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Stress and the timing of breeding: Glucocorticoid-luteinizing hormones relationships in an arctic seabird

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ABSTRACT

In birds, stressful environmental conditions delay the timing of breeding but the underlying mechanisms are poorly understood. The stress hormone corticosterone appears to be a good candidate for mediating the decision to breed and when to start egg-laying, via a possible inhibition of luteinizing hormone (LH) and sex-steroids production. We used luteinizing hormone releasing hormone (LHRH) challenge in pre-laying male and female Black-legged kittiwakes (*Rissa tridactyla*) to test whether LH and testosterone secretion were depressed by elevated corticosterone levels. Females bearing high baseline corticosterone levels showed reduced baseline LH levels and a low ability to release LH, following LHRH challenge. Further, females bearing low baseline LH levels and elevated baseline corticosterone levels were more likely to skip breeding. However, non-breeding females were physiologically primed for breeding, since they mounted high LHRH-induced LH release. Egg-laying date was advanced in good body condition females but was unaffected by hormones secretion. In males, corticosterone levels had no effect on LH and/or testosterone secretion and did not affect their decision to breed. Interestingly, males with high LHRH-induced testosterone release bred early. Our study highlights clear sex-differences in the HPG sensitivity to stress hormones in pre-laying kittiwakes. Because females have to store body reserves and to build up the clutch, they would be more sensitive to stress than males. Moreover, intrasexual competition could force male kittiwakes to acquire reproductive readiness earlier in the season than females and to better resist environmental perturbations. We suggest that high testosterone releasing ability would mediate behavioural adjustments such as courtship feeding, which would stimulate early egg-laying in females.

1. Introduction

Breeding at the right time is a key-component of fitness, as successful reproduction requires a good overlap between energy-demanding needs and the peak of available resources (*match-mismatch hypothesis*, Cushing, 1990; Visser et al., 1998; Stenseth and Mysterud, 2002; Durant et al., 2007; review in Visser, 2008). Hence, as adaptive responses to environmental variations, free-living organisms must exhibit considerable behavioural and physiological flexibilities in the timing of their seasonal activities (review in Wingfield, 2008). To do so, they integrated photoperiod, as a fixed cue (Wingfield and Kenagy, 1991; Dawson et al., 2001) and variable cues, such as ambient temperature, food supply, nest sites availability, and/or stimulatory social interactions (Wingfield, 1980; Wingfield and Kenagy, 1991; Ball, 1993; Visser et al., 1998; Wingfield et al., 2003; Schoech et al., 2004; Ball and Ketterson, 2008; Both et al., 2009). At the endocrine level, the onset of breeding involves the activation of the hypothalamic-pituitary-gonadal axis (hereafter HPG axis): increasing day length activates the expression of a neurohormone, the Gonadotropin Releasing Hormone (GnRH) that triggers the release of two pituitary gonadotrophins: the luteinizing hormone and the follicle-stimulating hormone (LH and FSH, reviewed in Dawson et al., 2001). In turn, gonadotrophins activate the gonadal development and the release of sex steroids such as estradiol and testosterone. A wide range of sex steroid hormone-dependent behaviours is then expressed (Ball, 1993), such as nest building, courtship, and mating. However, this hormonal cascade is not only driven by photoperiod, but could also be regulated by additional non-photoperiodic cues. Specifically, pre-laying energetic constraints, such as food shortage and/or environmental cues that enable individuals to anticipate food availability (see Shultz

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et al., 2009) should be involved in the down-regulation of the HPG axis (Wingfield et al., 2003).

At the individual level, the underlying mechanisms have received increased attention (review in Schoech et al., 2009). One potential mediator is the endocrine stress response, which is known to adjust life-history strategies in relation to environmental conditions and to individual physiological state (Ricklefs and Wikelski, 2002; Wingfield and Sapolsky, 2003). Indeed, the release of glucocorticoids during stressful perturbations triggers physiological and behavioural adjustments that shift energy investment away from reproduction and redirects it towards survival (Wingfield and Sapolsky, 2003). Relating this to the timing of breeding, elevated pre-laying glucocorticoids levels might postpone or even halt the immediate breeding event, as correlatively shown in Florida scrub-jays (*Aphelocoma coerulescens*, Schoech et al., 2009), Marine iguanas (*Amblyrhynchus cristatus*, Vitousek et al., 2010), and Snow petrels (*Pagodroma nivea*, Goutte et al., 2010). Moreover, experimental administration of glucocorticoids decreased the proportion of breeding females and delayed the onset of egg-laying in captive Zebra finches (*Taeniopygia guttata*, Salvante and Williams, 2003). However, the functional action of glucocorticoids on the HPG axis remains poorly understood in free-living vertebrates (reviewed in Schoech et al., 2009).

In domesticated animals, it has been better documented how stress and stress hormones can act on the HPG system via central and peripheral sites. First, glucocorticoids can suppress LH release by inhibiting pituitary responsiveness to GnRH (e.g. Breen and Karsch, 2006). Second, glucocorticoids can act directly at the hypothalamic level by disrupting the GnRH pulse frequency (e.g. Oakley et al., 2009). Third, glucocorticoids can impair testicular development but not through the inhibition of LH release (e.g. in Common carp, *Cyprinus carpio* L., Consten et al., 2002). A novel down-regulator of the HPG axis, the gonadotropin-inhibitory hormone (GnIH), appears to inhibit the release of LH, FSH and testosterone (review in Bentley et al., 2009; Tsutsui et al., 2009; McGuire and Bentley, 2010), and is especially expressed during stressful conditions and/or glucocorticoid release (Calisi et al., 2008; Kirby et al., 2009). Thus, stress hormones seem to be candidates for the stress-related inhibition of the reproductive axis and associated physiological pathways.

Despite these findings from captive or domesticated species, the functional action of glucocorticoids on gonadotropin release and timing of breeding remains unclear and controversial in free-living vertebrates (review in Schoech et al., 2009). For instance, experimental increased glucocorticoid levels did not suppress LH and testosterone levels in free-living male American Tree sparrows (*Spizella arborea*, Astheimer et al., 2000) and failed to delay breeding in food-supplemented Florida scrub jay (*Aphelocoma coerulescens*, Schoech et al., 2007). In that context, it is crucial to carefully distinguish the two levels of glucocorticoids. The baseline glucocorticoid level is a marker of activities, energetic state and food availability (Kitaysky et al., 1999; Love et al., 2004; review in Landys et al., 2006), while the stress-induced glucocorticoid level mirrors the sensitivity to stress and the commitment into current reproduction (review in Wingfield and Sapolsky, 2003; Lendvai et al., 2007). Moreover, it is difficult to draw a general picture since males and females are expected to differ in the down-regulation of the HPG axis in response to stressful events (review in Ball and Ketterson, 2008).

The aim of the present study was to address the functional action of corticosterone -the main glucocorticoid in birds- on the breeding decision and the adjustment of egg-laying date in an Arctic population of Black-legged kittiwake (*Rissa tridactyla*). At the population level, a long-term (38 years) study in Svalbard showed great inter-annual variation in the timing of breeding (Moe et al., 2009). Moreover, breeding success was higher in years when breeding was early, and high spring sea surface temperatures were associated with early breeding (Moe et al., 2009). This population is thus well-appropriated for our study, since at the individual level, early (pre-laying) environmental and physiological conditions should be decisive for the breeding schedule. In Svalbard, kittiwakes attend the colony during two months before egg-laying (i.e. pre-laying period), thus offering the opportunity to explore corticosterone/LH relationships in pre-laying males and females, by using luteinizing hormone releasing hormone (LHRH) challenge. LHRH is an equivalent of the GnRH, and a small injection of LHRH is commonly used to assess the readiness of a bird to breed and its ability to release temporary LH and sex-steroid (Schoech et al., 1996). We predicted that elevated baseline and/or stress-induced corticosterone levels during the pre-laying period would be linked to non-breeding decision or postponed egg-laying date, through the disruption of the HPG axis. First, we tested whether baseline and/or LHRH-induced levels of LH or testosterone decreased with increasing baseline and/or stress-induced corticosterone levels. Then, we investigated whether the breeding decision was related to hormonal levels (corticosterone, LH, testosterone). Finally, we tested whether the egg-laying date was related to hormonal levels (corticosterone, LH, testosterone).

2. Materials and methods

2.1. Study area and birds

Our study was conducted on a colony of Black-legged kittiwakes at Kongsfjorden, Svalbard (78°54′N, 12°13′E), 7 km southeast of Ny-Ålesund, Norway. Black-legged kittiwakes are colonial seabirds that breed on cliffs throughout the northern parts of the Pacific and Atlantic, including the Barents Sea region up to the Svalbard Archipelago (Anker-Nilssen et al., 2000). We studied kittiwakes in one plot of around 116 pairs breeding on cliff ledges at heights of 5–10 m.

2.2. Blood sampling and LHRH challenge

Male and female kittiwakes were sampled from 20 May to 6 June 2008, during the pre-laying period (i.e. copulations and nest building period). In Black-legged kittiwakes, baseline LH in males and females and testosterone in males reach maximal levels during the pre-laying period (Goutte et al., unpublished data). Seventy seven birds were caught on the nests with a noose at the end of a 5 m fishing rod. In 50 of them, a first blood sample (ca. 0.3 mL) was collected immediately after capture, from the alar vein with a 1 mL heparinised syringe and a 25-gauge needle to assess baseline LH, testosterone (in males only) and corticosterone levels. Bleeding time (i.e. time elapsed from capture to the end of the first blood sample: 3 min 31 ± 5 [SE] seconds) exceeded the time recommended by Romero and Reed (2005) for some birds. Corticosterone levels were thus related to bleeding time in males (estimate of the slope: 0.067 ± 0.019, $F_{1,25} = 14.540$, $p < 0.001$) but not in females ($F_{1,21} = 0.012$, $p = 0.914$). We performed statistical tests by using the residuals of corticosterone against bleeding time, then by excluding corticosterone levels, whose bleeding time exceeded 3 min, and then by using absolute corticosterone levels. The three approaches led to similar results. Hence, we reported the results for absolute corticosterone levels to facilitate the comparison with other published results.

Immediately after this first blood sampling, kittiwakes were injected with 0.1 mL of a solution of LHRH ([Gin^8], Sigma Lot 121H04314) to test the responsiveness of the pituitary gland and the gonads. The LHRH was dissolved in physiological solution to yield a final dosage of 0.6 μg/0.1 mL (1.5 μg/kg body mass in

1 mL of 0.9% saline solution). This dose of LHRH has been shown to be sufficient to elicit the maximal release of LH in other seabird species (Jouventin and Mauget, 1996). We administered 0.1 mL of LHRH solution (LHRH-injected birds, $N = 46$) or saline solution (control birds, $N = 31$) directly into the alar vein. Kittiwakes were then placed into cloth bags and subsequent blood samples (ca. 0.3 mL) were collected from the alar vein at 10 min and 30 min after the injection. Hence we assessed the LHRH-induced release of LH in males and females, as well as the LHRH-induced release of testosterone in males only.

Kittiwakes were individually marked with metal rings and PVC plastic bands engraved with a three-digit code and fixed to the bird's tarsus for identification from a distance. Birds were weighed to the nearest 2 g using a Pesola spring balance, and their skull length (head + bill) was measured to the nearest 0.5 mm with a sliding caliper. A scaled mass index (Peig and Green, 2009) was calculated individually for males and females separately, because of sex-difference in skull length (Moe et al., 2002). Kittiwakes were marked with spots of dye on the forehead to distinguish them from their partner during subsequent observation and were released. Using a mirror at the end of an 8 m fishing rod, we checked the whole plot (ca. 116 nests) every two days to monitor breeding decision (at least one egg is laid or no egg laid) and egg-laying dates. Breeding decision and egg-laying date were not influenced by the LHRH-injection ($p > 0.39$ for all tests).

2.3. Molecular sexing and hormone assay

Blood samples were centrifuged, and plasma and red blood cells were separated and stored at −20 °C until used, respectively in hormone assays or molecular sexing, at the Centre d'Etudes Biologiques de Chizé (CEBC). Molecular sexing was performed as detailed in Weimerskirch et al. (2005). LH radioimmunoassay was conducted following the methods previously described for other seabirds (Mauget et al., 1994; Chastel et al., 2005), and validated for Black-legged kittiwake plasma. Pooled plasma samples of kittiwakes produced dose–response curves that paralleled the chicken LH standard curves (''AGM 51122F", sources: LH, Prf. Ishii and Wakabayashi, Wadesa University, Japan, Fig. 1). Parallel curves indicate that the concentration-dependent binding of LH to antibody is similar in kittiwakes and chickens, and that this heterologous RIA can be used to assess relative levels of plasma LH in the Black-legged kittiwakes. The lowest detectable concentration for LH was 0.06 ng/mL and the intra-assay coefficient of variation was 8.7% ($N = 3$ duplicates). Plasma concentrations of testosterone were assayed for males only, by radioimmunoassay, at the CEBC as described by Chastel et al. (2003). The lowest detectable concentration for testosterone was 0.05 ng/mL and the intra-assay coefficient of variation was 7% ($N = 3$ duplicates). Plasma concentrations of corticosterone were determined by radioimmunoassay at the CEBC, as described by Lormée et al. (2003). The lowest detectable concentration for corticosterone was 0.5 ng/mL. Only one assay was performed and the intra-assay coefficient of variation was 6.7% ($N = 5$ duplicates).

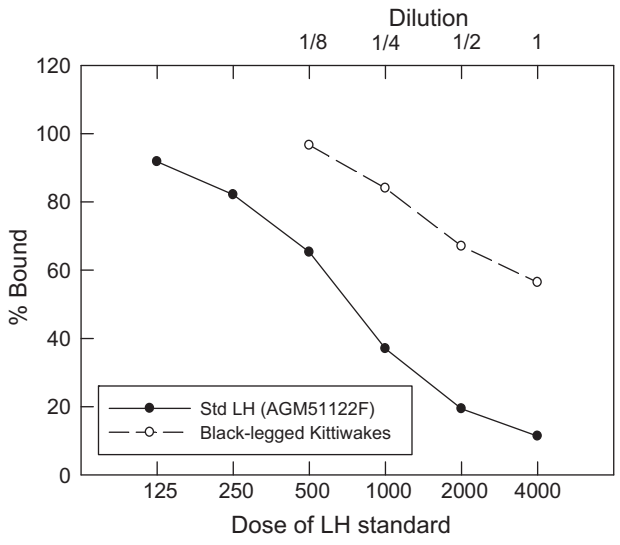

Fig. 1. Dose–response curve for LH in standard LH (AGM 51122F) and Black-legged kittiwakes. LH standard is expressed in pg per tube.

2.4. Statistical analyses

All statistical analyses were performed using R 2.8.0 (R Development Core Team, 2008). We used generalised linear mixed-effects models (GLMM) and included bird identity as a random effect, to test the individual variation of hormone levels over 10 and 30 min after the injection of LHRH or saline solution. Then we used generalised linear models (GLM) with a normal/binomial error distribution and an identity/logit link function to test our biological assumptions (Table 1). Baseline and LHRH-induced LH and testosterone levels were tested as a function of bleeding time, sampling date and scaled mass index in males and females separately (Table 1i). Then, effects for baseline and/or stress-induced corticosterone levels on baseline and LHRH-induced LH levels were tested in pre-laying male and female kittiwakes and on baseline and LHRH-induced testosterone levels in males only (Table 1ii). Lastly, the effects for hormone levels on breeding decision and on first egg-laying date were tested in males and females (Table 1iii and 1iv). Diagnostic plots were assessed whether the data sufficiently met the assumptions of the linear model, and dependent continuous variables were log-transformed when necessary.

3. Results

LHRH-injection had no effect on stress-induced corticosterone levels compared to controls (10 min: $F_{1,72} = 0.295$, $p = 0.589$, 30 min: $F_{1,70} = 0.621$, $p = 0.434$). Corticosterone levels significantly increased after 10 and 30 min (GLMM, $F_{2,143} = 615.911$, $p < 0.001$), without effect of sex (GLMM, $F_{1,75} = 2.217$, $p = 0.141$; interaction: $F_{2,141} = 1.511$, $p = 0.224$). Scaled mass index was neither correlated to sampling date (males: $F_{1,41} = 1.127$, $p = 0.295$, females: $F_{1,32} = 2.146$, $p = 0.153$). Before LHRH-injection, baseline (i.e. at 0 min) hormone levels did not differ between LHRH-injected and control kittiwakes (GLM, LH: $F_{1,41} = 0.690$, $p = 0.411$; testosterone: $F_{1,25} = 0.131$, $p = 0.721$, corticosterone: $F_{1,74} = 0.501$, $p = 0.481$). Following LHRH injection, LH levels reached maximum levels at 10 min and then returned to baseline after 30 min (GLMM, time as factor, $F_{2,50} = 41.684$, $p < 0.001$), without sex difference (sex: $F_{1,42} = 2.263$, $p = 0.140$; interaction: $F_{2,48} = 1.640$, $p = 0.205$, Fig. 2A and B). In controls birds, LH levels significantly decreased over 10 and 30 min of handling (GLMM, $F_{2,32} = 8.029$, $p = 0.002$, Fig. 2A and B) and were lower in males than in females at 30 min (GLM, $F_{1,22} = 12.161$, $p = 0.002$) but not at 10 min after the injection of saline solution ($F_{1,21} = 1.302$, $p = 0.267$). In LHRH-injected males, testosterone levels significantly increased over 30 min after injection (GLMM, $F_{2,32} = 5.958$, $p = 0.006$, Fig. 2C), while in control males, testosterone significantly decreased over 30 min (GLMM, $F_{2,28} = 5.648$, $p = 0.009$, Fig. 2C).

Concerning the factors influencing hormonal levels during the pre-laying period (Table 1i), baseline LH levels were not influenced by sampling date (males: $F_{1,25} = 1.135$, $p = 0.297$; females: $F_{1,21} = 3.080$, $p = 0.094$), by scaled mass index (males: $F_{1,24} = 0.200$,

Table 1
Biological assumptions tested and associated generalised linear models. Model selection was performed by a step down approach starting from the global model including all the independent variables. Sample sizes were given for males (Nm) and females (Nm). Corticosterone and luteinizing hormone levels were abbreviated as 'CORT' and 'LH'.

Dependant variable	Independant variables	Nm	Nf
i-Effect of sampling date, body condition and sex on hormone levels			
1-a Baseline LH	Bleeding time, sampling date, scaled mass index	27	23
1-b LHRH-induced LH	Sampling date, scaled mass index	23	17
1-c Baseline testosterone	Bleeding time, sampling date, scaled mass index	27	
1-d LHRH-induced testosterone	Sampling date, scaled mass index	22	
ii-Effect of corticosterone levels on LH and testosterone levels			
2-a Baseline LH	Baseline CORT	27	22
2-b Baseline LH	Stress-induced CORT	27	22
2-c LHRH-induced LH	Baseline CORT	23	16
2-d LHRH-induced LH	Stress-induced CORT	23	16
2-e Baseline Testosterone	Baseline CORT	27	
2-f Baseline Testosterone	Stress-induced CORT	26	
2-g LHRH-induced Testosterone	Baseline CORT	22	
2-h LHRH-induced Testosterone	Stress-induced CORT	22	
iii-Effect of hormone levels on breeding decision (breeding or skipped breeding)			
3-a Breeding decision	Body condition	43	32
3-b Breeding decision	Baseline CORT	27	21
3-c Breeding decision	Stress-induced CORT	42	29
3-d Breeding decision	Baseline LH	27	21
3-e Breeding decision	LHRH-induced LH	23	16
3-f Breeding decision	Baseline Testo	27	
3-g Breeding decision	LHRH-induced Testo	22	
iv-Effect of hormone levels on first-egg-laying date			
4-a First-egg-laying date	Body condition	31	20
4-b First-egg-laying date	Baseline CORT	20	15
4-c First-egg-laying date	Stress-induced CORT	30	20
4-d First-egg-laying date	Baseline LH	20	15
4-e First-egg-laying date	LHRH-induced LH	15	10
4-f First-egg-laying date	Baseline Testo	18	
4-g First-egg-laying date	LHRH-induced Testo	15	

$p = 0.659$; females: $F_{1,20} = 1.425$, $p = 0.247$), and by bleeding time (males: $F_{1,23} < 0.001$, $p = 0.997$; females: $F_{1,19} = 0.014$, $p = 0.908$). LHRH-induced LH levels (10 min) were not influenced by sampling date (males: $F_{1,21} < 0.001$, $p = 0.988$; females: $F_{1,15} = 1.195$, $p = 0.293$) and scaled mass index (males: $F_{1,20} = 0.653$, $p = 0.429$; females: $F_{1,14} = 0.529$, $p = 0.479$). In males, baseline testosterone levels were not related to bleeding time ($F_{1,25} = 0.134$, $p = 0.718$). Baseline and LHRH-induced (30 min) testosterone levels were neither influenced by sampling date ($F_{1,24} = 0.717$, $p = 0.406$ and $F_{1,19} = 0.927$, $p = 0.348$) nor by scaled mass index ($F_{1,25} = 2.355$, $p = 0.138$ and $F_{1,20} = 3.385$, $p = 0.081$).

3.1. Effect of corticosterone levels on LH and testosterone (Table 1ii)

Baseline LH levels decreased significantly with increasing baseline corticosterone levels in females (estimate: −0.405 ± 0.228, Table 2a, Fig. 3A) but not in males (estimate: 0.136 ± 0.099, Table 2a, Fig. 3B). In addition, LHRH–induced LH levels (10 min) decreased significantly with increasing baseline corticosterone levels in females (estimate: −0.929 ± 0.474, Table 2c, Fig. 3C) but not in males (estimate: −0.165 ± 0.224, Table 2c, Fig. 3D). There was no effect of stress-induced corticosterone levels on baseline and LHRH-induced LH levels in both sexes (Table 2b and d). In males, baseline testosterone levels did not vary with baseline or stress-induced corticosterone levels (Table 2e and f). LHRH-induced testosterone levels did not vary with baseline and/or stress-induced corticosterone levels (Table 2g and h).

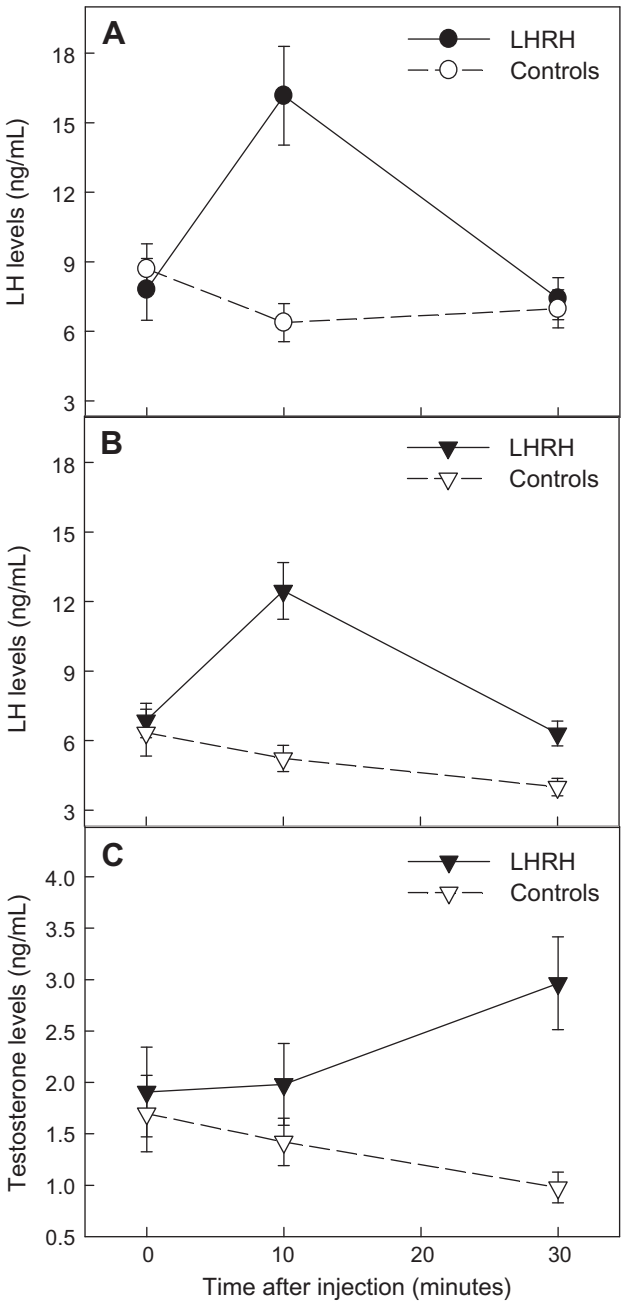

Fig. 2. Hormonal change after the injection of the LHRH solution (solid line, filled symbols) or of the saline solution (control, dashed lines, open symbols). LHRH-injected females (A, circle) and males (B, triangle) significantly released LH over the first 10 min then LH returned to the baseline levels over 30 min, while control levels decreased after injection. (C) Testosterone levels in males following the injection of LHRH (solid line) or saline solution (control, dashed lines). LHRH-injected males showed significantly elevated testosterone over 30 min, while control levels decreased after injection.

3.2. Hormonal levels and breeding decision (Table 1iii)

Breeding decision was not influenced by scaled mass index in both sexes (Table 3a). In females, breeding decision was related to pre-laying baseline LH levels and to pre-laying baseline corticosterone levels: females that will not breed show significantly lower pre-laying baseline LH levels and higher baseline corticosterone levels than females that will breed (Table 3a, Fig. 4A and B). LHRH-induced LH levels and stress-induced corticosterone levels were not linked to female breeding decision (Table 3a, Fig. 4C and D). In males, breeding decision was not related to levels of

Table 2
Modelling baseline (Hyp. 2a and 2b) and LHRH-induced (Hyp. 2c and 2d) LH levels, baseline (Hyp. 2e and 2f) and LHRH-induced (Hyp. 2 g and 2h) testosterone (T) levels as a function of baseline (or stress-induced) corticosterone levels (CORT), using GLMs (normal error distribution, identity link function) in pre-laying male and female kittiwakes.

Hyp	Dependant variable	Independant variables	d.f.	*F*	*p*-value
2a	Baseline LH levels	Baseline CORT (males)	1,25	1.880	0.183
		Baseline CORT (females)	**1,20**	**4.327**	**0.050**
2b	Baseline LH levels	Stress-induced CORT (males)	1,25	1.188	0.286
		Stress-induced CORT (females)	1,20	0.020	0.890
2c	LHRH-induced LH levels (log)	Baseline CORT (males)	1,21	0.224	0.641
		Baseline CORT (females)	**1,14**	**4.643**	**0.049**
2d	LHRH-induced LH levels (log)	Stress-induced CORT (males)	1,21	0.432	0.518
		Stress-induced CORT (females)	1,14	2.175	0.162
2e	Baseline T levels (log)	Baseline CORT (males)	1,25	1.370	0.253
2f	Baseline T levels (log)	Stress-induced CORT (males)	1,24	0.265	0.612
2g	LHRH-induced T levels (log)	Baseline CORT (males)	1,20	3.221	0.088
2h	LHRH-induced T levels (log)	Stress-induced CORT (males)	1,20	0.164	0.690

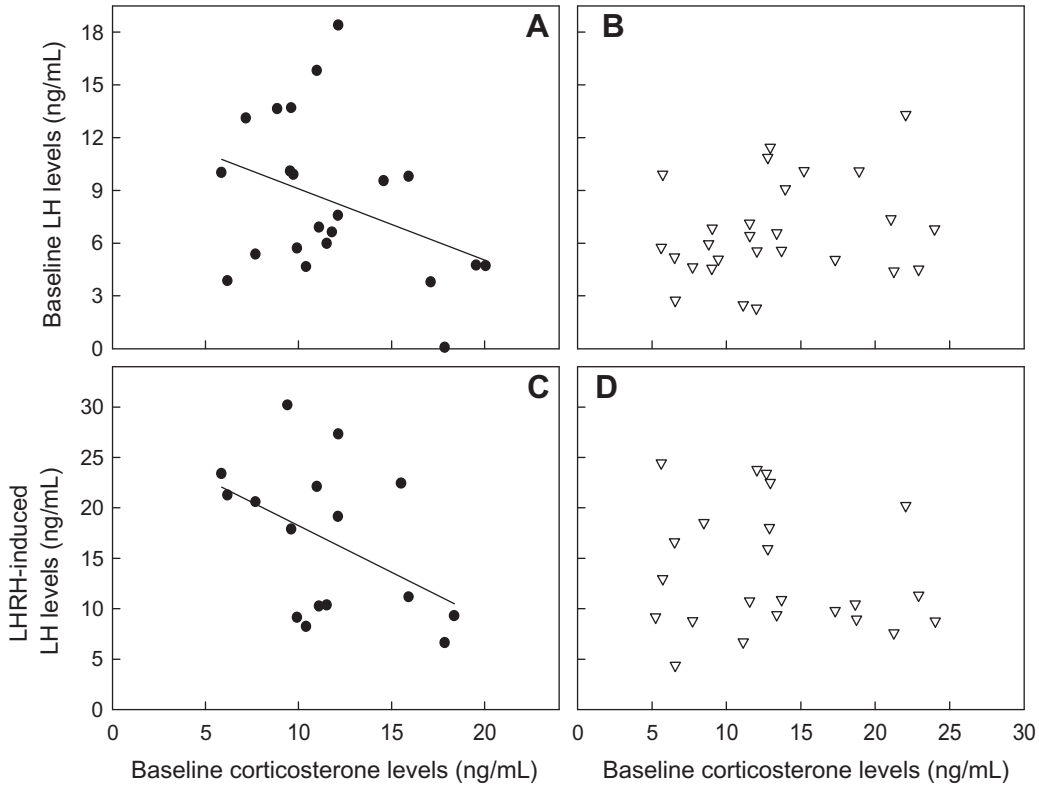

Fig. 3. In pre-laying females (filled circles), baseline (A) and LHRH-induced (C) LH levels decreased with increasing baseline corticosterone levels. In pre-laying males, baseline (B) and LHRH-induced (D) LH levels were not linked to baseline corticosterone levels.

baseline or LHRH-induced LH (Table 3a, Fig. 4A and C), baseline or stress-induced corticosterone (Table 3a, Fig. 4B and D), baseline or LHRH-induced testosterone (Table 3a).

3.3. Hormonal levels and egg-laying dates (Table 1iv)

In females, there was a significant effect of pre-laying scaled mass index on first egg-laying date (Table 3b): females with high scaled mass index start laying their first egg early (Fig. 5A). Males pre-laying scaled mass index had no effect on egg-laying date (Table 3b). Baseline or stress-induced corticosterone, baseline or LHRH-induced LH levels and baseline testosterone levels had no influence on first egg-laying date (Table 3b). On the other hand, males with high LHRH-induced testosterone levels bred significantly earlier than males with low LHRH-induced testosterone levels (Table 3b, Fig. 5B).

4. Discussion

In the present study we showed that elevated baseline, but not stress-induced, corticosterone levels have the potential to disrupt the HPG axis of female Black-legged kittiwakes. Furthermore, females with low baseline LH levels and elevated baseline corticosterone levels had the highest probability to skip breeding. On the contrary, elevated baseline and/or stress-induced corticosterone levels did not disrupt LH release in males, highlighting strong sex-difference in the HPG sensitivity to stress in Black-legged kittiwakes. Concerning the timing of breeding, females' scaled mass index, but not hormone levels, had a positive influence on egg-laying date. Males that exhibited high LHRH-induced testosterone release ability bred earlier than males with low ability to release testosterone.

Table 3
Modelling (a) breeding decision and (b) first-egg-laying date in 2008 as a function of scaled mass index and hormones levels using GLMs with binomial error distribution and logit link function (a) or normal error distribution and identity link function (b).

Independant variables	*N*	Chi^2	*p*-value	*N*	Chi^2	*p*-value
a-Breeding decision	MALES			FEMALES		
Scaled mass index	43	0.087	0.768	32	1.078	0.299
Baseline CORT	27	0.016	0.900	**21**	**7.656**	**0.006**
Stress-induced CORT	42	0.029	0.866	30	0.036	0.850
Baseline LH	27	0.173	0.678	**21**	**7.368**	**0.007**
LHRH-induced LH	23	1.599	0.206	16	0.614	0.433
Baseline Testosterone	27	0.704	0.402			
LHRH-induced Testosterone	22	0.443	0.506			
b-First egg-laying date	MALES			FEMALES		
Independant variables	d.f.	F	p-value	d.f.	F	p-value
Scaled mass index	1,29	0.486	0.491	**1,18**	**5.289**	**0.034**
Baseline CORT	1,18	1.622	0.219	1,13	0.004	0.953
Stress-induced CORT	1,28	0.650	0.427	1,18	0.003	0.959
Baseline LH	1,18	0.425	0.523	1,13	0.874	0.367
LHRH-induced LH	1,13	0.987	0.339	1,8	0.323	0.585
Baseline Testosterone	1,16	1.639	0.219			
LHRH-induced Testosterone	**1,13**	**7.365**	**0.018**			

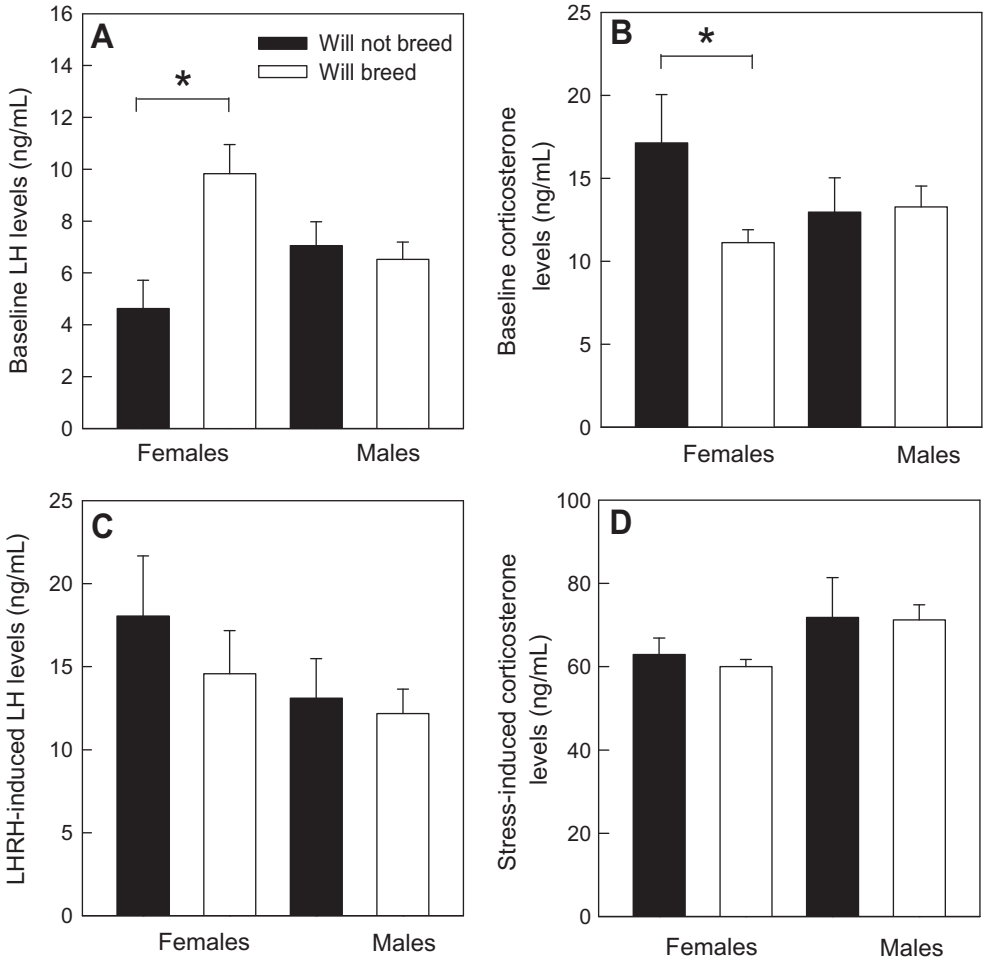

Fig. 4. Hormone levels in pre-laying male and female kittiwakes that will breed (white bar) and that will not breed (black bar): (A) baseline LH levels, (B) baseline corticosterone levels, (C) LHRH-induced LH levels and (D) stress-induced corticosterone levels.

4.1. Effect of corticosterone levels on LH and testosterone

Our study showed, for the first time in free-living birds, that elevated baseline corticosterone was paralleled by low baseline LH levels and by low ability to release LH after a LHRH-injection. Such down-regulation of the HPG axis by stress hormones was however strongly sex-specific in kittiwakes, since it was only observed in females. In free-living avian species, the link between LH and corticosterone levels has been seldom investigated and to our knowledge, studies were mainly biased towards males (Wilson and Follett,

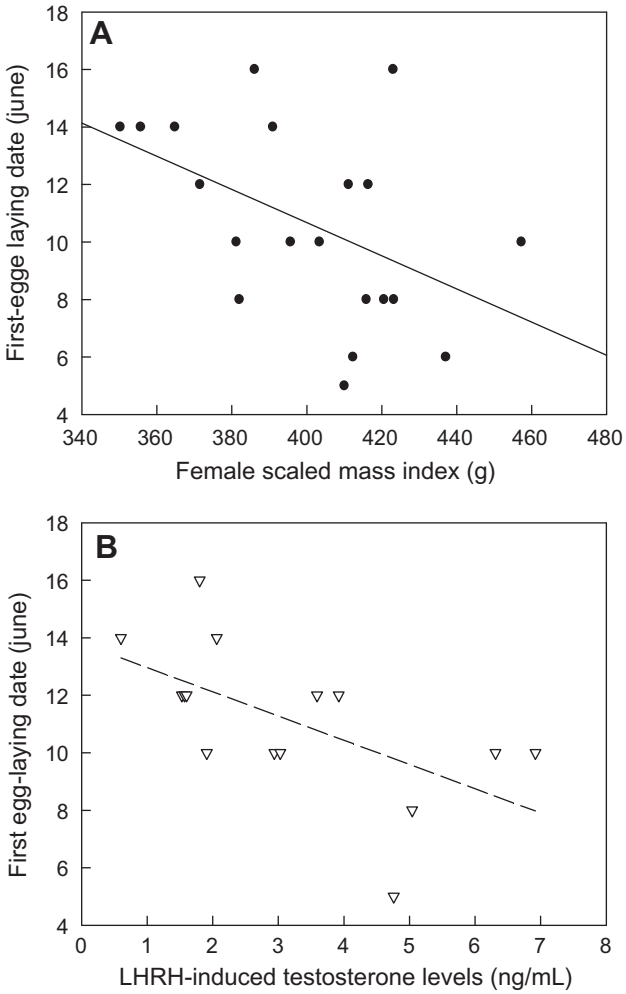

Fig. 5. (A) Egg-laying date (June 2008) advanced with increasing females' scaled mass index; (B) Egg-laying date (June 2008) advanced with increasing males' testosterone release ability.

1975; Astheimer et al., 2000; review in Schoech et al., 2009). Our results highlight that in female kittiwakes, plasma corticosterone may act at the pituitary level, though a down-regulation of LH production and/or a low responsiveness to the GnRH input. This physiological pathway could be related to the stress-induced expression of GnIH and its associated inhibition of LH release, as recently reported in captive house sparrows and laboratory rats (Calisi et al., 2008; Kirby et al., 2009).

In males, baseline corticosterone levels had no effect on baseline LH levels and on LH releasing ability after a LHRH challenge. This supports the idea that the sensitivity of the HPG axis to stressful cues may differ between sexes, as suggested by Ball and Ketterson (2008). For instance, intrasexual competition could force male kittiwakes to acquire reproductive readiness earlier in the season than females and to better resist environmental perturbations. Moreover, females have to extract and store a sufficient amount of energy to build up the eggs, hence being more sensitive to environmental stressors, such as poor food supply, than males (Ball and Ketterson, 2008). The low susceptibility to stress of male HPG axis was further supported by the lack of relationship between baseline corticosterone and testosterone levels as experimentally observed in male American Tree sparrows (Astheimer et al., 2000).

It is also important to notice that stress-induced corticosterone levels had no effect on LH in males and females and on testosterone levels in males. In kittiwakes, corticosterone may need time to suppress the HPG axis, therefore a short acute release of stress hormones could be inappropriate to trigger strong HPG disruption.

4.2. Hormonal levels and breeding decision

As previously found (Salvante and Williams, 2003; Vitousek et al., 2010; Goutte et al., 2010), elevated pre-laying baseline corticosterone levels were associated with the decision to skip breeding in females. Females bearing elevated baseline corticosterone levels may then be of lower quality (e.g. low foraging skills, Angelier et al., 2007; Kitaysky et al., 2010) and may be unable to cope with the energetic requirements for egg-formation and future incubation effort.

Moreover, LH levels were higher in females that did breed than in females that did not breed. Since the LHRH injection did not reveal an inability to release LH, non-breeding females had a fully functional HPG axis (Schoech et al., 1996) and thus appeared to be physiologically primed for breeding. In that context, how to explain low baseline LH levels in non-breeding females? Our results suggest that non-breeding females failed to express endogenous GnRH input that is required for high baseline LH levels. Poor nest site, weak social stimulations or low mate interactions in females that will not breed could explain their low baseline LH levels (Ball, 1993; Dawson, 2008 for review). This down-regulation of LH levels might have been associated with possible reduced release of other pituitary hormones (FSH) and/or estradiol, which play a major role in the growth of reproductive organs and the expression of sexual behaviours. Estradiol is known to stimulate female begging behaviour (Hunt and Wingfield, 2004) and courtship feeding (Eda-Fujiwara et al., 2003). In pre-laying Black-legged kittiwakes, courtship feeding by the males is commonly observed and is closely linked to successful copulation (Helfenstein et al., 2003; Kempenaers et al., 2007). Hence, females with low baseline LH levels would have exhibited low estradiol levels and low mating effort, thereby skipping the breeding attempt. Therefore, in pre-laying female kittiwakes, elevated corticosterone levels appear to be strongly involved in the disruption of the HPG axis and to negatively affect breeding decision. At the ultimate level, skipped breeding could be an adaptive response to promote females' own survival, especially for long-lived prudent parents (Drent and Daan, 1980).

4.3. Hormonal levels and egg-laying dates

Concerning the timing of breeding, females with low scaled mass index laid their first egg later than females with high one. However, baseline corticosterone levels were not related to the timing of breeding, contrary to recent correlative and experimental findings in another long-lived seabird, the Snow petrel (Goutte et al., 2010). Why is the hormonal regulation of the timing of breeding species-dependant? Energetic constraints associated with egg formation should differ between these two seabird species. Indeed, small Larids, like kittiwakes, build up an egg in about 6–8 days (Astheimer and Grau, 1990), while female petrels have to accumulate energy reserves at sea during an extended period of three weeks (pre-laying exodus, Warham, 1996). Therefore, the timing of breeding should be under strong energetic constraints in petrels, and thus should be greatly influenced by early environmental conditions and physiological state (baseline corticosterone levels, Goutte et al., 2010). On the contrary, kittiwakes should experience lower energetic constraints during egg formation, and in turn the timing of egg-laying would be poorly mediated by elevated baseline corticosterone levels. The functional action of corticosterone on the egg-laying date could also be environment-dependant. Indeed, favourable conditions may overcome the effect of stress hormones on the first egg-laying date, as it was shown in females Florida scrub-jays (Schoech et al., 2009). The observed inhibition of HPG axis by high baseline

corticosterone levels in females could postpone the breeding schedule, but only during poor years.

In male kittiwakes, corticosterone levels were not as decisive as for females concerning the breeding decision, thereby highlighting sex differences in the response to environmental cues related to seasonal events (Ball and Ketterson, 2008; Blas and Hiraldo, 2010; Goutte et al., 2010). Furthermore, baseline and/or LHRH-induced LH and testosterone levels did not differ between breeders and non breeders, as found in Florida scrub jays (Schoech et al., 1996). Therefore, in male kittiwakes, pre-laying energetic and hormonal states appear to poorly influence the HPG system and the breeding decision.

Interestingly, the timing of breeding was closely linked to males' testosterone profile. Female kittiwakes bred earlier when their mates exhibited higher testosterone release ability after a LHRH challenge. How could males' testosterone drive the egg-laying date? High testosterone levels after a LHRH input could mediate behavioural adjustments in males, such as aggressiveness to obtain and defend a high quality nest, and/or high quality courtship display. In turn, provisioning effort was suggested to be a sexually selected trait on which female kittiwakes base decisions about timing and frequency of copulations (Kempenaers et al., 2007). Additionally, males with high testosterone release ability may enhance high body condition in pre-laying female kittiwakes, thereby triggering early breeding.

In conclusion, our study highlights clear sex-differences in the HPG sensitivity to stress hormones in pre-laying kittiwakes. This may originate from the specific constraints faced by males and females during the pre-laying period. Experimental manipulations of corticosterone levels coupled with behavioural observations (courtship feeding effort and copulation rate in corticosterone-implanted males and females) have to be conducted to confirm this hypothesis.

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