

RESEARCH ARTICLE

Experimentally reduced corticosterone release promotes early breeding in black-legged kittiwakes

Aurélie Goutte^{1,*}, Céline Clément-Chastel¹, Børge Moe², Claus Bech³, Geir Wing Gabrielsen⁴ and Olivier Chastel¹

¹Centre d'Etudes Biologiques de Chizé, CNRS, F-79360, France, ²Norwegian Institute for Nature Research (NINA), Arctic Ecology Department, NO-9296 Tromsø, Norway, ³Department of Biology, Norwegian University of Science and Technology (NTNU), NO-7491 Trondheim, Norway and ⁴Norwegian Polar Research Institute, NO-9296 Tromsø, Norway

*Author for correspondence (aglod@locean-ipsl.upmc.fr)

Accepted 18 February 2011

SUMMARY

Breeding at the right time is important for successful reproduction. In birds, stressful environmental conditions are known to delay the timing of breeding but the underlying mechanisms are poorly understood. The stress hormone corticosterone appears to be a good candidate for mediating egg-laying date according to early environmental conditions and physiological state. By experimentally reducing the release of corticosterone in black-legged kittiwakes during the pre-laying period, we tested whether egg-laying date was mechanistically linked to corticosterone levels. Male and female kittiwakes were implanted with a low dose of exogenous corticosterone to inhibit endogenous corticosterone production. According to our predictions, the experimental reduction of corticosterone release was paralleled by a significant advancement of egg laying in females (around 4 days earlier). In addition, females with experimentally reduced corticosterone release gained mass during the pre-laying period compared with controls. Ultimately, the advancement of egg laying in females with experimentally reduced corticosterone levels was associated with an enhanced breeding success. This effect was strongly sex specific. In corticosterone-treated male kittiwakes, egg-laying date and reproductive success were not affected, but breeding probability was lower than in controls. This corticosterone treatment did not influence immediate clutch size, or return rate and breeding decision the following year. Our results support the hypothesis that corticosterone secretion during the pre-laying period mediates the timing of breeding in this long-lived seabird, possibly through the dynamics of energy reserves.

Key words: arctic seabird, fitness, *Rissa tridactyla*, stress hormones, timing of breeding.

INTRODUCTION

Breeding at the right time is one of the most important factors for successful reproduction in a fluctuating environment (e.g. Lack, 1968; Perrins, 1970; Lyon et al., 2008). In birds, late breeding is usually associated with low breeding success. This is mainly due to a possible mismatch between the offspring's energy needs and the peak of food supply (match–mismatch hypothesis) (Visser et al., 1998; Stenseth and Mysterud, 2002; Durant et al., 2007). In addition, the timing of egg laying has long-term fitness consequences because an experimental advancement and/or delay of breeding compromise future reproduction and survival (Nilsson and Svensson, 1996; Brinkhof et al., 2002). The adjustment of the timing of breeding is thus crucial and is possible through some behavioural and physiological flexibility in response to environmental and internal factors (reviewed in Wingfield, 2008; McNamara and Houston, 2008). In this context, free-living birds are thought to track pre-breeding environmental cues that enable them to anticipate the peak of food availability and then to adaptively adjust their breeding schedule (e.g. Frederiksen et al., 2004). However, this hypothesis is not always supported by correlative and experimental evidence (Hipfner et al., 2008; Shultz et al., 2009). The timing of breeding may also be imposed by pre-laying energetic constraints associated with the clutch formation (Durant et al., 2005; Visser and Both, 2005), depending on resource allocation strategies. Indeed, income-

breeding species and capital breeders are assumed to differ in their response to environmental cues regulating the onset of breeding (Drent and Daan, 1980). It is therefore essential to clarify the underlying mechanisms that link pre-laying environmental cues, physiological state and phenological decisions at the individual level.

What are the mechanisms underlying the onset of breeding? Increasing day length activates the hypothalamic–pituitary–gonadal axis (hereafter HPG axis). The expression of the gonadotropin releasing hormone (GnRH) triggers the release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) (reviewed in Dawson et al., 2001). In turn, gonadal development is promoted and sex steroids (estradiol and testosterone) are released, thereby mediating the adoption of sexual behaviours, such as courtship, mating and nest building (Ball, 1993). In addition to this fixed photoperiodic cue, many environmental variables, such as temperature, food supply and/or stimulatory social interactions may interact to modulate the onset of breeding (Wingfield, 1980; Wingfield and Kenagy, 1991; Ball, 1993; Wingfield et al., 2003; Dawson, 2008). The underlying mechanisms that regulate the HPG axis according to pre-laying non-photoperiodic cues have received growing attention, but it still remains poorly understood (review in Schoech et al., 2009). One potential mediator is the release of glucocorticoids in response to stressors (such as inclement weather, food shortage or predator attacks), which is known to adjust life-

history strategies according to environmental conditions and physiological state (Ricklefs and Wikelski, 2002; Wingfield and Sapolsky, 2003). In vertebrates, the acute secretion of glucocorticoids (corticosterone and/or cortisol) *via* the HPA axis promotes immediate survival through the mobilization of stored energy at the expense of current reproduction (reviewed in Breuner et al., 2008). During the pre-laying period, elevated corticosterone (hereafter CORT) levels appear to be associated with delayed breeding in females (Salvante and Williams, 2003; Schoech et al., 2009; Goutte et al., 2010a) *via* a possible perturbation of the HPG axis (reviewed in Schoech et al., 2009). In black-legged kittiwakes (*Rissa tridactyla*, Linnaeus 1758), a species showing large inter-annual variation in the timing of breeding and breeding success (Moe et al., 2009), we have shown that females with high baseline CORT levels had reduced baseline LH levels and a low ability to release LH, following GnRH challenge (Goutte et al., 2010b). In this species, reproductive success is higher in years when the timing of nesting is earlier (Byrd et al., 2008), and this also applies to our study population (Moe et al., 2009). Moreover, skipped breeding is related to high CORT levels in female kittiwakes (Lanctot et al., 2003; Kitaysky et al., 2010; Goutte et al., 2010b). Thus, CORT appears to be a potential candidate for mediating the timing of breeding according to pre-laying environmental and physiological conditions. In this respect, it is crucial to carefully distinguish the baseline and the stress-induced CORT levels. Baseline CORT levels are considered to be a marker of activity, energetic state and food availability (Kitaysky et al., 1999; Love et al., 2004) (reviewed in Landys et al., 2006) and are related to reproductive performance (reviewed in Bonier et al., 2009). Stress-induced CORT levels reflect the sensitivity to stress and the commitment to current reproduction (reviewed in Wingfield and Sapolsky, 2003; Lendvai et al., 2007; Bokony et al., 2009) and are related to the recent nutritional history of the kittiwake (Kitaysky et al., 2007; Kitaysky et al., 2010).

A low release of CORT in response to stress would be a possible mechanism to ensure early breeding through full activation of the HPG axis. In the present study, we tested this hypothesis by down-regulating the CORT release by black-legged kittiwakes during the pre-laying period. To do so, we implanted pre-laying male and female kittiwakes with a low dose of exogenous CORT to inhibit the HPA axis and endogenous CORT production. When exposed to low doses of CORT for a prolonged period, the HPA axis compensates by reducing adrenocorticotrophic hormone (ACTH) production, adrenal activity and CORT production (Akana et al., 1992; Wolkowitz, 1994). Following this CORT treatment, we addressed the following issues. (1) What are the phenological consequences of the CORT treatment? As the HPG axis of females appears to be more sensitive to stressful events than the males' in kittiwakes (Goutte et al., 2010b), we predicted that females with reduced CORT levels would breed earlier than control ones. (2) What are the fitness consequences of the CORT treatment? Hatching and breeding success the year of the treatment, as well as return rate and breeding decision the following year, were monitored. As early breeding is associated with high breeding success (Perrins, 1970), especially in kittiwakes (Byrd et al., 2008; Moe et al., 2009), and as an attenuated CORT stress response favours reproductive investment (Lendvai et al., 2007), we predict that female kittiwakes with reduced CORT levels would have a higher breeding success than controls.

MATERIALS AND METHODS

Study area and birds

Our study was conducted in 2009 between 19 May and 8 August in a colony of black-legged kittiwakes at Kongsfjorden, Svalbard

(78°54'N, 12°13'E), 7 km east 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. We studied a plot of *ca.* 100 pairs breeding on cliffs at a height of 5–10 m. Birds were individually marked with white PVC plastic bands engraved with a three-letter code and fixed to the bird's tarsus. Thus, kittiwakes could be identified from a distance without perturbation. During the 2009 pre-laying period, foraging trip durations were longer ($N=48$, 3.75 ± 0.27 days, mean \pm s.e.) than during the 2008 pre-laying period ($N=81$, 1.70 ± 0.13 days, $F_{1,126}=59.01$, $P<0.001$), suggesting low and/or distant food availability.

Experimental CORT treatment

The CORT implantation was conducted during the pre-laying period, from 19 to 31 May 2009. This was on average 20–30 days before the egg-laying date (19.2 ± 0.3 June). A total of 21 females and 20 males were captured at their nests with a noose on the end of a 5 m fishing rod (day 0). Immediately after capture (3 min and 2 ± 5 s), a blood sample was collected from the alar vein with a 1 ml heparinized syringe and a 25 gauge needle, to determine baseline CORT levels. In 8 birds, handling time exceeded 3 min, the time recommended by Romero and Reed (Romero and Reed, 2005) to assess baseline CORT levels. These values were thus excluded from the analysis. Immediately after this first blood sample, we implanted the kittiwakes subcutaneously (between the shoulders) with one 25 mm Silastic tube (internal diameter 1.47 mm, external diameter 1.96 mm; Dow Corning, Midland, MI, USA) sealed at the ends with medical grade silicon (Dow Corning) and either filled with crystallized CORT (C2505, Sigma Chemical Co., St Louis, MO, USA; $N=13$ CORT-blocked females and $N=10$ CORT-blocked males) or empty ($N=8$ control females and $N=10$ control males). The implant site was disinfected with povidone iodine and then glued (Vetbond, 3M Santé, Cergy Pontoise, France). Administration of exogenous CORT is known to down-regulate the endogenous CORT production in birds over a prolonged period (Vandenborne et al., 2005; Romero et al., 2005; Busch et al., 2008; Müller et al., 2009). Two 25 mm Silastic implants significantly elevate baseline CORT levels in black-legged kittiwakes during the first 3 days after implantation (Kitaysky et al., 2001; Angelier et al., 2007; Angelier et al., 2009). We chose half of this dose (one implant) to inhibit CORT release, without strongly elevating baseline CORT levels (Akana et al., 1992; Wolkowitz, 1994).

After implantation, birds were weighed to the nearest 2 g using a Pesola spring balance. The skull length (head+bill) was measured to the nearest 0.5 mm using a sliding calliper. An index of body condition was calculated as residuals from a regression of body mass against body size (GLM, $F_{1,38}=15.934$, $P<0.001$). Kittiwakes were then marked with spots of dye on the forehead to facilitate subsequent observations and were released.

Physiological effects of the CORT treatment

During the first 26 days after the CORT implantation (day 1 to day 26, still during the pre-laying period), 18 CORT-blocked kittiwakes and 19 controls were recaptured opportunistically. The number of days between the treatment and the recapture did not differ between CORT-blocked kittiwakes (11.72 ± 1.83 days) and control kittiwakes (11.11 ± 1.54 days, Student's *t*-test, $t=0.258$, d.f.=34.277, $P=0.798$). Body mass was recorded, and a blood sample was collected immediately (2 min 54 ± 5 s) after capture to assess baseline CORT levels. The rate of body mass change was calculated as the difference between the body mass on the day of recapture (M_t) and

the body mass on the day of implantation (M_0), relative to the number of days elapsed since the treatment and before egg laying; that is, $(M_t - M_0)/(t - 0)$. A second blood sample was collected 15 min (15 min 46 ± 19 s) after handling to assess the stress-induced CORT levels. Although maximum levels of CORT are reached 30 min after capture and handling in black-legged kittiwakes (Chastel et al., 2005), we decided to reduce the time of capture in order to minimize the level of disturbance. CORT levels and rate of body mass change were monitored before eggs were laid (still during the pre-laying period).

Timing of breeding and fitness consequences of the CORT treatment

During the egg-laying period, we checked the nests every 2 days, using a mirror on the end of an 8 m fishing rod. Breeding decision (laying egg or not), egg-laying date and clutch size (1 or 2 eggs) were thus monitored. Then, we checked the nests every 2 or 3 days to monitor the number of predated eggs per active nest, and the number of chicks that reached 12 days old per active nest (hereafter called breeding success). We assumed that CORT-blocked kittiwakes and control ones were exposed to the same level of disturbance. During the following year (from 20 May 2010 to 13 June 2010), we monitored the return rate and the breeding decision of CORT-blocked and control kittiwakes.

Hormone assay

The blood samples were immediately put on ice until centrifugation at the end of the day. Plasma was separated and stored at -20°C . Molecular sexing was performed as detailed elsewhere (Weimerskirch et al., 2005). Plasma concentrations of CORT were determined by radioimmunoassay at the Centre d'Etudes Biologiques de Chizé (CEBC), as previously described (Lormée et al., 2003). All samples were run in one assay. The coefficient of intra-assay variation was assessed using several reference plasmas within the assay (coefficient of variation = 5.8%, $N=5$ duplicates). The minimum detectable CORT level was 0.5 ng ml^{-1} .

Statistical analyses

All statistical analyses were performed using R 2.8.0. (R Development Core Team, 2008). Regarding baseline CORT levels, the first day after treatment was distinct from the succeeding days. Thus, we first tested for an effect of CORT treatment (CORT-blocked or control), sampling period (day 0, day 1 and period between day 2 and day 26), sex and the interactions CORT treatment \times period and treatment \times sex on baseline CORT levels, by using generalized linear mixed models (GLMMs), including bird identity as a random effect. Then we focused on the period from day 2 to day 26. We tested for an effect of treatment, day of sampling (day 2 to day 26), sex and the interactions treatment \times day of sampling, and treatment \times sex on baseline CORT levels, using generalized linear models (GLMs) with normal errors and an identity link function. Second, we investigated whether stress-induced CORT levels were dependent on CORT treatment, sampling period (day 1 and the period between days 2 and 26) or day of sampling (from the 1st to the 26th day after treatment), sex and the interactions CORT treatment \times day of sampling, and treatment \times sex by using a GLM with normal errors and an identity link function. Stress-induced CORT levels were not monitored on day 0.

Baseline CORT levels have been related to breeding decision and egg-laying date (Lancot et al., 2003; Goutte et al., 2010a; Goutte et al., 2010b; Kitaysky et al., 2010). Thus, we included the initial baseline CORT levels (prior to the CORT treatment) in our analyses. We tested for an effect of the CORT treatment, body condition,

initial baseline CORT levels and the interactions CORT treatment \times body condition and CORT treatment \times initial baseline CORT levels on breeding decision, using GLMs with binomial error distribution and a logit link function, and on laying date of the first egg, using GLMs with Poisson errors and a log link function. In addition, the CORT treatment may have affected other breeding performance in 2009 and in 2010. We thus tested for an effect of the CORT treatment, body condition and the interaction CORT treatment \times body condition on clutch size, number of lost eggs and breeding success, using GLMs with Poisson errors and a log link function, and on return rate and breeding decision the following year by using GLMs with binomial error distribution and a logit link function. Males and females were analysed separately. Finally, we tested for an effect of treatment on the rate of body mass change before egg laying, using Wilcoxon tests. Males and females were analysed separately. Only pre-laying females that did breed were analysed for body mass change, because non-breeding females are not constrained to acquire and store energy for egg building. Dependent variables were log transformed when necessary and we checked whether models met the assumptions.

RESULTS

Validation of the CORT treatment

On day 0, CORT-blocked kittiwakes and controls did not differ in baseline CORT levels ($F_{1,31}=2.209$, $P=0.148$) even when considering an interaction effect with sex (sex: $F_{1,30}=1.813$, $P=0.189$; treatment \times sex: $F_{1,29}=0.019$, $P=0.892$). Moreover, they did not differ in body condition (treatment: $F_{1,39}=0.002$, $P=0.964$; treatment \times sex: $F_{1,37}=0.530$, $P=0.471$), or in date of treatment (treatment: $F_{1,39}=0.330$, $P=0.569$; treatment \times sex: $F_{1,37}=1.557$, $P=0.220$).

CORT treatment affected baseline CORT levels, but the effect was influenced by sampling period (Table 1A, Fig. 1A): in CORT-blocked kittiwakes, baseline CORT levels increased from day 0 to day 1, but subsequently dropped below that of the control group (CORT-blocked group, $9.47 \pm 1.28 \text{ ng ml}^{-1}$; control group, $13.58 \pm 1.26 \text{ ng ml}^{-1}$, Table 1B). In addition, males had significantly lower baseline CORT than females during this period (Table 1B). In the control group, baseline CORT levels were not affected by the sampling period (GLMM, $F_{2,12}=0.342$, $P=0.717$) or by the day of sampling (GLMM, $F_{1,13}=1.1001$, $P=0.313$). After CORT treatment, stress-induced CORT levels were significantly lower in the CORT-blocked kittiwakes ($30.78 \pm 3.76 \text{ ng ml}^{-1}$) than in the controls ($50.35 \pm 3.61 \text{ ng ml}^{-1}$, Table 1C,D, Fig. 1B). Stress-induced CORT levels were unaffected by the sampling period or the day of sampling, sex and their interactions (Table 1C,D).

CORT treatment and immediate phenological and fitness consequences

In females, the breeding decision did not differ between CORT-blocked kittiwakes and control ones and was not influenced by initial baseline CORT levels (Table 2A, Fig. 2A). However, the proportion of non-breeding females increased with decreasing body condition (Table 2A). In males, CORT-blocked kittiwakes showed a higher probability to skip breeding than control ones, but body condition did not affect the breeding decision (Table 2A, Fig. 2A). In addition, males that skipped the 2009 breeding season had an initially lower baseline CORT level ($12.334 \pm 2.157 \text{ ng ml}^{-1}$, mean \pm s.e., $N=8$) than males that did breed ($23.062 \pm 3.476 \text{ ng ml}^{-1}$, mean \pm s.e., $N=10$, Table 2A). CORT-blocked females laid their eggs around 4 days earlier than controls (Table 2B, Fig. 2B), without any effect of body condition and/or initial baseline CORT levels (Table 2B). CORT treatment of males did not influence egg-laying date (Table 2B,

Table 1. Validation of the CORT treatment

Dependent variable	Independent variable	d.f.	F	P
A. Baseline CORT levels (ng ml ⁻¹), N=68	Treatment	1,35	1.531	0.224
	Period	2,25	7.436	0.003
	Treatment × period	2,25	6.503	0.005
	Sex	1,35	0.303	0.586
	Treatment × sex	1,35	0.003	0.959
B. Baseline CORT levels (ng ml ⁻¹) between days 2 and 26, N=32	Treatment	1,30	9.378	0.005
	Days after treatment	1,29	0.087	0.770
	Sex	1,28	13.743	0.001
	Treatment × day	1,27	0.002	0.962
	Treatment × sex	1,26	2.898	0.101
C. Stress-induced CORT levels (ng ml ⁻¹), N=29	Treatment	1,27	13.101	0.001
	Period	1,26	2.616	0.119
	Sex	1,25	0.096	0.759
	Treatment × period	1,24	1.604	0.218
	Treatment × sex	1,23	1.604	0.218
D. Stress-induced CORT levels (ng ml ⁻¹) between days 2 and 26, N=29	Treatment	1,27	11.174	0.003
	Days after treatment	1,26	1.318	0.263
	Sex	1,25	0.153	0.700
	Treatment × day	1,24	0.001	0.981
	Treatment × sex	1,23	0.180	0.675

Effect of corticosterone (CORT) treatment, period (day 0, day 1, days 2–26), sex and the interactions CORT treatment × period, CORT treatment × sex on the levels of (A) baseline CORT and (C) stress-induced CORT. Effect of CORT treatment, day (from the second to the 26th day after CORT treatment), sex and the interactions CORT treatment × day, CORT treatment × sex on the levels of (B) baseline CORT and (D) stress-induced CORT. We used GLMMs with bird identity as a random effect (A) or GLMs using normal errors and an identity link function (B,C,D).

Fig. 2B). Clutch size was not influenced by CORT treatment or body condition in male or female kittiwakes (Table 2C). In nests with a CORT-blocked female, around 25% of the eggs were predated by Glaucous gulls, *Larus hyperboreus*. This was far lower than in nests with control females, where all the eggs were predated (Table 2D, Fig. 2C). Breeding success was significantly higher in nests with CORT-blocked females than in control ones (Table 2E, Fig. 2D). In males, our experimental CORT treatment had no effect on egg predation and on breeding success (Table 2D,E, Fig. 2C,D).

During incubation, our CORT implantation treatment had a significant effect on the rate of body mass change: CORT-blocked breeding females gained mass whereas control breeding females lost mass (Wilcoxon $W=22.5$, $P=0.033$, Fig. 3). Body mass change did not differ between CORT-blocked males and controls ($W=11$, $P=0.181$).

CORT treatment, return rate and breeding decision the following year

The return rate in 2010, the year after the CORT implantation treatment, did not differ between CORT-blocked and control females or between CORT-blocked and control males (Table 2F, Fig. 2E). For the kittiwakes observed in the colony during the pre-laying period in 2010, the decision to breed did not differ between CORT-blocked and control male and female kittiwakes (Table 2G, Fig. 2F). Body condition during the pre-laying period in 2009 did not influence the return rate and the decision to breed in 2010, even when considering an interaction effect of treatment × body condition.

DISCUSSION

In this study of pre-laying kittiwakes, we found that a prolonged reduction of baseline and stress-induced CORT levels was accompanied by an advancement of the egg-laying date and by an improvement of breeding success. This effect was strongly sex

specific as it was only observed in females. Moreover, breeding female kittiwakes with experimentally reduced CORT levels gained body mass during the pre-laying period, whereas control females

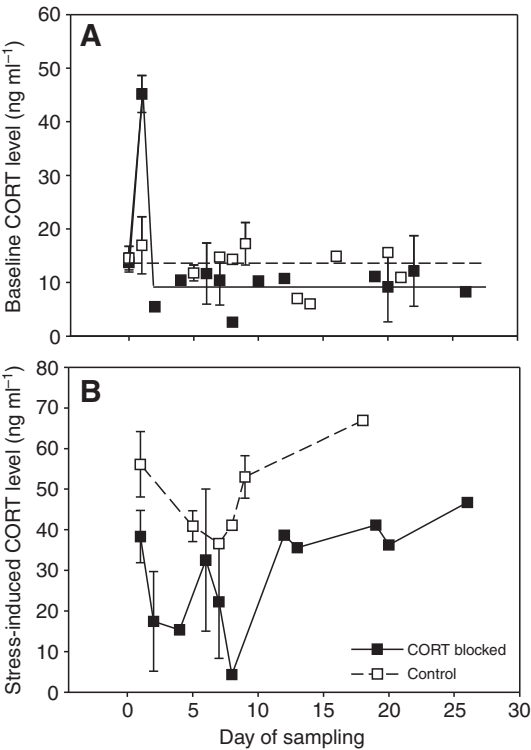


Fig. 1. Temporal change of (A) baseline corticosterone (CORT) levels and (B) stress-induced CORT levels with day of sampling after treatment (day 0 to day 26) for CORT-blocked kittiwakes and controls. Baseline CORT levels increased from day 0 to day 1 after CORT treatment, but subsequently dropped to levels below those of the control group.

Table 2. CORT treatment and fitness consequences

Dependent variable	Independent variable	Statistics (females)			Statistics (males)		
		<i>N</i>	χ^2	<i>P</i>	<i>N</i>	χ^2	<i>P</i>
A. Breeding decision in 2009	Treatment	21	1.318	0.251	19	4.466	0.035
	Body condition	21	4.566	0.033	19	1.715	0.190
	Treatment \times body condition	21	0.566	0.452	19	0.201	0.654
	Initial CORT	19	0.143	0.705	19	8.558	0.003
	Treatment \times initial CORT	19	0.212	0.645	19	0.076	0.783
B. Egg-laying date in 2009	Treatment	13	4.035	0.045	12	0.488	0.485
	Body condition	13	0.093	0.761	12	0.227	0.634
	Treatment \times body condition	13	0.544	0.461	12	0.052	0.820
	Initial CORT	12	0.284	0.594	10	0.189	0.664
	Treatment \times initial CORT	12	0.209	0.647	10	0.211	0.646
C. Clutch size in 2009	Treatment	13	0.036	0.849	12	0.080	0.777
	Body condition	13	<0.001	1.000	12	0.002	0.965
	Treatment \times body condition	13	0.002	0.964	12	0.009	0.925
D. Lost eggs in 2009	Treatment	13	4.010	0.045	12	0.439	0.508
	Body condition	13	<0.001	1.000	12	0.010	0.920
	Treatment \times body condition	13	0.125	0.723	12	0.205	0.650
E. Breeding success in 2009	Treatment	13	7.355	0.007	12	0.299	0.584
	Body condition	13	<0.001	1.000	12	0.350	0.554
	Treatment \times body condition	13	0.111	0.739	12	2.943	0.086
F. Return rate in 2010	Treatment	21	1.044	0.307	20	2.848	0.092
	Body condition	21	1.737	0.188	20	0.965	0.326
	Treatment \times body condition	21	2.648	0.104	20	0.185	0.667
G. Breeding decision in 2010	Treatment	16	0.760	0.383	15	0.043	0.835
	Body condition	16	2.497	0.114	15	0.161	0.689
	Treatment \times body condition	16	2.048	0.152	15	0.018	0.894

Modelling (A) breeding decision, (B) egg-laying date, (C) clutch size, (D) number of predated eggs, (E) breeding success in 2009, and (F) return rate and (G) breeding decision in 2010, as functions of treatment, body condition, initial baseline corticosterone (CORT) levels for A and B and the interactions treatment \times body condition, treatment \times initial CORT using GLMs with binomial error distribution and logit link function (A,F,G) and GLMs with Poisson error distribution, log link function (B–E), in CORT-blocked and control kittiwakes. Females and males were analysed separately. Significant variables are indicated in bold.

lost mass. Finally, the return rate and the breeding decision the following year were not affected by CORT treatment.

CORT treatment and CORT secretion

The aim of the present study was to reduce the release of CORT in kittiwakes during the pre-laying period. To do so, a low dose of exogenous CORT was used to down-regulate the HPA axis and the release of endogenous CORT in response to the stress of capture (Akana et al., 1992; Wolkowitz, 1994). Thus, in male and female kittiwakes, the stress-induced CORT levels were around 60% lower after CORT treatment than after sham treatment. This down-regulation of CORT release was long lasting as it was observed at least 26 days after CORT treatment. In addition, baseline CORT levels were reduced by about 70% from the second to the 26th day. On day 1, crystallised CORT was released into the bloodstream. As a result, basal CORT plasma levels were 3.5 times higher than before the CORT treatment. This short CORT increase was within the physiological range observed for an acute stress response in kittiwakes (Kitaysky et al., 1999; Chastel et al., 2005). It was much lower than the 10 times increase of baseline CORT levels observed in previous studies after the implantation of two CORT implants (Kitaysky et al., 2001; Angelier et al., 2007; Angelier et al., 2009).

It is important to note that we only measured total CORT levels, i.e. free and bound CORT levels. The binding protein corticosteroid binding globulin (CBG) binds CORT with high affinity in the circulation (e.g. Breuner and Orchinik, 2002; Shultz and Kitaysky, 2008). Although the primary role of CBG is under debate, the free hormone hypothesis suggests that the unbound, or free, hormone in the plasma is biologically active. In European kestrel (*Falco tinnunculus*) and barn owl (*Tyto alba*) nestlings, the increase of total CORT after CORT administration was paralleled by a concomitant short-lasting increase in CBG capacity (Müller et al., 2009). However, CBG capacity was not modified by CORT treatment after the peak of circulating baseline CORT. Similar increases in plasma and serum CBG were reported in house sparrows (*Passer domesticus*) and in mouse pups after glucocorticoid administration (Zhao et al., 1997; Breuner et al., 2003), but the opposite effect was found in rats (Feldman et al., 1979). In our study, it is conceivable that CBG, and thus the free fraction of CORT, could have been affected by the low-dose CORT experiment. For instance, plasma CBG capacity would have increased just after CORT treatment. However, the lack of clear patterns among published studies makes it difficult to interpret the data. Hence, our discussion should be viewed within the context of total CORT only. Future studies are needed to understand the effect of low-dose CORT treatment on

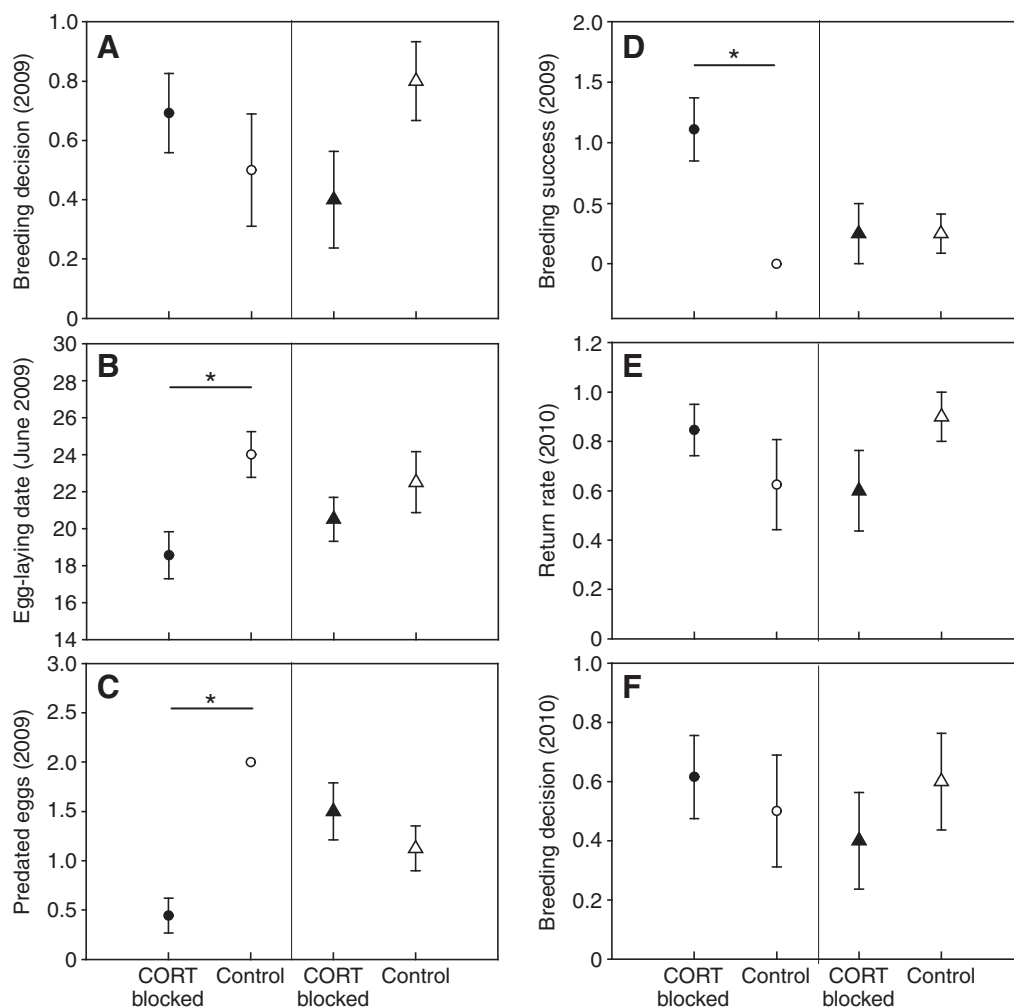


Fig. 2. (A) Breeding decision (at least one egg is laid: 1, no egg is laid: 0), (B) first egg-laying date, (C) number of predated eggs per active nest (0, 1 or 2), (D) breeding success (number of chicks >12 days old per active nest), (E) return rate the year following the treatment (not seen: 0, seen: 1), (F) breeding decision the year following the treatment for CORT-blocked and control kittiwakes. Circles denote females and triangles denote males. *Significant difference between the CORT-blocked and control birds.

CBG capacity, and on the total and free fractions of CORT in kittiwakes.

CORT treatment and the timing of breeding

In agreement with our predictions, we found that the experimental reduction of CORT release during the pre-laying period was paralleled by a significant advancement of egg laying in female kittiwakes (around 4 days earlier). This experimental CORT treatment strongly supports the hypothesis that CORT levels and the timing of egg laying are mechanistically linked. This has previously been suggested by correlative observations: high baseline CORT levels were associated with late breeding in female Florida scrub-jays (*Aphelocoma coerulescens*) (Schoech et al., 2009) and in snow petrels (*Pagodroma nivea*) (Goutte et al., 2010a). Moreover, in captive female zebra finches (*Taeniopygia guttata*) (Salvante and Williams, 2003), an experimental elevation of plasma CORT levels decreased the proportion of breeding females and delayed the onset of egg laying. However, during favourable conditions, high baseline CORT levels were not associated with delayed breeding in Florida scrub-jays (Schoech et al., 2009). Furthermore, experimental CORT administration did not delay the timing of breeding, when female Florida scrub-jays were fed *ad libitum* during the pre-laying period (Schoech et al., 2007). The link between CORT and phenology appears to be environment dependent (Schoech et al., 2009), as baseline CORT level is a marker of energetic state and food availability (Kitaysky et al., 1999; Love et al., 2004) (reviewed in

Landys et al., 2006). High CORT levels may mediate late breeding, especially during high energetic constraints. Indeed, the present study showed that experimentally reduced CORT levels during unfavourable pre-laying conditions had the potential to advance the timing of breeding in free-living birds.

What is the causal link between experimentally reduced CORT levels and early breeding in female kittiwakes? In a previous study, we found that high baseline CORT levels were associated with low baseline LH levels and low LH releasing ability after GnRH injection (Goutte et al., 2010b). By experimentally reducing CORT levels, LH secretion and gonadal development may be promoted, thereby triggering early breeding. In this respect, we also found a high rate of body mass gain in breeding females with reduced CORT levels. As this high rate of body mass gain could result from rapid gonadal growth, this would support the hypothesis of an accelerated reproductive physiology, induced by the experimentally reduced CORT levels. Alternatively, body mass gain could reflect the accumulation of energetic reserves in females with experimentally reduced CORT levels, instead of gonadal development. As high body condition in pre-laying female kittiwakes predicts early breeding (Goutte et al., 2010b), females with a high body mass gain would lay their eggs earlier in the season. Consequently, female kittiwakes with experimentally reduced CORT levels may breed earlier because of a higher accumulation of energy reserves compared with controls. As the primary function of CORT is to facilitate glucose release for utilization during challenges, female kittiwakes with experimentally

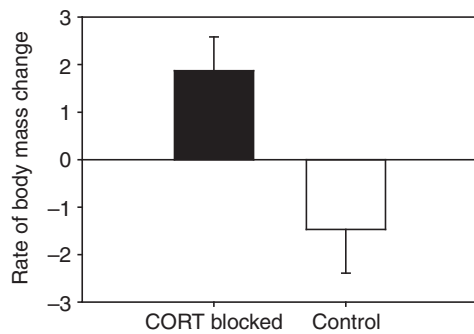


Fig. 3. Rate of body mass change between day of implantation and day of recapture, relative to the number of days elapsed since the treatment in CORT-blocked breeding females ($N=5$) and control breeding females ($N=4$). The number of days elapsed between the treatment and the recapture did not differ between these two groups (Wilcoxon test, $W=12.5$, $P=0.623$).

reduced CORT levels would have greater energy reserves than controls. Moreover, courtship feeding is a common behaviour in kittiwakes during the pre-laying period (Kempenaers et al., 2007). It is possible that females with experimentally reduced CORT levels were fed more and/or earlier than usual by their mates, hence allowing them to accumulate energy reserves and to breed earlier. Further behavioural observations must be conducted to test this hypothesis.

The effect of reduced CORT release on the timing of breeding was strongly sex specific as male kittiwakes with experimentally reduced CORT levels did not breed earlier than control birds. Indeed, the sensitivity of the HPG axis to stressful cues is thought to differ between sexes (Ball and Ketterson, 2008). Supporting this, we have recently shown that the inhibition of LH release by high baseline CORT levels was only observed in female kittiwakes and not in males (Goutte et al., 2010b). Pre-laying constraints should be strongly sex specific in free-living seabirds. Because of male–male competition, male kittiwakes must acquire reproductive readiness earlier in the season than females and must better resist environmental perturbations (Blas and Hiraldo, 2010). Moreover, females must extract and store a sufficient amount of energy to build up the eggs, and could be strongly sensitive to environmental stressors, such as a depletion of food supply early in the season (Ball and Ketterson, 2008; Blas and Hiraldo, 2010).

Fitness consequences of CORT treatment

First, it is important to note that the experimental reduction of CORT levels did not influence breeding decision in female kittiwakes. Only females with a low initial pre-laying body condition did not breed, which suggests that the reduction of CORT levels did not counteract energetic constraints. However, males with experimentally reduced CORT levels showed a lower breeding probability than controls, without an effect of body condition. In addition, low initial baseline CORT levels were associated with high laying failure in male kittiwakes. It is possible that male kittiwakes need to maintain relatively high baseline CORT levels to ensure sustained reproductive activity (Landys et al., 2006). During the pre-laying period, nest defence, foraging activity and courtship feeding would require moderate levels of baseline CORT in male kittiwakes; thus, a prolonged reduction of CORT levels could lead to nest and/or mate lost. This result highlights a strong sex difference in the hormonal regulation of breeding probability (Ball and Ketterson, 2008; Goutte et al., 2010b).

Second, female kittiwakes with experimentally reduced CORT levels had a higher breeding success than control birds, although clutch size did not differ between groups. Indeed, females with experimentally reduced CORT levels managed to successfully incubate their eggs (~ 0.5 vs 2 lost eggs in the control group). As frequently observed in birds, early breeders succeed better than late ones (review in Verhulst and Nilsson, 2008), particularly during the incubation period in seabirds (e.g. DeForest and Gaston, 1996). In this context, we suggest that females with experimentally reduced CORT levels would have benefited more from better environmental conditions than control birds, mainly because of the advancement of egg laying. Alternatively, being heavier, CORT-blocked females may have benefited from higher body reserves during the challenging brooding period compared with control females (Moe et al., 2002). The CORT treatment might also have lasted longer than the pre-laying period and might have been effective during the incubation and chick-rearing period. As the attenuation of the CORT stress response is known to favour high parental effort (brood value hypothesis) (Lendvai et al., 2007), females with experimentally reduced stress-induced CORT levels would have attended the nest more, hence having a higher reproductive success than control females. However, this should also have been observed in CORT-blocked males, given incubating and chick-rearing roles as well as CORT levels during these periods are largely similar for male and female kittiwakes (Kitaysky et al., 1999; Chastel et al., 2005). As male kittiwakes with experimentally reduced CORT levels did not show better reproductive success than control birds, the hypothesis of a very long attenuation of CORT release is contested.

Finally, the CORT treatment had no consequences on return rate and breeding decision the following year in female kittiwakes. However, males with experimentally reduced CORT levels tended to show a lower return rate in 2010 than control males. CORT manipulation could have led to negative effects on survival, as previously found in male kittiwakes implanted with two CORT implants during the chick-rearing period (Goutte et al., 2010c). Moreover, males with experimentally lower CORT levels may have dispersed in surrounding colonies in 2010 and/or may have skipped the 2010 breeding attempt, as a possible consequence of the high laying failure in 2009 (Naves et al., 2006). Further analyses (2 years of observation after the treatment and capture–mark–recapture analyses) are needed to confirm this tendency in males. Female kittiwakes with experimentally reduced CORT levels had an identical return rate and breeding decision the following year. Brinkhof and colleagues demonstrated that female coots (*Fulica atra*) with experimentally advanced breeding had a lower survival rate than controls (Brinkhof et al., 2002). However, the advancement of hatching date in coots was higher (10 days) than in our study (*ca.* 4 days). Moreover, the production of a second brood in the experimentally advanced coot pairs could have resulted in higher reproductive costs and a higher associated mortality rate (Brinkhof et al., 2002). Finally, the higher breeding success and, hence, the higher breeding investment in females with experimentally reduced CORT levels did not compromise their breeding opportunities the following year, contrary to the hypothesis of Golet and colleagues (Golet et al., 2004).

ACKNOWLEDGEMENTS

The present research project no. 330 was performed at Ny Ålesund Station and was supported by the French Polar Institute (IPEV). A.G. was supported by a BDI grant from CNRS/Région Poitou-Charentes. The authors thank the MariClim (165112/S30) project. We thank F. Amélineau, I. Egge Johnsen, T. Nordstad and E. Noreen for wonderful help during the 2009 season and F. Angelier, A. Lendvai and P. L. Pap for the 2010 return rate observations. At the CEBC, we thank C. Trouvé, A. Lacroix and S. Dano for their excellent technical assistance in hormone

assays and molecular sexing. Two anonymous reviewers provided constructive criticism of earlier drafts of the manuscript.

REFERENCES

- Akana, S. F., Scribner, K. A., Bradbury, M. J., Strack, A. M., Walker, C. D. and Dallman, M. F. (1992). Feedback sensitivity of the rat hypothalamo-pituitary-adrenal axis and its capacity to adjust to exogenous corticosterone. *Endocrinology* **131**, 585-594.
- Angelier, F., Shaffer, S. A., Weimerskirch, H., Trouvé, C. and Chastel, O. (2007). Corticosterone and foraging behavior in a pelagic seabird. *Physiol. Biochem. Zool.* **80**, 283-292.
- Angelier, F., Clément-Chastel, C., Welcker, J., Gabrielsen, G. W. and Chastel, O. (2009). How does corticosterone affect parental behaviour and reproductive success? A study of prolactin in black-legged kittiwakes. *Funct. Ecol.* **23**, 784-793.
- Ball, G. F. (1993). The neural integration of environmental information by seasonally breeding birds. *Am. Zool.* **33**, 185-199.
- Ball, G. F. and Ketterson, E. D. (2008). Sex differences in the response to environmental cues regulating seasonal reproduction in birds. *Philos. Trans. R. Soc. Lond. B* **363**, 231-246.
- Blas, J. and Hiraldo, F. (2010). Proximate and ultimate factors explaining floating behavior in long-lived birds. *Horm. Behav.* **57**, 169-175.
- Bokony, V., Lendvai, A. Z., Liker, A., Angelier, F., Wingfield, J. C. and Chastel, O. (2009). Stress response and the value of reproduction: are birds prudent parents? *Am. Nat.* **173**, 589-598.
- Bonier, F., Martin, P. R., Moore, I. T. and Wingfield, J. C. (2009). Do baseline glucocorticoids predict fitness? *Trends Ecol. Evol.* **24**, 634-642.
- Breuner, C. W. and Orchinik, M. (2002). Plasma binding proteins as mediators of corticosteroid action in vertebrates. *J. Endocrinol.* **175**, 99-112.
- Breuner, C. W., Wada, H., Shyu, J. and Love, O. P. (2003). Corticosteroid binding globulin capacity responds to chronic hormone treatment but not acute stressors. *Integr. Comp. Biol.* **46**, 1013.
- Breuner, C. W., Patterson, S. H. and Hahn, T. P. (2008). In search of relationships between the acute adrenocortical response and fitness. *Gen. Comp. Endocr.* **157**, 288-295.
- Brinkhof, M. W. G., Cave, A. J., Daan, S. and Perdeck, A. C. (2002). Timing of current reproduction directly affects future reproductive output in European coots. *Evolution* **56**, 400-411.
- Busch, D. S., Sperry, T. S., Wingfield, J. C. and Boyd, E. H. (2008). Effects of repeated, short-term, corticosterone administration on the hypothalamo-pituitary-adrenal axis of the white-crowned sparrow (*Zonotrichia leucophrys gambelii*). *Gen. Comp. Endocr.* **158**, 211-223.
- Byrd, G. V., Sydeman, W. J., Renner, H. M. and Minobe, S. (2008). Responses of piscivorous seabirds at the Pribilof Islands to ocean climate. *Deep-Sea Res. II* **55**, 1856-1867.
- Chastel, O., Lacroix, A., Weimerskirch, H. and Gabrielsen, G. W. (2005). Modulation of prolactin but not corticosterone responses to stress in relation to parental effort in a long-lived bird. *Horm. Behav.* **47**, 459-466.
- Dawson, A. (2008). Control of the annual cycle in birds: endocrine constraints and plasticity in response to ecological variability. *Philos. Trans. R. Soc. Lond. B* **363**, 1621-1633.
- Dawson, A., King, V. M., Bentley, G. E. and Ball, G. F. (2001). Photoperiodic control of seasonality in birds. *J. Biol. Rhythm.* **16**, 366-381.
- DeForest, L. N. and Gaston, A. J. (1996). The effect of age on timing of breeding and reproductive success in the thick-billed Murre. *Ecology* **77**, 1501-1511.
- Drent, R. H. and Daan, S. (1980). The prudent parent – energetic adjustments in avian breeding. *Ardea* **68**, 225-252.
- Durant, J. M., Hjermann, D. O., Anker-Nilssen, T., Beaugrand, G., Myrsterud, A., Pettorelli, N. and Stenseth, N. C. (2005). Timing and abundance as key mechanisms affecting trophic interactions in variable environments. *Ecol. Lett.* **8**, 952-958.
- Durant, J. M., Hjermann, D. O., Ottersen, G. and Stenseth, N. C. (2007). Climate and the match or mismatch between predator requirements and resource availability. *Clim. Res.* **33**, 271-283.
- Feldman, D., Mondon, C. E., Horner, J. A. and Weiser, J. N. (1979). Glucocorticoid and estrogen regulation of corticosteroid-binding globulin production by rat liver. *Am. J. Physiol. Endocrinol. Metab.* **237**, E493-E499.
- Frederiksen, M., Harris, M. P., Daunt, F., Rothery, P. and Wanless, S. (2004). Scale-dependent climate signals drive breeding phenology of three seabird species. *Glob. Change Biol.* **10**, 1214-1221.
- Golet, G. H., Schmutz, J. A., Irons, D. B. and Estes, J. A. (2004). Determinants of reproductive costs in the long-lived black-legged kittiwake: a multiyear experiment. *Ecol. Monogr.* **74**, 353-372.
- Goutte, A., Antoine, E., Weimerskirch, H. and Chastel, O. (2010a). Age and the timing of breeding in a long-lived bird: a role for stress hormones? *Funct. Ecol.* **24**, 1007-1016.
- Goutte, A., Angelier, F., Welcker, J., Moe, B., Clément-Chastel, C., Gabrielsen, G. W., Bech, C. and Chastel, O. (2010b). Long-term survival effect of corticosterone manipulation in black-legged kittiwakes. *Gen. Comp. Endocr.* **167**, 246-251.
- Goutte, A., Angelier, F., Clément-Chastel, C., Trouvé, C., Moe, B., Bech, C., Gabrielsen, G. W. and Chastel, O. (2010c). Stress and the timing of breeding: glucocorticoid- luteinizing hormones relationships in an arctic seabird. *Gen. Comp. Endocr.* **169**, 108-116.
- Hipfner, J. M., McFarlane-Tranquilla, L. A. and Addison, B. (2008). Do marine birds use environmental cues to optimize egg production? An experimental test based on relaying propensity. *J. Avian Biol.* **39**, 611-618.
- Kempnaers, B., Lancot, R. B., Gill, V. A., Hatch, S. A. and Valcu, M. (2007). Do females trade copulations for food? An experimental study in Black-legged Kittiwakes. *Behav. Ecol.* **18**, 345-353.
- Kitaysky, A. S., Wingfield, J. C. and Piatt, J. F. (1999). Dynamics of food availability, body condition and physiological stress response in breeding Black-legged Kittiwakes. *Funct. Ecol.* **13**, 577-584.
- Kitaysky, A. S., Wingfield, J. C. and Piatt, J. F. (2001). Corticosterone facilitates begging and affects resource allocation in the Black-legged kittiwake. *Behav. Ecol.* **12**, 619-625.
- Kitaysky, A. S., Piatt, J. F. and Wingfield, J. C. (2007). Stress hormones link food availability and population processes in seabirds. *Mar. Ecol. Prog. Ser.* **352**, 245-258.
- Kitaysky, A. S., Piatt, J. F., Hatch, S. A., Kitaikaia, E. V., Benowitz-Fredericks, Z. M., Shultz, M. T. and Wingfield, J. C. (2010). Food availability and population processes: severity of nutritional stress during reproduction predicts survival of long-lived seabirds. *Funct. Ecol.* **24**, 625-637.
- Lack, D. (1968). *Ecological Adaptations for Breeding in Birds*. London: Methuen.
- Lancot, R. B., Hatch, S. A., Gill, V. A. and Eens, M. (2003). Are corticosterone levels a good indicator of food availability and reproductive performance in a kittiwake colony? *Horm. Behav.* **43**, 489-502.
- Landys, M. M., Ramenofsky, M. and Wingfield, J. C. (2006). Actions of glucocorticoids at a seasonal baseline as compared to stress-related levels in the regulation of periodic life processes. *Gen. Comp. Endocr.* **148**, 132-149.
- Lendvai, A. Z., Giraudeau, M. and Chastel, O. (2007). Reproduction and modulation of the stress response: an experimental test in the house sparrow. *Proc. Roy. Soc. B. Biol.* **274**, 391-397.
- Lormée, H., Jouventin, P., Trouve, C. and Chastel, O. (2003). Sex-specific patterns in baseline corticosterone and body condition changes in breeding Red-footed Boobies *Sula sula*. *Ibis* **145**, 212-219.
- Love, O. P., Breuner, C. W., Vézina, F. and Williams, T. D. (2004). Mediation of a corticosterone-induced reproductive conflict. *Horm. Behav.* **46**, 59-65.
- Lyon, B. E., Chaine, A. S. and Winkler, D. W. (2008). Ecology: a matter of timing. *Science* **321**, 1051-1052.
- McNamara, J. M. and Houston, A. I. (2008). Optimal annual routines: behaviour in the context of physiology and ecology. *Philos. T. R. Soc. B* **363**, 301-319.
- Moe, B., Langseth, I., Fyhn, M., Gabrielsen, G. W. and Bech, C. (2002). Changes in body condition in breeding kittiwakes *Rissa tridactyla*. *J. Avian Biol.* **33**, 225-234.
- Moe, B., Stempniewicz, L., Jakubas, D., Angelier, F., Chastel, O., Dinessen, F., Gabrielsen, G. W., Hanssen, F., Karnovsky, N. J., Ronning, B., Welcker, J., Wojczulanis-Jakubas, K. and Bech, C. (2009). Climate change and phenological responses of two seabird species breeding in the high-Arctic. *Mar. Ecol. Prog. Ser.* **393**, 235-246.
- Müller, C., Almási, B., Roulin, A., Breuner, C. W., Jenni-Eiermann, S. and Jenni, L. (2009). Effects of corticosterone pellets on baseline and stress-induced corticosterone and corticosteroid-binding-globulin. *Gen. Comp. Endocr.* **160**, 59-66.
- Naves, L. C., Monnat, J. Y. and Cam, E. (2006). Breeding performance, mate fidelity, and nest site fidelity in a long-lived seabird: behaving against the current? *Oikos* **115**, 263-276.
- Nilsson, J. A. and Svensson, E. (1996). The cost of reproduction: a new link between current reproductive effort and future reproductive success. *Proc. R. Soc. Lond. B* **263**, 711-714.
- Perrins, C. M. (1970). Timing of birds breeding seasons. *Ibis* **112**, 242-255.
- R Development Core Team (2008). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL <http://www.R-project.org>.
- Ricklefs, R. E. and Wikelski, M. (2002). The physiology/life-history nexus. *Trends Ecol. Evol.* **17**, 462-468.
- Romero, L. M. and Reed, J. M. (2005). Collecting baseline corticosterone samples in the field: is under 3 min good enough? *Comp. Biochem. Physiol.* **140A**, 73-79.
- Romero, L. M., Strohlic, D. and Wingfield, J. C. (2005). Corticosterone inhibits feather growth: potential mechanism explaining seasonal down regulation of corticosterone during molt. *Comp. Biochem. Physiol.* **142A**, 65-73.
- Salvante, K. G. and Williams, T. D. (2003). Effects of corticosterone on the proportion of breeding females, reproductive output and yolk precursor levels. *Gen. Comp. Endocr.* **130**, 205-214.
- Schoech, S. J., Bowman, R., Bridge, E. S., Morgan, G. M., Rensel, M. A., Wilcoxon, T. E. and Boughton, R. K. (2007). Corticosterone administration does not affect timing of breeding in Florida scrub-jays (*Aphelocoma coerulescens*). *Horm. Behav.* **52**, 191-196.
- Schoech, S. J., Rensel, M. A., Bridge, E. S., Boughton, R. K. and Wilcoxon, T. E. (2009). Environment, glucocorticoids, and the timing of reproduction. *Gen. Comp. Endocr.* **163**, 201-207.
- Shultz, M. T. and Kitaysky, A. S. (2008). Spatial and temporal dynamics of corticosterone and corticosterone binding globulin are driven by environmental heterogeneity. *Gen. Comp. Endocr.* **155**, 717-728.
- Shultz, M. T., Piatt, J. F., Harding, A. M. A., Kettle, A. B. and Van Pelt, T. I. (2009). Timing of breeding and reproductive performance in murre and kittiwakes reflect mismatched seasonal prey dynamics. *Mar. Ecol. Prog. Ser.* **393**, 247-258.
- Stenseth, N. C. and Myrsterud, A. (2002). Climate, changing phenology, and other life history and traits: Nonlinearity and match-mismatch to the environment. *Proc. Natl. Acad. Sci. USA* **99**, 13379-13381.
- Vandenborne, K., De Groef, B., Geelissen, S., Kühn, E., Darras, V. and Van der Geyten, S. (2005). Corticosterone-induced negative feedback mechanisms within the hypothalamo-pituitary-adrenal axis of the chicken. *J. Endocrinol.* **185**, 383-391.
- Verhulst, S. and Nilsson, J. A. (2008). The timing of birds' breeding seasons: a review of experiments that manipulated timing of breeding. *Philos. Trans. R. Soc. Lond. B* **363**, 399-410.
- Visser, M. E. and Both, C. (2005). Shifts in phenology due to global climate change: the need for a yardstick. *Proc. R. Soc. Lond. B* **272**, 2561-2569.
- Visser, M. E., van Noordwijk, A. J., Tinbergen, J. M. and Lessells, C. M. (1998). Warmer springs lead to mistimed reproduction in great tits (*Parus major*). *Proc. R. Soc. Lond. B* **265**, 1867-1870.
- Weimerskirch, H., Lallemand, J. and Martin, J. (2005). Population sex ratio variation in a monogamous long-lived bird, the wandering albatross. *J. Anim. Ecol.* **74**, 285-291.
- Wingfield, J. C. (1980). Fine temporal adjustment of reproductive functions. In *Avian Endocrinology* (ed. A. Eppe and M. H. Stetson), pp. 367-389. New York, NY: Academic Press.

- Wingfield, J. C.** (2008). Comparative endocrinology, environment and global change. *Gen. Comp. Endocr.* **157**, 207-216.
- Wingfield, J. C. and Kenagy, G. J.** (1991). Natural regulation of reproductive cycles. In *Vertebrate Endocrinology: Fundamentals and Biomedical Implications* (ed. P. K. T. Pang and M. P. Schreibman), pp. 181-241. San Diego, CA: Academic Press.
- Wingfield, J. C. and Sapolsky, R. M.** (2003). Reproduction and resistance to stress: when and how? *J. Neuroendocr.* **15**, 711-724.
- Wingfield, J. C., Hahn, T. P., Maney, D. L., Schoech, S. J., Wada, M. and Morton, M. L.** (2003). Effects of temperature on photoperiodically induced reproductive development, circulating plasma luteinizing hormone and thyroid hormones, body mass, fat deposition and molt in mountain white-crowned sparrows, *Zonotrichia leucophrys oriantha*. *Gen. Comp. Endocr.* **131**, 143-158.
- Wolkowitz, O., M.** (1994). Prospective controlled-studies of the behavioral and biological effects of exogenous corticosteroids. *Psychoneuroendocrino.* **19**, 233-255.
- Zhao, X. F., Scrocchi, L. A. and Hammond, G. L.** (1997). Glucocorticoids induce corticosteroid-binding globulin biosynthesis by immature mouse liver and kidney. *J. Steroid Biochem.* **60**, 163-169.