothesis”, e.g., Perrins 1970; Martin 1987) or lower survival and recruitment of later fledged chicks (Verhulst et al. 1995; Verboven and Visser 1998). Therefore, some species reduce their effort: kittiwakes (*Rissa tridactyla*) reduce egg production irrespective of food supplementation (Gasparini et al. 2006) and Brünnich’s guillemots (*Uria lomvia*) produce smaller replacement eggs (Hipfner et al. 1999), whereas European starlings (*Sturnus vulgaris*) show no decline in clutch size or egg mass (Christians et al. 2001). However, many studies report that often only a small proportion of individuals relay and these birds are supposed to be high-quality individuals (Rooneem and Robertson 1997; Hegyi and Sasvári 1998; Wendeln et al. 2000; Arnold et al. 2010; Becker and Zhang 2011), which are able to meet the costs of an additional energy-consuming breeding period without jeopardizing their future survival or reproduction.

Breeding behavior is controlled by hormones, which often link environmental and body condition to reproductive decisions (Wingfield et al. 1998). One hormone crucially important for parental care in birds is prolactin. It is a pituitary hormone, mostly elevated during incubation (Buntin 1996), and high values are necessary for the expression of parental care such as constant incubation or guarding and feeding of chicks (Buntin 1996; Wang and Buntin 1999). Prolactin implants even enhance breeding behavior in non-breeding birds (Wang and Buntin 1999). Especially under stressful or unfavorable conditions, high levels become particularly important for parents to attend the clutch or the brood and not to forage for themselves (Chastel et al. 2005). However sooner or later, prolactin levels decrease with acute stress (Angelier and Chastel 2009; Schmid et al. 2011). After breeding, prolactin returns to basal level in most species (Goldsmith et al. 1984; Hall 1986; Sharp et al. 1988; Chastel and Lormee 2002).

Corticosterone, on the other hand, is the main avian stress hormone and rises immediately after exposure to stress (Romero and Romero 2002; Romero and Reed 2005). A chronically high stress level of corticosterone redirects behavior away from reproduction or territory defense to self-maintenance and survival, resulting in brood abandonment (Wingfield et al. 1998). The adrenocortical stress response is thought to be an adaptive mechanism especially in long-lived species, where adult survival is the main contributing factor to fitness. A slightly elevated baseline level, on the contrary, can be linked to higher foraging activity (Angelier et al. 2006) or

facilitation of energy via gluconeogenesis (Sapolsky et al. 2000). This helps an individual to cope with stressful situations especially in an unpredictable environment. If food availability declines and body mass is reduced, corticosterone increases (Kitaysky et al. 1999, 2007; Jenni-Eiermann et al. 2008) and ultimately prolactin level lowers (Criscuolo et al. 2005, 2006) leading to nest desertion.

We compared baseline prolactin and corticosterone between the first and the replacement clutch in common terns (*Sterna hirundo*) at the individual level. The common tern is a small long-lived seabird and an ideal model species, because it normally lays one clutch per season but is able to replace it in case of loss (Wendeln et al. 2000; Becker and Ludwigs 2004). Approximately, 25 % of birds initiate a replacement clutch after loss of the first one (Becker and Zhang 2011), and both parents invest in incubation and rearing of the brood (Wendeln et al. 2000).

We were able to analyze the change in prolactin and corticosterone from the first to the replacement clutch in a unique study colony, where we had detailed information on breeding experience, reproductive parameters and individual life history. We used longitudinal individual-based data to address the following questions: (1) do baseline prolactin and corticosterone change within an individual from incubation during the first clutch to the reneating attempt? As breeding is probably the most demanding phase during the year and the production and care of a replacement clutch increase it, we would expect lower prolactin and elevated corticosterone. (2) Are hormone values in replacement breeders similar to late laying breeders? Inexperienced birds lay later (Ezard et al. 2007) and have lower prolactin (Riechert et al. 2012). (3) Do replacement breeders differ in hormone values during their first clutch from those that did not reneate after the loss of the first clutch? We would expect that to be the case, as replacement breeders are often high-quality individuals characterized by higher experience and superior reproductive success (Becker and Zhang 2011).

## Materials and methods

### Study population and field work

The study was performed at the common tern colony “Banter See” in Wilhelmshaven, Germany (53°30′40″N, 8°06′20″E) in 2008 and 2009, when 350–380 breeding pairs were recorded. The colony is situated on six artificial islands (11 × 5 m, 1 m between islands), surrounded by low concrete walls, which are equipped with 44 resting boxes. During our study, 16 of the 44 resting boxes were additionally provided with electronic balances (Sartorius TE6100, accuracy ± 1 g) recording the weights of birds

remotely and automatically (see Wendeln and Becker 1996). The colony has been studied since 1980, and from 1992 onward all fledglings were individually marked with a subcutaneously injected passive transponder (TROVAN ID 100) in addition to a metal ring. Marked adults and subadults are automatically registered in the colony by special antennas on the resting boxes that read the transponder every 5 s (for details, see Becker and Wendeln 1997). Between 1992 and 1995, 101 adults were additionally trapped on the nest and fitted with transponders. During the early years of the project, terns were sexed by observation of copulations and since 1998 by standard molecular methods (Becker and Wink 2002).

The breeding colony was checked three times a week, new nests were marked and laying dates as well as clutch sizes were recorded (Wagener 1998). At every subsequent control, the fate of eggs and the causes of loss were determined (damaged, cold, not hatching). Once during incubation, each nest was equipped with a mobile antenna for 1 or 2 days to identify pair mates and assign nest site and reproductive parameters to the individuals. Immediately after hatching, chicks were ringed and followed until they fledged or died.

#### Blood sampling

For blood sampling of breeders, we used starved third stage larvae of blood-sucking bugs (*Dipetalogaster maximus*) in hollow eggs (Becker et al. 2006; Arnold et al. 2008; Bauch et al. 2010). Samples were taken in the middle of individual incubation (9–14 days after clutch completion). To minimize the possibility of diel variation in prolactin and corticosterone values, all blood samples were taken in the early morning between 5 and 10 a.m. Time of day was not significantly correlated with prolactin or corticosterone baseline values (prolactin:  $r = 0.113$ ,  $P = 0.156$ ,  $N = 160$ ; corticosterone:  $r = -0.146$ ,  $P = 0.066$ ,  $N = 158$ ). The colony is accustomed to researcher activities, and adults settle down immediately after a person had passed their clutch. Every morning before sampling, we equipped all focal clutches with antennas, and after the target bird was identified, we replaced the whole clutch with dummy eggs, one containing the bug (Becker et al. 2006). The common tern eggs were kept warm and safe in a box during the sampling procedure. After 20–30 min, the bugs had normally sucked enough blood (about 300  $\mu$ l) and we immediately punctured their abdomen and extracted the blood with a syringe. Blood samples were stored cool on ice until centrifugation in the laboratory (within 2–5 h).

In both years, the bugs successfully sucked blood in more than 80 % of the trials. There was no negative impact of the bug method on behavior or breeding success of the terns (Becker et al. 2006). This sampling method was

successfully validated in common terns for prolactin (Riechert et al. 2012) and corticosterone (Arnold et al. 2008) and has also been used to study blood physiology (Bauch et al. 2010), hormones (Voigt et al. 2004) or doubly labeled water (Voigt et al. 2003, 2005).

#### Hormone assay

Blood samples were centrifuged, and the plasma was separated and stored at  $-20$  °C until analysis in the laboratory. Baseline concentrations of prolactin and total (bound and free) corticosterone were measured in the Centre d'Études Biologiques de Chizé, France. Plasma level of prolactin was determined twice in each sample by a heterologous radio-immunoassay (RIA) as described in Cherel et al. (1994). Prolactin samples of common terns produced a dose-dependent response curve that paralleled the one of standard chicken plasma (source: Dr. A.F. Parlow, UCLA Medical Center, Los Angeles). Corticosterone was analyzed in the remaining plasma (normally 25  $\mu$ l) via RIA following Lormee et al. (2003, see also Riechert et al. 2012). The minimal detectable corticosterone level was 0.4 ng/ml (lowest measurement: 1.74 ng/ml). Due to large sample sizes in both years, corticosterone samples were run in different assays, although assay identity had no effect (unpaired  $t$  test: 2008:  $t = -0.836$ ,  $P = 0.406$ ,  $N = 65$ , 2009:  $t = -0.422$ ,  $P = 0.675$ ,  $N = 104$ ). Intra- and inter-assay variation for baseline prolactin and corticosterone was within a range of 7–11 % ( $n = 4$  duplicates for intra-assay variation).

#### Sampling schedule

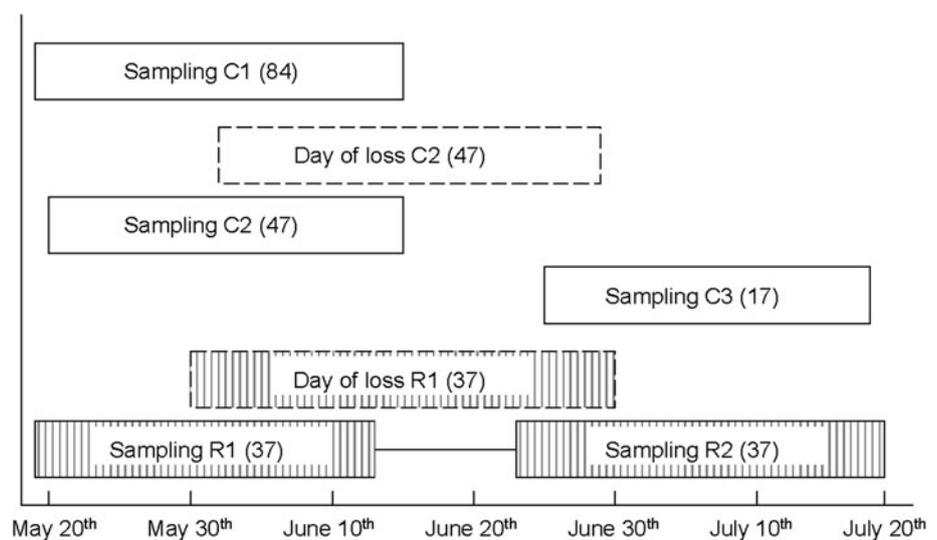
Many terns were sampled during incubation in 2008 or 2009 for different projects. It sometimes happened that terns lost their clutch or brood (due to damage of eggs, clutch or brood abandonment or death of chicks, mainly caused by starvation; see Table 1), comparable with an experimental removal of the nest contents. Some birds laid a replacement clutch, mostly in the same territory (González-Solís et al. 1999a), and were re-sampled (group R1, including one bird with a real second clutch,  $N = 37$ , Fig. 1). No bird changed its mate in the second breeding attempt (see also González-Solís et al. 1999b). The individual day of sampling (expressed as day after clutch completion) did not differ between the first and replacement clutch ( $11.1 \pm 1.8$  vs.  $11.3 \pm 1.8$ ,  $Z = -0.190$ ,  $P = 0.849$ ,  $N = 37$ ). Baseline prolactin and corticosterone values were not correlated between pair mates (prolactin:  $r = 0.207$ ,  $P = 0.498$ ,  $N = 13$ ; corticosterone:  $r = -0.168$ ,  $P = 0.566$ ,  $N = 14$ ) and, therefore, we used both partners for the comparison of hormones measured during incubation of the first and the replacement clutch.

**Table 1** Reproductive parameters (proportion of hatched chicks and breeding success as well as total losses from all eggs laid) and reason for nest failure (proportion of eggs lost, abandoned clutches and dead chicks related to total losses) for all analyzed groups

Reproductive parameters	Renesting (37)		Early breeders		Late breeders (C3, 17)
	First clutch (R1)	Second clutch (R2)	Successful (C1, 84)	Unsuccessful (C2, 47) <sup>a</sup>	
Hatching success	0.61	0.66	0.89	0.66	0.84
Breeding success	0.01	0.21	0.42	0	0.16
Total losses	0.99	0.79	0.58	1.00	0.84
Reason for nest failure					
Eggs lost	0.24	0	0.09	0.15	0.06
Abandoned clutch	0	0.22	0	0.06	0
Dead chicks	0.75	0.57	0.49	0.79	0.78

<sup>a</sup> Not renestingSample size *N* for each group in brackets

**Fig. 1** Range of sampling dates and dates of loss of the clutch or brood for birds, which relayed after loss (R) and control birds (C). *R1* Renesting birds sampled during incubation of their first clutch, *R2* renesting birds sampled during incubation of their replacement clutch. *C1* control birds that laid early in the season and did not lose the clutch or brood, *C2* control birds (non-renesters) sampled during incubation of their clutch, which was lost around the same time as the renesting group lost their first clutch, *C3* control birds sampled during incubation of their first clutch late in the season



To analyze a possible effect of the date, we also sampled a control group of terns, which laid their first clutch late in the season (C3,  $N = 17$ , Fig. 1). Laying date and sampling date of these control birds did not differ from respective dates of the replacement group (R2, Fig. 1) in 2008 or 2009 (2008, laying date:  $176 \pm 7$  vs.  $180 \pm 5$ , unpaired  $t$  test:  $t = 1.257$ ,  $P = 0.240$ ,  $N = 18$ ; sampling date:  $188 \pm 8$  vs.  $192 \pm 5$ ,  $t = 1.309$ ,  $P = 0.224$ ,  $N = 18$ ; 2009, laying date:  $174 \pm 6$  vs.  $176 \pm 6$ ,  $t = 1.373$ ,  $P = 0.249$ ,  $N = 36$ ; sampling date:  $184 \pm 7$  vs.  $187 \pm 6$ ,  $t = 1.429$ ,  $P = 0.162$ ,  $N = 36$ ). Within the replacement group (R2), we further differentiated between birds that abandoned their clutch during incubation ( $N = 8$ ) and those that lost their chicks ( $N = 29$ ).

Another objective was the characterization of individuals that renested after loss of the first brood. Therefore, we chose another group of birds from the pool of sampled individuals (C2,  $N = 47$ , Fig. 1), which lost the first clutch or brood around the same time as the replacement group

but did not relay. Even after brood loss, common terns stay for several weeks in the colony and do not reneest elsewhere (Szwierczynski 2005). Additionally, a control group (C1,  $N = 84$ ) was chosen, which laid around the same time as R1 but did not lose their clutch or brood. As reproductive data from pair mates are identical and as data from individuals sampled in both years are not independent, we chose only one partner per nest or 1 year of sampling by chance for C1–C3. The date of loss did not differ between C2 and R1 in both years (2008:  $168 \pm 6$  vs.  $166 \pm 4$ , unpaired  $t$  test:  $t = -1.062$ ,  $P = 0.296$ ,  $N = 36$ ; 2009:  $164 \pm 6$  vs.  $164 \pm 9$ ,  $t = -0.209$ ,  $P = 0.896$ ,  $N = 48$ ).

Some birds lost their clutch in 2008 and 2009, but produced a replacement clutch in only 1 year. The date of loss did not differ between the years (renesting:  $165 \pm 5$ , non-renesting:  $167 \pm 6$ , paired  $t$  test:  $t = -1.974$ ,  $P = 0.099$ ,  $N = 7$ ). For each individual within these groups, we calculated hatching success as the proportion of hatched eggs from the clutch and breeding success as the

proportion of fledged chicks from all laid eggs. Then we determined the mean hatching and breeding success for these groups, respectively (see Table 1).

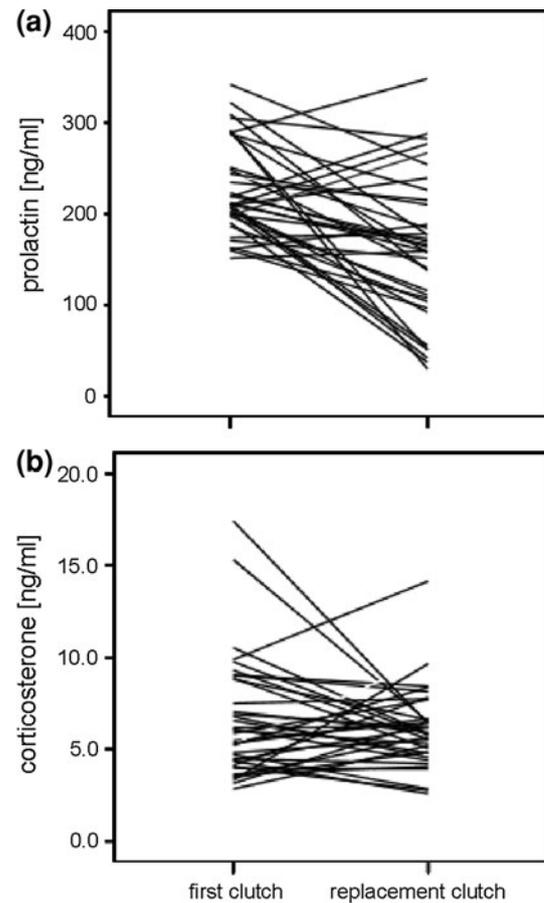
#### Definitions and statistical analyses

We used a general linear model for repeated measurements (GLMM) to analyze the change in baseline prolactin and corticosterone between first and replacement clutch (within-subject factor) and the influence of year, sex (between-subject factors) and breeding experience (covariate) as well as their interaction terms. We used type III sum of squares and, in case of post hoc comparisons, Bonferroni corrections. Breeding experience of terns was defined as the number of years the birds had reproduced at the ‘Banter See’ colony before the sampling year (experience at recruitment = 0, range: 0–17 years; see also Limmer and Becker 2010). By one-way ANOVA or Kruskal–Wallis test, we compared prolactin and corticosterone values between early (R1, C1) and late (C3) first clutches. Using an unpaired *t* test, we compared both hormone values between replacement and late first clutches (R2 and C3). By Pearson correlation, we related hormone values and date of sampling. The date was expressed as day of the year. By an unpaired *t* test, we compared prolactin and corticosterone values between individuals that lost their eggs (due to damaged/unhatched eggs or abandoned clutches) and individuals that lost their chicks (mainly due to starvation) within the groups R1, R2 and C2.

Next, we performed a binary logistic regression investigating the decision to relay or not with both hormone values of the first clutch, the phase in which they lost their brood (1 = incubation, 2 = nestling phase) and their interaction terms as independent variables. The analysis was done separately for males and females to avoid pseudo replication as we often sampled both pair mates. Breeding experience was not used because (1) it is linked to hormone values in common terns (Riechert et al. 2012) and (2) previous breeding experience was not significantly different between birds which produced a replacement clutch and individuals that did not ( $7 \pm 1$  year vs.  $6 \pm 1$  year,  $t = 1.407$ ,  $P = 0.163$ ,  $N = 88$ ). We chose a backward stepwise approach and report the last step of the model in the results.

Within an individual, we compared the year with a renesting attempt to the one without. Therefore, we used a paired *t* test to compare prolactin and corticosterone values, breeding experience and date of loss during incubation of the first clutch between years.

All statistical analyses were two-tailed, results were expressed as mean  $\pm$  1 SE and level of significance was set to  $P \leq 0.05$ . All analyses and graphs were performed with SPSS 18.0.



**Fig. 2** Individual baseline prolactin (a) and corticosterone profiles (b) for common terns sampled during incubation of their first and replacement clutch within one season

## Results

### Hormonal change from first to replacement clutch

On average, baseline prolactin significantly decreased in most individuals from the first to the replacement clutch ( $227.4 \pm 8.9$  vs.  $162.3 \pm 12.7$  ng/ml, Fig. 2a; Table 2a) with a significant positive correlation between the two values ( $r = 0.377$ ,  $P = 0.022$ ,  $N = 37$ ). Corticosterone on the other hand did not differ significantly between the samples ( $6.6 \pm 0.5$  vs.  $6.2 \pm 0.4$  ng/ml, Fig. 2b; Table 2b) nor were levels between the first and replacement clutch correlated ( $r = 0.239$ ,  $P = 0.153$ ,  $N = 37$ ). In the first breeding attempt, most birds lost their brood during the chick phase and, therefore, hatching success did not differ between the two breeding attempts ( $P = 0.749$ , Table 1).

The change in baseline prolactin values between first and replacement clutch was not correlated with any analyzed variable (Table 2a), whereas corticosterone was slightly higher in males than in females (Table 2b). All interactions between hormones and between-subject effects

**Table 2** Outcome of the GLMRM analyzing the change in prolactin (a) and corticosterone (b) during incubation of the first and the replacement clutch in an individual tern

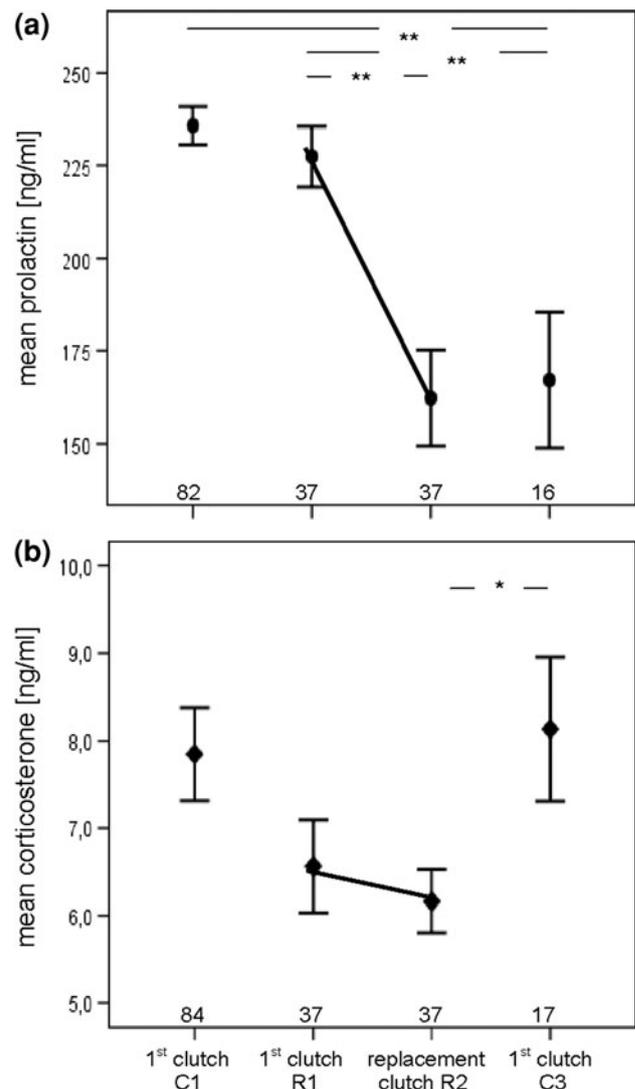
Explanatory variable	<i>F</i>	<i>df</i>	<i>P</i>
<b>(a) Prolactin</b>			
Clutch	4.916	1	0.034
Sex	2.645	1	0.114
Experience	0.012	1	0.913
Year	0.561	1	0.460
Sex × experience	2.786	1	0.105
Sex × year	0.019	1	0.892
Experience × year	0.214	1	0.647
<b>(b) Corticosterone</b>			
Clutch	0.071	1	0.792
Sex	4.006	1	0.054
Experience	0.006	1	0.940
Year	1.964	1	0.171
Sex × experience	2.206	1	0.148
Sex × year	3.048	1	0.091
Experience × year	0.306	1	0.585

The change in hormone levels and the influence of between-subject effects and their interaction terms are shown. Significant variables are given bold

were not significant ( $P > 0.05$  in all cases). Prolactin decreased from first to replacement clutch in birds, which abandoned their replacement clutch ( $216.6 \pm 20.4$  ng/ml vs.  $127.7 \pm 27.2$  ng/ml,  $t = 2.246$ ,  $P = 0.060$ ,  $N = 8$ ) as well as in those that did not ( $230.2 \pm 9.3$  ng/ml vs.  $171.6 \pm 14.1$  ng/ml,  $t = 4.351$ ,  $P < 0.001$ ,  $N = 29$ ). But individuals which abandoned their replacement clutch (on average  $8 \pm 2$  days after sampling) had prolactin values that were 25 % smaller, although that was not a statistically significant difference, during incubation of the renesting attempt than individuals that cared for their clutch and brood afterward ( $127.7 \pm 27.2$  vs.  $171.3 \pm 14.7$  ng/ml,  $t = -1.279$ ,  $P = 0.125$ ,  $N = 37$ ). During the first breeding attempt, no bird abandoned its clutch. Corticosterone on the other hand did not differ significantly (abandoning vs. caring parents:  $6.7 \pm 1.3$  vs.  $6.1 \pm 0.3$  ng/ml,  $t = 0.543$ ,  $P = 0.603$ ,  $N = 37$ ).

#### Seasonal influence

Control birds (C3) sampled during incubation of their first clutch late in the season had similar prolactin values as birds sampled during incubation of their replacement clutch ( $t = -0.211$ ,  $P = 0.834$ ,  $N = 53$ , Fig. 3a), but significantly higher baseline corticosterone values ( $t = -2.184$ ,  $P = 0.040$ ,  $N = 54$ , Fig. 3b). On the other hand, the late controls C3 showed lower prolactin concentrations



**Fig. 3** Mean baseline prolactin (a) and corticosterone concentration (b) in birds sampled twice, during incubation of their first (R1) and their replacement clutch (R2), connected by a line. The control groups C1 and C3 were sampled during incubation of their clutches; C1 clutches were laid simultaneously to first clutches of renesting birds, and C3 simultaneously to replacement clutches. Sample size *N* at the bottom of the graph, asterisks mark significant differences between samples ( $* < 0.05$ / $** < 0.01$ )

than the replacement group (R1) and the early controls C1 (ANOVA,  $F_{2,132} = 11.976$ ,  $P < 0.001$ , post hoc R1 vs. C3:  $P = 0.001$ ,  $N = 53$ , Fig. 3a; C1 vs. C3:  $P < 0.001$ ,  $N = 98$ ; C1 vs. R1:  $P = 0.712$ ,  $N = 119$ , Fig. 3a), but we found no difference in corticosterone levels between these three groups (Kruskal–Wallis test,  $\chi^2 = 3.217$ ,  $P = 0.200$ ,  $N = 138$ , Fig. 3b).

Prolactin values were significantly correlated with date of sampling, which corresponds to laying date: birds sampled later in the season had lower baseline prolactin than earlier sampled individuals ( $r_s = -0.195$ ,  $P = 0.004$ ,  $N = 265$ ), whereas corticosterone was not correlated with date ( $r_s = 0.001$ ,  $P = 0.994$ ,  $N = 264$ ). Hatching success

was slightly higher in late first clutches compared to replacement clutches ( $Z = -1.832$ ,  $P = 0.067$ ,  $N = 54$ , Table 1), whereas breeding success showed no significant difference ( $Z = -0.453$ ,  $P = 0.450$ ,  $N = 54$ , Table 1).

Characterization of relaying birds

84 birds lost their clutch between end of May and beginning of July, 16 during incubation and 68 during the chick phase. 44 % produced a replacement clutch on average  $14 \pm 2$  days after loss of the first clutch or brood. Neither within renesting birds during their first (R1) or second breeding attempt (R2) nor in unsuccessful early breeders (C2) were prolactin and corticosterone values related to the causes of failure ( $P \geq 0.100$  in every case, see Online Resource 1).

Regarding the decision to relay, the interaction of both hormones proved to be the most influential variable in males (Wald = 4.8424,  $P = 0.028$ ,  $B = 0.999$ ). Fathers with higher baseline prolactin and higher corticosterone during their first reproductive attempt were more likely to initiate a replacement clutch after loss of the first one (prolactin:  $231.7 \pm 38.7$  vs.  $201.9 \pm 67.2$ ,  $t = 1.611$ ,  $P = 0.106$ , Fig. 4a; corticosterone:  $7.5 \pm 4.0$  vs.  $5.7 \pm 2.3$ ,  $t = 1.720$ ,  $P = 0.091$ , Fig. 4b). In females, the last model revealed an influence of prolactin  $\times$  corticosterone and prolactin level alone (prolactin  $\times$  corticosterone: Wald = 5.012,  $P = 0.025$ ,  $B = 1.002$ ; prolactin: Wald = 3.014,  $P = 0.083$ ,  $B = 0.986$ ). Females initiating a replacement clutch were characterized by significantly lower corticosterone ( $5.7 \pm 2.3$  vs.  $7.4$  vs.  $2.5$ ,  $t = -2.330$ ,  $P = 0.027$ , Fig. 4b) and insignificantly higher prolactin values ( $224.8 \pm 48.9$  vs.  $205.1 \pm 38.9$ ,  $t = 1.379$ ,  $P = 0.175$ , Fig. 4a).

Some birds lost their clutch in both 2008 and 2009, but relaid only in 1 year. Though the date of loss did not differ significantly (relaying vs. non-relaying:  $164 \pm 2$  vs.  $167 \pm 2$ ,  $t = -1.974$ ,  $P = 0.099$ ,  $N = 7$ ), baseline prolactin values were marginally higher during incubation of the first clutch in the year when they renested ( $232.5 \pm 11.5$  vs.  $178.7 \pm 28.5$  ng/ml,  $t = 2.128$ ,  $P = 0.077$ ,  $N = 7$ ). Breeding experience and corticosterone did not differ significantly (experience:  $6 \pm 1$  vs.  $6 \pm 1$ ,  $t = 0.354$ ,  $P = 0.736$ ; corticosterone:  $7.1 \pm 1.5$  ng/ml vs.  $8.3 \pm 1.5$  ng/ml,  $t = -1.166$ ,  $P = 0.288$ ).

Discussion

After nest failure, which is comparable with an experimental removal of the clutch or brood, as expected common terns demonstrated a clear decrease in baseline prolactin level from incubation of the first clutch to the replacement clutch, whereas corticosterone did not differ in

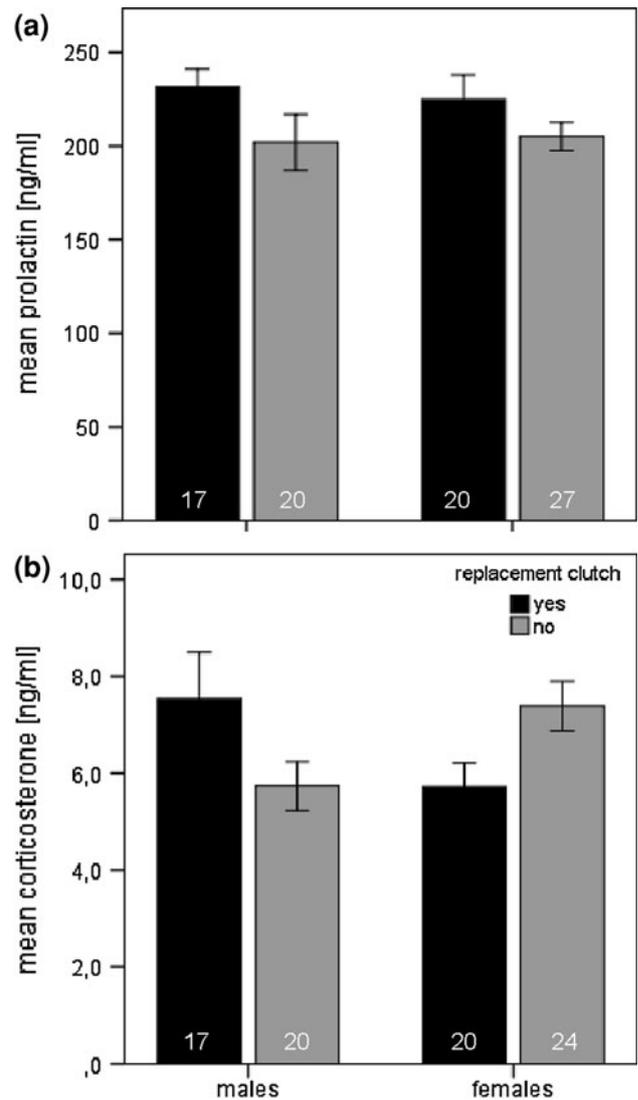


Fig. 4 Mean baseline prolactin (a) and corticosterone value (b) measured during incubation of the first clutch compared between birds which renested after loss of the first clutch and those that did not. Males are represented in black bars, and females in gray bars. Sample size N is given at the bottom of columns. Asterisks mark significant differences based on a t test (\*<0.05)

contrast to our suggestion. Neither breeding experience nor sex or year was related to the hormonal change from first to replacement clutches. Late-laying terns, however, had low prolactin and increased corticosterone levels. The reason for nest failure was not related to prolactin or corticosterone values. Interestingly, one factor driving the probability to relay after loss of the first clutch seems to be a high baseline prolactin value.

Contrary to our finding that prolactin decreased during incubation of the replacement clutch was the result in the Gentoo penguin (*Pygoscelis papua*), where prolactin values were still very high (Mauget et al. 1995). A possible explanation could be provided by the incubation behavior

of penguins: they spend weeks or even month foraging at sea, but maintain high prolactin values (Lormee et al. 1999). In these species, prolactin is probably controlled endogenously to make sure they do not abandon their clutch or brood after long absences (Garcia et al. 1996). In great tits (*Parus major*), Silverin (1991) also found lower prolactin values measured during care of the replacement clutch. However, these were cross-sectional studies, and longitudinal individual-based data were lacking. To the best of our knowledge, this is the first study in birds comparing prolactin and corticosterone values between the first and renesting attempt on the individual level.

#### Hormone values during renesting

Pooling abandoned clutches and those with lost eggs and contrasting them to broods with dead chicks, the reason for nest failure was not related to baseline prolactin or corticosterone values within renesting (R1, R2) and non-renesting birds (C2). However, contrasting birds that successfully incubated until hatching with birds that deserted their replacement clutch a few days after sampling, the latter had lower prolactin titers. Declining prolactin levels could be associated with a disruption in incubation behavior and ultimately trigger nest desertion (Cherel et al. 1994; Chastel and Lormee 2002; Groscolas et al. 2008). Hence, nest abandonment might, besides other factors, be triggered by declining prolactin values a few days previously. Decreased prolactin during incubation of the replacement clutch could be linked to a lower investment, for instance due to a lower survival probability for later fledged chicks (Gasparini et al. 2006). However, this seems not to be the case in common terns: clutch size and hatching success were similar between the first and the renesting attempt within an individual, suggesting that terns do not lower their effort for the replacement clutch in relation to these parameters. Furthermore, recent work on common terns by Becker and Zhang (2011) and on magpies (*Pica pica*, De Neve et al. 2004) showed that renesting attempts were more successful than late first breeding attempts in most of the analyzed years. These results might support the quality hypothesis rather than the timing hypothesis (Christians et al. 2001), which claims that low-quality birds with minor success lay later in the season, whereas according to the timing hypothesis low success late in the season is caused by unfavorable environmental conditions. In our study, we too demonstrated a slightly higher breeding success for replacement clutches compared to late first clutches. Therefore, we suggest that the decrease in prolactin from the first to the renesting attempt might not have negative consequences on behavior or the success parameters we measured, but this should be clarified in future investigations. Instead, the decrease in

prolactin could be linked to some other internal or external processes.

Corroborating the hypothesis that common terns were in good condition, despite managing a second demanding breeding phase, we found similar corticosterone levels between the two breeding attempts. However, our expectation was an increased corticosterone level, evoked by a second stressful breeding phase and reduced body reserves (Cherel et al. 1988; Schoech et al. 1996; male common terns Wendeln et al. 2000). In earlier studies, it was suggested that common terns spend a high level of parental care on replacement clutches (Wendeln et al. 2000; Arnold et al. 2004); males lose more weight during the second courtship phase than during the first, while females gain more weight through courtship feeding (Wendeln et al. 2000). The comparatively low corticosterone value might suggest that renesting birds are high-quality individuals, which are able to meet the costs of additional egg production and incubation without compromising their breeding success or survival (Becker and Zhang 2011). Common terns do not face a predictable decline in food availability at the end of the season, unlike other species (Perrins 1970; Daan et al. 1988), and later fledged young do not suffer from reduced survival or recruitment probability (Nisbet 1996; Braasch et al. 2009). This suggests that a replacement attempt could be worthwhile for terns to increase their reproductive output, which was confirmed by Becker and Zhang (2011) with individual-based data.

#### High quality and hormones

Are renesting breeders characterized by different prolactin or corticosterone values compared to non-renesting ones? Renesting individuals indeed had slightly higher prolactin during incubation of the first clutch than birds that did not reneest after loss of the first clutch or brood. Birds laying early in the season and not losing their clutch or brood showed comparably high prolactin values as renesting individuals, but had clearly higher values than non-renesters. Their high prolactin titer could also be a sign of high quality. An increased prolactin level could be a sign of high quality: House sparrows (*Passer domesticus*) with higher prolactin during rearing of young fledged more chicks (Ouyang et al. 2011). Furthermore, Miller et al. (2009) demonstrated a positive relationship between baseline prolactin and parental effort. Therefore, elevated prolactin levels could be an additional sign of the higher quality of relaying common terns. However, individual quality of birds could not explain why six out of seven individuals, which had lost their first clutch in 2 years but relayed only once, had higher prolactin during incubation of the first clutch when they reneested afterward. However, we have no

explanation for the lower prolactin concentration during the year when they not renested.

The possible link between baseline corticosterone and relaying as an indicator of quality in common terns was not as clear as for prolactin: we found significantly lower baseline corticosterone in females that produced a replacement clutch, whereas it was the opposite in males. In the house sparrow, increased corticosterone during the nestling phase was positively linked to breeding success possibly due to higher energy transfer to the nestlings (Miller et al. 2009). However, studies of the relationships between corticosterone and fitness have yielded conflicting results (reviewed by Bonier et al. 2009; Angelier et al. 2010) and are difficult to interpret. The link might not be causal, but arise from a relationship between corticosterone and other factors like food availability or body condition.

### Seasonal influence

The lower prolactin level of common terns measured during incubation of the renesting attempt might be linked to a seasonal process, as we found decreasing values with advancing date. Young, inexperienced birds lay later in the season (Ezard et al. 2007) and Riechert et al. (2012) demonstrated lower prolactin in inexperienced terns. However, the fact that older birds, which initiated a replacement clutch around the same time as young individuals initiated their first one, had comparably low prolactin suggest a possible influence of date irrespective of experience (calendar effect). In the house sparrow, prolactin values also declined with advancing laying date (Ouyang et al. 2011), whereas in the wandering albatross (*Diomedea exulans*), Angelier et al. (2006) did not find a link with the season. Other processes related to the season are photorefractoriness and molt and for both we found evidence in the literature for a relation with baseline prolactin levels (Proudman and Siopes 2002, 2006; Dawson et al. 2009). Proudman and Siopes (2002) suggested that prolactin could be responsible for fine-tuning the end of the breeding season by facilitating gonadal regression or molt.

Baseline corticosterone on the other hand was not affected by date of sampling, comparable to Lapland longspurs (*Calcarius lapponicus*, Romero et al. 1998) or snow petrels (*Pagodroma nivea*, Angelier et al. 2007a). Wandering albatrosses on the contrary exhibited decreasing values with advancing date (Angelier et al. 2006). Common terns initiating their first clutch late in the season exhibited higher baseline corticosterone values than replacement breeders during incubation of their early first clutch or replacement clutch. Late breeders are probably young, inexperienced birds, which could be characterized by higher corticosterone (Angelier et al. 2007b), perhaps due to poor foraging success (Kitaysky et al. 2011) or high energy expenditure (Welcker

et al. 2009). The replacement breeders were older, more experienced birds, which are probably more successful in foraging and managing their reproductive period and therefore, contrary to our prediction, they were characterized by lower corticosterone values.

### Conclusion

We demonstrated declining prolactin values from the first to the renesting attempt, using longitudinal data. However, this decrease was not linked to reduced success, but might be induced by end of breeding season processes like gonadal regression or molt. Birds that had abandoned their clutch a few days after sampling exhibited an already steeper decrease in prolactin values than successful ones. Our results suggest that common terns could manage a second energy-demanding breeding period within one season without diminishing their body reserves or hazarding their future survival and success. Renesting individuals were characterized by higher baseline prolactin during the first breeding attempt than non-renesting birds, which could be related to higher quality of these terns. Corticosterone did not rise during incubation of the replacement clutch, maybe suggesting that renesting individuals were in good condition despite the second demanding breeding phase. The higher prolactin level of renesting birds could be an indicator of their superior quality, whereas corticosterone showed no clear pattern.

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