The prolactin response to an acute stressor in relation to parental care and corticosterone in a short-lived bird, the Eurasian hoopoe

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ABSTRACT

Prolactin plays an important role in mediating parental care in birds, but little is known about changes in prolactin levels when animals disrupt their reproductive behaviour during emergency life-history stages. We investigated the variation of prolactin levels with breeding stage, sex, body condition and as a response to a standardized acute stressor in a small short-lived bird, the Eurasian hoopoe *Upupa epops* under natural field conditions. We found higher baseline levels of prolactin in females during the brooding phase than in their mates which feed them and their chicks at this stage. Moreover, this is the first report of a differential prolactin stress-response between sexes with contrasting parental care within a breeding phase. Capture, handling and restraint induced a clear decrease of prolactin levels which was less pronounced in females at the very early stage of brooding compared to females in later stages. In contrast, the prolactin stress response in males remained nearly constant over the breeding stages and was stronger than in females. Baseline levels of prolactin, but not handling-induced levels, were positively correlated with body condition. We found a weak relationship between the decrease in prolactin due to acute handling stress and handling-induced levels of corticosterone. Taken together, both baseline and stress response levels of prolactin were related to the amount of parental care, although we found no relationship with reproductive success. It appears that the response to an acute stressor in prolactin levels is finely tuned to parental duties and investment. Hence, prolactin appears to be involved in mediating the trade-off between current reproduction *versus* self-maintenance and future reproduction.

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1. Introduction

Prolactin plays a central role in regulating parental care in birds and mammals [10]. Therefore prolactin is likely to be involved in mediating the trade-off between current reproduction *versus* self-maintenance and future reproduction [1]. From life-history theory, it follows that individuals should adaptively adjust prolactin levels according to the prevailing conditions in the course of the breeding cycle (long-term conditions in the order of days; e.g. [18,25] as well as during unpredictable events triggering an acute stress response (short-term conditions in the order of hours [17] in order to maximize fitness.

It is well known that avian prolactin levels increase during the egg laying stage, at the onset of parental activity, to reach a peak upon hatching [50]. After hatching prolactin levels decrease in some species (e.g. in precocial species with little parental care after hatching), while in other taxa (mainly altricial species which feed nestlings until fledging) prolactin levels stay elevated into the feeding period or until fledging [50]. In particular, pelagic seabirds are thought to maintain high prolactin levels throughout the nestling phase to maintain nest-bonds during their very long foraging trips (king penguin *Aptenodytes patagonicus* [24], red-tailed tropicbird *Phaeton rubricauda* [32], Emperor penguin *Aptenodytes forsteri* [31], while in other altricial species with similar parental duties (feeding chicks until fledging) prolactin levels decrease during the nestling phase (masked booby *Sula sula*, red-footed booby *Sula dactyla* [32], pied flycatcher *Ficedula hypoleuca* [52], ring dove *Streptopelia risoria* [13]. Hence, there seems to be considerable variation between species in the pattern of prolactin levels during the nestling phase. Parental duties in altricial species during the nestling phase comprise brooding (i.e. warming chicks unable to thermoregulate by themselves) and foraging. We know of no study investigating prolactin levels with respect to both brooding and foraging (for nestlings and for self-maintenance). Brooding competes more strongly with foraging for self-maintenance than foraging for nestlings, because brooding birds have to leave the nest, while parents feeding nestlings can feed for themselves at the same as they collect food for nestlings [16,36]. The mediation of this trade-off is particularly crucial for the sex that broods solely.

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Indeed, elevated levels of baseline prolactin have been found to coincide with a high intensity of parental care, e.g. during incubation, whereas lower prolactin levels enable individuals to desert the brood and forage to replenish their body reserves [18,25,5]. Hence, we expect higher prolactin levels in the sex providing more care, as has been generally found among bird species [50,1].

At the individual level, the intensity of parental care is thought to be mediated by prolactin levels [1] and is expected to vary with the condition of the parent. Indeed higher baseline levels of prolactin have been found in parents in good body condition [1] and in experienced breeders [2,3]. These studies have all been done in seabirds and geese and await confirmation in small short-lived species; they describe the adaptation of the prolactin levels to long-term, predictable variation of prevailing breeding condition.

However, an unexpected stressor may suddenly disturb the balance in the trade-off between current reproduction and self-maintenance and may call for a transitional reduction in parental care in order to facilitate survival of the parent [61,59]. Therefore, we would expect a decrease in prolactin levels as a response to an acute stressor. This is the pattern generally observed in birds [17,1,35,28,45], in contrast to mammal studies and a study in non-breeding birds which generally described an increase of the prolactin levels under stressful events [22,45]. Nonetheless, there are relatively few studies that investigated the response to acute stress of prolactin levels in birds [1], most on long-lived seabirds, and it is known that life-history traits may affect the stress response [9]. Hence it is timely to investigate stress-related prolactin levels in shorter lived species. Moreover, it remains to be studied whether and how the prolactin stress response is modulated according to differences in parental duties between sexes, the brood stage and the condition of the parent.

As part of the typical response to an acute stressor, glucocorticoid levels, corticosterone in birds, increase [48]. The activation of the hypothalamo–pituitary–adrenal (HPA) axis plays an essential role in the response to acute stress (i.e. during emergency life-history stages, *sensu* [61], modifying hormone levels and triggering physiological and behavioural changes. Corticosterone inhibits processes that are not immediately vital such as part of the immune system or reproductive activities and favours survival [61,48,40,54]. Hence, corticosterone is involved in the regulation of the trade-off between current reproduction *versus* future survival and reproduction [44,59,30,9]. Both corticosterone and prolactin seem to act concomitantly in parental birds experiencing chronic stress (a decrease in prolactin levels paralleled by an increase in corticosterone levels during acute or prolonged environmental perturbations and energetic constraints; [20,61,4,25], a pattern confirmed by experimental studies with corticosterone administration [19,5]. Furthermore, baseline corticosterone and prolactin levels are sometimes negatively correlated in free-living birds [4,6]. However, the short term secretory pathways of these two hormones rely on different mechanisms [50,59] and there is no evidence for a mechanistic link between the prolactin and the corticosterone response to an acute stressor [1]. Thus, the mechanisms that drive the secretion or clearance of the two hormones prolactin and corticosterone to act concomitantly during emergency life-history stages in breeding birds remain unknown.

At the ultimate level, life-history theory would predict a higher reproductive success or at least a higher rearing success in individuals with a higher prolactin level, i.e. in those individuals thought to invest more into reproduction than others, all other things being equal. This however may only hold up to a certain level of prolactin, depending on the environmental situation, and hence the pattern of the relationship between prolactin level and reproductive success may be more complex. Surprisingly, we found only a few studies in free-living birds investigating this aspect [43,38,3,5,35].

The aim of this correlative study was to investigate the questions raised above, focussing on the relationship between plasma prolactin concentration and breeding stage, sex, body condition and as a response to a standardized acute stressor in a small short-lived bird under natural field conditions. The Eurasian hoopoe is particularly well suited because of the strong asymmetry in parental care [33]. In particular the following five predictions were examined. (1) If baseline levels of prolactin reflect the amount of parental care, we expect females, which are brooding the chicks alone during the first 10 days after hatching, to have higher prolactin levels than males and to decrease prolactin levels with increasing age of the nestlings, i.e. with decreasing brooding activity. (2) We hypothesised that an acute stressor (20–28 min of handling and restraint) induces a fall of prolactin levels which should be smaller in birds with high parental care than in birds with low parental care; more precisely we predict females which perform most of brooding to down regulate the prolactin response to acute stress. (3) We predicted that birds in good condition have higher levels of baseline prolactin and a smaller decrease with handling than leaner birds, because birds in good condition would have a better capability of enduring parental care. (4) Because prolactin and corticosterone would concomitantly affect the reproductive physiology and behaviour with antagonistic effects, we expected a negative correlation between baseline and handling-induced prolactin and corticosterone levels. (5) The reproductive success is expected to be positively correlated with baseline and handling-induced prolactin levels.

2. Materials and methods

2.1. Study site and study species

The study was carried out in the upper Rhone valley in the canton Valais, Switzerland, between Sierre and Martigny (46°11′N, 7°18′E, about 62 km^2, 1.9 × 43 km) in 2008. The hoopoe population of the Valais breeds almost entirely in nest boxes provided during a conservation project [7].

The Eurasian hoopoe is an altricial bird species with asynchronous hatching [33]. The female incubates alone and broods the chicks (mean brood size in this study 5.5 ± 1.7 SD; $n = 90$) during 8–10 days after the first egg hatched, while food for the nestlings and the female is provided by the male. Thereafter, both male and female provision the brood, as long as the female is not incubating a new clutch [8]. The chicks fledge at the age of 23–25 days. Individuals of the study population attempt 1 or 2, exceptionally 3 broods.

2.2. Capture, blood sampling and body condition

From 96 adults attending a brood (mean body mass 70.9 g ± 5.7 SD; $n = 120$), two blood samples (28–150 µl) were taken by puncturing the alar veins with disposable needles. The first blood sample was taken as soon as possible after capture (mean ± SD: 101 s ± 39; range 39–240 s) to obtain baseline levels of prolactin and corticosterone [17,46]. Plasma baseline prolactin levels were independent of handling time within the first 4 min following capture (linear mixed model with individual identity nested into brood identity as random intercept; males: handling time 128 s ± 32 SD, $F_{1,44} = 2.56$, $p = 0.12$; females: handling time 83 s ± 33, $F_{1,71} = 0.67$, $p = 0.4$) and sampling date (linear mixed model with individual identity nested into brood identity as random intercept; $F_{1,29} = 1.23$, $p = 0.28$). Because corticosterone levels were significantly related to handling time, we used the residuals of a linear mixed model of log-normal transformed baseline corticosterone levels (dependent variable) and handling time (explanatory

variable) as explanatory variable and individual identity nested into brood identity as random intercept (effect of handling time: $1.66\ \text{ng} \times \text{ml}^{-1} \pm 0.07$ SE, $F_{1,29} = 37.60$, $p < 0.0001$).

After weighing and measuring, the bird was kept in a cloth bag until a second blood sample was taken within 20–28 min (mean ± SD: 1326 s ± 107) after capture. These samples provided values induced by the capture, handling and restraint stress (handling-induced samples, [60,57]. Handling-induced plasma prolactin levels were independent of handling time (linear mixed model with individual identity nested into brood identity as random intercept; effect of handling time on prolactin levels: $F_{1,29} = 3.01$, $p = 0.09$). Handling-induced plasma corticosterone levels were weakly but significantly related to handling time (effect of handling time on log-normal transformed corticosterone levels: $0.0009\ \text{ng} \times \text{ml}^{-1} \pm 0.0003$ SE, $F_{1,29} = 5.70$, $p = 0.02$). Because the effects of handling time within handling-induced levels of corticosterone were weak, we used the raw handling-induced corticosterone levels in all further tests.

Blood was collected in heparinized microcapillary tubes. Within about 5 min after the second blood sample, the plasma was separated from the blood cells after 5 min of centrifugation and stored in liquid nitrogen in the field, then conserved at −20 °C until analysis.

The sex of adults was determined by inspection of the uropygial gland. The gland of breeding females is filled with a brown and smelly secretion, whereas the gland of males is small and empty [34]. Nestling age at capture ranged from 2 to 9 days after hatching. Almost all females (70 out of 73) were caught by hand in the nest box while brooding, i.e. within the first 9 days after hatching. All males were captured at the nest box entrance while provisioning their broods.

As an indicator of body size, we measured tarsus length, length of the 5th primary feather and wing length. Because these variables were highly correlated, we used only the length of the 5th primary as an indicator of the body size. Body mass was measured using a 100 g spring balance.

As an indicator of body condition we used the residuals of a linear mixed model with body mass as the dependent variable and sex with body size, defined as the 5th primary length, as independent variables, and individual identity as random factor (effect size of primary length $0.38\ \text{g} \pm 0.15$ SE, $p = 0.02$, effect size of sex $4.04\ \text{g} \pm 1.45$ SE, $p = 0.006$). The sexes differed in body size (primary length of males 119.3 ± 0.5 SEM, $n = 47$, females 112.8 mm ± 0.5, $n = 73$). Body condition decreased significantly with advancing nestling age in females (linear mixed model with body condition as the dependent, nestling age as the independent and individual identity as random variable: $F_{1,14} = 15.97$, $p = 0.001$, effect size −2.05 ± 0.51 SE) but not in males ($F_{1,8} = 1.47$, $p = 0.2$, effect size −0.35 ± 0.29 SE).

2.3. Hormone assay

Plasma prolactin concentration was determined by a heterologous competitive radio-immuno assay (RIA) at the Centre d'Etude Biologique de Chizé-CNRS in France following the method described by Cherel et al. [18]. All reactants were diluted with 0.025 M barbital–bovine serum albumin (BSA) buffer. 25 µl standard prolactin (ranging from 250 to 16,000 pg/25 µl) or 15 µl hoopoe plasma was incubated with 250 µl antibody solution and 250 µl labelled chicken prolactin (AFP 4444B; Dr. Parlow, NHPP Harbor-UCLA Medical Center, Torrance, CA, USA) for 2 days at 4 °C. An anti-antibody solution was incubated for an additional day and then the free fraction was discarded. The radioactivity of the precipitate was measured directly with an automatic gamma counter (Cobra autogamma, Packard®). Inter-assay variation was 5.91% and intra-assay variation did not exceeded 10%. Hoopoe

plasma cross reacted in the Parlow chicken prolactin assay and diluted out parallel to the standard curve (Fig. 1).

Plasma corticosterone concentration was determined by an enzyme immuno assay at the Swiss Ornithological Institute in Sempach as described in Müller et al. [37]. Briefly, 5–10 µl of plasma was extracted and analysed in triplicates. The inter-assay variation ranged from 14.4% to 16.8% and the intra-assay variation ranged from 9.6% to 18.4%.

2.4. Data analyses

All statistical analyses were performed using the program R [21] (version 2.10.1). The effect of several factors on plasma levels of prolactin (dependent variable) was evaluated with a linear mixed model as implemented in the package nlme [42]. This procedure is appropriate for the analysis of repeated measures in an unbalanced design. Estimates of fixed effects were computed with maximum likelihood estimation. Individual identity nested into the brood identity were introduced as random intercept using restricted maximum likelihood estimation and likelihood-ratio for significance testing [62], thus accounting for the fact that 37.5% of the individuals (33 of 88 individuals) were investigated at two breeding attempts and that we often captured both adults of a brood (64% of the broods; 47 broods with both adults captured, 26 broods with only one adult captured). We tested the dependence of the prolactin levels on disturbing variables (handling time, date) and in a second step for dependence on variables known to influence levels of circulating prolactin (sex, nestling age, body condition with all two-ways interactions). The significance of the fixed effects was tested sequentially in the order specified in the model (hierarchical calculation of p-values) with conditional F-tests [41]. The predicted values presented in the graphs were computed from the reduced model containing only significant terms with $p < 0.05$.

To assess whether handling-induced levels of prolactin differed between the two subsequent blood samples and between the sexes, we conducted a linear mixed model with prolactin levels as the dependent variable, and blood sample (baseline *versus* stress
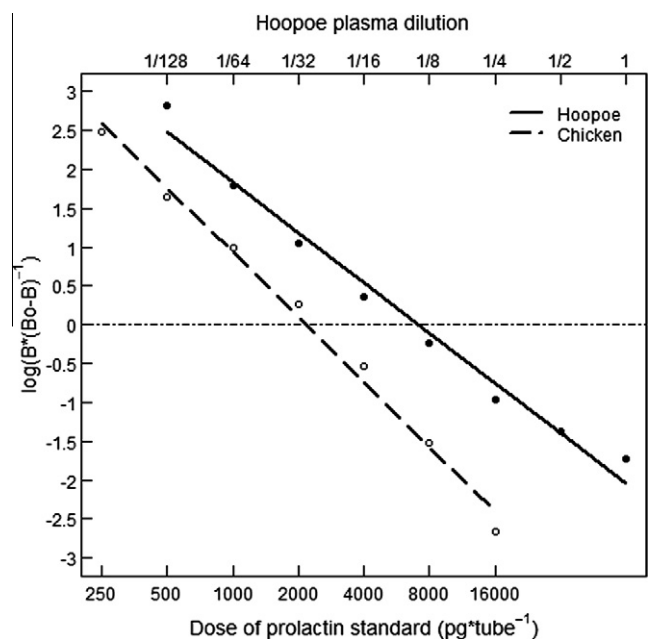

Fig. 1. Dose response curve of standard chicken prolactin (open circle and dashed line) and hoopoe plasma dilutions (solid circle and solid line).

Table 1
Effect of baseline prolactin level, sex, nestling age, body condition and their two-way interaction terms on handling-induced plasma levels of prolactin. The significant terms (in bold) remained significant ($p < 0.05$) when eliminating backwards the non-significant variables from the models.

Variables	df	Effects ± SE	F-value	p-value
Intercept	1,94	54.01 ± 34.37	800.10	<.0001
Baseline prolactin	1,11	−0.21 ± 0.32	171.41	<.0001
Sex	1,94	−69.16 ± 44.32	5.55	0.02
Nestling age	1,11	−8.04 ± 5.22	7.27	0.02
Body condition	1,11	−1.31 ± 4.47	1.54	0.24
Baseline prolactin × sex	1,11	0.99 ± 0.37	3.45	0.09
Baseline prolactin × nestling age	1,11	0.08 ± 0.05	4.63	0.06
Baseline prolactin × body condition	1,11	0.01 ± 0.04	1.16	0.30
Sex × nestling age	1,11	14.08 ± 8.32	6.52	0.03
Sex × body condition	1,11	6.92 ± 3.98	1.01	0.34
Nestling age × body condition	1,11	−0.31 ± 0.66	0.87	0.37
Baseline prolactin × sex × nestling age	1,11	−0.19 ± 0.07	6.71	0.03
Baseline prolactin × sex × body condition	1,11	−0.04 ± 0.03	3.22	0.10
Baseline prolactin × nestling age × body condition	1,11	0.01 ± 0.01	0.58	0.46
Sex × nestling age × body condition	1,11	−0.43 ± −0.51	0.72	0.41

induced), sex and their interaction as fixed factors and individual identity nested into the brood identity as random intercept.

Handling-induced levels of prolactin were dependent on baseline levels (linear mixed model: $F_{1,23} = 136.5$, $p < 0.0001$). We therefore included baseline levels as a covariable in the linear mixed models with handling-induced prolactin levels as the dependent variable (Table 1).

The influence of prolactin level on rearing success and on fledgling success was tested separately with generalized linear models using a quasibinomial or poisson distribution, respectively. Rearing success was calculated as the proportion of fledglings on the total number of eggs laid in a brood. The independent variables were hatching date to take account of seasonal variation and prolactin levels of both sexes including their two-way interaction term. We used the residuals of the prolactin levels computed with the linear mixed model containing all significant terms for baseline and handling-induced levels, respectively. Chi-square was used for significance tests of the single terms and terms with $p > 0.05$ were eliminated from the model.

Because prolactin and corticosterone act concomitantly [1], we compared the baseline levels of the two hormones and the handling-induced levels of the two hormones, respectively. Additionally, we tested for a correlation between baseline levels of prolactin and handling-induced levels of corticosterone, since high baseline levels of prolactin and a dampened corticosterone stress response are associated with parental care. In turn, we tested the influence of corticosterone on the decrease of prolactin levels [ln(baseline levels) – ln(handling-induced levels), the transformation is necessary in order to respect the normal distribution of the residuals], since the secretion of corticosterone may influence prolactin levels. We tested for the correlation between prolactin and corticosterone levels with linear mixed models.

3. Results

3.1. Baseline prolactin levels

Plasma baseline prolactin concentration ranged between 21.0 ng ml^{-1} and 276.2 ng ml^{-1}. Baseline prolactin levels were significantly related to sex, nestling age and body condition, while all interaction terms were not significant (Intercept: 147.5 ng ml^{-1} ± 15.5 SE, $F_{1,89} = 971.7$, $p < 0.0001$; sex (female effect): 36.3 ng ml^{-1} ± 8.8, $F_{1,27} = 34.8$, $p < 0.0001$; nestling age: −9.1 ng ml^{-1} ± 2.6, $F_{1,27} = 21.0$, $p = 0.0001$; body condition: 2.4 ng ml^{-1} ± 0.8, $F_{1,27} = 9.7$, $p = 0.004$; interaction terms: $p > 0.3$; random effects were not significant, $p > 0.7$). Females had higher baseline prolactin levels
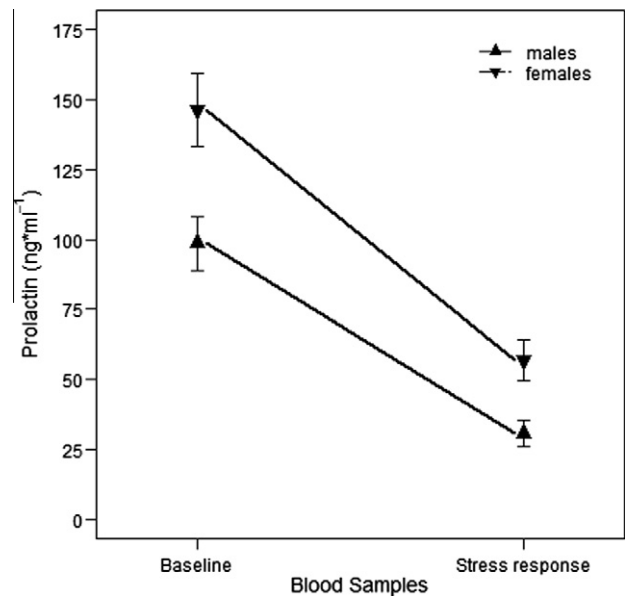

Fig. 2. Baseline and handling-induced levels of prolactin for foraging males (point-up triangles, $n = 47$) and brooding females (point-down triangles, $n = 73$). The stress response was induced by 20–28 min of handling and restraint. The means of the raw data are represented with 0.95 confidence intervals.

than males (Fig. 2). Baseline levels of prolactin dropped to 50% within 7 days after hatching (model predicted estimate from day 2 to day 9 after hatching) without a difference between the sexes (interaction term sex × nestling age not significant). At a given nestling age, baseline levels of prolactin were higher for birds in good body condition than for lean birds (predicted prolactin levels in females at nestling age 2: 182.5 ng ml^{-1} at the 3rd quantile and 158.2 ng ml^{-1} at the 1st quantile of the body condition frequency distribution).

For individuals captured at two subsequent breeding attempts, we observed no significant difference of the prolactin levels between the two captures ($F_{1,18} = 0.24$, $p = 0.63$). We did not observe any correlation in baseline prolactin levels between the mates of a brood. Baseline prolactin levels (corrected for nestling age) were not correlated between the parents of a brood (one-sided correlation test with Pearson estimates: $t = 0.59$, df = 28, $p = 0.56$, $R^2 = 0.11$). Rearing success and fledgling success were independent of baseline levels of females, males or their interaction ($p_{Chi} > 0.1$).

Baseline levels of prolactin were independent of baseline levels of corticosterone for both sexes combined ($F_{1,23} = 0.43$, $p = 0.52$)
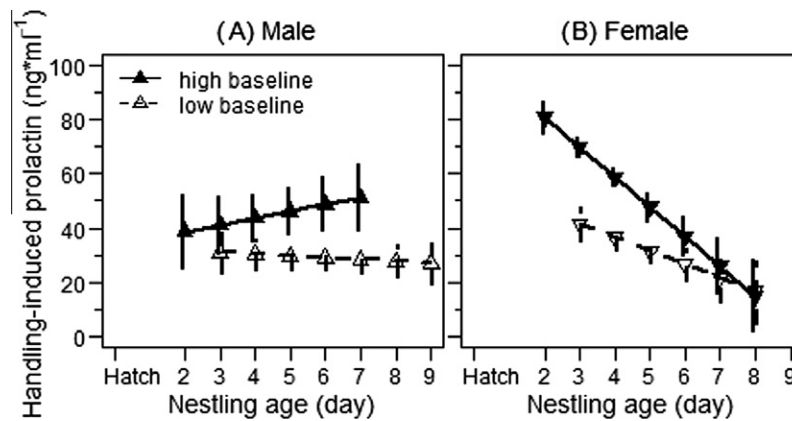
Fig. 3. Dependence of handling-induced plasma prolactin levels on nestling age for males (A) and females (B), as predicted by the model presented in Table 1 with 0.95 confidence intervals. For each sex, two relationships are given, one for individuals with low baseline prolactin levels (88.6 ng ml^{-1} which is the 1st quantile of the frequency distribution, filled triangles and solid lines) and one for individuals with high baseline prolactin levels (158.5 ng ml^{-1} which is the 3rd quantile of the frequency distribution, open triangles and dashed lines).

and for each sex separately (males: $F_{1,8}$ = 1.73, p = 0.22; females: $F_{1,14}$ = 0.04, p = 0.89). Baseline levels of prolactin did not correlate with handling-induced levels of corticosterone (for both sexes combined $F_{1,23}$ = 0.43, p = 0.52; males: $F_{1,8}$ = 1.73, p = 0.22; females: $F_{1,14}$ = 0.04, p = 0.89).

3.2. Handling-induced prolactin levels

Plasma prolactin concentration of samples taken 20–28 min (mean 1326 s ± 107 SD) after capture (handling-induced prolactin) ranged between 6.9 ng ml^{-1} and 182.6 ng ml^{-1}. The decrease of prolactin levels between the first (baseline) and the second (handling induced) blood sample was highly significant and greater in females than in males (linear mixed model with individual identity as random intercept: intercept: 97.94 ng ml^{-1} ± 6.00 SE; difference between baseline and handling-induced samples: −67.98 ng ml^{-1} ± 6.91, $F_{1,142}$ = 351.94, p < 0.001; difference between sexes: 49.68 ng ml^{-1} ± 7.70, $F_{1,94}$ = 38.07, p < 0.001; interaction term: −21.67 ng ml^{-1} ± 8.86, $F_{1,142}$ = 5.98, p = 0.016; the random effect individual identity was significant: LR = 16.33, p < 0.001; Fig. 2).

Handling-induced levels of prolactin were dependent on baseline levels of prolactin, sex, nestling age and the interaction of baseline levels with sex and nestling age, while body condition had no significant influence (Table 1, Fig. 3). Females with the highest baseline levels of prolactin had the strongest decrease of handling-induced prolactin with progressing nestling age (Fig. 3). When the nestlings became thermo-independent (at around 8 days), all females showed similar handling-induced levels of prolactin, independently of their baseline level. In contrast, males with high baseline prolactin levels showed an increase of handling-induced prolactin levels with progressing nestling age, while males with low baseline prolactin levels did not (Fig. 3).

Males decreased prolactin as a response to handling by about 70% throughout the first 9 days after hatching (at 1st quantile of nestling age distribution [2–3d]: baseline levels 118 ng ml^{-1} ± 23 SDM, handling induced levels 33 ng ml^{-1} ± 8 SDM; at 3rd quantile of nestling age distribution [6–9 days]: baseline levels 84 ng ml^{-1} ± 34 SDM, handling-induced levels 24 ng ml^{-1} ± 11 SDM). In contrast, in females the proportional decrease of prolactin levels with handling progressed from 55% just after hatching to 77% at day 9 after hatching (at 1st quantile of nestling age distribution [2–3 days]: baseline levels 170 ng ml^{-1} ± 54 SDM, handling induced levels 77 ng ml^{-1} ± 35 SDM; at 3rd quantile of nestling age distribution [6–9 days]: baseline levels 98 ng ml^{-1} ± 35 SDM, handling-induced levels 23 ng ml^{-1} ± 5 SDM). Therefore, even

though prolactin baseline levels were lower at a later stage of the brood, the handling-induced response in females was proportionally stronger when nestlings reached thermo-independence than close to hatching date (see the significant three-way interaction baseline prolactin × sex × nestling age in Table 1).

Handling-induced levels of prolactin (corrected for baseline levels, sex and nestling age) were not correlated between the parents of the same brood (one-sided correlation test with Pearson estimates: t = 0.18, df = 28, p = 0.86, R^2 = 0.03). Rearing success and fledgling success were not correlated with handling-induced prolactin levels (p_{Chi} > 0.2).

Handling-induced levels of prolactin were weakly correlated with the handling-induced levels of corticosterone (Intercept: 54.42 ng ml^{-1} ± 6.44 SE, $F_{1,95}$ = 301.5, p < 0.0001; handling-induced corticosterone effect: −0.38 ng ml^{-1} ± 0.21, $F_{1,23}$ = 3.41, p = 0.08). In turn, the difference between baseline levels and handling-induced levels of prolactin was weakly but significantly correlated with handling-induced levels of corticosterone (log-normal transformed prolactin levels: Intercept: −0.910 ng ml^{-1} ± 0.079 SE, $F_{1,95}$ = 132.2, p < 0.0001; handling-induced corticosterone effects: −0.007 ng ml^{-1} ± 0.003, $F_{1,23}$ = 7.0, p = 0.015; random effects: p > 0.2; Fig. 4).

4. Discussion

We found higher baseline levels of prolactin in females during the brooding phase than in males. Capture, handling and restraint induced a clear decrease of prolactin levels which was less pronounced in females at the very early stage of brooding compared to females in later stages and to males. Baseline levels of prolactin, but not handling-induced levels, were positively correlated with body condition. Although we hypothesised a negative correlation between prolactin and corticosterone levels, we found only a weak relationship between the decrease in prolactin levels due to handling and the handling-induced levels of corticosterone. Finally, we found no relationship between prolactin levels and reproductive success. In the following we discuss these findings with respect to the five predictions presented in the introduction.

4.1. Variation of baseline prolactin with sex and brooding behaviour

As predicted, baseline levels of prolactin were positively correlated with the type and amount of parental care. Only females brood the chicks with decreasing intensity during the first 10 days after hatching. Brooding females had higher baseline prolactin
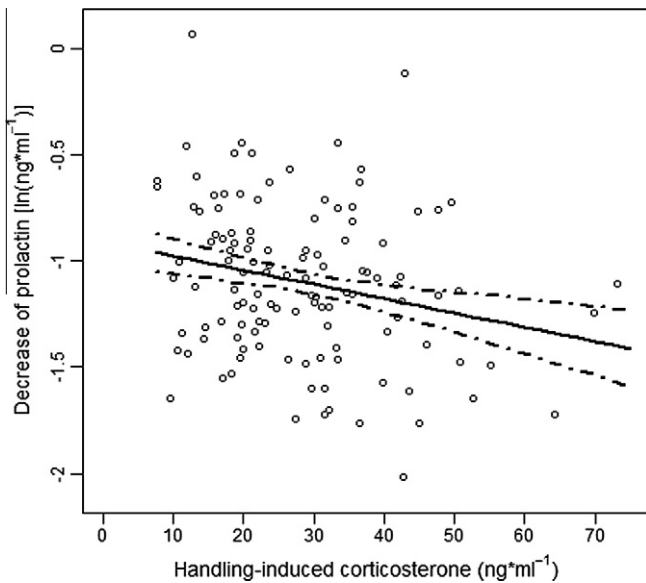
Fig. 4. Dependence of the decrease of prolactin due to handling [ln(baseline levels) – ln(handling-induced levels)] on handling-induced levels of corticosterone. Raw data are represented with the predicted mean (solid line) and 0.95 confidence intervals (dashed lines) of the model presented in the text.

levels than males which provision both females and chicks (Fig. 2). These results agree with most, if not all, studies comparing prolactin levels among mates with asymmetric parental care [29,39].

One may argue that provisioning activity represents as much effort as brooding and therefore both sexes would provide a similar amount of parental care [8]. However, during brooding, the female has little opportunity to forage, while the male can feed himself while provisioning his family, hence the bond to the nest is stronger in females than males. At the mechanistic level, the higher baseline levels of prolactin in females compared to males may result from the positive feedback on prolactin secretion of the permanent contact with the newly hatched nestling's during brooding, similar to the tactile stimulation provided by eggs during incubation (e.g. [11,26]). The decline of baseline levels of prolactin with advancing nestling age corresponds with the time course of change in prolactin secretion found in altricial species (pied flycatcher *F. hypoleuca* [52], ring dove *S. risoria* [13] and three albatross species [27] in which the peak of prolactin secretion generally occurs at hatching and in the presence of newly hatched chicks. Concentrations of prolactin, thereafter, decrease with the progressive decrease of the contact with the chicks until parental care is completed [50]. Experimentally repeated exposure to newly hatched chicks delayed the decrease of baseline prolactin levels in pied flycatchers [52] and suggests that the presence of newly hatched chicks is involved in the regulation of the baseline levels of prolactin. Hence, in the female hoopoe, the baseline level of prolactin decreases with the decrease of brooding intensity and contact frequency with the offspring. The decrease in brooding intensity is coupled with an increase in foraging for self-maintenance and for the offspring. The concomitant decrease in prolactin levels does not support the idea that high prolactin promotes foraging and food intake, as found in non-breeding ring doves [12,14]. Furthermore, the decrease of baseline prolactin in females may have a preparatory effect to quickly breed anew, since elevated prolactin levels may inhibit the hypothalamo–pituitary–gonadal axis and thus may hinder the start of a new breeding cycle [15,47,53,49]. However, comparative studies give little support to that hypothesis, e.g. [51,24] but see [58,56,27].

4.2. Prolactin stress response

This study is the first reporting handling-induced prolactin changes in a non-sea bird species. As predicted, 20–28 min of handling and restraint induced a decrease of prolactin levels (Fig. 2). This agrees with growing evidences that prolactin levels decrease in response to acute stress in breeding birds [17,1,45,28]. Moreover, a stress-induced decrease of prolactin is generally observed in life-history stages characterized by elevated baseline levels [23]. Nonetheless, the decrease by 50–80% within 20–28 min of handling is the largest so far reported.

The magnitude of the prolactin stress response was negatively correlated with the amount of parental care. Females at an early stage of the brood (when the asynchronously hatching nestlings have not yet all hatched) showed the smallest stress-induced decrease of prolactin (Fig. 3). In contrast, females brooding older offspring (partly thermo-independent) showed the largest stress-induced decrease in prolactin levels. Furthermore, females in late brooding stage (nestlings are 7–9 days old) reached similar handling-induced prolactin levels independently of their baseline levels. Finally, males had generally an intermediate decrease of prolactin level throughout the first 9 days after hatching (Fig. 3). Occasionally, the female starts a subsequent brood before the offspring of the current brood fledge, sometimes even immediately after brooding is completed, while only the male provisions the nestlings until fledging. Hence, the prolactin stress response was lowest when the female's presence at the nest was imperative (i.e. brooding), but highest when her presence was facultative. Nonetheless, additional studies are needed to elucidate whether individual variation of prolactin levels drive the decision of females to desert the current brood to start a new subsequent brood. In turn, probably because males provide parental care throughout the nestling phase, they showed a constant prolactin stress response over the first 10 days of the nestling period. However, males with a higher prolactin baseline level decreased prolactin less as a response to handling and restraint (Fig. 3). Hence, the handling-induced decrease of prolactin level may depend on the investment in parental-care, since males with high baseline prolactin levels potentially invest more in parental care [35].

These results agree with previous studies that interpreted the handling-induced decrease of prolactin level as part of the global response to an acute stressor, mediating life-history trade-offs between current reproduction and future survival [17,3,6,55].

4.3. Body condition and baseline and handling-induced levels of prolactin

Independently of the decrease of prolactin with nestling age, baseline levels were higher in birds in better body condition. High prolactin levels are supposed to strongly bind the parent to its brood. Therefore, when the energy stores of the parent becomes depleted, lowering the baseline level of prolactin permits the parent to change the balance between parental care and self-maintenance and redirect more energy from offspring to self-maintenance [18]. As in previous studies, we found no correlation between the prolactin stress response and body condition, e.g. [17,18].

4.4. Correlations between prolactin and corticosterone

We observed only a weak but significant correlation between the decrease of prolactin levels with handling (difference between baseline and handling-induced levels) and handling-induced levels of corticosterone (Fig. 4). This confirms the conclusion of an earlier study in which no evidence was found that the response to an acute stressor is under the direct influence of the HPA-axis [6].

However, both hormones were negatively correlated in some studies, but not in all, because of the concomitant effects in response to environmental and energetic perturbations [1,28]. We propose that uncoupled mechanisms dedicated to the regulation of the prolactin and corticosterone stress response enable a differential processing of the influencing factors. Uncoupled physiological mechanisms can provide a greater flexibility of the regulation of parental behaviour. Nonetheless, to interpret the variation in prolactin levels and its effects on parental decisions, there is a need to elucidate which physiological processes modulate the prolactin stress response and whether they interact with the HPA-axis and corticosterone secretion.

4.5. Prolactin levels and reproductive success

Reproductive success was predicted to be positively correlated with prolactin levels because prolactin levels are positively correlated with parental care. However, we observed no significant relationships between rearing or fledging success and either baseline or handling-induced prolactin levels. To our knowledge, very few studies examined the relationship between natural prolactin level and reproductive success of the current brood [43,38,4]. Only Angelier et al. [4] found that the magnitude of the prolactin stress response predicted incubation success in snow petrels *Pagadroma nivea*. In the same species, exogenous corticosterone decreased the prolactin level and chick survival [5]. Further, when the brood was lost, i.e. when parental care was no longer required, baseline and handling-induced prolactin levels changed in the black-legged kittiwake *Rissa tridactyla* [17].

In this present study, we blood sampled the individuals only once per breeding attempt and used this value as an indicator of parental care in the correlation with rearing or fledging success. However, little is known about within-individual variation of prolactin levels. Hence, the study of the effects of prolactin levels on parental care and subsequent breeding success would need further data from blood samples taken repeatedly at several standardised nestling ages. In addition, a relationship between prolactin and breeding success may be apparent only under more severe environmental conditions.

5. Conclusion

This study confirmed the relationship between baseline prolactin levels and parental care (dependent on body condition) in a species with strongly asymmetric parental duties. Moreover, this study is the first reporting a differential prolactin stress-response between sexes with contrasting parental care within a breeding phase. It appears that the response to acute stress in prolactin levels is finely tuned to the prevailing parental duties (which depend on the sex and the age of the nestlings) and to the individual parental investment (expressed as baseline prolactin level). Similarly as the well-studied stress-response of corticosterone, the stress-response of prolactin appears to be involved in mediating the trade-off between current reproduction *versus* self-maintenance and future reproduction [30,9].

Futures studies are needed to investigate interactions between the corticosterone and the prolactin stress-response which appear to be uncoupled in terms of mechanisms. It seems that the two hormones have differential effects on reproduction which are only beginning to be elucidated [26].

References

[1] F. Angelier, O. Chastel, Stress prolactin and parental investment in birds: a review, Gen. Comp. Endocrinol. 163 (2009) 142–148.
[2] F. Angelier, S.A. Shaffer, H. Weimerskirch, O. Chastel, Effect of age, breeding experience and senescence on corticosterone and prolactin levels in a long-lived bird: the Wandering Albatross, Gen. Comp. Endocrinol. 149 (2006) 1–9.
[3] F. Angelier, B. Moe, H. Weimerskirch, O. Chastel, Age-specific reproductive success in a long-lived bird: do older parents resist stress better?, J Anim. Ecol. 76 (2007) 1181–1191.
[4] F. Angelier, H. Weimerskirch, S. Dano, O. Chastel, Age, experience and reproductive performance in a long-lived bird: a hormonal perspective, Behav. Ecol. Sociobiol. 61 (2007) 611–621.
[5] F. Angelier, C. Clément-Chastel, J. Welcker, G.W. Gabrielsen, O. Chastel, How does corticosterone affect parental behaviour and reproductive success? A study of prolactin in Black-legged kittiwakes, Funct. Ecol. 23 (2009) 784–793.
[6] F. Angelier, B. Moe, S. Blanc, O. Chastel, What factors drive prolactin and corticosterone responses to stress in a long-lived bird species: the snow petrel (*Pagadroma nivea*)?, Physiol. Biochem. Zool. 82 (2009) 590–602.
[7] R. Arlettaz, M. Schaub, J. Fournier, T.S. Reichlin, A. Sierro, J.E.M. Watson, V. Braunisch, From publications to public actions: when conservation biologists bridge the gap between research and action, Bioscience 60 (2010) 835–842.
[8] R. Arlettaz, M. Schaad, T.S. Reichlin, M. Schaub, Impact of weather and climate variation on Hoopoe reproductive ecology and population growth, J. Ornithol. 15 (2010) 889–899.
[9] V. Bókony, Á.Z. Lendvai, A. Licker, F. Angelier, J.C. Wingfield, O. Chastel, Stress response and the value of reproduction: are birds prudent parents?, Am Nat. 173 (2009) 589–598.
[10] C.M. Book, J.R. Millam, M.J. Guinan, R.L. Kitchell, Brood patch innervation and its role in the onset of incubation in the turkey hen, Physiol. Behav. 50 (1991) 281–285.
[11] J.D. Buntin, Neural and hormonal control of parental behaviour in birds, in: J.S. Rosenblatt, C.T. Snowdon (Eds.), Advances in the Study of Behavior, vol. 25, Academic Press, New York, 1996, pp. 161–213.
[12] J.D. Buntin, G.R. Figge, Prolactin and growth hormone stimulate food intake in ring doves, Pharmacol. Biochem. Behav. 31 (1989) 533–540.
[13] J.D. Buntin, R.M. Hnasko, P.H. Zuzick, D.L. Valentine, J.G. Scammell, Changes in bioactive prolactin-like activity in plasma and its relationship to incubation behavior in breeding ring doves, Gen. Comp. Endocrinol. 102 (1996) 221–232.
[14] J.D. Buntin, R.M. Hnasko, P.H. Zuzick, Role of the ventromedial hypothalamus in prolactin-induced hyperphagia in ring doves, Physiol. Behav. 66 (1999) 255–261.
[15] P.M. Camper, W.H. Burke, The effect of prolactin on reproductive function in female Japanese quail (*Coturnix coturnix japonica*), Poult. Sci. 56 (1977) 1130–1134.
[16] O. Chastel, M. Kersten, Brood size and body condition in the House Sparrows *Passer domesticus*: the influence of brooding behaviour, Ibis 144 (2002) 284–292.
[17] O. Chastel, A. Lacroix, H. Weimerskirch, G.W. Gabrielsen, Modulation of prolactin but not corticosterone responses to stress in relation to parental effort in a long-lived bird, Horm. Behav. 47 (2005) 459–466.
[18] Y. Cherel, R. Mauget, A. Lacroix, J. Gilles, Seasonal and fasting-related changes in circulating gonadal steroids and prolactin in king penguins, *Aptenodytes patagonicus*, Physiol. Zool. 67 (1994) 1154–1173.
[19] F. Criscuolo, O. Chastel, F. Bertile, G.W. Gabrielsen, Y. Le Maho, T. Raclot, Corticosterone alone does not trigger a short term behavioural shift in incubating female Common Eiders but does modify long term reproductive success, J. Avian Biol. 36 (2005) 306–312.
[20] D.J. Delehanty, J.W. Oring, A.J. Fivizzani, M.E. El Halawani, Circulating prolactin of incubating male Wilson's phalaropes corresponds to clutch size and environmental stress, Condor 99 (1997) 397–405.
[21] R Development Core Team, R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria (2009), Available from: <http://www.R-project.org>.
[22] M.E. Freeman, B. Kanyicska, A. Lerant, G. Nagy, Prolactin: structure, function and regulation of secretion, Physiol. Rev. 80 (2000) 1523–1631.
[23] R.R. Gala, The physiology and mechanisms of the stress induced changes in prolactin secretion in the rat, Life Sci. 46 (1990) 1407–1420.
[24] V. Garcia, P. Jouventin, R. Mauget, Parental care and the prolactin secretion pattern in the king penguin: an endogenously timed mechanism?, Horm Behav. 30 (1996) 259–265.

[25] R. Groscolas, A. Lacroix, J.P. Robin, Spontaneous egg or chick abandonment in energy-depleted king penguins: a role for corticosterone and prolactin?, Horm Behav. 53 (2008) 51–60.

[26] M.R. Hall, A.R. Goldsmith, Factors affecting prolactin secretion during breeding and incubation in the domestic duck (Anas platyrhynchos), Gen. Comp. Endocrinol. 49 (1983) 270–276.

[27] J.A.L. Hector, A.R. Goldsmith, The role of prolactin during incubation: comparative studies of three Diomedea albatrosses, Gen. Comp. Endocrinol. 60 (1985) 236–243.

[28] B.J. Heidinger, O. Chastel, I.C.T. Nisbet, E.D. Ketterson, Mellowing with age: older parents are less responsive to a stresor in a long-lived seabird, Funct. Ecol. 24 (2010) 1037–1044.

[29] E.D. Ketterson, V. Nolan Jr., L. Wolf, A.R. Goldsmith, Effect of sex, stage of reproduction, season, and mate removal on prolactin in dark-eyed juncos, Condor 92 (1990) 922–930.

[30] Á.Z. Lendvai, M. Giraudeau, O. Chastel, Reproduction and modulation of the stress response: an experimental test in the house sparrow, Proc. R. Soc. B 274 (2007) 391–397.

[31] H. Lormée, P. Jouventin, O. Chastel, R. Mauget, Endocrine correlates of parental care in an Antarctic winter breeding seabird, the Emperor Penguin, Aptenodytes forsteri, Horm. Behav. 35 (1999) 9–17.

[32] H. Lormée, P. Jouventin, A. Lacroix, O. Chastel, Reproductive endocrinology of tropical seabirds: sex-specific patterns in LH, steroids, and prolactin secretion in relation to parental care, Gen. Comp. Endocrinol. 117 (2000) 413–426.

[33] M. Martín-Vivaldi, J.J. Palomino, M. Soler, J.J. Soler, Determinants of reproductive success in the Hoopoe Upupa epops a hole-nesting non-passerine bird with asynchronous hatching, Bird Study 46 (1999) 205–216.

[34] M. Martín-Vivaldi, M. Ruiz-Rodríguez, J.J. Soler, J.M. Peralta-Sánchez, M. Méndez, E. Valdivia, A.M. Martín-Platero, M. Martínez-Bueno, Seasonal sexual and developmental differences in hoopoe preen gland morphology and secretions. Evidence for a role of bacteria, J. Avian Biol. 40 (2009) 191–205.

[35] D.A. Miller, C.M. Vleck, D.L. Otis, Individual variation in baseline and stress-induced corticosterone and prolactin levels predicts parental effort by nesting mourning doves, Horm. Behav. 56 (2009) 457–464.

[36] J. Moreno, Strategies of mass change in breeding birds, Biol. J. Linn. Soc. 37 (1989) 297–310.

[37] C. Müller, S. Jenni-Eiermann, L. Jenni, Effects of a short period of elevated circulating corticosterone on postnatal growth in free-living Eurasian kestrels Falco tinnunculus, J. Exp. Biol. 212 (2009) 1405–1412.

[38] T.W. O'Dwyer, W.A. Buttemer, D.M. Priddel, J.A. Downing, Prolactin, body condition and the costs of good parenting: an interyear study in a long-lived seabird, Gould's petrel (Pterodroma leucoptera), Funct. Ecol. 20 (2006) 806–811.

[39] L.W. Oring, A.J. Fivizzani, M.A. Colwell, M.E. El Halawani, Hormonal changes associated with natural and manipulated incubation in the sex-role reversed Wilson's Phalarope, Gen. Comp. Endocrinol. 72 (1988) 247–256.

[40] S.F. Perry, K.M. Gilmour, Respiratory and cardiovascular system during stress, in: P.H.M. Balm (Ed.), Stress Physiology in Animals, Sheffield Academic Press, Sheffield, 1999, pp. 52–107.

[41] J. Pinheiro, D. Bates (Eds.), Mixed-Effects Models in S and S-Plus, Springer, New York, 2000.

[42] J. Pinheiro, D. Bates, S. DebRoy, D. Sarkar, The R Core Team, nlme: Linear and Nonlinear Mixed Effects Models, 2009, R package version 3.1–96.

[43] M. Préault, O. Chastel, F. Cézilly, B. Faivre, Male bill colour and age are associated with parental abilities and breeding performance in blackbirds, Behav. Ecol. Sociobiol. 58 (2005) 497–505.

[44] R.E. Ricklefs, M. Wikelski, The Physiology/Life-History Nexus, Tr. Ecol. Evol. 17 (2002) 462–468.

[45] S. Riou, O. Chastel, A. Lacroix, C.H. Hamer, Stress and parental care: prolactin response to acute stress throughout the breeding cycle in a long-lived bird, Gen. Comp. Endcrinol. 168 (2010) 8–13.

[46] L.M. Romero, J.M. Reed, Collecting baseline corticosterone samples in the field: is under 3 min good enough?, Gen Comp. Endocrinol. 140 (2005) 73–79.

[47] I. Rozemboim, C. Tabibzadeh, M.E. El Halawani, Effect of ovine prolactin administration on hypothalamic vasoactive intestinal peptide (VIP), onadotropin releasing hormone I and II content, and anterior pituitary VIP receptors in laying turkey hens, Biol. Reprod. 48 (1993) 1246–1250.

[48] R.M. Sapolsky, L. Romero, A. Munck, How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions, Endocr. Rev. 21 (2000) 55–89.

[49] P.J. Sharp, M.C. MacNamee, R.J. Sterling, R.W. Lea, H.C. Pedersen, Relationship between prolactin, LH and broody behaviour in bantam hens, J. Endocrinol. 118 (1988) 279–285.

[50] P.J. Sharp, A. Dawson, R.W. Lea, Control of luteinizing hormone and prolactin secretion in birds, Comp. Biochem. Physiol. C 119 (1998) 275–282.

[51] B. Silverin, Annual changes in plasma levels of LH, and prolactin in free-living female great tits (Parus major), Gen. Comp. Endocrinol. 83 (1991) 425–431.

[52] B. Silverin, A.R. Goldsmith, Plasma prolactin concentration in breeding pied flycatchers (Ficedula hypoleuca) with an experimentally prolonged brooding period, Horm. Behav. 24 (1990) 104–113.

[53] A.J. Smith, M. Mondain-Monval, P. Simon, B.K. Andersen, O.P.F. Clausen, P.O. Hofmo, R. Scholler, Preliminary studies of the effects of bromocriptine on testicular regression and the spring moult in a seasonal breeder, the male blue fox (Alopex lagopus), J. Reprod. Fert. 80 (1987) 517–524.

[54] K.S. Stier, B. Almasi, J. Gasparini, R. Piault, A. Roulin, L. Jenni, Effects of corticosterone on innate and humoral immune functions and oxidative stress in barn owl nestlings, J. Exp. Biol. 212 (2009) 2085–2091.

[55] J. Verreault, N. Verboven, G.W. Gabrielsen, R.J. Letcher, O. Chastel, Changes in prolactin in a highly organohalogen contaminated Arctic top predator seabird, the Glaucous gull, Gen. Comp. Endocrinol. 156 (2008) 569–576.

[56] C.M. Vleck, L. Ross, D. Vleck, T.L. Bucher, Prolactin and parental behavior in Adélie penguins: effect of absence from nest, incubation length, and nest failure, Horm. Behav. 38 (2000) 149–158.

[57] J.C. Wingfield, Modulation of the adrenocortical response to stress in birds, in: K.G. Davey, R.E. Peter, S.S. Tobe (Eds.), Perspective in Comparative Endocrinology, National Research Council of Canada, Ottawa, 1994, pp. 502–528.

[58] J.C. Wingfield, A.R. Goldsmith, Plasma levels of prolactin and gonadal steroids in relation to multiple-brooding and renesting in free-living populations of the song sparrow, Melospiza melodia, Horm. Behav. 24 (1990) 89–103.

[59] J.C. Wingfield, R.M. Sapolsky, Reproduction and resistance to stress: when and how?, J Neuroendocrinol. 15 (2003) 711–724.

[60] J.C. Wingfield, C.M. Vleck, M.C. Moore, Seasonal changes in adrenocortical response to stress in birds of the Sonoran desert, J. Exp. Zool. 264 (1992) 419–428.

[61] J.C. Wingfield, D.L. Maney, C.W. Breuner, J.D. Jacobs, S. Lynn, M. Ramenofsky, R.D. Richardson, Ecological bases of hormone–behavior interactions: the emergency life history stage, Am. Zool. 38 (1998) 191–206.

[62] A.F. Zuur, E.N. Ieno, N.J. Walker, A.A. Saveliev and G.M. Smith (Eds.), Mixed Effects Models and Extensions in Ecology, R. Springer Science+Business Media, New York, 2009.