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Protracted treatment with corticosterone reduces breeding success in a long-lived bird

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Abstract

Determining the physiological mechanisms underpinning life-history decisions is essential for understanding the constraints under which life-history strategies can evolve. In long-lived species, where the residual reproductive value of breeders is high, adult survival is a key contributor to lifetime reproductive success. We therefore expect that when adult survival is compromised during reproduction, mechanisms will evolve to redirect resources away from reproduction, with implications for reproductive hormones, adult body mass, nest attendance behaviour and breeding success. We investigated whether manipulating corticosterone, to simulate exposure to an environmental stressor, affected the secretion of prolactin and breeding success in the black-legged kittiwake *Rissa tridactyla*. We used implanted Alzet® osmotic pumps to administer corticosterone to incubating kittiwakes at a constant rate over a period of approximately eight days. Manipulated birds were compared with sham implanted birds and control birds, which had no implants. There was no significant difference in the body mass of captured individuals at the time of implantation and implant removal. Corticosterone-implanted males showed lower nest attendance during the chick rearing period compared to sham-implanted males; the opposite pattern was found in females. Corticosterone treated birds showed a marginally significant reduction in breeding success compared to sham-implanted individuals, with all failures occurring at least one week after implant removal. However, prolactin concentrations at implant removal were not significantly different from initial values. We were unable to measure the profile of change in corticosterone during the experiment. However, our results suggest a delayed effect of elevated corticosterone on breeding success rather than an immediate suppression of prolactin concentrations causing premature failure.

Keywords

Prolactin; Physiology; Parental care; Reproduction; Black-legged kittiwake; Rissa tridactyla

1 Introduction

Life-history theory predicts that, when resources are limiting, trade-offs occur between reproductive investment in the current breeding opportunity and self-maintenance to preserve future breeding opportunities (Stearns, 1977). In long-lived species, in which the success of any one breeding event is a relatively small component of lifetime reproductive success, allocation decisions that favour parent rather than offspring survival are expected (reviewed in Linden and Møller, 1989). The emergency life-history stage modulates the physiology and behaviour of organisms, through redirecting energy away from non-essential physiology and behaviours, such as reproduction or immune response, towards those needed for survival (reviewed in Wingfield et al., 1998).

The emergency life-history stage involves the elevation of corticosterone—the main glucocorticoid in birds—in response to the activation of the hypothalamic-pituitary-adrenal axis (Wingfield et al., 1998). Chronic stress results from long-term exposure to a stressor, often resulting in negative fitness consequences such as low productivity, suppressed immunity and inhibited growth, rather than the short-term benefits of the emergency life-history stage, associated with acute stress. During chronic stress the duration of both the stressor and the consequences for an animal's physiology are long-lasting (Boonstra, 2013), resulting in sustained elevations of corticosterone. However, when corticosterone concentrations increase above baseline, the rate of passive clearance increases and active negative feedback reduces endogenous production (Sapolsky et al., 2000, Rich and Romero, 2005, Romero et al., 2005). This means that corticosterone is not maintained at stress-induced concentrations for long periods of time and therefore reduces the negative fitness consequences of long-lasting elevated corticosterone (Sapolsky et al., 2000, Romero, 2002).

Changes in corticosterone concentrations have a variety of implications for parental behaviour (reviewed in Crossin et al., 2012, Crespi et al., 2013). Baseline concentrations of corticosterone can be positively correlated with parental behaviour ('corticosterone-adaptation hypothesis') through stimulatory effects on foraging behaviour, which enhance provisioning to chicks (Kitaysky et al., 2001, Angelier and Chastel, 2009). On the other hand, protracted stress-induced elevations of corticosterone suppress parental behaviour ('corticosterone-induced reproductive conflict'), causing the redirection of resources away from breeding and towards self-maintenance (Love et al., 2004).

Changes in corticosterone also have implications for the body mass of breeders. However, there is no clear prediction as to how chronic stress affects body mass. Correlational studies often show that increases in baseline corticosterone concentrations are associated with declines in body mass or body condition (i.e. size-corrected body mass e.g. Cherel et al., 1988, Cherel et al., 1994, Kitaysky et al., 1999). However, Schultner et al. (2013a) showed that initial increases in baseline corticosterone concentrations were associated with an increase in fat reserves. Corticosterone secretion has also been found to be independent of changes in body condition in some species (e.g. Silverin and Wingfield, 1982, Wingfield et al., 1999, Lormée et al., 2003).

Evidence regarding the mechanistic process that modulates the stress response during breeding is currently inconclusive. One potential mediator of the stress response is via changes in prolactin concentrations (Chastel et al., 2005, reviewed in Angelier and Chastel, 2009). Prolactin has a wide variety of roles throughout the vertebrates (Norris, 1980). In birds it promotes incubation and parental care, and is secreted in response to long photoperiods and further by the presence of eggs and young in the nest (Dawson and Goldsmith, 1985, El Halawani et al., 1986). Chronic stress is expected to cause declines in reproductive hormones (Sapolsky, 2000) and, therefore, it is possible that corticosterone may disrupt prolactin secretion and reduce breeding behaviour such as nest attendance (Angelier et al., 2009a).

Studies to date that have looked for relationships between corticosterone and prolactin in long-lived seabirds have reached a range of often conflicting conclusions: some studies have found a negative correlation between the two hormones whilst others have found no relationship (reviewed in Angelier and Chastel, 2009, Riou et al., 2010). It has been suggested that, whilst the responses of corticosterone and prolactin to acute stress are not mechanistically linked, their responses to chronic stress are, with prolactin mediating the effect of corticosterone on breeding behaviour (reviewed in Angelier and Chastel, 2009, Angelier et al., 2013). Implantation of corticosterone in black-legged kittiwakes *Rissa tridactyla* breeding in Svalbard, Norway caused a reduction in prolactin concentrations, breeding success and nest attendance (Angelier et al., 2009a). In that study corticosterone was not successfully manipulated over a protracted period but rather increased to a peak on day one and had returned to baseline concentrations by day three (Angelier et al., 2009a). However, the responses of corticosterone and prolactin to chronic stress have not, to our knowledge, been tested.

The kittiwake is a long-lived seabird with a typical bi-parental care system, which has been studied in both the Atlantic and the Pacific with regards to its breeding biology and physiology (e.g. Golet et al., 2004, Angelier et al., 2009a, Goutte et al., 2010, Kitaysky et al., 2010). In this study, we used Alzet® osmotic pumps, which release substances at a constant rate over a number of days, in an attempt to elevate corticosterone concentrations over several days in a North Sea population of black-legged kittiwakes (hereafter 'kittiwake'). We hypothesised that this protracted elevation of corticosterone via Alzet® osmotic pumps would have a disruptive effect on breeding, specifically causing a short-term reduction in prolactin concentration and body mass, a reduction in nest attendance during the chick-rearing period, and lower breeding success by the end of the season.

2 Methods

2.1 Nest activity

270 kittiwake nests from 15 different plots on the Isle of May, National Nature Reserve, Firth of Forth, south-east Scotland (56° 11' N, 02° 33' W) were observed daily from laying to fledging in 2011 and for each nest we recorded the lay date, clutch size and either date of failure or number of chicks fledged (breeding success; Fig. 1).

2.2 Blood sampling of kittiwakes

38 kittiwakes (27 females and 11 males) were captured, each from different nests, during late incubation (May 25–May 30; 18 ± 5.4 (mean ± SD) days after laying; all birds were incubating at the time of initial capture; Fig. 1) using an eight metre long noose pole. A maximum of 1 ml of blood was taken from the wing vein using sterile 1 ml non-heparinised syringes. Samples were centrifuged, once the blood had been allowed to clot, and frozen as separate red blood cells and serum.

A dictaphone was used to precisely record the elapsed time between capture (i.e. when the noose was placed over a individual's head) and the end of blood sampling (individual sampling time), which we aimed to complete within three minutes of capture so that baseline, or near baseline, concentrations of corticosterone would be obtained (Romero and Reed, 2005). Individual sampling time was 2.7 ± 0.6 (mean ± SD) min, with 15 % of samples being obtained within two minutes and 75 % within three minutes. All samples were collected within 5.7 min. We also recorded the elapsed time between the extension of the noose pole towards the target individual and the lifting of the captured individual off its nest (capture

time). Capture time was 1.2 ± 0.8 (mean ± SD) min with 56 % of captures occurring within one minute, 88 % within two minutes and 98 % within three minutes. In order to assess any impact of disturbance to individuals during the catching of other individuals at the same colony, individuals were ranked in the order that they were captured at a given site, on a given catching attempt. If a site was returned to later that day (> 6 hours later) or on the subsequent day, this was assumed to be a new catching attempt. There was a maximum of eight individuals captured per site on a given catching attempt.

2.3 Corticosterone manipulation in kittiwakes

For individuals already carrying a British Trust for Ornithology (BTO) metal ring the unique ring number was recorded and remaining individuals were ringed. Individuals were weighed to the nearest gram using a Pesola. Previous studies have used open-ended silastic tubes or self-degradable pellets to deliver corticosterone. These are unlikely to maintain protracted elevation such as that experienced during adverse environmental conditions (Romero et al., 2005, Newman et al., 2010, Thierry et al., 2013). Instead, we used Alzet® osmotic pumps (length: 3.0 cm; diameter: 0.7 cm; mass: 1.1 g; nominal volume: 200 µl; delivery period: 14 days; rate of delivery: 0.5 $\mu l.h^{-1}$). These were inserted subcutaneously on the flank immediately anterior to the thigh, under local anaesthesia. These osmotic pumps are used widely in a range of studies including pharmacology, biotechnology and immunology (> 13,500 publications in the Alzet® bibliography) and have been successfully used to administer corticosterone in white throated sparrows *Zonotrichia albicollis* (Horton et al., 2007). A small incision was made using a sterile scalpel blade and this was closed with suture. The pumps contained either corticosterone dissolved in polyethylene glycol 400 (PEG) at a concentration of 28 $mg.ml^{-1}$ (corticosterone-implanted individuals; n = 17) or PEG only (sham-implanted individuals; n = 21). Kittiwakes weigh on average 380 g and therefore two pumps were necessary to deliver the required dose of corticosterone, which we estimated using data from Horton et al. (2007). We matched experimental groups for location to account for potential plot effects. At the time of implantation there were no significant differences between corticosterone-implanted and sham-implanted groups with respect to clutch size (linear model: $t = 1.71$, $df = 36$, $P = 0.10$, $R^2 = 0.07$), lay date (linear model: $t = 0.89$, $df = 36$, $P = 0.38$, $R^2 = 0.02$) or sex ratio (Binomial generalized linear model: $z = 1.23$, $P = 0.22$) Individuals were marked with picric acid on the head or tail feathers to aid identification during attempted recapture.

Implanted individuals were recaptured 8 ± 1 (mean ± SD) days later (26 ± 1 days after laying; 40 % of nests had hatched at least one egg by the time of recapture). This interval was chosen to be long enough to maximise recapture probability but to be before all the corticosterone had been delivered. 71 % of implanted individuals were successfully recaptured (n = 27; 14 sham-implanted individuals; 13 corticosterone-implanted individuals). Recaptured individuals were blood sampled, as described above, weighed and the implants removed using the same technique as for insertion. Examination of all the removed implants indicated that they were empty. At the time of implant removal, an additional seven individuals (controls) that had not been implanted, and whose partners had not been implanted, were captured and blood sampled; these controls enabled us to test for any adverse effects of the initial capture and implantation of the osmotic pumps on the experimental birds. In order to reduce disturbance at the colonies and maximise the chances of recapture, individuals were only captured twice and we did not record nest attendance between initial capture and attempted recapture. The individuals used in this study were not part of any other study and therefore remained relatively undisturbed outside of our experimental protocols, minimising their exposure to acute stress.

All work was carried out under Home Office personal licences (Bethany Nelson: PIL 60/12426 and Alistair Dawson: PIL 70/1697) and associated project licences (Alistair Dawson: PPL 60/4176 and Francis Daunt: PPL 60/4001) and Scottish Natural Heritage research permit (MON/RP/131).

2.4 Nest attendance

We recorded nest attendance by identifying which members of the corticosterone-implanted and sham-implanted pairs were present at the nest using the unique pattern of white and black markings on the tips of the wing feathers (Chardine, 2002) and the picric acid markings. Data were collected from 37 out of the 38 nests included in the corticosterone manipulation experiment, during early to mid chick-rearing (9 June to 30 June; Fig. 1). One nest was excluded because it was in a colony that was easily disturbed and therefore was hard to access for individual identification purposes. Nest attendance was checked up to three times daily (total: 824 checks; time of day: 8:00–20:40).

2.5 Molecular sexing

DNA was extracted from the red blood cells of all samples using a Qiagen DNeasy® Blood and Tissue Kit (QIAGEN Ltd., West Sussex, UK), following the manufacturer's instructions.

Individuals were sexed as described by Griffiths et al. (1998) using the primers described by Albores-Barajas et al. (2010). Samples from individuals of known sex were included as controls in all PCR amplifications and agarose gels. A negative control was also included containing no DNA. 50 % of the samples were repeated to check for consistency and no contradictory results were found.

2.6 Hormone assays

Prolactin concentrations were determined by a heterologous RIA using a primary antibody raised in rabbit against recombinant starling prolactin and a donkey anti rabbit secondary antibody (Bentley et al., 1997). Duplicate 20 µl samples were assayed. All samples were run in one assay and the intra-assay variation was 4.5 %. Serial dilutions of serum samples were parallel to the serial dilutions of the standard.

Corticosterone concentrations were determined by a quantitative competitive enzyme-immuno assay (EIA). Serum samples were equilibrated with 2000 cpm ¬3 H-CORT to measure recovery and extracted using diethyl ether. Extracted samples were analysed in duplicate using an EIA kit (Wada et al., 2007). A dose response curve of kittiwake serum ran parallel to the standard curve. Values were corrected for sample dilution and recovery. The average extraction efficiency was 80 ± 0.9 %. The mean inter-assay variation was 6.4 % and the intra-assay variation ranged from 5.6 to 6.8 %.

2.7 Statistical methods

All statistical analyses were performed in the R computing environment (version: 2.10.1, R Development Core Team, 2009). Eight separate analyses were conducted. For each analysis we selected the “best” model, and then assessed the significance of individual variables within this model. In each case we selected the "best" model to be the model with the lowest Akaike Information Criterion (AIC) or AICc (AIC with correction for small sample sizes) value (Burnham and Anderson, 2002). AICc was used for all analyses that did not contain random effects, because sample sizes were small. AIC was used for analyses that contained random effects, due to the difficulties in calculating the effective sample size *n* (which AICc requires) within mixed models

2.7.1 Body mass

To examine differences between treatment groups in adult body condition, we used mass rather than size-corrected mass because previous studies disagree about which, if any,

methods for calculating body condition are valid (Green, 2001, Schulte-Hostedde et al., 2005, Schamber et al., 2009). We used linear mixed models; models were fitted by maximum likelihood (ML) for the purposes of calculating AIC values and by REML for the purposes of obtaining parameter estimates and standard errors, and P values were calculated using Markov chain Monte Carlo simulations (Function *pvals.fnc*; Package *languageR* version 1.4). We only included individuals that had been successfully recaptured and therefore had both pre- and post-implant data: including individuals that were only captured once would have biased the pre-implant data because these individuals were probably more susceptible to disturbance. We included individual as a random factor in all models to account for repeated measures. The full model contained fixed effects for treatment (i.e. sham-implanted or corticosterone-implanted), pre/post-implant (i.e. 0 = pre-implant sample; 1 = post-implant sample) and the interaction between these (which was the key variable of interest). The full model also included date relative to lay date (i.e. date of sampling an individual relative to that individual's lay date), because mass tends to decline during the season (e.g. Mrosovsky and Sherry, 1980, Wendeln and Becker, 1999, Moe et al., 2002). Since evidence suggests that daily energy expenditure, which may affect body mass, varies between males and females (higher in female kittiwakes, Fyhn et al., 2001; higher in male kittiwakes, Thomson et al., 1998), we included sex, the interaction between treatment and sex, the interaction between pre/post-implant and sex and the three-way interaction between treatment, pre/post-implant and sex.

We also ran an additional analysis with body mass as the response variable to compare the post-implant measurements of sham-implanted individuals with control individuals, which were measured at the time of implant removal (Fig. 1). We did not need to include individual as a random effect in this analysis, because only one sample was being analysed per individual, and therefore used linear models. The full model contained treatment (i.e. sham-implanted or control), which was the key variable of interest, as well as date relative to lay date, sex and the interaction between treatment and sex.

2.7.2 Nest attendance

We used a generalized linear mixed model with a binomial distribution to test for any effect of treatment on the presence of implanted individuals at the nest during the chick-rearing period (i.e. after implant removal; Fig. 1): we used an explanatory variable with individuals coded as present (1) or absent (0). Nest was included as a random effect. Treatment was the

main fixed effect of interest and the full model also included day since implant and the interaction between day since implant and treatment (to assess whether any differences became more or less apparent over time). We included brood size as a fixed effect and the interaction between brood size and treatment due to previous studies suggesting that larger broods are more likely to be left unattended (Wanless and Harris, 1989). Sex and the interaction between sex and treatment were both also included. We treated each nest check as an independent event as no autocorrelation was detected in our model (estimated autocorrelation parameter of zero when we attempted to include this in the full model).

2.7.3 Breeding success

We use a generalized linear with an overdispersed binomial distribution to compare breeding success across groups. The binomial denominator was clutch size at the time of treatment – this means that differences in clutch size prior to treatment were effectively removed from the analysis. We considered four models: a null model in which all three groups (i.e. control, sham-implanted or corticosterone-implanted) were assumed to have the same mean, a model in which the treatment and sham groups were pooled, a model in which the sham and control groups were pooled, and a model in which the three groups were assumed to all have different means. The former two models imply that corticosterone has no effect on breeding success, and the latter two models imply that it does have an effect.

2.7.4 Hormone concentrations

We found no effect of individual sampling time (linear model: $t = 0.87$, $P = 0.39$), capture time ($t = 0.67$, $P = 0.51$), rank order of capture ($t = 0.31$, $P = 0.76$), an interaction between individual sampling time and capture time ($t = 0.85$, $P = 0.40$) or an interaction between individual sampling time and rank order of capture ($t = 0.24$, $P = 0.81$) on corticosterone concentrations (full model: $F_{5,66} = 0.67$, $R^2 = 0.05$). There was also no effect of total time (total = individual sampling time + capture time; linear model: $t = 1.01$, $P = 0.32$), rank order of capture ($t = 0.79$, $P = 0.43$) or the interaction between total time and rank ($t = 0.76$, $P = 0.45$) on corticosterone concentrations (full model: $F_{3,68} = 0.38$, $R^2 = 0.02$). Therefore, we included all samples in our analyses as representative of baseline concentrations.

When analysing the effect of treatment on corticosterone and prolactin concentrations, we used linear mixed models—the models for hormone concentrations had the same structure as those for body mass and were fitted in the same way. The full model included date relative to

lay date as a fixed effect, because prolactin cycles seasonally, rising during incubation (Dawson, 2006, 2008). We included sex as a fixed effect, the interaction between treatment and sex, the interaction between pre/post-implant and sex and the three-way interaction between treatment, pre/post-implant and sex, because of sex-specific patterns in corticosterone (Lormée et al., 2003), and sex-specific responses of prolactin to stress (Angelier et al., 2009b). As corticosterone and prolactin concentrations were constrained by being positive and the residuals were not normally distributed, we transformed these two response variables by taking the logarithm to base ten.

We also ran two additional models with corticosterone and prolactin as response variables to compare the post-implant measurements of sham-implanted individuals with control individuals, which were measured at the time of implant removal (Fig. 1). These two additional models had the same structure as the model comparing the post-implant body mass of sham and control individuals.

3 Results

There were no signs of any adverse effects of the implants at the time of recapture and in all cases the incision area had completely healed. None of the implanted birds disappeared during the experiment and there was no significant difference in breeding success between sham-implanted individuals and controls (see section 3.3).

3.1 Body mass

For the comparison of corticosterone-implanted and sham-implanted individuals the model with lowest AIC (Fig. 2a; see supplementary data, Table S1) contained sex, treatment and the interaction between sex and treatment, but within this model there was no significant effect of sex ($t = 1.27$, $P = 0.21$), treatment ($t = 0.20$, $P = 0.84$), or the interaction between sex and treatment ($t = 0.04$, $P = 0.97$) on body mass.

For the comparison of post-implant body mass of sham-implanted and control individuals the model with lowest AICc (see supplementary data, Table S2) only contained sex, reflecting a tendency for males to have higher body mass than females, but this effect was non-significant ($t = 1.75$, $P = 0.10$, $R^2 = 0.14$).

3.2 Nest attendance

The best model for nest attendance, according to AIC (see supplementary data, Table S3), contained sex, treatment, the sex-by-treatment interaction and time since implant. The main effect of treatment on the presence of a previously implanted individual at the nest was non-significant ($t = 0.38$, $df = 33$, $P = 0.71$) but there was a significant effect of sex ($t = 2.95$, $df = 33$, $P = 0.01$), with females attending more than males (mean ± SD percentage of time spent attending nest; females: 45 ± 50 %; males: 37 ± 48 %). There was a significant interaction between treatment and sex ($t = 2.42$, $df = 33$, $P = 0.02$; Fig. 2b), with corticosterone-implanted females attending more than sham-implanted females and corticosterone-implanted males attending less than sham-implanted males. There was also a significant effect of time since implant ($t = 2.19$, $df = 784$, $P = 0.03$) with a 4.1 ± 1.3 % (mean ± SE) reduction in nest attendance per day since implantation irrespective of whether the individual was corticosterone-implanted or sham-implanted.

3.3 Breeding success

The best model for breeding success, according to AIC (see supplementary data, Table S4), was that in which the sham and control groups were the same as each other but different from the corticosterone-treated group. There was marginally significant evidence that corticosterone treated individuals fledged a lower proportion of chicks (34 %) than individuals that were not treated with corticosterone (61 % (including sham-implanted birds and controls); difference of 26 ± 12 % points (mean ± SE); $t = 2.01$, $P = 0.05$; Fig. 2c). Breeding failures, i.e. losses of all remaining chicks, occurred on average on June 21 ± 9 (mean ± SD), which was 16 ± 9 (mean ± SD) days after implant removal (Fig. 1). There was no significant difference between the timing of failure of corticosterone- and sham-implanted individuals (Student's t test: $t = 0.33$, $df = 11$, $P = 0.74$).

3.4 Hormone concentrations

For the comparison of corticosterone concentrations of treated and sham individuals the model with lowest AIC (see supplementary data, Table S5) contained sex, which had a marginally significant effect (linear mixed effects model: $t = 1.90$, $P = 0.06$). The corticosterone data were largely sensitive to one value (corticosterone-implanted male; pre-implant: 7.7 ng/ml; post-implant: 169.9 ng/ml; Mahalanobis distance: $MD = 45.9$, $df = 9$;

368 critical value = 27.9), which resulted in a large standard error associated with the post-
369 implant mean for corticosterone-implanted individuals (23.6 ± 12.3; Fig. 3a). However, the
370 results of the model did not change substantively if this value was excluded.

371 For the comparison of prolactin concentrations of treated and sham individuals, the model
372 with lowest AIC (Fig. 3b; see supplementary data, Table S6) contained sex, pre/post implant
373 and the sex-by-pre/post implant interaction. There was a marginally significant effect of sex
374 (linear mixed effects model: $t = 2.04$, $P = 0.05$) and a significant effect of pre/post-implant (t
375 $= 2.87$, $P < 0.01$) on prolactin concentrations. Prolactin concentrations were 12.8 ± 25.7
376 ng/ml (mean ± SD) lower at the time of the post-implant blood sample compared to the pre-
377 implant sample. There was no significant effect of the interaction between sex and pre/post-
378 implant ($t = 1.56$, $P = 0.13$).

379 For the comparisons of post-implant corticosterone and prolactin concentrations of sham and
380 control individuals the models with lowest AICc were the null models (Figs. 3a and 3b; see
381 supplementary data, Tables S7 and S8 respectively), suggesting that there was no empirical
382 support for a relationship between hormone concentrations and any of the variables
383 considered.

384

385 ## 4 Discussion

386 High corticosterone concentrations, as seen during chronically stressful conditions, have been
387 associated with reductions in prolactin concentrations and body condition (e.g. Angelier and
388 Chastel, 2009), and decreased reproduction (e.g. Thierry et al., 2013) and survival (e.g.
389 Crespi et al., 2013). Our results support these findings in part, with the experimental elevation
390 of corticosterone being associated with a decrease in breeding success. However, we found
391 no evidence for an effect on circulating prolactin or body mass after eight days of treatment.

392 The fact that there was no significant difference in corticosterone concentrations at implant
393 removal compared to implantation, yet there was a marginally significant difference in
394 overall breeding success between the treatment groups, suggests that corticosterone had been
395 elevated, albeit for a shorter duration than we expected. Because our results show a negative
396 effect of treatment on breeding success, it is unlikely that endogenous corticosterone was
397 blocked, as has been found in other studies (e.g. Goutte et al., 2011). However, it is possible
398 that, after implantation, endogenous corticosterone dropped precipitously, impacting an

individual's ability to maintain homeostatic functioning by down-regulating the glucocorticoid stress response (reviewed in Busch and Hayward, 2009). Such an inhibition of the stress response could have resulted in the observed reductions in breeding success later in the season. Sustained low and medium doses of corticosterone were successfully administered to white throated sparrows using Alzet® osmotic pumps (Horton et al., 2007); however, in both cases this initially resulted in elevated circulating concentrations followed by a decline. A similar pattern of change could have occurred in our experiment. We were unable to monitor change in corticosterone during the experiment by recapturing individuals repeatedly as this would have jeopardised recapturing birds after eight days to remove implants and also caused additional acute stress, the effect of which could have been indistinguishable from our corticosterone manipulation. Therefore, our conclusions must be made with caution in light of the unknown effects, in terms of duration and magnitude, of our manipulation on corticosterone concentrations.

Schultner et al. (2013a) suggested that energy allocation is more dynamic than previous studies have often assumed, with an initial increase in baseline corticosterone concentrations being related to increases in body condition, used as a proxy for endogenous energy reserves, until a threshold level is reached. A subsequent decline in body mass, below a critical threshold level, may cause breeding failure, as documented in Arctic terns *Sterna paradisaea* facing natural chronic stress (Monaghan et al., 1992). Thierry et al. (2013) found that poor weather conditions caused greater nest desertion in corticosterone treated birds. It is possible that we manipulated corticosterone concentrations within the range of values that maintained body mass rather than inducing a decline and that some further physiological or environmental stress, after our corticosterone treatment had finished, may have caused our corticosterone-implanted individuals to subsequently decline in body mass and fail their breeding attempt. Our results are also limited by a relatively small sample size, which reduces the likelihood of detecting an effect over and above the natural variations in body mass.

Male and female seabirds have previously been shown to have differing patterns of change in baseline corticosterone concentrations, body condition, provisioning rates and nest attendance during the breeding season (e.g. Lormée et al., 2003, Harding et al., 2004, Leclaire et al. 2011). In our study, females showed higher nest attendance during chick-rearing than males. We predicted that corticosterone treatment would disrupt breeding behaviour and reduce nest attendance; however, we found that whilst this was true for male kittiwakes, the opposite was true for females. Schultner et al. (2013b) recently showed that kittiwakes from an Atlantic

breeding population showed higher nest attendance in response to a short-term increase in corticosterone, which was explained by their faster life-history strategy compared to Pacific breeding populations. However, the sex differences that we found in attendance behaviour could be driven primarily by one or two data points and higher sample sizes would be required for a robust conclusion about within-population sex differences in attendance behaviour.

The lack of a reduction in prolactin concentration after eight days of treatment suggests that corticosterone does not have an immediate effect on prolactin. This is compatible with our results that suggest a delayed effect on breeding success rather than an immediate abandonment of breeding. We speculate that prolactin would have declined following failure, when the stimulatory effects of the nest and chicks would have been removed (Hall and Goldsmith, 1983), and may have declined prior to failure when we were unable to catch kittiwakes. The study of kittiwakes breeding in Svalbard, Norway by Angelier et al. (2009a) showed that when corticosterone was administered using silastic tubes, its concentration was elevated to supra-physiological levels 24 hours later, and whilst this caused a small but significant decline in prolactin concentrations, the decline did not occur until after corticosterone had returned to baseline values. We might have found a similar decline in prolactin had we been able to blood sample individuals at a later date. However, from our results we cannot assume that prolactin concentrations fall as a result of chronically raised corticosterone, or that low prolactin concentrations are necessary to induce breeding failure. Recently, Angelier et al. (2013) showed a lack of a mechanistic link between corticosterone and prolactin after an acute capture-restraint stress response. Our results indicate that the same is true after one week of corticosterone treatment, suggesting that corticosterone and prolactin may be mediating different aspects of the response to environmental perturbation.

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Figure legends

Fig. 1 Timing of experimental procedures and how these correspond to key events within the breeding season are indicated along a timeline of day of year. For events signified by a single point with a vertical hatched line (mean), horizontal lines show the range of the data. For events signified by a grey rectangle between two single points each with vertical hatched lines, the horizontal line joining the two points indicates the duration of time which these events lasted.

Fig. 2 (a) Body mass (mean ± standard error) for kittiwakes given sham implants (Sham: n = 14), corticosterone implants (Cort: n = 12) and no implants (Control: n = 7). Only implanted individuals that were successfully recaptured are included and only samples taken at the time of post-implant are available for control individuals. (b) Nest attendance (proportion of visits; mean ± standard error) during chick-rearing of female (filled bars) and male (open bars) corticosterone- and sham-implanted kittiwakes. (c) Breeding success (mean ± standard error) calculated as number of chicks fledged for kittiwakes given no implants (Control: n = 7), sham implants (Sham: n = 21) and corticosterone implants (Cort: n = 17). Letters above bars indicate statistical significance between groups (mean values of bars with the same letter are not significantly different (a; $P > 0.05$); mean values of bars with different letters are significantly different (b; $P < 0.05$).

Fig. 3 (a) Corticosterone concentration and (b) prolactin concentration (mean ± standard error) for kittiwakes given sham implants (Sham: n = 14), corticosterone implants (Cort: n = 12) and no implants (Control: n = 7) during pre-implant (filled bars) and post-implant (open bars. Only implanted individuals that were successfully recaptured are included and only samples taken at the time of post-implant are available for control individuals.

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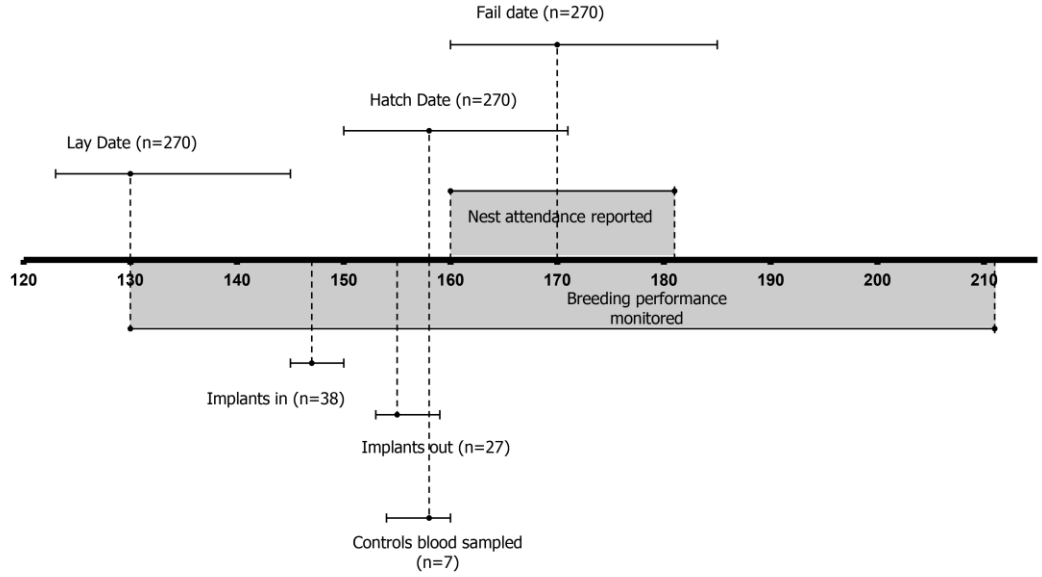

641

642 **Fig. 1.** Timing of experimental procedures and how these correspond to key events within the
643 breeding season are indicated along a timeline of day of year. For events signified by a single
644 point with a vertical hatched line (mean), horizontal lines show the range of the data. For
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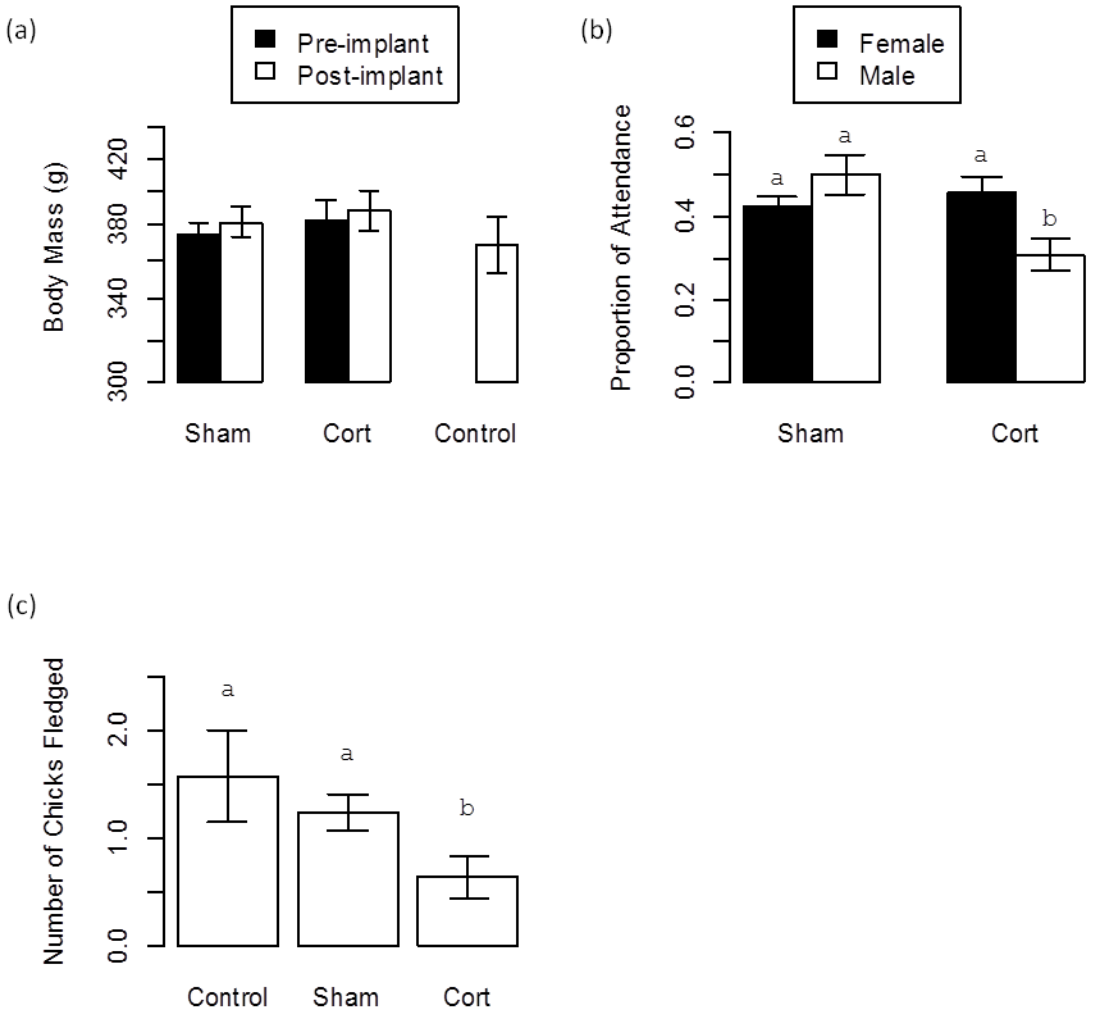

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652 **Fig. 2.** (a) Body mass (mean ± standard error) for kittiwakes given sham implants (sham: n =
653 14), corticosterone implants (cort: n = 12) and no implants (control: n = 7). Only implanted
654 individuals that were successfully recaptured are included and only samples taken at the time
655 of post-implant are available for control individuals. (b) Nest attendance (proportion of visits;
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657 corticosterone- and sham-implanted kittiwakes. (c) Breeding success (mean ± standard error)
658 calculated as number of chicks fledged for kittiwakes given no implants (control: n = 7),
659 sham implants (sham: n = 21) and corticosterone implants (cort: n = 17). Letters above bars
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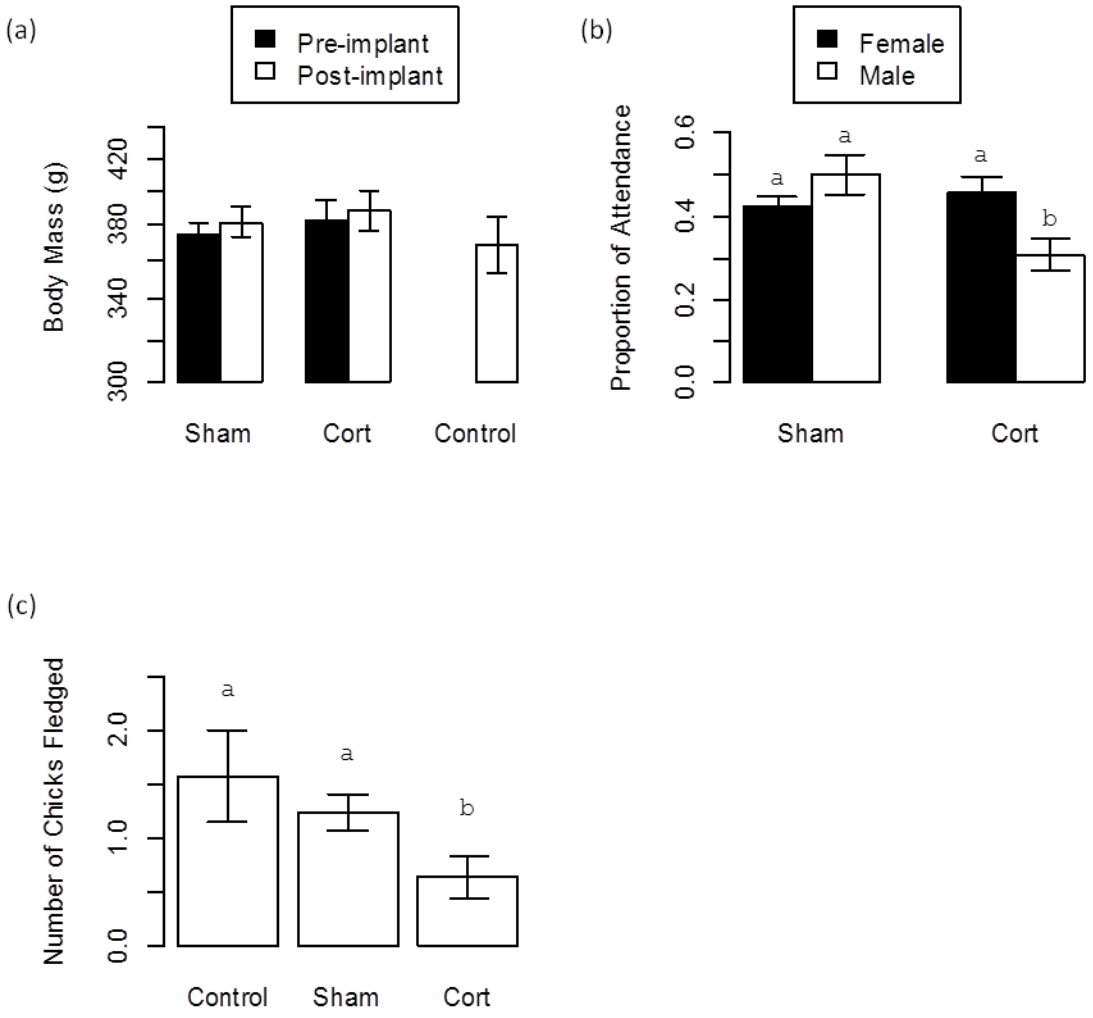

664

665 **Fig. 3.** (a) Corticosterone concentration and (b) prolactin concentration (mean ± standard
666 error) for kittiwakes given sham implants (sham: n = 14), corticosterone implants (cort: n =
667 12) and no implants (control: n = 7) during pre-implant (filled bars) and post-implant (open
668 bars. Only implanted individuals that were successfully recaptured are included and only
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671