



Article (refereed) - postprint

Nelson, Bethany F.; Daunt, Francis; Monaghan, Pat; Wanless, Sarah; Butler, Adam; Heidinger, Britt J.; Newell, Mark; Dawson, Alistair. 2015. **Protracted treatment with corticosterone reduces breeding success in a long-lived bird.**

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

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23 **Abstract**

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

46 **Keywords**

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

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53 **1 Introduction**

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

63 The emergency life-history stage involves the elevation of corticosterone—the main
64 glucocorticoid in birds—in response to the activation of the hypothalamic-pituitary-adrenal
65 axis (Wingfield et al., 1998). Chronic stress results from long-term exposure to a stressor,
66 often resulting in negative fitness consequences such as low productivity, suppressed
67 immunity and inhibited growth, rather than the short-term benefits of the emergency life-
68 history stage, associated with acute stress. During chronic stress the duration of both the
69 stressor and the consequences for an animal's physiology are long-lasting (Boonstra, 2013),
70 resulting in sustained elevations of corticosterone. However, when corticosterone
71 concentrations increase above baseline, the rate of passive clearance increases and active
72 negative feedback reduces endogenous production (Sapolsky et al., 2000, Rich and Romero,
73 2005, Romero et al., 2005). This means that corticosterone is not maintained at stress-induced
74 concentrations for long periods of time and therefore reduces the negative fitness
75 consequences of long-lasting elevated corticosterone (Sapolsky et al., 2000, Romero, 2002).

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

84 Changes in corticosterone also have implications for the body mass of breeders. However,
85 there is no clear prediction as to how chronic stress affects body mass. Correlational studies
86 often show that increases in baseline corticosterone concentrations are associated with
87 declines in body mass or body condition (i.e. size-corrected body mass e.g. Cherel et al.,
88 1988, Cherel et al., 1994, Kitaysky et al., 1999). However, Schultner et al. (2013a) showed
89 that initial increases in baseline corticosterone concentrations were associated with an
90 increase in fat reserves. Corticosterone secretion has also been found to be independent of
91 changes in body condition in some species (e.g. Silverin and Wingfield, 1982, Wingfield et
92 al., 1999, Lormée et al., 2003).

93 Evidence regarding the mechanistic process that modulates the stress response during
94 breeding is currently inconclusive. One potential mediator of the stress response is via
95 changes in prolactin concentrations (Chastel et al., 2005, reviewed in Angelier and Chastel,
96 2009). Prolactin has a wide variety of roles throughout the vertebrates (Norris, 1980). In birds
97 it promotes incubation and parental care, and is secreted in response to long photoperiods and
98 further by the presence of eggs and young in the nest (Dawson and Goldsmith, 1985, El
99 Halawani et al., 1986). Chronic stress is expected to cause declines in reproductive hormones
100 (Sapolsky, 2000) and, therefore, it is possible that corticosterone may disrupt prolactin
101 secretion and reduce breeding behaviour such as nest attendance (Angelier et al., 2009a).

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

116 The kittiwake is a long-lived seabird with a typical bi-parental care system, which has been
117 studied in both the Atlantic and the Pacific with regards to its breeding biology and
118 physiology (e.g. Golet et al., 2004, Angelier et al., 2009a, Goutte et al., 2010, Kitaysky et al.,
119 2010). In this study, we used Alzet® osmotic pumps, which release substances at a constant
120 rate over a number of days, in an attempt to elevate corticosterone concentrations over
121 several days in a North Sea population of black-legged kittiwakes (hereafter ‘kittiwake’). We
122 hypothesised that this protracted elevation of corticosterone via Alzet® osmotic pumps
123 would have a disruptive effect on breeding, specifically causing a short-term reduction in
124 prolactin concentration and body mass, a reduction in nest attendance during the chick-
125 rearing period, and lower breeding success by the end of the season.

126 **2 Methods**

127 **2.1 Nest activity**

128 270 kittiwake nests from 15 different plots on the Isle of May, National Nature Reserve, Firth
129 of Forth, south-east Scotland (56° 11' N, 02° 33' W) were observed daily from laying to
130 fledging in 2011 and for each nest we recorded the lay date, clutch size and either date of
131 failure or number of chicks fledged (breeding success; Fig. 1).

132 **2.2 Blood sampling of kittiwakes**

133 38 kittiwakes (27 females and 11 males) were captured, each from different nests, during late
134 incubation (May 25–May 30; 18 ± 5.4 (mean \pm SD) days after laying; all birds were
135 incubating at the time of initial capture; Fig. 1) using an eight metre long noose pole. A
136 maximum of 1 ml of blood was taken from the wing vein using sterile 1 ml non-heparinised
137 syringes. Samples were centrifuged, once the blood had been allowed to clot, and frozen as
138 separate red blood cells and serum.

139 A dictaphone was used to precisely record the elapsed time between capture (i.e. when the
140 noose was placed over a individual’s head) and the end of blood sampling (individual
141 sampling time), which we aimed to complete within three minutes of capture so that baseline,
142 or near baseline, concentrations of corticosterone would be obtained (Romero and Reed,
143 2005). Individual sampling time was 2.7 ± 0.6 (mean \pm SD) min, with 15 % of samples being
144 obtained within two minutes and 75 % within three minutes. All samples were collected
145 within 5.7 min. We also recorded the elapsed time between the extension of the noose pole
146 towards the target individual and the lifting of the captured individual off its nest (capture

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

154 **2.3 Corticosterone manipulation in kittiwakes**

155 For individuals already carrying a British Trust for Ornithology (BTO) metal ring the unique
156 ring number was recorded and remaining individuals were ringed. Individuals were weighed
157 to the nearest gram using a Pesola. Previous studies have used open-ended silastic tubes or
158 self-degradable pellets to deliver corticosterone. These are unlikely to maintain protracted
159 elevation such as that experienced during adverse environmental conditions (Romero et al.,
160 2005, Newman et al., 2010, Thierry et al., 2013). Instead, we used Alzet® osmotic pumps
161 (length: 3.0 cm; diameter: 0.7 cm; mass: 1.1 g; nominal volume: 200 μ l; delivery period: 14
162 days; rate of delivery: 0.5 μ l.h⁻¹). These were inserted subcutaneously on the flank
163 immediately anterior to the thigh, under local anaesthesia. These osmotic pumps are used
164 widely in a range of studies including pharmacology, biotechnology and immunology ($>$
165 13,500 publications in the Alzet® bibliography) and have been successfully used to
166 administer corticosterone in white throated sparrows *Zonotrichia albicollis* (Horton et al.,
167 2007). A small incision was made using a sterile scalpel blade and this was closed with
168 suture. The pumps contained either corticosterone dissolved in polyethylene glycol 400
169 (PEG) at a concentration of 28 mg.ml⁻¹ (corticosterone-implanted individuals; n = 17) or PEG
170 only (sham-implanted individuals; n = 21). Kittiwakes weigh on average 380 g and therefore
171 two pumps were necessary to deliver the required dose of corticosterone, which we estimated
172 using data from Horton et al. (2007). We matched experimental groups for location to
173 account for potential plot effects. At the time of implantation there were no significant
174 differences between corticosterone-implanted and sham-implanted groups with respect to
175 clutch size (linear model: $t = 1.71$, $df = 36$, $P = 0.10$, $R^2 = 0.07$), lay date (linear model: $t =$
176 0.89 , $df = 36$, $P = 0.38$, $R^2 = 0.02$) or sex ratio (Binomial generalized linear model: $z = 1.23$,
177 $P = 0.22$) Individuals were marked with picric acid on the head or tail feathers to aid
178 identification during attempted recapture.

179 Implanted individuals were recaptured 8 ± 1 (mean \pm SD) days later (26 ± 1 days after laying;
180 40 % of nests had hatched at least one egg by the time of recapture). This interval was chosen
181 to be long enough to maximise recapture probability but to be before all the corticosterone
182 had been delivered. 71 % of implanted individuals were successfully recaptured ($n = 27$; 14
183 sham-implanted individuals; 13 corticosterone-implanted individuals). Recaptured
184 individuals were blood sampled, as described above, weighed and the implants removed
185 using the same technique as for insertion. Examination of all the removed implants indicated
186 that they were empty. At the time of implant removal, an additional seven individuals
187 (controls) that had not been implanted, and whose partners had not been implanted, were
188 captured and blood sampled; these controls enabled us to test for any adverse effects of the
189 initial capture and implantation of the osmotic pumps on the experimental birds. In order to
190 reduce disturbance at the colonies and maximise the chances of recapture, individuals were
191 only captured twice and we did not record nest attendance between initial capture and
192 attempted recapture. The individuals used in this study were not part of any other study and
193 therefore remained relatively undisturbed outside of our experimental protocols, minimising
194 their exposure to acute stress.

195 All work was carried out under Home Office personal licences (Bethany Nelson: PIL
196 60/12426 and Alistair Dawson: PIL 70/1697) and associated project licences (Alistair
197 Dawson: PPL 60/4176 and Francis Daunt: PPL 60/4001) and Scottish Natural Heritage
198 research permit (MON/RP/131).

199 **2.4 Nest attendance**

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

208 **2.5 Molecular sexing**

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

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

216 **2.6 Hormone assays**

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

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

229 **2.7 Statistical methods**

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

239 **2.7.1 Body mass**

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

242 methods for calculating body condition are valid (Green, 2001, Schulte-Hostedde et al., 2005,
243 Schamber et al., 2009). We used linear mixed models; models were fitted by maximum
244 likelihood (ML) for the purposes of calculating AIC values and by REML for the purposes of
245 obtaining parameter estimates and standard errors, and P values were calculated using
246 Markov chain Monte Carlo simulations (Function *pvals.fnc*; Package *languageR* version 1.4).
247 We only included individuals that had been successfully recaptured and therefore had both
248 pre- and post-implant data: including individuals that were only captured once would have
249 biased the pre-implant data because these individuals were probably more susceptible to
250 disturbance. We included individual as a random factor in all models to account for repeated
251 measures. The full model contained fixed effects for treatment (i.e. sham-implanted or
252 corticosterone-implanted), pre/post-implant (i.e. 0 = pre-implant sample; 1 = post-implant
253 sample) and the interaction between these (which was the key variable of interest). The full
254 model also included date relative to lay date (i.e. date of sampling an individual relative to
255 that individual's lay date), because mass tends to decline during the season (e.g. Mrosovsky
256 and Sherry, 1980, Wendeln and Becker, 1999, Moe et al., 2002). Since evidence suggests that
257 daily energy expenditure, which may affect body mass, varies between males and females
258 (higher in female kittiwakes, Fyhn et al., 2001; higher in male kittiwakes, Thomson et al.,
259 1998), we included sex, the interaction between treatment and sex, the interaction between
260 pre/post-implant and sex and the three-way interaction between treatment, pre/post-implant
261 and sex.

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

269 2.7.2 Nest attendance

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

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

282 2.7.3 Breeding success

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

292 2.7.4 Hormone concentrations

293 We found no effect of individual sampling time (linear model: $t = 0.87$, $P = 0.39$), capture
294 time ($t = 0.67$, $P = 0.51$), rank order of capture ($t = 0.31$, $P = 0.76$), an interaction between
295 individual sampling time and capture time ($t = 0.85$, $P = 0.40$) or an interaction between
296 individual sampling time and rank order of capture ($t = 0.24$, $P = 0.81$) on corticosterone
297 concentrations (full model: $F_{5,66} = 0.67$, $R^2 = 0.05$). There was also no effect of total time
298 (total = individual sampling time + capture time; linear model: $t = 1.01$, $P = 0.32$), rank order
299 of capture ($t = 0.79$, $P = 0.43$) or the interaction between total time and rank ($t = 0.76$, $P =$
300 0.45) on corticosterone concentrations (full model: $F_{3,68} = 0.38$, $R^2 = 0.02$). Therefore, we
301 included all samples in our analyses as representative of baseline concentrations.

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

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

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

318

319 **3 Results**

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

324 **3.1 Body mass**

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

330

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

335

336 **3.2 Nest attendance**

337 The best model for nest attendance, according to AIC (see supplementary data, Table S3),
338 contained sex, treatment, the sex-by-treatment interaction and time since implant. The main
339 effect of treatment on the presence of a previously implanted individual at the nest was non-
340 significant ($t = 0.38$, $df = 33$, $P = 0.71$) but there was a significant effect of sex ($t = 2.95$, $df =$
341 33 , $P = 0.01$), with females attending more than males (mean \pm SD percentage of time spent
342 attending nest; females: 45 ± 50 %; males: 37 ± 48 %). There was a significant interaction
343 between treatment and sex ($t = 2.42$, $df = 33$, $P = 0.02$; Fig. 2b), with corticosterone-
344 implanted females attending more than sham-implanted females and corticosterone-implanted
345 males attending less than sham-implanted males. There was also a significant effect of time
346 since implant ($t = 2.19$, $df = 784$, $P = 0.03$) with a 4.1 ± 1.3 % (mean \pm SE) reduction in nest
347 attendance per day since implantation irrespective of whether the individual was
348 corticosterone-implanted or sham-implanted.

349

350 **3.3 Breeding success**

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

361

362 **3.4 Hormone concentrations**

363 For the comparison of corticosterone concentrations of treated and sham individuals the
364 model with lowest AIC (see supplementary data, Table S5) contained sex, which had a
365 marginally significant effect (linear mixed effects model: $t = 1.90$, $P = 0.06$). The
366 corticosterone data were largely sensitive to one value (corticosterone-implanted male; pre-
367 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 \pm 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

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

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

425 Male and female seabirds have previously been shown to have differing patterns of change in
426 baseline corticosterone concentrations, body condition, provisioning rates and nest attendance
427 during the breeding season (e.g. Lormée et al., 2003, Harding et al., 2004, Leclaire et al.
428 2011). In our study, females showed higher nest attendance during chick-rearing than males.
429 We predicted that corticosterone treatment would disrupt breeding behaviour and reduce nest
430 attendance; however, we found that whilst this was true for male kittiwakes, the opposite was
431 true for females. Schultner et al. (2013b) recently showed that kittiwakes from an Atlantic

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

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

456 **Acknowledgements**

457 We would like to thank the Natural Environment Research Council (NERC) for funding this
458 work (NERC Doctoral Training Grant number: NE/H525346/1 and the European Research
459 Council AdG 268926 to Pat Monaghan) and Scottish Natural Heritage (SNH) for access to
460 the island. We would like to thank Hanna Granroth-Wilding and Emi Takahashi for help
461 collecting fledging success data, Annika Telford for assistance in the molecular sexing
462 laboratory, Rebecca Watson for assistance with the EIA assay and Deena Mobbs for help

463 with the figures. We would also like to thank our anonymous reviewers for their helpful
464 comments, which have improved this manuscript.

465 **Figure legends**

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

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

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

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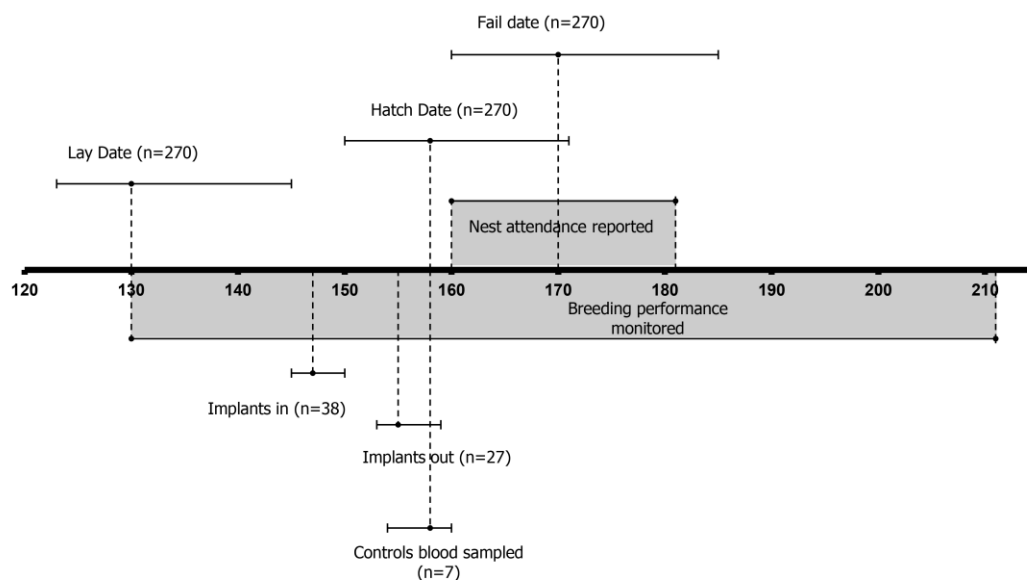
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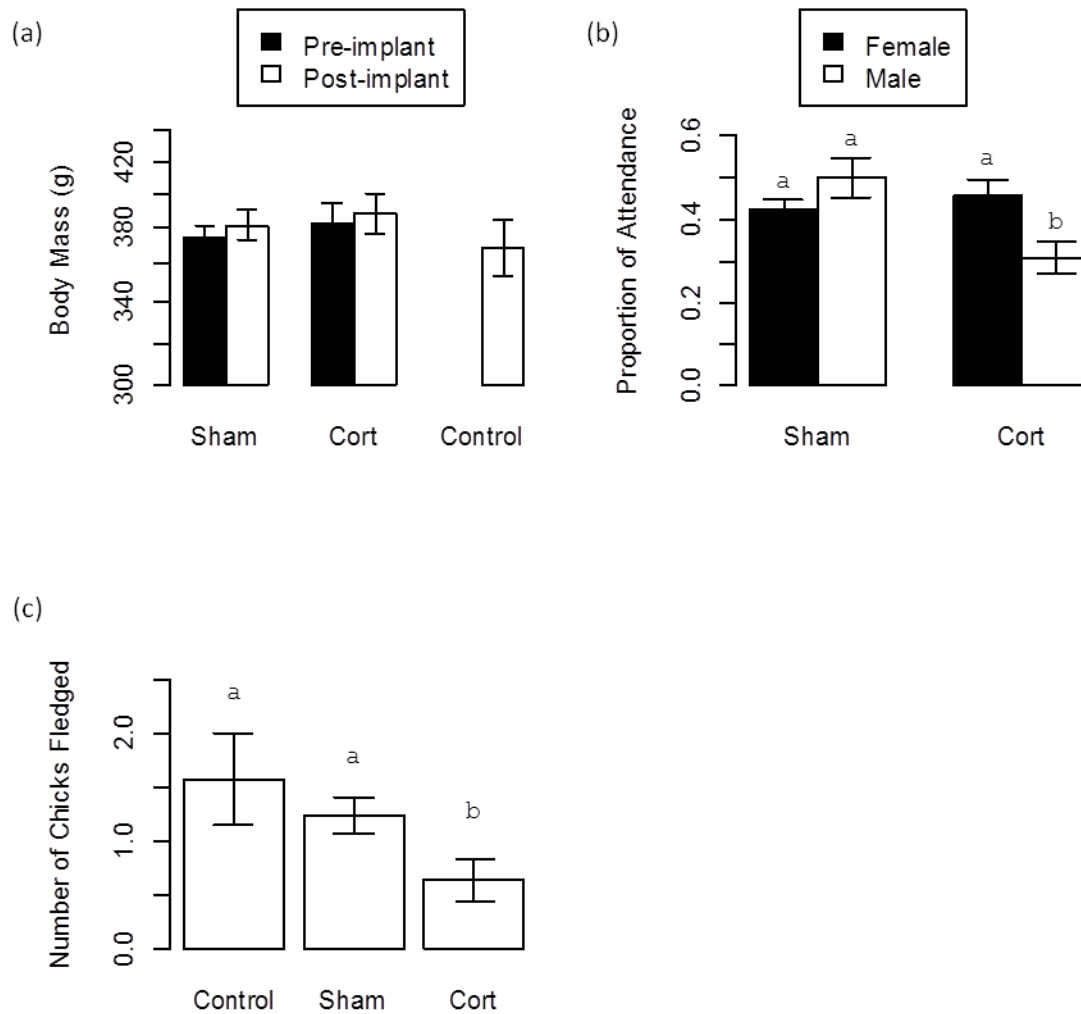
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642 **Fig. 1.** Timing of experimental procedures and how these correspond to key events within the
643 breeding season are indicated along a timeline of day of year. For events signified by a single
644 point with a vertical hatched line (mean), horizontal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

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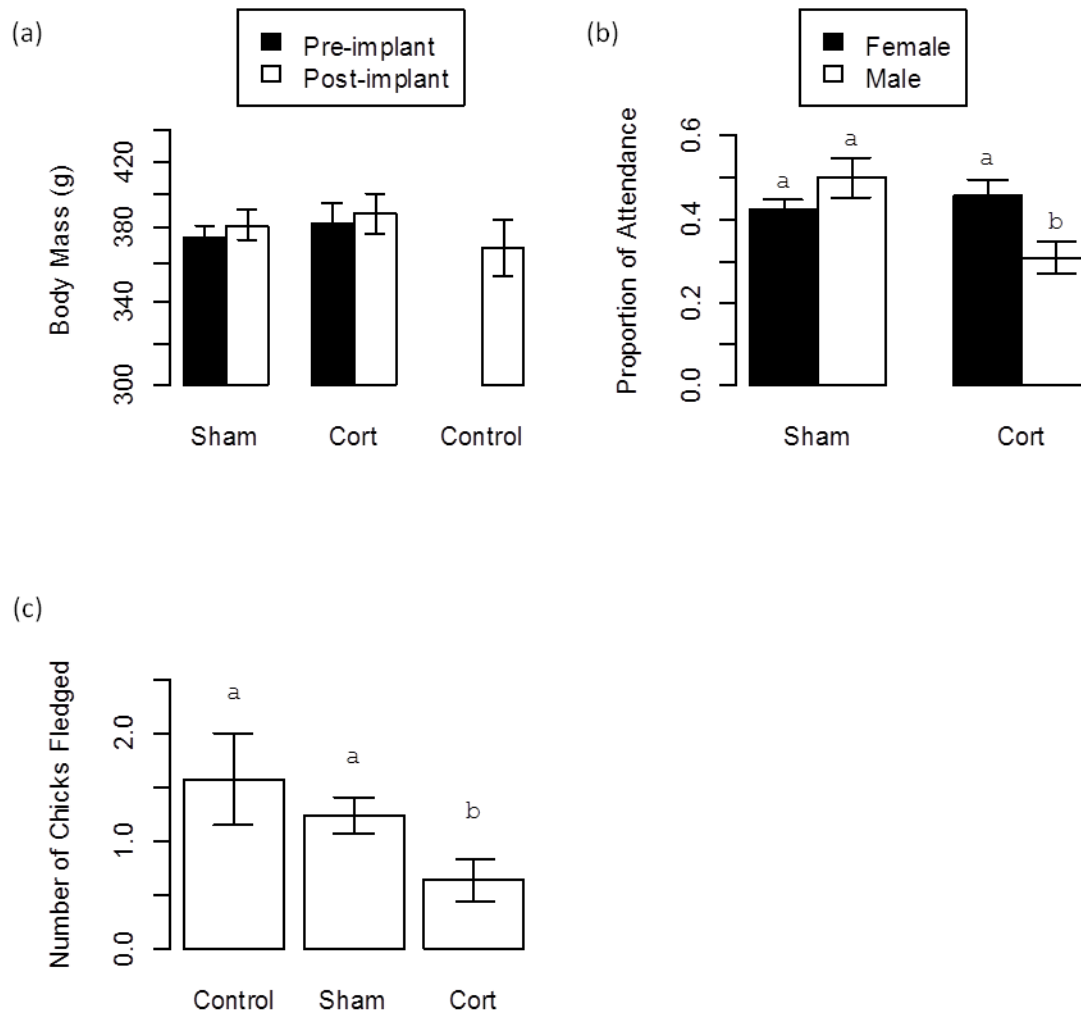
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652 **Fig. 2.** (a) Body mass (mean \pm standard error) for kittiwakes given sham implants (sham: n =
653 14), corticosterone implants (cort: n = 12) and no implants (control: n = 7). Only implanted
654 individuals that were successfully recaptured are included and only samples taken at the time
655 of post-implant are available for control individuals. (b) Nest attendance (proportion of visits;
656 mean \pm standard error) during chick-rearing of female (filled bars) and male (open bars)
657 corticosterone- and sham-implanted kittiwakes. (c) Breeding success (mean \pm 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
660 indicate statistical significance between groups (mean values of bars with the same letter are
661 not significantly different (a; $P > 0.05$); mean values of bars with different letters are
662 significantly different (b; $P < 0.05$).

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664

665 **Fig. 3.** (a) Corticosterone concentration and (b) prolactin concentration (mean \pm 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
 669 samples taken at the time of post-implant are available for control individuals.

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