Corticosterone levels of tufted puffins vary with breeding stage, body condition index, and reproductive performance

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ARTICLE INFO

ABSTRACT

Corticosterone (CORT) levels in free-living animals are seasonally modulated and vary with environmental conditions. Although most studies measure total CORT concentrations, levels of corticosteroid binding globulin (CBG) may also be modulated, thus altering the concentration of CORT available for diffusion into tissues (free CORT). We investigated the seasonal dynamics of CBG, total CORT, and free CORT in breeding tufted puffins (*Fratercula cirrhata*) during 2 years characterized by high rates of nestling growth and survival. We then compared concentrations of total CORT in this population to levels in chick-rearing puffins at another colony during 2 years with low productivity. At the high productivity colony, levels of CBG, total baseline CORT, free baseline CORT, and total maximum CORT were all higher prior to egg-laying than during late incubation and late chick-rearing. Levels of CBG were positively correlated with body condition index (BCI) and free baseline CORT was negatively correlated with BCI. Total baseline levels of CORT during chick-rearing were two to four times higher at the colony with low rates of nestling growth and survival. Our results demonstrate the need for long-term datasets to disentangle seasonal trends in CORT levels from trends driven by changes in environmental conditions. Given the negative effects associated with chronic elevation of CORT, our results indicate the cost of reproduction may be higher during years characterized by low productivity.

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1. Introduction

In birds, activity of the hypothalamus–pituitary–adrenal (HPA) axis is reflective of predictable life history events and unpredictable environmental conditions (Wingfield et al., 1997; Romero, 2002). Activation of the HPA axis results in increased plasma concentrations of corticosterone (CORT), a steroid released by the adrenal cortex, which stimulates mobilization of stored energy reserves and elicits behavioral changes that promote survival (reviewed in Sapolsky et al., 2000). In the marine environment, climate-driven changes in primary productivity affect ecosystem structure and prey available to foraging seabirds. Although reduced prey availability is known to depress the reproductive output of seabirds (e.g. Suryan et al., 2002; Frederiksen et al., 2006), the physiological response of breeding adults to such changes is currently unclear. Baseline levels of CORT have been shown to correlate with reproductive success and body condition in black-legged kittiwakes (*Rissa tridactyla*; Kitaysky et al., 1999; Buck et al., 2007) as well as with food availability in common murres (*Uria aalga*; Kitaysky et al., 2007). Kitaysky et al. (2007) also found that maximum stress-

induced levels of CORT in murres were correlated with food availability in the recent past (one month prior). Because the reproductive output of seabirds is tightly correlated with prey availability (Suryan et al., 2002; Frederiksen et al., 2006), these studies suggest that access to food may be one of the dominant factors affecting HPA activity in seabirds. However, Lanctot et al. (2003) suggest that CORT levels will reflect forage availability in only some situations.

Activation of the HPA axis is not without costs because chronic elevation of glucocorticoids can have deleterious effects including muscle wasting, impaired cognitive abilities, and compromised immune function (Saino et al., 2003; Sapolsky et al., 2000). The cost of reproduction is generally measured as the effect of current reproductive effort on the probability of future survival and fecundity (e.g. Golet et al., 2004) although other metrics, such as CORT, may be useful if they are correlated with survival and/or future fecundity (e.g. Golet et al., 2004; Kitaysky et al., 2007). Seabirds are relatively long-lived and have low annual fecundity; it is possible that in some species adults are unwilling to endure greater reproductive costs when food availability is low. Under this scenario, consequences of reduced prey availability are borne entirely by their offspring and adults may even elect to forego reproduction entirely rather than experience greater reproductive costs. This appears to be the case for some adult seabirds that seem unwilling to sacrifice body con-

dition to maintain chick provisioning rates when flight costs are experimentally increased (e.g. Saether et al., 1993; Weimerskirch et al., 1999; Duriez et al., 2000). Adults may also avoid the costs associated with chronic elevation of CORT by prioritizing their own maintenance over the survival of their young. If this is the case, then adult CORT levels may not accurately reflect colony productivity.

Most field studies measure total levels of circulating CORT in the blood which includes hormone bound to corticosteroid binding globulin (CBG) as well as an unbound fraction (free CORT). It has been argued that only free hormone is available to tissues and may therefore be a more meaningful measure of physiological stress (Breuner and Orchinik, 2002; Love et al., 2004). CBG binds CORT specifically and reversibly, and therefore functions to transport this insoluble hormone to target tissues. CBG may also serve to buffer target tissues from negative effects of elevated CORT and to slow metabolic clearance rates (reviewed in Breuner and Orchinik, 2002). Circulating levels of CBG may be modulated for other functions; CBG serves as a sex–steroid transporter, may be internalized by some target cells, can possibly activate intracellular second-messenger systems, and functions in hormone delivery to inflammatory sites (reviewed in Breuner and Orchinik, 2002).

The adrenocortical response to environmental perturbations is likely species-specific and the role of CBG in modulating free CORT has only recently been addressed in seabirds (Dempsey, 2006; Shultz and Kitaysky, 2008). Furthermore, plasma CORT and CBG concentrations may be affected by stage of reproduction which could confound use of CORT as a measure of physiological stress (Breuner and Orchinik, 2002; Romero, 2002; Love et al., 2004). In this paper, we investigate total CORT, CBG, and free CORT in free-living tufted puffins (Fratercula cirrhata) during three stages of reproduction: prior to egg-laying, late incubation, and late chick-rearing. We also include body condition index as a covariate in our analyzes to determine whether CORT and CBG vary with endogenous stores of energy. We then compare total baseline and maximum stress-induced CORT levels in chick-rearing adults from a high productivity colony to levels in adults captured at a different colony during years of low productivity. We assume that rates of nestling growth and survival reflect prey availability, as has been shown in other seabirds (e.g. Becker et al., 2007; Thayer and Sydeman, 2007). We predicted that total CORT would be negatively correlated with nestling growth and survival rates, indicating costs associated with changes in environmental conditions were incurred by adults as well as nestlings.

2. Methods

2.1. Study site and species

This study was carried out on East Amatuli Island (EAI) in the Barren Islands group, AK (58°55′N, 152°00′W) in 1996–1997 and on Chiniak Island (CI) in Chiniak Bay on the northeast side of Kodiak Island, AK (57°40′N, 152°20W) in 2004–2005. At both locations, tufted puffins nest colonially in single-pair burrows excavated in the soil. Puffins are monogamous and exhibit bi-parental care with males and females sharing incubation and chick-rearing duties. A single egg is laid between late May and early June, hatching occurs from mid to late July, and chicks fledge between late August and early September. Rates of nestling growth and age at fledge varies widely across years and colonies (Piatt and Kitaysky, 2002), presumably due to differences in prey availability.

2.2. Colony productivity and growth rates

We began collecting data on nestling growth rates and fledging success on 23 July in 1996 (N=73 nestlings monitored), 28 July in 1997 (N=57), 24 July in 2004 (N=34), and 22 July in 2005 (N=44). Nestlings that could not be reached through the entrance were accessed using holes excavated in previous years and sealed with either plywood or flat rocks. We weighed chicks using spring scales (±2 g) and measured flattened wing-length from the wrist to the wingtip (±1 mm) every 4–5 days throughout the nestling period. The final day we examined burrows for the presence of a chick and weighed chicks still remaining in the nest was between 10 and 12 September in all years. When hatch date was unknown, we estimated age using a wing-length vs. age regression derived from known-age nestlings on CI. We

calculated growth rate for each nestling as the slope of the linear regression equation relating mass and age between ages 10 and 30 days, the near-linear portion of the growth curve (Gjerdrum et al., 2003). Data from known-age nestlings were lacking for EAI; therefore, we estimated age of all EAI chicks based on the CI regression. Because of the lower growth rates on EAI (see Section 3), nestling age may have been underestimated for this island. However, expanding or contracting the age-range used in our analysis had little effect on estimates of growth rates for EAI chicks, indicating our analysis was robust against slight bias in age estimates. We defined fledging success as the number of chicks reaching a minimum wing-length of 130 mm per egg hatched (Gjerdrum et al., 2003) and assumed that chicks died if they disappeared before attaining this minimum.

2.3. Capture and blood sampling

All birds were captured between 10:00 and 17:00. At EAI, we captured adults by hand in their burrows during early chick-rearing (1996: 28 July; 1997: 1 August) and determined breeding status at the time of captures; only actively breeding birds were sampled. At CI, we captured adults during four time-periods: prior to egg-laying (22 May–2 June), late incubation (1–11 July), early chick-rearing (2005 only; 4–12 August), and late chick-rearing (23 August–4 September). During egg-laying and incubation, puffins were captured with a 7 × 10 m net draped over a cluster of 20–30 burrow entrances. We could not verify the nesting status of most individuals captured using this method and it is possible that some "pre-lay" birds had commenced incubation and that some "incubating" birds were brooding very young nestlings. All birds captured during incubation had a brood patch and most burrows were active indicating we likely caught very few non-breeders. During early and late chick-rearing, adults were captured by hand in their burrows. We captured most adults in late chick-rearing immediately after they delivered bill-loads of food to their young. If an adult was captured more than once, we included only data from the first capture in our analysis to ensure all observations were independent.

We obtained blood samples by puncturing either the alar or tarsal vein with a 23-guage needle and collected blood in heparinized capillary tubes. An initial blood sample was taken within 3 min of reaching into burrows or capture in a net, before circulating levels of CORT increase in response to handling induced stress (Romero and Reed, 2005). We did not find a significant relationship between time after capture and CORT concentrations within 0–3 min, confirming our assumption that these samples are indicative of baseline levels. Birds were restrained in cloth bags and additional samples were collected at 10, 30, and 50 min. We defined the maximum stress-induced CORT level as the highest concentration measured across the time-series of samples (following Kitaysky et al., 2007). Only baseline samples were obtained from birds captured during early chick-rearing on CI. Blood was transferred immediately into 0.5 or 1.5 ml microcentrifuge tubes which were stored on ice for several hours until they were centrifuged and separated. Plasma and blood cells were stored frozen (−70 °C) until laboratory analyzes were performed. We extracted DNA from blood cells using a DNeasy tissue kit (QIAGEN Inc., Valentia, CA) and determined sex according to the methods of Griffiths et al. (1998). We did not determine sexes of puffins captured on EAI.

At CI, we measured adult wing-chord length using a ruler (±1 mm), and bill and straight tarsus lengths using dial calipers (±0.1 mm). Body mass was determined using a spring scale (±2 g). We regressed mass against the first principle component (PC1) of a principle component analysis on the three morphometric measures and used the output residuals as a body condition index (BCI; see details in Williams et al., 2007). We calculated BCI separately for each sex because the relationship between mass and structural size is sex-specific in this species. The relationship between mass and PC1 was determined for each sex using datasets consisting of 69 males and 107 females (Williams et al., 2007). Puffins in the present study are a subset of the birds captured and analyzed for body condition index in the study of Williams et al. (2007). Puffins captured at EAI were not sexed and BCI was not calculated.

2.4. Laboratory techniques

For each plasma sample, we determined CORT concentration in duplicate following extraction in dichloromethane using direct radioimmunoassay as detailed in Wingfield et al. (1992). We determined recovery values and used these to adjust final assayed concentrations of CORT. Mean recovery was 92 ± 7% (SD). Four assays were conducted; inter-assay variation was 7% and intra-assay variation ranged between 2% and 3%. For puffins captured on CI in 2005 and 2006, we randomly selected between 8 and 12 plasma samples from each stage of reproduction (excluding early chick-rearing) in each year and determined CBG concentrations as described in Love et al. (2004). We determined optimal incubation time (2 h), final plasma concentration (1:450) and CORT affinity (K_d = 2.21 ± 0.257). Exogenous CORT was stripped from plasma by incubating each 10 μl sample with 20 μl dextran-coated charcoal solution for 20 min at room temperature prior to centrifugation at 4000 rpm for 10 min at 4 °C. We analyzed samples for total binding in triplicate and simultaneously determined non-specific binding in duplicate using unlabeled CORT. Following incubation, we separated bound and free radioligand using rapid vacuum filtration (Brandel Harvester) over glass fiber filters (Whatman GF/B, soaked in 25 mM Tris with 0.3% PEI for 1 h before filtering). All plasma samples used for determination of CBG concentrations were obtained from blood

samples collected within 3 min of capture. Free CORT was estimated using the equation of Barsano and Baumann (1989). For CBG analysis, all samples were run in a single assay; intra-assay variation was 6%.

2.5. Statistical analyzes

We performed all statistical analyzes using SAS 9.1 (SAS Institute 2006). We tested for differences in nestling mortality between all 4 years (all pairwise comparisons) using continuity-adjusted chi-squared tests with alpha adjusted to 0.0083 using a Bonferonni correction. We compared rates of nestling growth between all 4 years using ANOVA followed by post-hoc Tukey HSD tests. Because of the large number of chicks still remaining in their nests on the day of final nest checks in 1996 and 1997 at EAI (see Section 3), we compare fledging masses between years only at CI (using a Student's t-test).

For puffins captured on CI, we investigated the seasonal dynamics of total baseline CORT, free baseline CORT, CBG capacity, and total maximum CORT using four separate ANCOVAs. We log-transformed total baseline CORT, total maximum CORT, free baseline CORT, and CBG capacity to meet the assumptions of normality and homoscedasticity required for parametric tests. One individual from late chick-rearing in 2004 was an outlier in terms of low levels of CBG (101.5 nM) and extremely high levels of free CORT (3.2 ng/ml). For this reason, we excluded this individual from analyzes of CBG and free CORT; exclusion of this individual did not affect the results of statistical tests. We included the main effects year, sex, and stage of reproduction along with body condition index as a continuous covariate. The initial global model included all two-way interactions; interaction terms were subsequently dropped when $P > 0.10$. Similar to previous seabird studies (Buck et al., 2007; Kitaysky et al., 2007), we found no evidence for an effect of sex on free CORT, total CORT, or CBG, and therefore we subsequently excluded sex from all models. Significant stage of reproduction effects were examined using post-hoc Tukey HSD tests.

We compared total baseline CORT in adults captured during early chick-rearing to adults captured in late chick-rearing at CI in 2005 using a Mann–Whitney U test. We compared chick-rearing levels of total baseline and total maximum CORT between all 4 years using Kruskal–Wallis tests (because variance was unequal between years), followed by Tukey HSD tests. For this analysis, adults were sampled during late chick-rearing at CI and early chick-rearing at EAI. However, because baseline CORT levels were not significantly different between early vs. late chick-rearing birds at CI in 2005 (see Section 3) we assumed that observed differences were not a function of stage of chick-rearing.

3. Results

3.1. Colony productivity and growth rates

Fledging was delayed at EAI relative to CI. At EAI, 25% (18 of 73) and 39% (22 of 57) of the study chicks remained in the nest on the final day of monitoring in 1996 and 1997, respectively. In contrast, only 3% (1 of 34) and 5% (2 of 44) of chicks were still in their nests on the final day of monitoring at CI in 2004 and 2005. At EAI, 63% (46 of 73) and 30% (17 of 57) of the monitored nestlings were either found dead or were presumed to have died (disappeared from nest when wing-chord <130 mm) in 1996 and 1997, respectively. Mortality was lower on CI in 2004 (12%; 4 of 34) and in 2005 (5%; 2 of 44), although the difference between 2004 on CI and 1997 on EAI was not significant ($X^2 = 2.96$, $p = 0.09$). Mortality rates did not differ ($p = 0.45$) between years on CI, but on EAI mortality was significantly higher ($p < 0.0003$) in 1996 than in 1997.

The mean ($\pm$SE) rate of mass increase during the linear growth phase was 4.4 ± 0.4 g/day ($N = 59$) in 1996, 8.7 ± 0.6 g/day ($N = 48$) in 1997, 14.3 ± 0.5 g/day ($N = 28$) in 2004, and 16.2 ± 0.5 g/day ($N = 41$) in 2005. Growth rates of puffin nestlings were significantly different between years ($F_{3,174} = 121.18$, $p < 0.0001$). Post-hoc Tukey tests indicated growth was significantly ($p < 0.05$) more rapid in 2004 and 2005 than in 1996 and 1997; growth in 1997 was also more rapid than in 1996. Although growth rates at CI did not differ between 2004 and 2005, nestlings obtained a higher peak mass and had lower levels of mass recession in 2005 which ultimately led to a higher mass at fledging in that year (2004: 467.3 ± 11.9 g, $N = 28$; 2005: 563.6 ± 8.2 g, $N = 38$; $t = -6.87$, $p < 0.0001$; Fig. 1).

3.2. CBG and total and free corticosterone

For CI adults, total baseline CORT was significantly affected by stage of reproduction ($F_{2,94} = 22.28$, $p < 0.0001$), but not by BCI ($F_{1,94} = 3.35$, $p = 0.07$), or by year ($F_{1,94} = 0.76$, $p = 0.38$). Total baseline CORT was significantly higher prior to egg-laying compared to late incubation and late chick-rearing (Tukey HSD test, $p < 0.05$, Fig. 2a). CBG capacity was significantly affected by stage of reproduction ($F_{2,56} = 11.31$, $p < 0.0001$) and was positively affected by BCI ($F_{1,56} = 7.51$, $p = 0.008$); the effect of year was not significant ($F_{1,56} = 0.82$, $p = 0.37$). Similar to total baseline CORT, CBG capacity was higher during pre-lay compared to late incubation and late chick-rearing (Tukey HSD test, $p < 0.05$, Fig. 2b). Free baseline CORT was also significantly affected by stage of reproduction ($F_{2,54} = 9.74$, $p < 0.001$), and was negatively affected by BCI ($F_{1,54} = 12.13$,
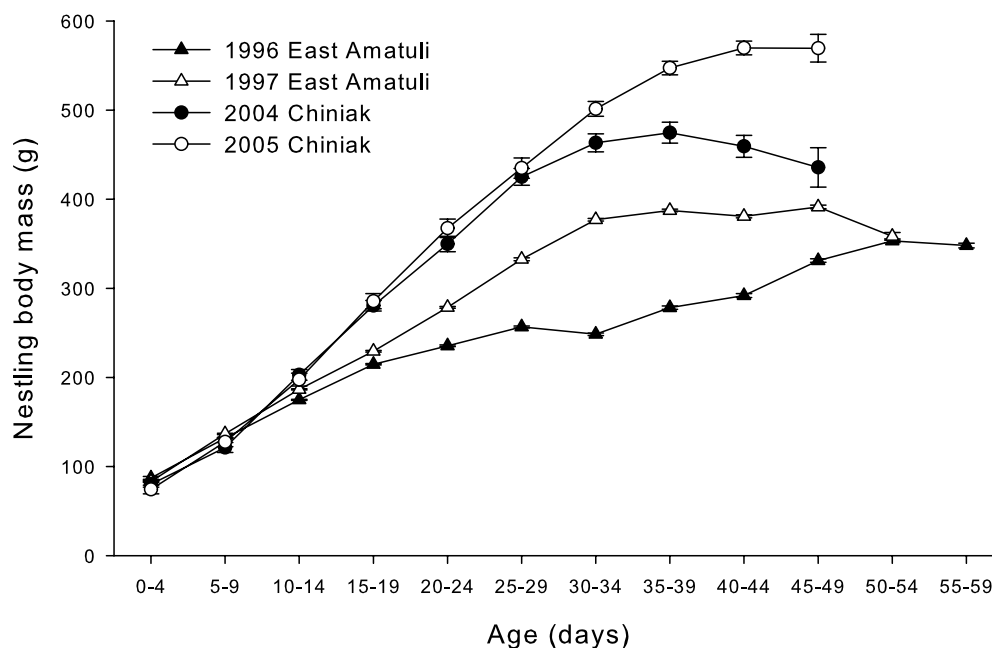

Fig. 1. Mean mass ($\pm$SE) of nestlings in 5-day age-bins at East Amatuli Island in 1996 ($n = 73$) and 1997 ($n = 58$), and at Chiniak Island in 2004 ($n = 34$) and 2005 ($n = 43$). Sample sizes in each age-bin averaged 34 (range 10–55) in 1996, 33 (9–47) in 1997, 27 (13–34) in 2004 and 34 (12–43) in 2005.
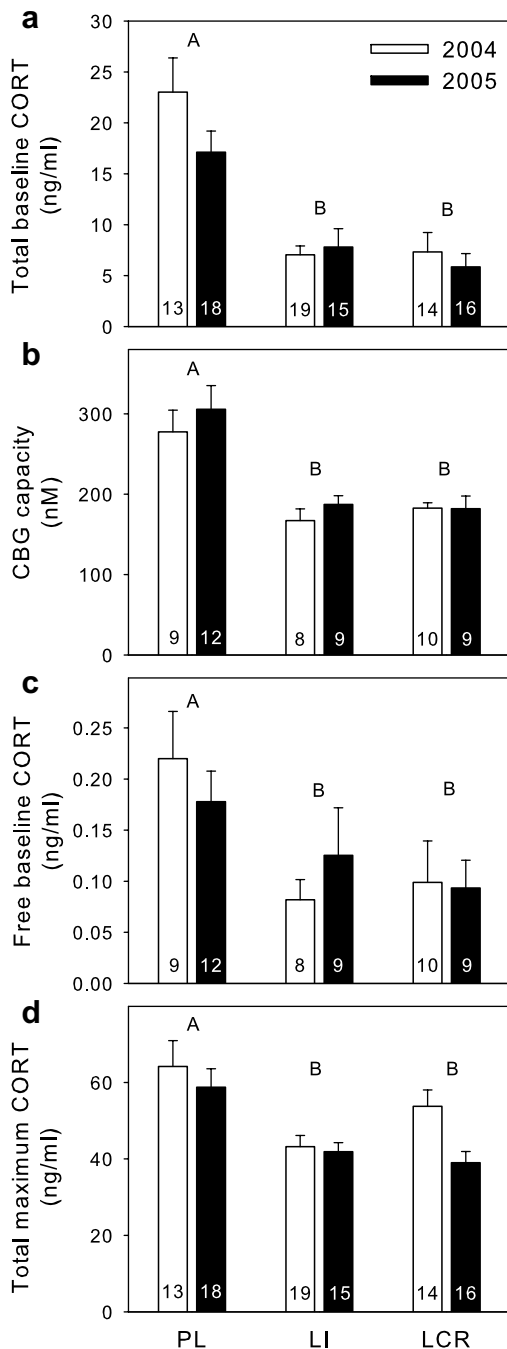
Fig. 2. Plasma levels (±SE) of (a) total baseline CORT, (b) CBG, (c) free baseline CORT, and (d) total maximum CORT in tufted puffins captured at Chiniak Island in 2004 and 2005 during three breeding stages: prior to egg-laying (PL), late incubation (LI), and late chick-rearing (LCR). Sample sizes for each year and breeding stage are denoted on the bars. Different letters above the bars designate significant differences ($p < 0.05$) between breeding stages. One outlier is excluded from CBG and free baseline CORT (see Section 2).

$p = 0.001$); there was no significant year effect ($F_{1,54} = 0.04$, $p = 0.83$). Tukey HSD test revealed that free baseline CORT was significantly higher prior to egg-laying than during late incubation or late chick-rearing ($p < 0.05$, Fig. 2c). Capture and handling protocol resulted in a robust stress response during all time-periods (Fig. 3). Total maximum CORT was significantly affected by stage of reproduction ($F_{2,94} = 10.61$, $p < 0.0001$). The effect of year on maximum CORT was also significant ($F_{1,94} = 5.44$, $p = 0.02$) whereas the effect of BCI was not ($F_{1,94} = 0.22$, $p = 0.64$). Post-hoc Tukey tests revealed pre-lay puf-

fins had higher maximum CORT than birds captured in late incubation or late chick-rearing ($p < 0.05$; Fig. 2d). Total maximum CORT appeared to be elevated during the chick-rearing period of 2004.

At CI in 2005, total baseline CORT in early chick-rearing (2.7 ± 0.7 ng/ml (SE), $N = 6$) was not significantly different from levels measured in late chick-rearing (6.1 ± 1.5 ng/ml, $N = 16$; $U = 51$, $p = 0.2$). Total Baseline CORT during chick-rearing was significantly different between the 4 years ($F_{3,41} = 10.34$, $p < 0.0001$). Post-hoc Tukey tests revealed that total baseline CORT levels from EAI in 1996 and 1997 were significantly higher than levels from CI in 2004 and 2005 ($p < 0.05$; Fig. 4). Total maximum CORT during chick-rearing differed significantly between the 4 years ($F_{3,40} = 6.36$, $p = 0.001$). Tukey tests revealed that total maximum CORT levels in 1997 and 2004 were significantly higher than in 2005 ($p < 0.05$; Fig. 4). Levels of total baseline CORT were higher in the 2 years characterized by low rates of nestling growth, but there was no clear relationship between total maximum CORT and chick growth (Fig. 4).

4. Discussion

We examined whether total CORT, free CORT, and/or CBG were correlated with stage of reproduction, body condition index, or colony productivity in a relatively long-lived seabird, the tufted puffin. At CI, tufted puffins exhibited baseline levels of total CORT that were more than 2.5-times higher prior to egg-laying compared to late incubation and late chick-rearing. The observed pattern for CBG mirrored that of total CORT, yet free CORT was also significantly elevated during the pre-lay period. Total maximum CORT was elevated prior to egg-laying compared to late incubation and late chick-rearing but this seasonal change was less pronounced compared to total baseline levels. Although plasma CORT levels of adult puffins from CI suggest a stage-specific trend, CORT levels from chick-rearing birds captured at EAI during 2 years characterized by low rates of nestling growth and survival indicate that high baseline CORT levels can also occur during chick-rearing. Long-term datasets are needed to establish whether CORT and CBG levels are seasonally modulated or simply reflect changes in environmental conditions. Assuming chronic elevation of CORT is costly in terms of survival probability and/or future reproductive output, the elevated total baseline CORT levels observed in chick-rearing birds during the low-productivity years (at EAI) are consistent with the hypothesis that the cost of reproduction varies with environmental conditions.

4.1. Seasonal variation in CORT and CBG: endogenous or exogenous effects?

The seasonal dynamics of CBG and total and free CORT in tufted puffins was similar between the 2 years at CI, yet it is not clear if this is indicative of an endogenous seasonal rhythm. For other seabird species, the data are sparse and equivocal. In a 5-year study of common murres, Kitaysky et al. (2007) found no consistent seasonal patterns for total baseline or total maximum CORT. During a 1-year study of red-footed boobies (*Sula sula*), Lormée et al. (2003) found that total baseline CORT levels were lower during pre-lay than during incubation and chick-rearing. No consistent differences were found in total CORT, free CORT, or CBG levels between incubation and chick-rearing stages for either black-legged kittiwakes (Shultz and Kitaysky, 2008) or red-legged kittiwakes (*Rissa brevirostris*; Dempsey, 2006). Additionally, total baseline and maximum CORT levels during the chick-rearing period at our low productivity site were comparable to levels observed prior to egg-laying at our high productivity colony. Assuming low productivity was driven by low food availability, as is typical for puffin species (Barrett, 2002; Durant et al., 2006; Frederiksen et al., 2006), our results indicate that a shortage of food during chick-rearing could mask an
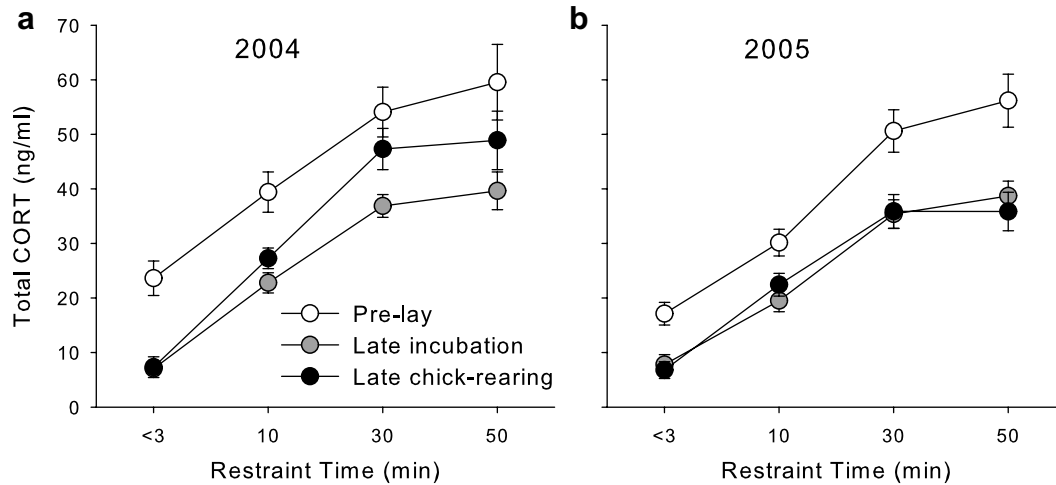
Fig. 3. Seasonal pattern in stress-induced levels of CORT (mean±SE) of adult tufted puffins captured in (a) 2004 and (b) 2005 during three breeding stages: pre-lay, late incubation, and late chick-rearing.

endogenous seasonal rhythm in total CORT, if one exists. Alternatively, factors other than food availability may have differed between the two colonies in this study.

It is also possible that low food availability during the pre-lay period in 2004 and 2005 was the proximate driver of elevated levels of total CORT we observed prior to egg-laying. Assuming that limited food availability elicited elevated CORT during pre-lay, the physiological basis for simultaneously increasing plasma CBG levels is not apparent. However, CBG is not solely a glucocorticoid transporter; CBG also functions as a sex–steroid transporter, is thought to be internalized in some cells, and may activate intracellular second-messenger systems (reviewed in Breuner and Orchinik, 2002). CBG is also cleaved by activated neutrophils, releasing CORT at sites of inflammation (Pemberton et al., 1988) and elevated CBG may therefore be useful in providing a greater plasma reservoir of

CORT. Given the multiple roles of CBG, long-term studies and captive experiments are needed to disentangle ultimate rhythms in CBG and CORT from effects of proximate factors.

Love et al. (2004) suggested that increased free baseline CORT during the chick-rearing phase in starlings may be adaptive and necessary to increase the foraging activity of parents so they can meet the energetic demands of chicks. In contrast, levels of total and free baseline CORT in chick-rearing puffins from CI are the same as birds captured during incubation even though BCI declines between late incubation and late chick-rearing (Williams et al., 2007). Alcids typically lose body mass during reproduction and this mass loss is thought to be primarily due to depletion of lipid stores (e.g. Niizuma et al., 2002). It has been suggested that loss of body mass during chick-rearing may be an intrinsic process in seabirds (Jones, 1994). Nevertheless, studies indicate that adult body mass is positively correlated with parental provisioning rate and with rates of nestling growth and survival in some species (Weimerskirch et al., 2001; Gaston and Hipfner, 2006; Williams et al., 2007). Ultimately, whether a concomitant increase in CORT is driven by the loss of body mass may depend on the degree of mass loss and depletion of endogenous lipid stores. In fasting penguins, for example, energy is derived primarily from lipid stores and CORT levels remain low and stable until lipids are depleted beyond some threshold level at which point CORT levels and protein catabolism increase (Robin et al., 1998).

Our results indicate mass loss during chick-rearing is not necessarily associated with an increase in total CORT in puffins. We postulate that in low productivity years, food availability is reduced and adults lose more body mass and deplete their lipid reserves, eventually resulting in significantly elevated CORT levels. However, we lack data on adult BCI from EAI so we are unable to determine whether elevated CORT at this location was accompanied by low BCI. Additionally, total and free levels of CORT were elevated during the pre-lay period at CI yet BCI of birds during the pre-lay period was not different from that of incubating adults (Williams et al., 2007). Although free CORT was correlated with BCI, this effect was only present if stage of reproduction was included in the model. This lack of concordance between CORT and BCI across the breeding season suggests a sliding set-point exists for body mass or body condition that depends on the stage of reproduction. That is, birds may be endure a loss in body mass during the energetically demanding chick-rearing period without a concomitant increase in circulating levels of free or total CORT.
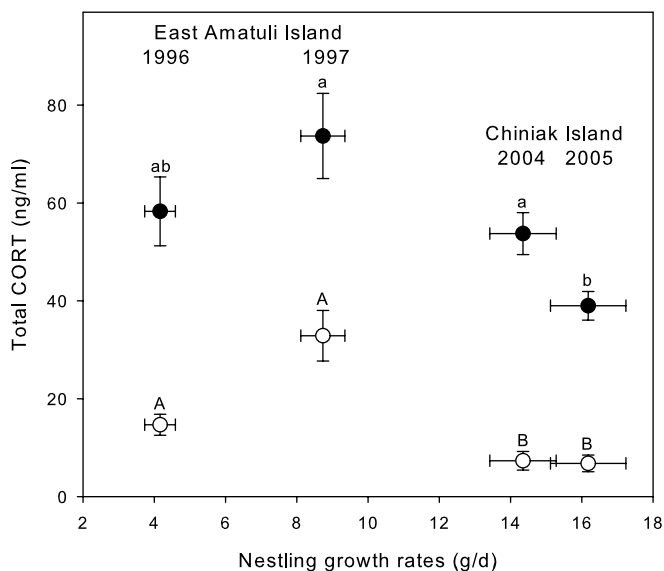

Fig. 4. Mean (±SE) total plasma baseline (open circles) and maximum (filled circles) CORT in chick-rearing tufted puffins vs. mean (±SE) rates of nestling growth. Data are from East Amatuli Island in 1996 (baseline and maximum CORT: $N=6$) and 1997 (baseline CORT: $N=6$; maximum CORT: $N=5$) and from Chiniak Island in 2004 ($N=14$) and 2005 ($N=16$). Different upper-case letters above circles indicate significant differences ($p<0.05$) in mean total baseline CORT, whereas different lower-case letters designate significant differences in mean total maximum CORT.

4.2. Free vs. total CORT

The free hormone hypothesis posits that only unbound CORT is available to tissues (Mendel, 1989) and may therefore be a more valid measure of physiological stress (Breuner and Orchinik, 2002). For instance, Love et al. (2004) found free baseline CORT was higher in European starlings (*Sturnus vulgaris*) that abandoned nests compared to those that did not abandon, yet total baseline and total stress-induced levels showed no such relationship. Although we found no evidence that CBG altered the seasonal dynamics of CORT at CI, we did find a positive relationship between CBG and BCI which resulted in a negative relationship between free CORT and BCI. Assuming our BCI is an accurate measure of stored energy reserves, this result is consistent with studies that found nutritional state affects CBG capacity and consequently free CORT. Rat pups subject to perinatal food restriction (Léonhardt et al., 2002), white-crowned sparrows (*Zonotrichia leucophrys gambelii*) subjected to fasts (Lynn et al., 2003), and red-legged kittiwake chicks subject to a low calorie low-protein diet (Dempsey, 2006) all exhibit reduced CBG levels compared to controls. Ultimately, long-term studies are needed to determine whether free CORT reflects seabird productivity more accurately than does total CORT.

4.3. Elevated CORT and the cost of reproduction

Experimental manipulations of reproductive effort in long-lived seabirds demonstrate a cost of reproduction in terms of adult survival (Golet et al., 1998) and future fecundity (Wernham and Bryant, 1998). Although adult seabirds may buffer nestlings from consequences of reduced prey availability by increasing foraging effort (e.g. Burger and Piatt, 1990), it is currently unclear whether doing so compromises their probability of survival and/or reproduction in future years (i.e. increases their cost of reproduction; Erikstad et al., 1998). Because chronically elevated CORT has negative physiological consequences that may directly affect the probability of survival, it may be correlated with the cost of reproduction. Elevated CORT in murres is correlated with reduced prey availability, a greater probability of skipping breeding in subsequent years, and a lower probability of survival (Kitaysky et al., 2007). Furthermore, experimental elimination of reproductive effort in another long-lived seabird, the black-legged kittiwake, resulted in reduced plasma CORT concentrations and increased survival (Golet et al., 2004). Levels of total CORT in chick-rearing puffins were much higher during years characterized by low rates of nestling growth and survival. However, we were unable to detect a difference in total baseline CORT between the two low productivity years, despite the relatively large differences in nestling growth and survival rates between these years. Though the difference in CORT was non-significant, the result was opposite to what we expected: baseline CORT was lower in the lowest productivity year (1996) than in the year of moderate productivity and chick growth (1997). These results may indicate a non-linear relationship between CORT and productivity. Alternatively, this non-significant trend may be a function of our small sample size for CORT measures in both years at EAI.

Assuming that chronic elevation of CORT negatively affects survival and/or future fecundity, results of our study and previous studies (e.g. Buck et al., 2007; Kitaysky et al., 2007) indicate the cost of reproduction in murres, kittiwakes, and puffins may change with current foraging conditions. In contrast, some species may exhibit a fixed cost of reproduction. Handicapping adult prions and petrels with leg weights results in increased foraging trip duration and decreased chick meal mass, but adult body mass was unaltered (Saether et al., 1993; Weimerskirch et al., 1999; Duriez et al., 2000). However, in the yellow-nosed albatross (Weimerskirch et al., 2001) and the thick-billed murre (*Uria lomvia*; Gaston and Hipfner, 2006) adult body mass is positively correlated with productivity supporting the premise that the cost of reproduction may be higher in low productivity years. We contend that elevated CORT in low productivity years also provides evidence that reproductive costs reflect changing environmental conditions. However, more studies are needed to determine whether CORT and/or body mass (or BCI) are actually indicative of a cost to future reproductive output.

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